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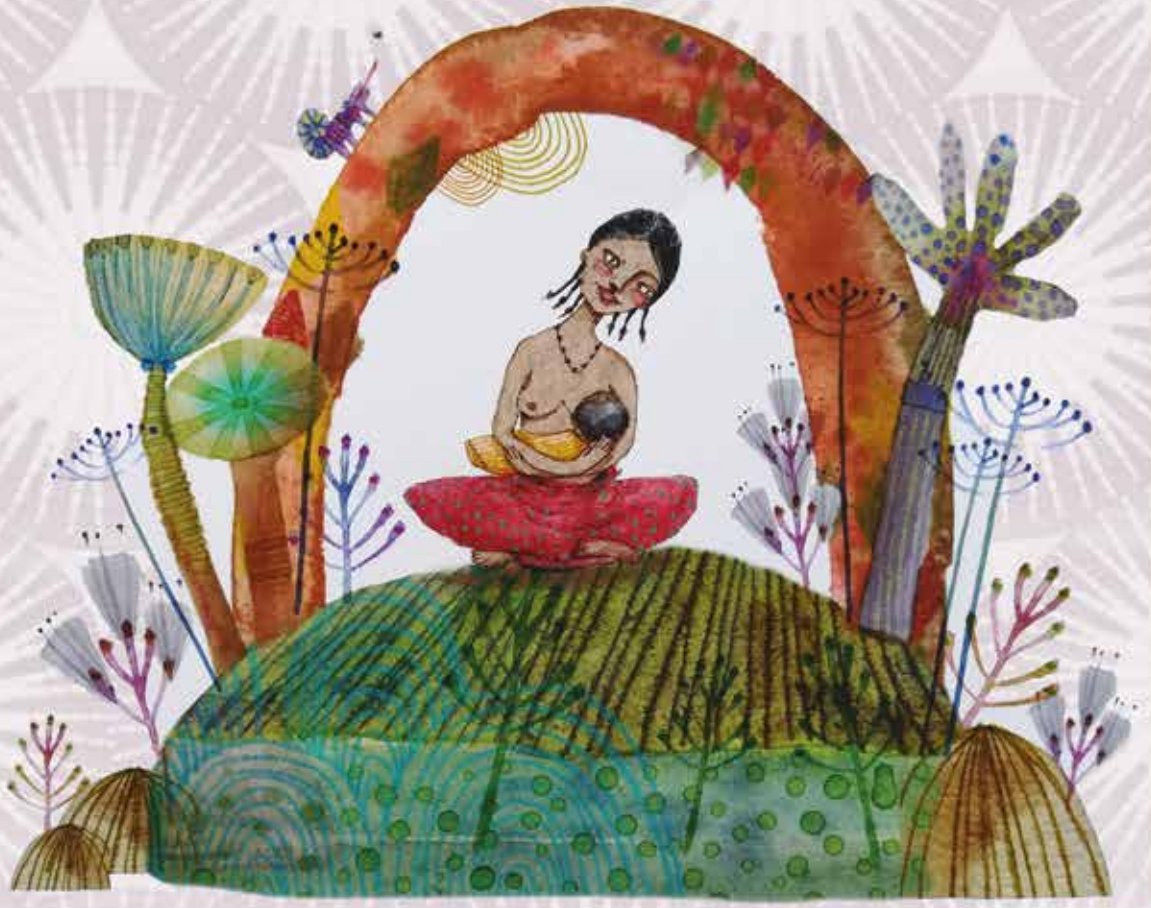

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UNDERSTANDING DIFFERENT OPPORTUNITIES TO PREVENT SEVERE DISEASE AND REDUCE MORTALITY IN AFRICAN INFANTS AND CHILDREN

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OPORTUNIDADES PARA PREVENIR ENFERMEDADES GRAVES Y REDUCIR LA MORTALIDAD EN LACTANTES Y NIÑOS AFRICANOS

Prevención de la transmisión vertical y mejora del diagnóstico de enfermedades que amenazan la vida de los niños

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El Dr Quique Bassat, investigador del Instituto de Salud Global de Barcelona y del Centro de Investigação em Saúde de Manhiça,

hace constar

que la tesis titulada
UNDERSTANDING DIFFERENT OPPORTUNITIES TO PREVENT SEVERE DISEASE AND REDUCE MORTALITY IN AFRICAN INFANTS AND CHILDREN

presentada por María Dolores Madrid Castillo ha sido realizada bajo su dirección, y cumple todos los requisitos que dicta la normativa vigente para la presentación de tesis doctorales como un compendio de artículos en la Facultad de Medicina de la Universitat de Barcelona,

y considera,

que la memoria resultante es apta para optar al grado de Doctor en Medicina con mención Internacional por la Universidad de Barcelona

Y para que quede constancia, firma el presente documento

Quique Bassat
Barcelona, 15 de Enero de 2018

A mis padres, a mis hermanas, a toda mi gente,
por su apoyo continuo e incondicional.

It always seems impossible...
until it is done.

Nelson Mandela

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Authors: Lola Madrid, Rosauro Varo, Quique Bassat.

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Authors: Lola Madrid, Anna C. Seale, Maya Kohli-Lynch, Karen M. Edmond, Joy E. Lawn, Paul T. Heath, Shabir A. Madhi, Carol J. Baker, Linda Bartlett, Clare Cutland, Michael G. Gravett, Margaret Ip, Kirsty Le Doare, Craig E. Rubens, Samir K. Saha, Ajoke Sobanjo-ter Meulen, Johan Vekemans, and Stephanie Schrag; for the Infant GBS Disease Investigator Group.

Published in *Clinical Infectious Diseases,* 2017
2016 Impact factor 8.22 , D1

3. Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique.

Authors: Lola Madrid, Sónia Amós Maculuve, Alba Vilajeliu, Emma Sáez, Sergio Massora, Anelsio Cossa, Rosauro Varo, Antonio Sitoe, Noraida Mosqueda, Rui Anselmo, Khatia Munguambe, Sara M Soto, Cinta Moraleda, Eusebio Macete, Clara Menéndez, Quique Bassat.

Accepted in *Pediatric Infectious Diseases Journal,* 2017
2017 Impact factor 2.49, Q1

4. Congenital and perinatally-acquired infections in resource-constrained settings.

Authors: Lola Madrid, Rosauro Varo, Antonio Sitoe and Quique Bassat.

Published in E*xpert Review of Anti-infective Therapy* 2016
2016 Impact factor 3.14, Q2

5. Congenital cytomegalovirus, parvovirus and enterovirus infection in Mozambican newborns at birth: a cross-sectional survey.

Authors: Lola Madrid, Rosauro Varo, Sonia Maculuve, Tacilta Nhampossa, Carmen Muñoz-Almagro, Enrique Calderón, Cristina Esteva, Carla Carrilho, Mamudo Ismail, Begoña Vieites, Vicente Friaza, María del Carmen Lozano-Dominguez, Clara Menéndez, Quique Bassat.

Accepted in *PLOS One*, 2018
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6. Hypoglycemia and Risk Factors for Death in 13 Years of Pediatric Admissions in Mozambique.

Authors: Lola Madrid, Sozinho Acacio, Tacilta Nhampossa, Miguel Lanaspa, Antonio Sitoe, Sónia Amós Maculuve, Helio Mucavele, Llorenç Quintó, Betuel Sigaúque, and Quique Bassat.

Published in *The American Journal of Tropical Medicine and Hygiene,* 2016
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7. Post-discharge mortality in children admitted to a rural Mozambican hospital: development of a prediction model to identify children at risk of dying.

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Under review

ABBREVIATIONS AND ACRONYMS

- **AECID**: Agencia Española de Cooperación Internacional y Desarrollo
- **ANC**: antenatal care
- **ANISA**: Aetiology of Neonatal Sepsis in South Asia
- **ARR**: annual reduction rate
- **B19V**: parvovirus B19
- **CA**: complete autopsy
- **cCMV**: congenital cytomegalovirus
- **CFR**: case fatality risk
- **CHAMPS**: Mortality Prevention Surveillance Network
- **CHERG**: Child Health Epidemiology Reference Group
- **CISM**: Centro de Investigação em Saúde de Manhiça
- **CMV**: cytomegalovirus
- **CNBS**: Mozambican Ethics Committee
- **CNS**: central nervous system
- **CoD**: cause of death
- **CRVS**: civil registration and vital statistics
- **CYAR**: child years at risk
- **DALYs**: disability adjusted life-years
- **DSS**: Demographic Surveillance System
- **DUC**: dried umbilical cord
- **E. coli**: Escherichia coli
- **EPI**: expanded programme of immunization
- **EOD**: early onset disease
- **EOGBS**: early-onset disease due to GBS
- **EV**: enteroviruses
- **GA**: gestional age
- **GAVI**: Global Vaccine Alliance
- **GBD**: Global Burden Disease
- **GBS**: Group B streptococcus
- **GEMS**: Global Enteric Multicenter Study
- **GPS**: global positioning system
- **HH**: households
- **HIV**: Human immunodeficiency virus
- **IAP**: intrapartum antibiotic prophylaxis
- **ICD**: International classification of Diseases
- **IgG**: immunoglobulin G
- **ISGLOBAL**: Barcelona Institute for Global Health
- **IPTp**: intermittent preventive treatment during pregnancy
- **ISCIII**: Instituto de Salud Carlos III
- **IUGR**: intrauterine growth restriction
- **HAART**: highly active antiretroviral therapy
- **HBV**: hepatitis B
- **HIC**: high income countries
- **HSV**: herpes simplex virus
- **K. Pneumonie**: Klebsiella pneumoniae
- **LB**: live births
- **LBW**: low birth weight
- **LIC**: low income countries
- **LMIC**: lower-middle income countries

- **LOD**: late onset disease
- **LOGBS**: late-onset disease due to GBS
- **LSHTM**: London School of Hygiene and Tropical Medicine
- **MCBIRs**: minimum community-based incidence rates
- **MDH**: Manhiça District Hospital
- **MIA**: minimally invasive autopsy
- **MCV**: measles-containing vaccine
- **MITS**: minimally invasive tissue sampling
- **MTCT**: mother-to-child-transmission
- **MCEE**: Maternal and Child Epidemiology Estimation
- **MDGs**: Millennium Development Goals
- **MR**: mortality rate
- **MSS**: Morbidity Surveillance System
- **NMR**: neonatal mortality rate
- **NDI**: neurodevelopment impairment
- **NPA**: nasopharyngeal aspirate
- **PCR**: polymerase chain reaction
- **PCV**: anti-pneumococcal vaccine
- **PDM**: post-discharge mortality
- **PERCH**: Pneumonia Etiology Research for Child Health
- **Perm-ID**: permanent identification number
- **PPROM**: preterm premature rupture of the membranes
- **PSBI**: possible serious bacterial infection
- **RSV**: respiratory syncytial virus
- **RT-PCR**: real time polymerase chain reaction
- **SA**: South Asia
- **SDGs**: Sustainable Development Goals
- **SEA**: South East Asia
- **SGA**: small-for-gestational-age
- **SSA**: Sub-Saharan Africa
- **S. aureus**: Staphylococcus aureus
- **S. pneumoniae**: Streptococcus pneumoniae
- **T. gondii**: Toxoplasma gondii
- **TB**: Tuberculosis
- **U5**: under five years old
- **U5MR**: under five mortality rate
- **UN**: United Nations
- **UMIC**: upper-middle income countries
- **US**: United States
- **VA**: verbal autopsy
- **VZV**: varicella zoster virus
- **WAZ**: weigh-for-age
- **WHO**: World Health Organization

SUMMARY

Background and rationale

Infant and child mortality have played a crucial role in health transition patterns and both have been considered as good indicators of development and demographic modernization. The establishment of the Millennium Development Goals (MDGs) meant a significant injection of funds and resources for health, and a national and international commitment to child health, which led to an unprecedented progress in the reduction of child mortality worldwide.

From 2000 to 2015, the implementation and scale-up of many life-saving interventions targeting various leading causes of under-5 (U5) deaths as primary accelerants of child survival, such as malaria, pneumonia, diarrhoea, or measles led to a reduction by more than 60% of the overall under-5 mortality rate (U5MR). However, this decrease in child mortality was not enough to achieve the specific target for child survival established by MDGs, namely a two-thirds reduction of U5MR by 2015 in relation to the 1990's figures. A significant barrier for achieving such an ambitious objective relates to the fact that neonatal mortality decreased by a much slower rate than mortality among children aged 1-59 months, especially in low-income countries (LIC) in Sub-Saharan Africa (SSA).
These data are consistent with trends on the main causes of death (CoD) in children over time. Estimates have suggested that those diseases for which life-saving strategies have been implemented (measles, malaria, diarrhoea or pneumonia) have been or are being replaced as main CoD among children U5 by those diseases for which interventions to reduce them have not been fully developed or widely implemented (i.e neonatal deaths).

Neonatal deaths accounted for almost half of all deaths among children U5 in 2016. Within those, preterm birth complications are the leading CoD in the neonatal period, being birth asphyxia and neonatal infections other significant causes of mortality in newborns. However, caution is

required when interpreting data regarding main CoD as data sources used to produce such estimates in poor countries are unreliable, including accurate vital statistics, verbal autopsies or hospital records. Innovative tools such as the minimally invasive autopsy (MIA), currently being explored for mortality surveillance purposes, appear as potentially useful tools that will improve our current understanding of what is really killing neonates or children in LMIC.

In terms of neonatal infections, neonatal sepsis and meningitis are well-known leading causes of mortality and severe sequelae in term and preterm babies in industrialized countries but information about them and their burden in LIC is more limited. Early-onset disease (EOD, 0-6 days), is usually vertically transmitted, and mainly caused by Group B streptococcus (GBS) and Escherichia coli (E. coli). Late-onset disease (LOD, 7-89 days) is generally due to horizontal infections (either community-acquired or hospital-acquired) and most frequently associated pathogens include E. coli, Klebsiella spp, GBS and other Gram-positive organisms (Staphylococcus aureus and Coagulase-negative staphylococci). Maternal colonization by these pathogens is crucial for transmission from mother to child to occur, presenting in such cases generally as EOD. Prevalence of maternal colonization may be affected by Human Immunodeficiency Virus (HIV), maternal nutritional status and other maternal risk factors. Prevention strategies such as maternal screening of GBS at the end of the pregnancy and intrapartum antibiotic prophylaxis (IAP) for those mothers found to be carriers have demonstrated their efficacy in dramatically reducing EOD. However, IAP as we know it is not currently reaching all women in need in poor settings, possibly in relation to the fragility of health systems, precariousness of laboratory infrastructures, or more simply because a significant proportion of deliveries in these settings still occurs at home. In addition, other preventive strategies against E. coli or those bacterial causing LOD have yet to be developed.

Other infectious diseases also considered as important contributors to neonatal, infant and even child morbidity and mortality include infections encompassed under the TORCH syndrome. The HIV pandemic and the Zika virus outbreak have raised great concern across the world and have highlighted the importance of other pathogens for infants, including viruses, parasites or fungi. Screening of some of these diseases such as syphilis or HIV is routinely offered to women attending antenatal care (ANC) clinics

of LIC. However, further screening of other potential vertically transmitted pathogens, such as cytomegalovirus (CMV), rubella, toxoplasma gondii (T. gondii), enterovirus (EV), parvovirus B19 (B19V), herpes simplex virus (HSV) or hepatitis B virus (HBV), among others is not offered. Congenital CMV infection (cCMV) is the most prevalent and principal cause of deafness in developed countries. However, and similarly to other congenital infections, it remains fundamentally unnoticed and therefore largely neglected in resource-constrained settings, thus also hindering the characterization of its real burden and impact in these settings.

An interesting approach to prevent many of these congenital and neonatal infections now includes maternal vaccination during pregnancy under the assumption that maternal transfer of antibodies to the newborn will be more feasible, effective and rapidly protective than waiting for the generation of neonatal immune responses to vaccines administered directly to them. This “vertical vaccination” strategy has already been successfully implemented for tetanus and pertussis control, and is being explored against other pathogens.

There are other well-known causes of severe disease and mortality in children, although not usually listed as direct CoD and therefore often forgotten in global estimates. Malnourished children, neonates born with a low weight or small for their gestational age, may be prone to suffering life-threating conditions or developing significant sequelae. In many cases, the fatal outcome is determined by the underlying condition rather than the acute disease. On the other hand, the emergence of certain complications such as for instance hypoglycaemia, relatively common in the evolution of many different diseases or conditions (malaria, sepsis, diarrhoea cases, malnutrition or neonates in general), may also adversely determine the prognosis of these cases, although the real incidence and significance of such complications remains to be described and addressed as part of their management strategies.

In many LMIC, at least half the child deaths occur at home, often without having been seen by a clinician. In some cases, though, deaths occur also at home, but soon after a contact with the health system. Inpatient mortality is a well-understood portion of the global overall child mortality estimates, and interventions to reduce it have been developed and successfully implemented. However, post-discharge mortality (PDM) could be as

high as inpatient mortality in those constrained-resource settings, but is clearly a neglected and understudied phenomenon. Indeed, no guidelines, recommendations or further strategies have been developed to address the care of patients during the days immediately following discharge, a critical period for child survival. The fragility of the health systems and scarcity of the health force in LIC, the unaffordable high costs to provide care for acute illness and the generalised lack of awareness about the burden and implications of PDM by health workers and policy makers partly explain this neglect.

In order to achieve the new targets for child mortality established by the Sustainable Development Goals (SDGs) by 2030, there is an urgent need to improve our current understanding of the main contributors to child mortality in those countries with higher morbidity and mortality, and as a consequence, to develop and implement interventions to tackle those diseases mainly contributing to premature and preventable mortality of children.

This thesis aims to address issues related with those severe diseases that cause the majority of morbidity and deaths among neonates, infants and children under five and for which no specific interventions have yet been implemented. Most of the studies conforming this thesis have been conducted in Manhiça, a semi-rural area in Southern Mozambique, and a paradigmatic example of a resource-constrained setting.

Materials and Methods

This thesis is based on research conducted by the Barcelona Institute for Global Health (ISGlobal)/ Hospital Clinic-Universitat de Barcelona in Spain, the Centro de Investigação em Saude de Manhica (CISM) in Mozambique. The thesis also includes work done in collaboration with the London School of Hygiene and Tropical Medicine (LSHTM).

This thesis is structured around seven articles: three published in peer reviewed international journals, two accepted but not yet published, and two additional articles currently under review:

i. A comment on the burden and importance of neonatal mortality globally, and discussion on main causes of death among this age group;
ii. A systematic review on the global incidence of infant invasive GBS disease, its case fatality risk (CFR) and the associated serotypes, updating previous global estimates;

iii. A cross-sectional study investigating the epidemiology of GBS and E. Coli infections in Manhiça, semi-rural area from southern Mozambique, so as to determine the prevalence and risk factors for maternal carriage of these pathogens, and acceptability from mothers around the use of rectal swabs for GBS and E. coli detection;
iv. A review of the epidemiology of congenital infectious disease in resource-constrained settings;
v. A hospital based survey to determine the prevalence of vertical transmission of CMV, EV and B19V in Manhiça;
vi. A retrospective data-analysis using the morbidity surveillance system (MSS) ongoing in Manhiça district hospital to describe the prevalence and incidence of hypoglycaemia among admitted Mozambican children during 13 years, its distribution among age groups and associated CFR;
vii. A retrospective data-analysis using hospital data of 16 years linked to the demographic surveillance system (DSS) ongoing in Manhiça district, designed to investigate the burden of paediatric PDM in the area, to identify predictors of mortality following discharge and to derive models that could efficiently stratify children according to PDM risk.

All seven manuscripts have been written by this thesis' candidate as first author, and encompass variable methodologies, including original research, reviews, and meta-analyses.

Key Results

The studies included in this thesis provide important results and highlight knowledge gaps that could contribute to improve our understanding of different diseases causing severe morbidity, sequelae and death among African infants and children and for which specific interventions have not yet been implemented in Mozambique.

The first article reviews the overall burden and significance of mortality during the neonatal period, highlighting the importance of reducing NMR in order to achieve SDGs target for child survival by 2030, as neonatal deaths currently account for nearly half of the global deaths in children U5 years of age. National and international efforts should now focus on this particularly vulnerable population group, so as to achieve universal coverage of those interventions having shown efficacy to reduce neonatal deaths, especially in those countries with high burden in order to achieve the ambitious SDG target for child mortality by 2030 (<25 deaths per 1000 live births).

The meta-analysis and review on *GBS* invasive disease represents an important update of a previous systematic review since it includes new data from low and middle-income contexts (18 new studies from 10 countries). The pooled incidence of invasive GBS disease in infants <90 days was estimated at 0.49 per 1000 live births (95%CI 0.43-0.56), and was highest in Africa (1.12) and lowest in Asia (0.30). EOD incidence was 0.41 (95% CI 0.36-0.47) and LOD incidence was 0.26 (95% CI 0.21-0.30). Overall CFR was 8.4%, being highest in Africa (18.9%, 95% CI 13.7-24.0), a figure four times higher than that of developed countries (4.7%, 3.3-6.1). EOD CFR was 10.0% (95% CI 7.0-12.0) ranging from 5.0% in developed countries to 27.0% in Africa. LOD CFR was 7.0% (95% CI 4.0-9.0) also lower in developed countries (4.0%) compared to Africa (12.0%). Serotype III (61.5%) dominated, with 97% of invasive disease being caused by just five serotypes (Ia, Ib, II, III and V).

The third article focuses on *GBS* and *E. coli* carriage in a semi-rural Mozambican setting. The study found that 21.3% of 320 women recruited in Manhiça were *GBS* carriers, while 16.3% were positive for *E. coli*. Prevalence of HIV among pregnant women participating in the study was 36.6%. No association was found between being colonized by *GBS* and *E. coli* and maternal risk factors. *GBS* isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had immunoglobulin G (IgG) antibodies against *GBS*. *E. coli* isolates showed resistance to ampicillin and co-trimoxazole in 28.9% and 61.3% of the cases, respectively.

The review on the epidemiology of congenital infections in lower-middle and low-income countries covered a period of 45 years and highlighted the concept that the overall burden of congenital infections appears to be much higher in these regions than in industrialized countries, although data gaps remain rather important.

The fifth article aimed to describe the prevalence of certain congenital infections such as CMV, Parvovirus B19 or enterovirus among a cohort of Mozambican mother-newborn dyads. The study found that 37 of the 118 women recruited were HIV positive. Prevalence of congenital CMV infection, detected by real-time polymerase chain reaction (RT-PCR) through dried umbilical cord samples was 2.6% (3/115) and 6.3% (3/96) when assessed by RT-PCR in nasopharyngeal aspirates obtained from neonates. The

concordance of the RT-PCR assay through DUC with their correspondent through NPA was moderate (Kappa = 0.42 and p<0.001. No differences on prevalence of congenital CMV infection were found among HIV-exposed and unexposed (OR 1.26, 95% CI 0.16–9.89, p=0.83). All (100%) mothers were seropositive for CMV IgG. RT-PCR of EV and B19V in dried umbilical cord samples were both negative in all screened cases. No histological specific findings were found in placental tissues. No risk factors associated to vertical transmission of these viral infections were found.

The sixth article aimed at characterizing hypoglycaemia among all paediatric inpatients admitted to a Mozambican district hospital, during a period of 13 years. The overall prevalence of hypoglycaemia was 3.2% (<3mmol/L) among 45 573 children <15 years hospitalized due to any illness. Of them two thirds (972) with levels <2.5mmol/L. Hypoglycaemic children were significantly more likely to die (OR 7.11; p<0.001), with an associated CFR of 19.3% (245/1267). Independent risk factors for hypoglycaemia on admission and death among hypoglycaemic children included prostration, unconsciousness, oedema, malnutrition and bacteraemia. Overall Minimum community-based incidence rate of hypoglycaemia was 1.57 episodes/1000 child years at risk (CYAR), significantly decreasing throughout the study period. Newborns showed the highest incidence (9.47 episodes/1000 CYAR, p<0.001).

The last article included in this thesis assessed the burden of post-discharge mortality among hospitalized Mozambican children <15 years at Manhiça District Hospital. It found an overall post-discharge mortality of 3.6%, with half of the deaths occurring in the first 30 days after discharge. One primary predictive model for all ages (<15 years) included young age, malnutrition, history of diarrhoea, clinical pneumonia symptoms, prostration, HIV and/or malnutrition associated symptoms, bacteraemia, positive *HIV* status, rainy season and transfer or absconding, with an area under the curve (AUC) ~80% during the whole follow-up. Alternative models based on the primary model were simplified in order to be adaptable to different contexts and had a similar performance. A model specific to infants <3 months identified as predictors being a neonate, low weight-for-age score, difficulty in breathing, hypothermia or fever, oral candidiasis and a history of absconding or transfer to another hospital, with an AUC ~80% during the 90-days of follow-up. Applying this model as an algorithm at the time of discharge, 80% of children with high risk of dying at home would be

identified and a better discharge planning could be done trying to prevent the fatal event occurring at home.

Conclusions and Recommendations

In order to achieve the specific SDG target for child mortality by 2030, an accelerated progress in reducing neonatal mortality in countries with higher burden is urgently required. If this is not considered a priority, the world may fail to significantly reduce overall child mortality. Universal coverage of essential interventions has the potential to reduce neonatal deaths, benefit women and children after the first month of life and additionally reduce stillbirths.

GBS disease is an important cause of infant sepsis and meningitis despite the limitations in the data. In Africa, where the incidence is highest, the mortality associated to *GBS* is also the highest and four times higher than in developed countries, suggesting this is the region where prevention strategies are most critical to implement. Serotype III accounted for over half of all disease-causing isolates. Existing preventive strategies using IAP are not usually available in low-income contexts and, with a higher number of home deliveries and late presentation to health facilities for delivery, IAP may be more difficult to implement. Maternal vaccination offers an alternative strategy, and the data we have suggest that a pentavalent conjugate vaccine (including Ia/Ib/II/III/V) would cover almost all disease-causing serotypes (97%) in young infants worldwide.

In Southern Mozambique, prevalence of *GBS* and *E. coli* colonization among pregnant women is high and comparable to those reported in similar settings and in high-income countries. HIV infection was not a risk factor for *GBS* or *E. coli* colonization. Serotype V was the most prevalent in Manhiça, Mozambique differs from those found in the majority of studies conducted in other countries. Therefore, it is essential to identify prevalent serotypes in each region in order to know the potential coverage, impact and implementation requirements of future anti *GBS* vaccination strategies.

Other infections vertically transmitted may be also an important cause of severe disease and lead to mortality in poor settings. Estimates from low and middle-income countries indicate that the burden of congenital infections may be higher in these regions than in industrialized countries. Vertical transmission of *CMV* in southern Mozambique is higher than in HIC and at least as high as other similar settings in SSA. Larger studies are needed to

evaluate the true burden, clinical relevance and consequences of congenital infections of *CMV*, *B19V* and *EV* in resource-constrained settings.

Hypoglycaemia is a common complication of many conditions causing hospitalization in Mozambican children, especially among newborns and children with severe infections, and is associated with an excessive and unacceptable risk of death, considering it is a treatable condition. A single determination on admission is not enough to rule out hypoglycaemia among admitted patients, and glycaemia should be recurrently screened during hospitalization. Better, cheaper and more innovative diagnostic and therapeutic alternatives need to be urgently investigated to better address the diagnosis, prevention and management of hypoglycaemia in developing countries.

Finally, interventions to address those child deaths occurring at home should be developed. Mortality following discharge is an important although poorly recognized contributor to child mortality. Post-discharge mortality in the first 90 days after hospital discharge is higher than inpatient mortality, especially in the first 30 days post-discharge, as shown by the study conducted in a semi-rural hospital in southern Mozambique. A simple predictive algorithm based on easy to collect variables may readily identify most infants and children at high risk of dying after discharge. Future research should consider validation of predictive models in different contexts and prospectively assessing their accuracy to identify children at risk of dying after discharge when are applied at hospital discharge time in resource-constrained settings. This could allow designing a better post-discharge planning, health education to the families and follow-up care.

Continued investment in child mortality data collection and understanding circumstances of child death is needed, not only for those deaths after a hospital discharge, but also for those children dying at home without ever entering in contact with the health system. Such data may be useful to design innovative, effective and feasible strategies to reduce infant and child mortality.

RESUMEN

Antecedentes y Justificación

La mortalidad de lactantes y niños ha jugado un papel crucial en los patrones de transición de la salud y es considerado un buen indicador de desarrollo y modernización demográfica a lo largo del tiempo. El establecimiento de los Objetivos de Desarrollo del Milenio (MDGs) supuso una importante inyección de fondos y recursos para la mejora de la salud y un compromiso nacional e internacional con la salud infantil, que condujo a un progreso sin precedentes en la reducción de la mortalidad infantil en todo el mundo.

Entre 2000 y 2015, la implementación y ampliación de intervenciones para salvar vidas dirigidas contra las principales causas de muerte (CoD) en menores de 5 años, como la malaria, la neumonía, la diarrea o el sarampión, redujeron en más de un 60% la tasa global de mortalidad en niños menores de 5 años (U5MR). Sin embargo, esta disminución en la mortalidad infantil no fue suficiente para alcanzar el objetivo específico de supervivencia infantil establecido por los MDGs: reducir en dos terceras partes la U5MR en 2015 respecto a las cifras de 1990. Una significante barrera para lograr tal ambicioso objetivo fue el hecho de que la mortalidad neonatal disminuyó a un ritmo mucho más lento que la mortalidad en niños entre 1 y 59 meses, especialmente en países empobrecidos (LIC) del África Subsahariana (SSA).

Esta información es consistente con el cambio que se ha producido en la clasificación de CoD a lo largo del tiempo. Las estimaciones sugieren que algunas de las enfermedades para las cuales se han implementado estrategias (sarampión, malaria, diarrea o neumonía) han sido o están siendo reemplazadas como principal CoD entre los niños menores de 5 años por aquellas enfermedades cuyas intervenciones para reducir su impacto no se han desarrollado o implementado completamente (muertes neonatales).

Las muertes neonatales representaron casi la mitad de todas las muertes entre los niños menores de 5 años en 2016 y, dentro de ellas, las complicaciones

de los niños nacidos prematuros fue la CoD principal. La asfixia perinatal e infecciones neonatales son otras causas importantes de mortalidad prematura de los recién nacidos. Sin embargo, debemos ser cautelosos interpretando datos sobre CoD ya que las fuentes de datos utilizadas para producir estimaciones en países pobres no son fiables, incluyendo registros vitales y hospitalarios disponibles y las autopsias verbales. Algunas herramientas innovadoras, como las autopsias mínimamente invasivas (MIA), que están siendo exploradas actualmente con motivo de vigilancia de mortalidad, parecen ser potenciales herramientas que mejorarán nuestra compresión sobre qué está realmente matando a neonatos y niños en países de renta media y baja.

En términos de infecciones neonatales, la sepsis neonatal y la meningitis son bien conocidas CoD y enfermedades que pueden producir secuelas graves tanto en los bebés prematuros y como aquello a término en países industrializados. Sin embargo, la información sobre estas enfermedades y su impacto en LIC es más limitada. La sepsis precoz (EOD, 0-6 días), generalmente es transmitida verticalmente de la madre al hijo y es causada principalmente por el estreptococo del grupo B (*GBS*) y por Escherichia coli (*E. coli*). La sepsis tardía (LOD, 7-89 días) normalmente se debe a una infección de transmisión horizontal (adquirida en la comunidad o adquirida en el hospital) y los patógenos más frecuentes que la producen son *E. coli*, *Klebsiella spp.*, *GBS* y otros organismos Gram-positivos (*Staphylococcus aureus* y *Estafilococos coagulasa negativos*). La colonización materna por estos patógenos es crucial para ser verticalmente transmitida a sus descendientes, presentándose en esos casos como EOD. La prevalencia de esta colonización materna puede verse afectada por el Virus de la Inmunodeficiencia Humana (*VIH*), el estado nutricional y otros factores de riesgo maternos. Las estrategias de prevención como la detección materna de *GBS* al final del embarazo y la profilaxis antibiótica intraparto (IAP) han demostrado su eficacia para reducir drásticamente la EOD. Sin embargo, IAP actualmente no está llegando a todas las mujeres necesitadas, debido a la fragilidad de los sistemas de salud en países empobrecidos, a la escasez de estructuras de laboratorios necesarias o simplemente porque una significante proporción de partos en estos contextos todavía se producen en el hogar. Además, no se están desarrollando otras estrategias preventivas contra *E. coli* u otras bacterias que causan LOD.

Otras enfermedades infecciosas también consideradas como contribuyentes importantes a la morbi-mortalidad neonatal, del lactante e incluso de niños más mayores son las infecciones englobadas en el síndrome TORCH. La pandemia del *VIH* y el brote del virus del *Zika* han suscitado gran preocupación en todo el mundo y han destacado la importancia de otros patógenos que afectan a los niños incluyendo virus, parásitos y hongos. La detección de algunas de estas enfermedades, como la sífilis o el *VIH*, se ofrece de forma rutinaria a las mujeres atendidas en las consultas prenatales (ANC) de países empobrecidos. Sin embargo, no ocurre lo mismo con la detección de otros posibles patógenos potencialmente transmisibles al feto durante el embarazo y parto, como *citomegalovirus* (*CMV*), *rubéola*, *toxoplasma*, *enterovirus* (*EV*), *parvovirus B19* (*B19V*), *virus del herpes simple* (*HSV*) o *hepatitis B* (HBV), entre otros. La infección congénita por *CMV* (cCMV) es la más frecuente y la principal causa de sordera en los países desarrollados. Sin embargo, como sucede con la mayoría de las infecciones congénitas, permanece olvidada en entornos con recursos limitados, y se desconoce en gran parte su carga real y su impacto.

Un enfoque interesante para prevenir muchas de estas infecciones congénitas y neonatales ahora incluye la vacunación materna durante el embarazo bajo la suposición de que la transferencia materna de anticuerpos al recién nacido será más factible, eficaz y rápidamente más protectora que esperar la generación de respuestas inmunes neonatales a las vacunas administradas directamente a ellos. Esta estrategia de "vacunación vertical" ya se ha implementado con éxito para el control del tétanos y la tos ferina, y se está explorando contra otros patógenos.

Existen otras causas bien conocidas de enfermedad grave y mortalidad en los niños, aunque generalmente no figuran como causas directas de muerte y, por lo tanto, a menudo se olvidan en las estimaciones mundiales. Los niños desnutridos, los recién nacidos con bajo peso o pequeños para la edad gestacional pueden sufrir enfermedades o complicaciones que amenazan su vida y tener secuelas graves. En muchos casos, el pronóstico y resultado final está determinado por la condición subyacente en vez de por la enfermedad aguda. Por otro lado, la emergencia de ciertas complicaciones como por ejemplo la hipoglucemia, relativamente común en la evolución de muchas enfermedades diferentes (malaria, sepsis, diarrea, malnutrición o neonatos en general), pueden determinar el pronóstico de estos casos, aunque la incidencia real y la significancia de tales complicaciones continua siendo descrita como parte de las enfermedades de base.

En muchos países de renta media o baja, al menos la mitad de las muertes de niños se producen en casa, a menudo sin haber sido visitados por un clínico. Sin embargo, en algunos casos, los niños mueren en casa justo después de haber sido atendidos en un centro sanitario. La mortalidad hospitalaria es un contribuyente bien conocido de la mortalidad infantil y se han desarrollado e implementado con éxito guías e intervenciones para reducirla. Aunque la mortalidad posterior al alta hospitalaria (PDM) puede ser tan alta como la mortalidad hospitalaria en entornos con recursos limitados, es un fenómeno olvidado y poco estudiado. De hecho no se han desarrollado guías, recomendaciones o estrategias adicionales para abordar los días inmediatamente posteriores a alta hospitalaria, el cual es un período crítico para la supervivencia infantil. La fragilidad de los sistemas de salud en LIC, los altos costos inasequibles para atender enfermedades agudas y la falta generalizada de conciencia sobre la carga y las implicaciones del PDM por parte de los trabajadores de salud y los responsables políticos explican en parte que la mortalidad durante el periodo inmediato al alta hospitalaria permanezca relegada al olvido.

Para alcanzar el nuevo objetivo de mortalidad infantil establecido por los Objetivos de Desarrollo Sostenible (SDGs) para 2030, es necesario mejorar el conocimiento en enfermedades graves en aquellos países con mayor morbi-mortalidad y desarrollar e implementar intervenciones para abordar las enfermedades que causan el mayor número de muertes entre los niños menores de 5 años.

Esta tesis pretende abordar cuestiones relacionadas con las enfermedades graves que causan la mayoría de las muertes en lactantes y niños menores de cinco años y para las cuales no se han implementado intervenciones específicas en Manhiça, un área semirural en el sur de Mozambique.

Materiales y Métodos

Esta tesis se basa en investigaciones realizadas en el Instituto de Salud Global de Barcelona (ISGlobal) / Hospital Clínic-Universitat de Barcelona en España, el Centro de Investigación en Salud de Manhica (CISM) en Mozambique y en la colaboración con la London School of Hygiene and Tropical Medicine de Londres.

Esta tesis está estructurada en siete artículos: tres publicados en revistas internacionales peer-reviewed, uno aceptado pero todavía no publicado y tres artículos adicionales actualmente en revisión:

i. Un comentario sobre la carga y el impacto de la mortalidad neonatal a nivel mundial y una discusión sobre las principales causas de muerte en este grupo de edad;
ii. Una revisión sistemática sobre la incidencia global de la enfermedad invasiva por GBS en lactantes menores de 3 meses, el riesgo de mortalidad asociado (CFR) y los serotipos causantes de enfermedad, actualizando las estimaciones previas;
iii. Un estudio transversal que investiga la epidemiología de las infecciones por GBS y E. Coli en Manhiça, un área semirural del sur de Mozambique, con el fin de determinar la prevalencia y los factores de riesgo de la colonización materna y la aceptabilidad por parte de las mujeres del uso de hisopos vaginales y rectales para la detección de GBS y de E. coli;
iv. Una revisión de la epidemiología de las infecciones congénitas en países empobrecidos;
v. Un estudio transversal para determinar la prevalencia de transmisión vertical de CMV, EV y B19V en Manhiça;
vi. Un análisis retrospectivo de los datos utilizando el sistema de vigilancia de la morbilidad (MSS) en curso en el hospital distrital de Manhiça para describir la prevalencia e incidencia de hipoglucemia entre los niños mozambiqueños ingresados, la distribución entre los grupos de edad y el CFR asociado;
vii. Un análisis de datos retrospectivo utilizando datos del MSS y del sistema de vigilancia demográfica (DSS) en curso en el distrito de Manhiça, diseñado para conocer la carga del mortalidad post-alta pediátrica en el área, identificar predictores de mortalidad después del alta y desarrollar modelos que podrían estratificar eficientemente a los niños según el riesgo del PDM .

Los siete artículos han sido escritos por el autor de esta tesis como primer autor y abarcan metodologías variables, que incluyen investigación original, revisiones y meta-análisis.

Resultados Clave

Los estudios incluidos en esta tesis proporcionan resultados importantes y resaltan las lagunas de conocimiento que contribuirán a mejorar nuestra comprensión de las enfermedades graves que causan la muerte entre lactantes y niños africanos y para las cuales todavía no se han implementado intervenciones en Mozambique.

El primer artículo revisó la carga general de mortalidad neonatal, destacando la importancia de reducir la tasa de mortalidad neonatal (NMR) para alcanzar el objetivo específico para la supervivencia infantil establecido por los SDGs para el año 2030, ya que las muertes neonatales representan casi la mitad de las muertes entre los niños menores de cinco años. Los esfuerzos nacionales e internacionales deberían enfocarse en este grupo vulnerable y por lo tanto, lograr una cobertura universal de aquellas intervenciones que han demostrado ser efectivas reduciendo la mortalidad neonatal, especialmente en aquellos países con mayor carga para alcanzar el ambicioso objetivo de reducir la mortalidad infantil a 25 por 1000 recién nacidos vivos (LB) para el año 2030.

La revisión sistemática y el meta-análisis en enfermedad invasiva por GBS representan una actualización importante de la revisión previa ya que incluyen nuevos datos países de renta media y baja (18 nuevos estudios de 10 países). La incidencia combinada de enfermedad *GBS* invasiva en recién nacidos <90 días fue de 0,49 por 1000 LB (IC del 95%: 0,43-0,56), y fue más alta en África (1,12) y más baja en Asia (0,30). La incidencia de EOD fue de 0,41 (IC del 95%: 0,36 a 0,47) y la incidencia de LOD fue de 0,26 (IC del 95%: 0,21 a 0,30). El CFR global fue del 8,4%, siendo más alto en África (18,9%, IC del 95%: 13,7-24,0), cuatro veces mayor que en los países desarrollados (4,7%, 3,3-6,1). El CFR de los casos de EOD fue del 10.0% (95% CI 7.0-12.0) siendo del 5.0% en los países desarrollados y del 27.0% en África. El CFR entre los casos de LOD fue del 7.0% (95% CI 4.0-9.0) también más bajo en los países desarrollados (4.0%) en comparación con África (12.0%). El serotipo III (61.5%) fue el más frecuente, con 97% de los casos causados por los serotipos Ia, Ib, II, III y V.

El tercer artículo está enfocado a la colonización materna por *GBS* y *E. coli* en un área semi-rural de Mozambique. El estudio encontró que el 21.3% de las 320 mujeres reclutadas en el estudio eran portadoras de *GBS*, mientras que el 16.3% eran positivas para *E. coli*. La prevalencia de *VIH* entre las

mujeres embarazadas que participaron en el estudio fue del 36,6%. No se encontró asociación entre estar colonizado por *GBS* y *E. coli* y factores de riesgo maternos. Todos los aislamientos de *GBS* fueron completamente susceptibles a penicilina y ampicilina. Los serotipos V (32.4%), Ia (14.7%) y III (10.3%) fueron los más comúnmente encontrados y el 69.2% de las mujeres evaluadas tenían inmunoglobulina G (IgG) contra *GBS*. Los aislados de *E. coli* mostraron resistencia a la ampicilina en 28.9% y co-trimoxazol en 61.3% de los casos.

La revisión de la epidemiología de las infecciones congénitas en países de renta media y baja abarcó un período de 45 años y destacó que la carga de infecciones congénitas puede ser mayor en estas regiones que en los países industrializados, aunque existen lagunas importantes respecto a los datos.

El quinto artículo incluído en esta tesis tenía como objetivo describir la prevalencia de varias infecciones congénitas en una cohorte de recién nacidos. El estudio encontró que 37 de 118 madre de estos recién nacidos eran VIH positivas. La prevalencia de infección congénita por CMV, detectada por reacción en cadena de la polimerasa a tiempo real (RT-PCR) a través de muestras de sangre seca de cordón umbilical, fue del 2.6% (3/115) y del 6.3% (3/96) cuando se evaluó mediante RT-PCR en aspirados nasofaríngeos obtenidos de neonatos al nacimiento. La concordancia entre los resultados encontrados mediante RT-PCR en ambas muestras fue moderada (Kappa = 0,42 yp <0,001). No se encontraron diferencias en la prevalencia de la infección congénita por CMV entre los expuestos al VIH y los no expuestos (OR 1.26, 95% CI 0.16–9.89, p=0.83). Todas (100%) madres fueron IgG seropositivas para CMV. RT-PCR de EV y B19V en las muestras de cordón fueron negativos en todos los casos evaluados. No se encontraron hallazgos histológicos específicos en los tejidos de la placenta ni tampoco se encontraron factores de riesgo asociados a la transmisión vertical de estas infecciones virales.

El artículo sexto pretendía caracterizar los episodios de hipoglucemia entre todos los ingresos pediátricos en un Hospital distrital de Mozambique por un periodo de 13 años. La prevalencia global de hipoglucemia del 3.2% (<3 mmol/L) entre los 45 573 niños <15 años hospitalizados debido a cualquier enfermedad. De ellos 2/3 (972) tuvieron niveles <2.5mmol/L. Los niños con hipoglucemia tenían una probabilidad significativamente mayor de

morir (OR 7.11; p <0.001), con un CFR asociada de 19.3% (245/1267). Los factores de riesgo independientes para presentar hipoglucemia al ingreso asociados a mortalidad incluyeron la postración, la pérdida del conocimiento, el edema, la malnutrición y la bacteriemia. La tasa mínima de incidencia de hipoglucemia basada en la comunidad fue de 1.57 episodios/1000 niños-años a riesgo (CYAR), disminuyendo significativamente a lo largo del período de estudio. Los recién nacidos mostraron las incidencias más altas (9.47 episodios/1000 CYAR, p <0.001).

El último artículo incluido en esta tesis evaluó la carga de mortalidad post-alta entre niños mozambiqueños menores de 15 años hospitalizados en el Hospital distrital de Manhiça. El estudio encontró una mortalidad post-alta global del 3.6%, con la mitad de las muertes ocurriendo en los primeros 30 días. Un modelo predictivo primario para todas las edades (<15 años) incluía: menor edad, desnutrición, antecedentes de diarrea, síntomas de neumonía clínica, postración, *VIH* y/o síntomas asociados a la desnutrición, bacteriemia, estado *VIH* positivo, estación lluviosa y transferencia o fuga del hospital, con un área bajo de la curva (AUC) ~ 80% durante todo el seguimiento. Los modelos alternativos basados en el primario pero simplificados para ser adaptados a diferentes contextos, tuvieron un rendimiento similar. Un modelo específico para lactantes <3 meses identificó como predictores de mortalidad: ser recién nacido, un bajo peso para la edad, dificultad para respirar, hipotermia o fiebre, candidiasis oral y antecedentes de fuga o traslado a otro hospital, con un AUC ~ 80% durante los 90 días de seguimiento. Al aplicar estos modelos como un algoritmo en el momento del alta, se identificarían alrededor del 80% de los niños con alto riesgo de mortalidad en los primeros 90 días después del alta hospitalaria y se podría hacer una mejor planificación del alta tratando de evitar la muerte en el hogar.

Conclusiones y Recomendaciones

Para alcanzar el objetivo específico para la mortalidad infantil establecido por los SGDs para el año 2030, se necesita un progreso acelerado en la reducción de la mortalidad neonatal en los países con mayor mortalidad infantil. Si este objetivo no es considerado una prioridad, el mundo no logrará reducir significativamente la mortalidad infantil global. La cobertura universal de las intervenciones esenciales tiene el potencial de reducir las muertes neonatales, beneficiar a las mujeres y los niños después del primer mes de vida y reducir el número de mortinatos.

La enfermedad *GBS* es una causa importante de sepsis infantil y meningitis a pesar de las limitaciones en los datos. En África, donde la incidencia es más alta, la mortalidad asociada al *GBS* es también la más alta y cuatro veces más alta que en los países desarrollados, lo que sugiere que esta es la región donde las estrategias de prevención son más importantes de introducir. El serotipo III representó más de la mitad de todos los aislamientos causantes de enfermedad invasiva en los lactantes menores de 3 meses. Las estrategias preventivas existentes que usan IAP generalmente no están disponibles en países empobrecidos, los cuales también presentan un mayor número de partos en el hogar y un acceso tardío a las instalaciones sanitarias en el momento del parto, haciendo más difícil de implementar la IAP. La vacunación materna es una estrategia alternativa muy prometedora ya que los datos que tenemos sugieren que una vacuna conjugada pentavalente (que incluya los serotipos Ia/Ib/II/III/V) cubriría casi todos los serotipos causantes de enfermedad (97%) en niños pequeños en todo el mundo.
En el sur de Mozambique, la prevalencia de la colonización por *GBS* y *E. coli* entre las mujeres embarazadas es alta y comparable a las reportadas en entornos similares y en países de rentas altas. La infección por VIH no fue un factor de riesgo para la colonización por *GBS* o *E. coli* en este estudio. El serotipo V fue el más prevalente en Manhiça, Mozambique y difiere de los encontrados en la mayoría de los estudios realizados en otros países. Por lo tanto, es esencial identificar los serotipos prevalentes en cada región con el fin de conocer los posibles requisitos de cobertura, impacto e implementación de futuras estrategias de vacunación anti-GBS.

Otras infecciones transmitidas de la madre al hijo también pueden ser una causa importante de enfermedad grave y conducir a la mortalidad en entornos de recursos limitados. Las estimaciones en los países de renta media y baja indican que la carga de las infecciones congénitas puede ser mayor en estas regiones que en los países industrializados. La transmisión vertical de *CMV* en el sur de Mozambique es más alta que en países ricos y al menos tan alta como en otros entornos similares en SSA. Se necesitan estudios adicionales y con mayor muestra para evaluar el verdadero impacto, la relevancia clínica y las consecuencias de las infecciones congénitas de *CMV*, *B19V* y *EV* en entornos con recursos limitados.

La hipoglucemia es una complicación común de muchas enfermedades que causan la hospitalización en niños en Mozambique, especialmente entre recién nacidos y niños con infecciones graves y está asociada con un riesgo

de muerte excesivo e inaceptable, considerando que es una complicación tratable. Una sola determinación de glucemia al ingreso no es suficiente para descartar la hipoglucemia entre los pacientes ingresados y debería ser evaluada de manera recurrente durante la hospitalización. Se deben investigar urgentemente alternativas diagnósticas y terapéuticas mejores, más baratas y más innovadoras para abordar de forma más eficiente las consecuencias de la hipoglucemia en los países empobrecidos.

Finalmente, se deben desarrollar intervenciones para abordar las muertes infantiles ocurridas en el hogar. La mortalidad después del alta es un contribuyente importante aunque poco reconocido de la mortalidad infantil. La mortalidad en los primeros 90 días después del alta hospitalaria es mayor que la mortalidad durante el ingreso, especialmente en los primeros 30 días posteriores al alta, como se ha mostrado por el estudio llevado a cabo en un hospital semi-rural del sur de Mozambique. Un algoritmo predictivo simple basado en variables fáciles de recoger podría identificar fácilmente a la mayoría de lactantes y niños con alto riesgo de morir después del alta. Las perspectivas futuras de investigación deberían considerar la validación de modelos predictivos en diferentes contextos y la evaluación prospectiva de su precisión para identificar a los niños en riesgo de morir después del alta cuando se aplican al momento del alta hospitalaria en entornos con recursos limitados. Esto podría permitir diseñar una mejor planificación posterior al alta, educación en higiene y salud para las familias y estrategias de un mejor y más accesible seguimiento.

Para todo ello, es necesario continuar invirtiendo en la recopilación de datos sobre mortalidad infantil y comprender las circunstancias que rodean a la muerte de un niño tanto después de un alta hospitalaria como aquellas ocurridas en casa sin haber tenido acceso a un hospital. Estos datos pueden ser muy útiles para diseñar estrategias innovadoras, efectivas y viables para reducir la mortalidad infantil.

01 | INTRODUCTION

1. Historical perspective of Under-five child mortality

1.1 The pre-Millennium Development Goals era

Child mortality has been the scope of study by multiple disciplines over time and the large number of studies published focusing on this subject testifies its long-established and enduring appeal. The topic has been important not only for the Medical Sciences but also for other fields such as Demography, Anthropology and Social Sciences1. The first attempt to measure child mortality was conducted in the 17th century, when infant and child deaths accounted for one third of the total number of deaths every year[2]. However, the modern features of statistical analysis of infant and child mortality were not assumed until the second half of the 19th century and the first decades of 20th century when it started to be recognized as a national social problem and an obstacle to population growth in Europe[3]. It was at that time when the use of infant and child mortality as an indicator of development and modernization acquired greater relevance, since it played a crucial role in health transition patterns and in demographic modernization of Western countries[1].

Analysing this historical perspective, it is not surprising that considerable research has focused on infant and child mortality in terms of temporal evolution, geographical differences and causes of death (CoD).

Focusing on more recent data, the Global Burden disease (GBD) and the Child Health Epidemiology Reference Group (CHERG, now called the MCEE, Maternal and Child Epidemiology Estimation group) approach had made huge endeavours to measure disability and mortality rates, analysing the causes worldwide since the early 1990s. In that year, more than a quarter century ago, 12.1 million (12.0-12.2) children under five years of age (U5) were estimated to have died, being the mortality rate (MR) 87.1 (84.5-90.1) per 1000 live births (LB). Of them, 4.6 million (4.5- 4.6) were neonates (babies in the first 28 days of life after birth), accounting for 37.6% of the total under five mortality rate (U5MR) in the 90s, while 31.2% of deaths

were among infants aged 1-12 months and also 31.2% among children between 1-4 years[4]. Child mortality rates were different across regions, from 10.9 and 15.4 per 1000 LB in North America and Europe respectively to the much higher U5MR of 125.9 per 1000 LB in South Asia (SA) or 182.6 per 1000 LB in Sub-Saharan Africa (SSA), with many specific countries having a mortality rate over 200 per 1000 LB[4].

Global U5MR decreased by ~1% from 1990 to 2000 (average 77.8 per 1000 LB) with the highest burden remaining in SA and SSA (91.8 and 156.9 per 1000 LB, respectively). However these regions achieved an important child mortality reduction and many fewer countries had a U5MR over 200 per 1000 LB compared to previous decade[4,5] (figure 1A and 1B)[6]. Demographic studies demonstrated that such a gain in life-expectancy over time has mainly occurred through improvement in child survival[7,8]. Under this landscape the world progressed towards the end of the millennium, and such a landmark encouraged the United Nations (UN) and 149 heads of states to engage in signing a global commitment with the aim of upholding human dignity, equality and equity and with special emphasis on promoting the survival of children as the most vulnerable. The Millennium Declaration signed in 2000, containing eight objectives for the international agenda, established the Millennium Development Goals (MDGs). MDG 4 specifically targeted a two thirds reduction of U5MR by 20159, while MDG6 aimed at reducing significantly the burden and impact of some of the major killers of children.

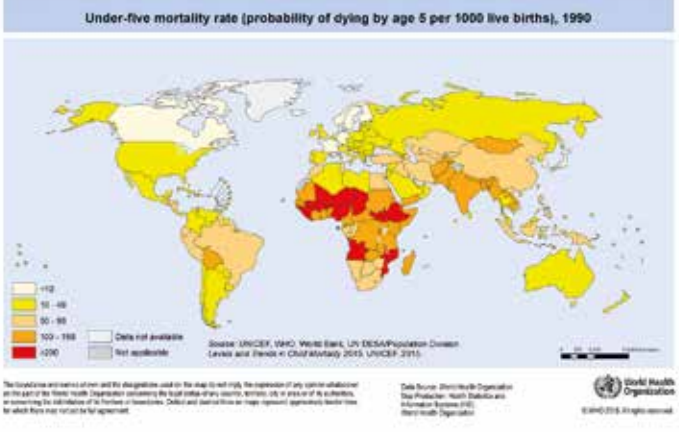

Figure 1. Global under five mortality rate in 1990. Source World Health Organization (WHO) 2015, adapted from WHO website[6]

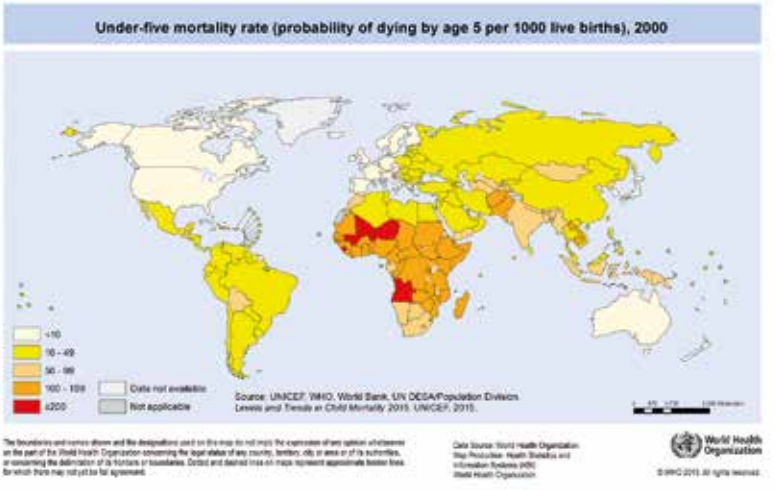

Figure 2. Global under five mortality rate in the year 2000. Source World Health Organization (WHO) 2015, adapted from WHO website[6].

1.2 The MDGs era

Since the establishment of the MDGs, the Global health community has increased in size and number, with some organizations becoming exclusively focused on the specific health areas targeted by the MDGs such as Newborn and child health[10]. This has meant a significant injection of funds and international resources for health, but also an amplified policy attention in child health from national governments, which have encouraged the increase in their domestic funding and financial commitments towards child health[4,10].

Amid such development assistance for health, the implementation and scale-up of many life-saving interventions targeting various leading causes of under-5 deaths as primary accelerators of child survival during this time, has triggered an unprecedented progress in the reduction of child mortality worldwide[4,11].

At the end of the MDG era, 2015, 5.9 million of children U5 died, yielding an U5MR of 42.5 per 1000 LB. 30.1% of these deaths took place in SA, 25.3% in Western SSA, and 15.6% in Eastern SSA. The U5MR ranged between 1.9 per 1000 LB in Andorra and 130.5 per 1000 LB in Chad among 194 countries. The ten countries with the highest U5MR were all in SSA and

had U5MRs above 90 per 1000 LB. In 2015, almost half of all U5 deaths occurred in the neonatal period (2.7 million, 45.1%)[11]. Neonatal mortality rate (NMR) generally was superior to mortality rate for older children in many regions. For instance, in SA, NMR nearly tripled the post-neonatal MR in 2015 (29.8 per 1000 LB vs. 12.1 per 1000 LB)[4]. For other regions such as western and central SSA, mortality rates were higher in children aged 1–4 years. India recorded the largest number of U5 deaths in that year, followed by Nigeria and Pakistan. Mali had the highest NMR with 40.6 per 1000 LB closely followed by Central African Republic and Pakistan[4].

From 1990 to 2015, U5MR thus decreased by ~50%, with 16,000 fewer daily deaths in 2015 in comparison to the 35,000 in 1990, and overall 4 million fewer U5 annual deaths than those occurring in 2000[4,11]. Despite this huge achievement, the estimated global annual reduction rate (ARR) of U5MR in 1900-2015 was 3.0%[4] and 4.0%[11] in the period 2000–15, both falling below the 4.4% per year pre-defined milestone required to achieve the MDG4 during the interval 1990–2015. In this period, of the 195 countries with data available, 58 met or surpassed this MDG4 target, most of them in North Africa and the Middle East, Central Europe, South East Asia (SEA), and Western Europe. Only two were in SSA. Among those achieving 4.4% ARR, 32 countries were classified as lower-middle income (LMIC) or upper-middle-income countries (UMIC) by the World Bank, and only four (Cambodia, Ethiopia, Liberia, and Nepal) were categorised as low-income countries (LIC)[4]. However, an acceleration of progress in reducing under-5 deaths has been observed during the 2000-2015 period, in which 16 countries in SSA achieved the MDG4 target, compared with the only two that had achieved that rate of reduction between 1990 and 2000. Many others countries who failed to achieve the 4.4% ARR from 1990 to 2015, including Russia, and other countries in Eastern Europe and Central Asia, also reached or surpassed this milestone during the period 2000-2015[4] (Figure 3).

This improvement in child survival is considered one of the most notable success stories of Global Health in recent times, highlighting the effect of international and national partnerships focusing on ending preventable mortality[4]. However, by the end of 2015, MDG4 target had globally not been achieved[11,12].

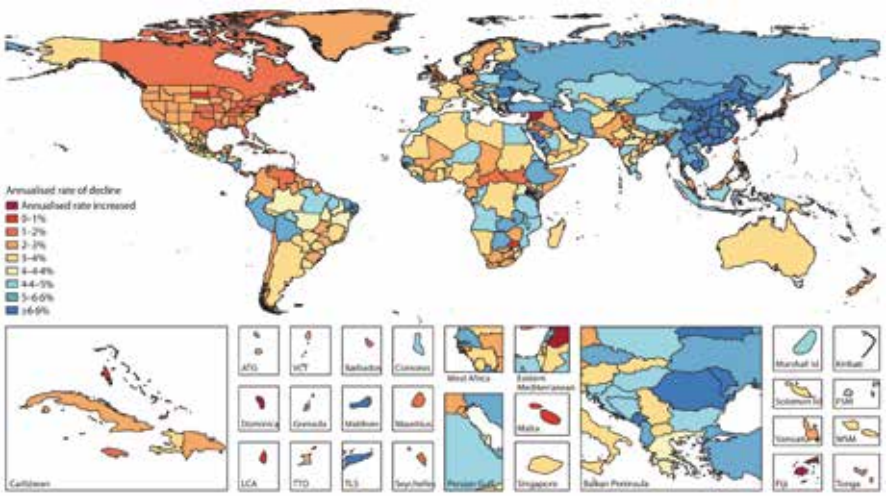

Figure 3. Geographical distribution of annualised rates of decrease in under-5 mortality, both sexes combined, 2000–15. Adapted from GBD 2015 Child Mortality Collaborators Lancet 2016[11]

2. Causes of global under-five child mortality

2.1 Sources for mortality estimates

Information on CoD is essential for policy definition, program designing and monitoring of its implementation and eventual success. Although data quantity and quality on mortality, especially in LIC and LMIC, have been strengthened and improved in recent years[13] allowing more accurate and reliable U5MR estimates, data gaps remain large for high burden countries where 90% of U5 deaths occur[4,11]. In these countries, the two conventional sources of information on CoD are civil registration and vital statistics (CRVS) and verbal autopsies (VA), two methodologies showing both a significant level of imprecision[14].

CRVS in resource-constrained settings, especially in rural or remote areas where most of the people died at home, fail to provide timely, complete and accurate vital statistics, and are not able to ensure a sufficient proportion of certified CoD by a physician[14]. Most CoD estimates available in these contexts thus derive mainly from deaths occurring in health facilities and in urban settings, being poorly representative of mortality in the general population and insufficiently reliable for public health purposes[15].

The World Health Organization (WHO) currently recommends the use of non-invasive indirect methods such as the VA, a protocolised procedure, which allows the classification of CoD by the analysis of data derived from structured interviews of family, friends, and caretakers of a deceased person

to collect information on signs and symptoms experienced prior to death. This information is then analysed, either by a physician or, more innovatively, by automated computer algorithms, to yield a likely CoD that is coded according to International Classification of Diseases (ICD) standards[16,17]. To date, VA has been primarily used in research contexts. However, the accuracy of VA remains its Achilles' heel as it depends in large part on the quality of the diagnostic criteria, the type of diseases involved, the place where death occurs, and the delay between death and VA. Deaths associated with non-specific signs and symptoms are especially problematic, an issue particularly frequent for perinatal and neonatal deaths[18]. Notwithstanding these important limitations, VAs remain in many settings the only source of CoD data, and their practice and improvement should therefore be encouraged[14].

An additional source of data for our current understanding of the main causes of child mortality, particularly among poor countries, is derived from hospital records. However, several studies have shown that in settings where diagnostic tools are scarce, and clinical diagnosis is seldom supported by further diagnostic methods, the reliability of these pre-mortem diagnoses may be low. When compared to diagnoses resulting from a pathological autopsy, clinico-pathological discrepancies are often significant, and major diagnostic errors very frequent[19,20].

Reliable ascertainment of CoD would require the use of post-mortem methods, including as the gold-standard the performance of a complete autopsy (CA). Such a method is seldom feasible in resource-constrained settings, both because of the scarcity of trained staff, infrastructures or resources, or due to its virtually nil acceptability on account of cultural and/or religious beliefs[19]. Additionally, the fact that a large proportion of deaths occur at home, outside of the health system, renders most deaths "invisible"[21] to the official statistics, and not prone to such kind of investigations. Therefore, due to the unreliability of the source of data used, the derived estimates are prone to error and should be interpreted with caution.

2.2 Causes of child mortality over time

From the pre-MDG era to the end of the MDG era, estimates of the main causes of child deaths have gradually changed at the global level[11]. Leading causes of U5 deaths in the 90s were lower respiratory infections followed by neonatal preterm complications and diarrhoeal diseases in the second and third positions, respectively (figure 4).

The implementation of preventive, diagnostic and treatment interventions strategies targeting various leading causes of U5 deaths such as malaria[22-24], pneumonia[25], diarrhoea[26], measles[27] or Human Immunodeficiency virus (HIV)[28] have likely contributed to reduce U5MR in many places, also significantly modifying the CoD rankings over time[4].

From 2000 to 2015, MR associated to pneumonia, diarrhoea, neonatal intrapartum related events, malaria, and measles were all reduced by more than 30% (figure 4)[4,18]. Collectively, reductions in these causes accounted for more than 60% of the total reduction in U5MR[11]. In 2015, neonatal preterm birth replaced lower respiratory infections as the principal cause of U5MR. Different groups considered different diseases as the second most common cause of U5MR in that year. Estimates produced by the MCEE and the WHO reported lower respiratory infections as the second main cause[11], but the GBD group proposed in this position neonatal encephalopathy[4]. The third position for these two different study groups included neonatal intrapartum complications by Liu and colleagues[11,18] and lower respiratory infections for the GBD group[4]. According to this same group, diarrhoea was downgraded from the third leading cause in 1990 and 2000 to the fourth in 2015 (figure 4 and 5)[4,11].

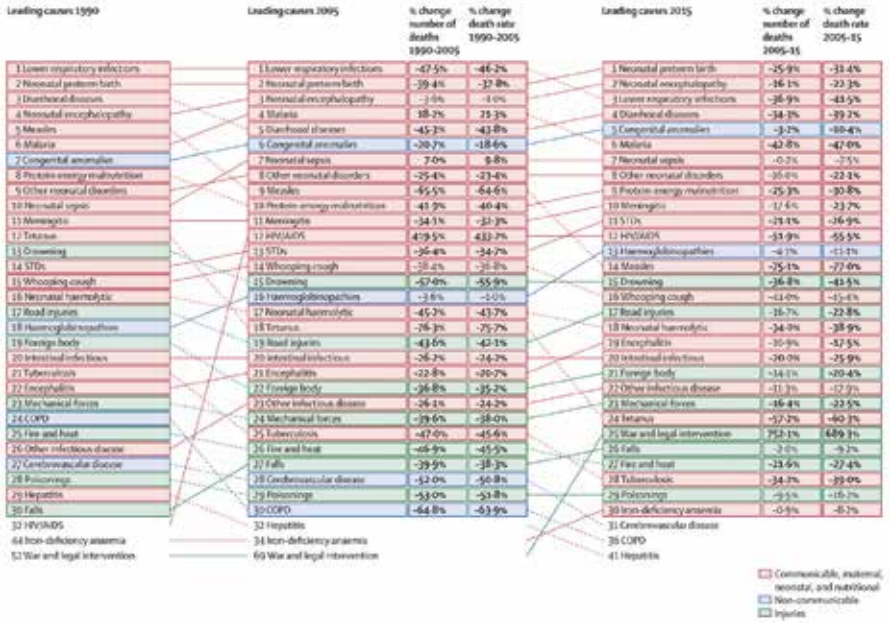

Figure 4. Leading 30 causes of global under-5 deaths for both sexes combined for 1990, 2005, and 2015. Adapted from ***GBD 2015 Child Mortality Collaborators Lancet*** 2016[4]

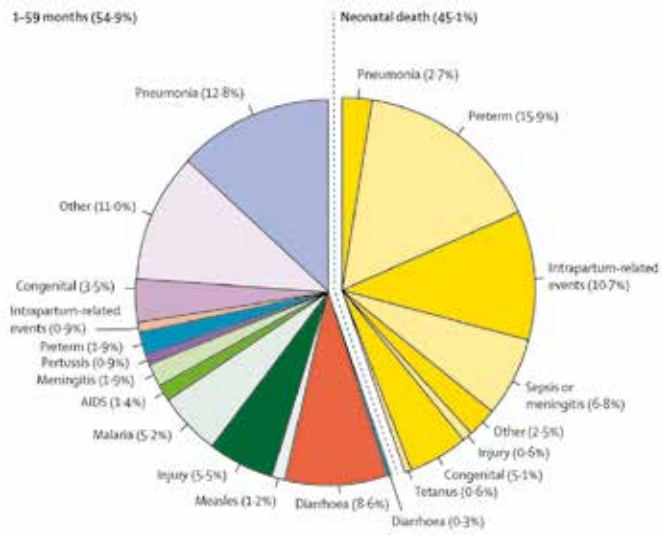

Figure 5. Global causes of under-5 deaths in 2015. Adapted from Liu et al, *Lancet* 2016[11].

Although global and national estimates of CoD are an important input to global health policy making, they are complex and need to have full transparency and reproducibility to ensure reliability of the results. However, most child deaths occur in LIC with sparse or absent data and hence, estimates of CoD in children are inaccurate. Proof of that is the comparison of estimates produced by the two main groups investigating overall CoD, GBD and MCEE, where the number of annual deaths attributable to a single disease calculated by each group may vary by more than 100 000 cases (figure 6)[29]. Thus, these estimates should be interpreted with a certain degree of scepticism; although they may be the only information on CoD available.

	1–59 months			0–27 days		
	MCEE	GBD	Relative difference	MCEE	GBD	Relative difference
Pneumonia	800 (681–923)	709 (629–791)	11	136 (84–219)*	197 (169–225)*	−45*
Diarrhoea	558 (479–731)	475 (398–545)	15	30 (12–33)†	45 (37–53)†	−125†
Malaria	456 (353–546)*	570 (438–733)*	−25*	0†	17 (11–26)†	NA†
Injury	324 (258–391)	321 (278–372)	1	–	–	–
Meningitis	151 (125–185)	121 (90–157)	20	–	–	–
AIDS	103 (76–142)†	64 (59–72)†	38†	–	–	–
Measles	102 (74–166)	82 (42–145)	20	–	–	–
Pertussis	60 (43–94)	56 (25–127)	7	–	–	–
Preterm birth complications	–	–	–	965 (615–1537)*	693 (554–854)*	28*
Intrapartum complications	–	–	–	662 (423–1054)	612 (492–774)	8
Sepsis	–	–	–	423 (269–688)	342 (295–475)	19
Congenital	–	–	–	276 (175–438)	247 (219–280)	11
Tetanus	–	–	–	49 (32–79)*	26 (12–39)*	47*
Other	967 (781–1134)*	1268 (NA)*	−31*	232 (145–373)*	453 (NA)*	−95*
Total	3571	3666	−4	2764	2614	5

Data are N in thousands (uncertainty range). GBD=Global Burden of Disease. MCEE=Maternal and Child Epidemiology Estimation. NA=not available. *Causes where the absolute value of the relative difference, defined as difference between the MCEE and GBD estimates divided by the MCEE estimates and multiplied by 100, is at least 25. †Causes where the 95% uncertainty ranges do not overlap between the MCEE and GBD estimates.

Table: MCEE and GBD estimates of causes of deaths among children aged 1–59 months and 0–27 days, worldwide, in 2013

Figure 6. Discrepancies for cause-specific attributable under-5 mortality between the two major groups producing child mortality estimates. Adapted from Liu et al, Lancet 2015[29].

Excluding neonatal deaths, main conditions causing deaths among U5 children include pneumonia, diarrhoea and malaria[11].

Pneumonia was estimated to cause 12.8% of death among children aged 1-59 months in 2015. The Pneumonia Etiology Research for Child Health (PERCH) Study has investigated in comprehensive manner pneumonia aetiology in children aged 1-59 months in seven African and Asian sites[30]. Preliminary results among HIV negative patients have reported that viral infections are the most common cause of pneumonia. Among them, respiratory syncytial virus (RSV) was the most common in 6/7 sites. Among bacterial cases, Mycobacterium tuberculosis (TB) and streptococcus pneumoniae (S. pneumoniae) were the most commonly found pathogens. Ten pathogens accounted for ~80% of all pneumonia cases. CFR was 9.6% and main pathogens causing death were TB, staphylococcus aureus (S. aureus), Pneumocystis jirovecii and S. pneumoniae. Five of seven sites had already implemented the highly effective anti-pneumococcal vaccine (PCV), although some of them after the study started (PERCH study group, personal communication). PCV has shown dramatic reductions in disease and mortality rates in those countries in which it has been introduced25 and together with an improved case management have led to a decrease of ~40% on number of deaths due to lower respiratory infections from 2000 to 20154.

Diarrhoea was the estimated CoD in 8.6% of children among 1-59 months in 2015. The Global Enteric Multicenter Study (GEMS) aimed to identify the aetiology and population-based burden of paediatric diarrhoeal disease in SSA and SA. Most attributable cases of moderate-to-severe diarrhoea were due to four pathogens: rotavirus, Cryptosporidium, enterotoxigenic E. coli and Shigella. CFR was 2.0% and most deaths were due to E. coli and cryptosporidium. Interventions targeting these pathogens, an improvement in case management such as for instance guaranteeing the rapid availability of oral rehydration therapy[31] or the wide implementation of the rotavirus vaccine[26] have the potential to substantially reduce the burden of moderate-to-severe diarrhoea. Altogether these factors have likely contributed to the observed decrease by more than 30% of diarrhoea related deaths from 2000 to 2015[4].

In 2015, there were 50 million less malaria cases compared with estimated numbers in 2000[32] and deaths due to malaria dropped by more than 40%

in the same period4. Today, malaria is still responsible for an estimated 5.2% of all child deaths, according to global estimates[4]. The implementation of different preventive measures against malaria, including the use of indoor residual spraying[33], the massive deployment of insecticide-treated nets[22,23], the implementation of intermittent preventive treatment during pregnancy[34], in addition to the improvement of case management through early diagnosis using rapid diagnostic tests35,36 or artemisinin-based combination therapies24 have significantly contributed to the reduction of the burden of this disease, historically one of the main child killers.

There are other well-known causes of child mortality although not usually listed as direct causes of death and therefore often forgotten in global estimates analyses. Some of diseases described above may affect malnourished children, neonates born with a low weight or small for gestational age (SGA)[12]. In many cases, the fatal outcome is determined by the underlying condition rather than the acute disease.

Around 45% of deaths among children U5 are linked to undernutrition. These mostly occur in LIC and LMIC. However, this condition does not appear sufficiently reflected among global estimates. Malnutrition affects all regions of the world. In 2017, WHO reported that there are still 155 million children U5 affected by chronic malnutrition and 52 million by acute malnutrition, most of them living in Africa and Asia. The high and middle-income countries have managed to halve the incidence of chronic malnutrition since 2000, while the LIC have more children with chronic malnutrition now than they did 15 years ago. Severe malnutrition is associated to higher mortality than chronic malnutrition. The highest incidence of acute malnutrition occurs in Asia and Africa, where ~30-35% of the children U5 who suffer from acute malnutrition meet criteria of severe acute malnutrition[37].

Being born too soon has been already mentioned as the leading CoD among neonates, but being born too small is also a factor that increases the risk of death or disability[38]. Being born small might be due to prematurity or SGA, or a combination of the two. Traditionally, low birth weight (LBW) defined as weigh at birth <2500 g has been more used as a marker for highest mortality and morbidity risk than SGA[12,38]. SGA babies might have grown healthily but be constitutionally small, or might have suffered intrauterine growth restriction (IUGR) due to placental insufficiency (e.g.,

pre-eclampsia or placental malaria), fetal reasons (such as multiple birth), environmental exposures, or nutritional factors especially driven by maternal pre-pregnancy nutritional status[12]. SSA and SA are the regions which concentrate higher numbers of SGA (32 million per year). Two thirds of SGA neonatal deaths are term LBW babies, which double the risk of neonatal mortality in comparison to their term-appropriate for gestational age peers. Preterm-SGA babies are 15 times more likely to die, with ongoing mortality risk after the neonatal period[12].

On the other hand, the emergence of certain complications, such as for instance hypoglycaemia, are a proxy of the severity of illness in childhood, as they may appear in the course of any severe or life-threatening disease (malaria, sepsis, diarrhoea cases, malnutrition or neonates in general). Hypoglycaemia may affect both, children and adults[39-41], being newborns and malnourished children the most vulnerable groups[42-44]. In SSA, its prevalence among paediatric admissions has been estimated to range between 1.8% and 7.3% [45,46]. Severe and prolonged hypoglycaemia can result in mental retardation, neurological deficits and recurrent seizures or even death[47,48]. In the developing world, hypoglycaemia remains an insufficiently recognized killer of children, as it is seldom diagnosed and whenever detected, often poorly managed, mainly in relation to the lack of simple equipment or trained staff.

In many resource-constrained settings, at least half the child deaths occur at home, often without having been attended by a clinician. In some cases, though, deaths occur also at home, but soon after a contact with the health system. Inpatient mortality is a well-understood contributor to overall child mortality and algorithms for diagnosis and treatment of acute diseases have been developed in the last decades, in order to address the management of diseases causing more U5 deaths during the acute phase49. However, post-discharge mortality (PDM) could be as high as inpatient mortality in LIC, but is also a neglected and understudied CoD. Indeed, no guidelines, recommendations or further strategies have been developed to address the care of patients during the days immediately following discharge, a critical period for child survival. Reasons to remain neglected are likely multifactorial: huge burden and high costs to provide care for acute illness, limited resources in the settings with higher incidence of post-hospital deaths and lack of awareness about its burden by health care workers[50,51].

Mortality following discharge in industrialized countries is limited to certain small high-risk groups[52-54]. However, in resource-constrained settings, children appear to be at increased risk of mortality following hospitalisation for any illness[55-59]. Some of these studies have explored risk factors associated to higher risk of dying after a hospital discharge. The most important predictors of PDM in LIC are history of previous hospitalizations, young age, HIV infection and hospitalizations related to malnutrition or pneumonia but no algorithms to identify children with higher risk of dying after a hospital discharge have been developed so far[51].

3. The most vulnerable time for a child´s survival: The neonatal period

3.1 Progress on neonatal care from the pre-MDG era to end of MDGs

Between 1990 and 2015, global neonatal deaths decreased by 42.4%, from 4.6 million deaths in 1990 to 2.7 million in 2015[4,11]. Despite of such an unprecedented progress, the relative contribution of neonatal mortality to the overall burden of U5 deaths is increasing. Due to the slower decline of neonatal mortality relative to mortality in older children, the fraction of neonatal deaths relative to overall U5 deaths increased from 37% in 1990 to 45% in 2015, a fact that has been considered by the expert community as "the unfinished global agenda in the MDG era"[60]. Whereas reduction of mortality in infants aged 1-12 months in the same period was 50%, or 59.8% among children aged 1-4 years, reduction on NRM has been lagging behind mortality among older age groups, with an annual decrease of 3.1% compared to 4.7% in older children[4,11,60].

Some countries and regions have achieved rapid NMR reductions between 1990 and 2015 and other countries and regions only small changes. The highest NMR remains in SA (29.8 per 1000 LB) and SSA (27.8 per 1000 LB), rates more than 10 times higher than those in western Europe (1.9 per 1000 LB)4,5,12. Such regions with high NMR now account for more than 75% of the neonatal death burden and tend to have the slowest progress in reducing NMR. Continuing with this trend of declining rate, it will be over a century before a neonate from SSA has the same survival probability as one born in Europe (figure 7)[12]. Approximately 7000 newborns die in the first month of life every day, most of these deaths occurring in the first week, with about 1 million deaths taking place during the first 24 hours of life, and an additional million deaths within the following six days[5,11].

The targeted MDG4 consisting in two-thirds reduction of U5MR by 2015 would have been possible if neonatal causes had been declining at a similar rate to that witnessed in the 1–59 month age group[11,12].

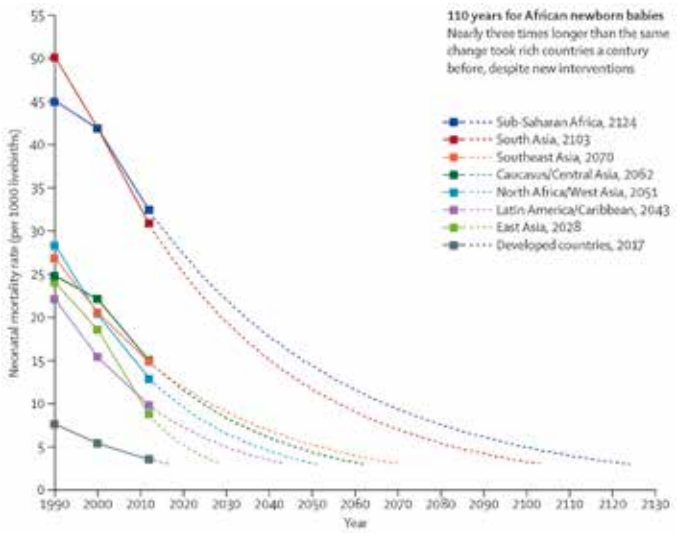

Figure 7. Time for each region to reach the same chance of neonatal survival as in 2012 for newborn babies in high-income countries. Adopted from Lawn et al Lancet 2014[12].

3.2. Leading causes of neonatal death

The majority of neonatal deaths result from preventable causes such as preterm birth complications (accounting for 35% of neonatal deaths), complications during labour and delivery (24%), and infectious diseases (15% sepsis/meningitis, 6% pneumonia, 1% tetanus, 1% diarrhoea) and congenital abnormalities (11%)[61]. Intrapartum-related conditions (also called birth asphyxia) and preterm birth dominate as causes of death in the early neonatal period (0-6 days), and infections are common in the later period (7-27 days), although they can also occur in the first days of life (figure 8)11,12. Approximately 99% of all child deaths are now circumscribed to these settings[4,12].

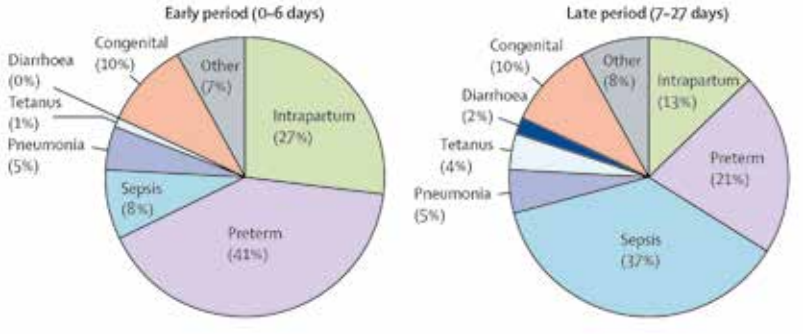

Figure 8. Leading causes of death by early and late-onset disease during neonatal period. Adapted from Lawn et al Lancet 2014[12].

3.2.1 Preterm birth complications

Preterm deliveries are those that occur before the completion of at least 37 weeks of gestational age. Over the past 20 years, the prevalence of premature birth has risen worldwide. The burden of being born preterm is greatest in LIC and LMIC, where an estimated 13.7 million infants were born too soon[62].

Direct complications of preterm birth were responsible for an estimated 36% of the world's 2.7 million neonatal deaths in 2015, making preterm birth the most common single CoD in children U5[11]. Preterm birth also increases the risk of death due to other causes, especially neonatal infections[63]. Hypothermia and malnutrition secondary to poor feeding further increase vulnerability being particularly important in resource-constrained settings[64]. In addition to its direct contribution to mortality, preterm birth can have lifelong effects on neurodevelopment, with increased risks of cerebral palsy and neurological impairment. Preterm birth is estimated to be responsible for 77 million disability-adjusted life-years (DALYs), 3.1% of the global total, an estimate similar to the burden of HIV or malaria[64].

Since complications of preterm birth are the first cause of neonatal deaths, it is essential to further characterize what are the risk factors associated with preterm birth. Delivery for maternal or foetal indications (such as pre-eclampsia or eclampsia, IUGR, among others), spontaneous preterm labour with intact membranes and preterm premature rupture of the membranes (PPROM) are the main causes of preterm birth and addressing them should be a priority in order to reduce NMR[65].

3.2.2 Infectious diseases

According to the global estimates of causes of neonatal deaths in 2015 and 2016, infections occupy the third position in terms of importance[11,61]. However, in regions such as SSA the estimated number of neonatal deaths secondary to infections remains unacceptably high (figure 9)[60]. Importantly, some studies specifically describing causes of neonatal death have reported infectious diseases as the principal cause among this age group. A multicentre study including countries from Asia, SSA and Latin America used a specific algorithm for identifying CoD designed to be applied in LIC and LMICs. Among more than 3000 neonatal deaths, infectious diseases were reported as the first global CoD, being the main in SSA and American sites, with prematurity being the most important in the Asian sites[66]. Main neonatal

CoD determined through minimally invasive autopsy (MIA) and complete autopsy in 41 neonates in Southern Mozambique included infectious diseases (66%), preterm complications (12%), congenital abnormalities or malformations (10%) and intrapartum-related complications (7%)[67]. Unpublished data on CoD also obtained through minimally invasive tissue sampling (MITS) methods in South Africa found that 73% of deaths among preterm babies were due to infections (S. Madhi, personal communication). Importantly, the two latter studies were able to identify the entire chain of biomedical events leading to the death, thus allowing for a more clearly identified evaluation of the underlying cause of death.

Sepsis, meningitis and pneumonia are the main syndromes causing neonatal mortality, especially neonatal sepsis[11,61]. Although different age cut-offs have been used for considering neonatal sepsis, the most accepted one is 0-89 days, a period that includes the totality of the neonatal period but also goes beyond it. Early-onset disease (EOD, 0-6 days) is usually vertically transmitted from mother to child causing EOD after an initial maternal colonization of the vagina, rectum or urinary tract, and can be passed to the offspring through the infection of the amniotic fluid, after membrane rupture, or during passage of the neonate through the vaginal canal during delivery[68-70] and usually presents as bacteremia without a focal source[71]. Late-onset disease (LOD, 7-89 days) is commonly due to horizontal infection (either community-acquired or hospital-acquired)[72] and may be acquired from nosocomial sources, from breast milk or from care providers[73-76] and includes more commonly meningitis[71].

Incidence of all causes of culture-confirmed neonatal sepsis varies between regions, ranging from 0.77 per 1000 LB in US[77] to 5.46 per 1000 LB in Kenya[78] or 5.9 per 1000 LB in South Africa[79]. This incidence may increase up to 36 per 1000 LB if only considering HIV infected infants.

Group B streptococcus (GBS) and Escherichia coli (E. coli) are the leading causes of EOD and together other gram negative and positive microorganisms, also cause LOD[68,77-81]. Around two-thirds of young infant disease is EOD[68,82]. Mother-to-child transmission (MTCT) of these pathogens is particularly associated to neonatal sepsis, meningitis and pneumonia, but also with preterm birth and very-low-birth-weight delivery, also associated to neonatal mortality[68,69,81,83,84].

Figure 9. Geographical distribution of main causes of neonatal deaths. Adapted from Liu et al Lancet 2015[85].

Maternal GBS carriage during the period closely related to the delivery has consistently demonstrated to determine the risk of vertical transmission, and thus of neonatal ensuing disease[86]. GBS maternal colonization worldwide is 18% according to a recent systematic review, being the highest in the Caribbean (35%) and Southern Africa (25%), although most of the studies were conducted in South Africa[87]. A systematic review published in 2012, reported an overall incidence of invasive GBS disease among infants of 0.53 per 1000 LB, with the highest incidence being documented in Africa (1.21 per 1000 LB), followed by the Americas (0.67 per 1000 LB) and the lowest incidence in Southeast Asia (0.016 per 1000 LB). Data from LIC are limited, although in such settings higher case fatality risk (CFR) have been described, such as for instance in Africa (22%) compared with the Americas (11%) or Europe (7%) and almost two-fold higher among EOD cases (12.1%) compared with LOD cases (6.8%)[68]. GBS was also associated to 1% of all stillbirths in developed countries and 4% in those occurring in Africa[88].

The primary intervention to reduce GBS-associated EOD involves the administration of intrapartum antibiotic prophylaxis (IAP) to women identified as either 1) being GBS carriers through microbiological screening (35-37 weeks' gestation)[89] of samples obtained from their genito-urinary

or gastrointestinal lower tract; or 2) fulfil any of the different risk factors associated to neonatal disease[90-92]. In high-incomes countries (HIC), the widespread implementation of the IAP strategy has essentially decimated *GBS* EOD among those babies born to women in whom it was correctly applied[93]. In those settings with IAP policy implemented, coverage of IAP is linearly associated to the risk of *GBS* EOD. AS IAP coverage increases the risk of GBS EOD decreases (from 1.1% in those settings with no IAP policy to 0.03% in those with varying coverage of IAP combined)[86]. The reduction of risk of invasive *GBS* EOD reaches up to 79% in setting with high coverage of microbiological-screening based policy (e.g. United States (US) or Spain) but decreases to only 50% when IAP consists in risk-based strategy, even with an adequate implementation[86]. In LIC and LMIC, the fragility of the health systems and the generalized lack of microbiology facilities, in the absence of a reliable rapid point of care test for GBS, hinder the applicability of the IAP strategy, therefore jeopardising the prevention of life-threatening *GBS* neonatal infections[94].

The IAP strategy has however not demonstrated any impact on GBS-associated LOD[94,95]. Late-onset *GBS* infection typically presents as bacteraemia, pneumonia or meningitis and less frequently as septic arthritis, cellulitis, or osteomyelitis. Although LOD is less commonly fatal than EOD, a recent systematic review on neurodevelopment impairment (NDI) secondary to *GBS* meningitis found that among meningitis survivors, 32% had NDI at 18 months of follow-up, including 18% with moderate to severe NDI[96].

Despite Africa having the highest incidence risk of neonatal sepsis worldwide[59], the real burden of morbidity and mortality due to this condition has been poorly quantified, on account of multiple reasons. Among them, low case ascertainment, particularly among those born in home deliveries as 80-90% of GBS EOD cases typically occur during the first 24 hours of life[97], inadequate access to care and/or high rapid CFR, particularly if cerebrospinal fluid sampling is not obtained and cases of meningitis are thus not detected[84].

E. coli is the second pathogen more commonly isolated among neonatal sepsis cases in those settings equipped with proper microbiology surveillance systems[77]. However, epidemiological data on maternal colonization by *E. coli* and its consequent impact in their offspring in this region are scarce. Maternal *E. coli* colonization seems to be lower in Europe and Asia than in SSA,

where limited data on the prevalence of vaginal carriage for this pathogen ranges from 9.1% in Nigeria to 46.5% in South Africa[79,98-100]. The prevalence of perinatal transmission of *E. coli* during delivery ranges between 21 to 50%[101], being a clear predisposing factor to develop neonatal infections[102]. *E. coli* can be responsible for both EOD and LOD[103], although EOD has been better studied[77,78]. Incidence of EOD due to this pathogen is 0.18-0.28 per 1000 LB although almost all articles published were conducted in HIC[77,81]. It is considered the most frequent cause of invasive bacterial infection in preterm infants, and the combination of infection and prematurity, as it has already been highlighted, increases the risk of death81. It is also the most common cause of stillbirths in developing countries[104].
For *E. coli*, contrarily to what occurs with *GBS*, no clearly defined preventive strategies have been proposed to reduce the burden of MTCT of this pathogen, and more research is needed to investigate the mechanisms involved in its pathogenicity and potential strategies to prevent its burden81. Moreover, antibiotic resistance is emerging a public health problem all over the world, however is insufficiently recognized in SSA, where antibiotic resistance rates are increasing and empiric and effective antibiotherapy may become challenging[72,105,106].

Besides *GBS* and *E. coli*, some studies conducted in LMIC and LIC have shown other microorganism that commonly cause invasive disease among infants <3 months. A recently published study of the incidence of invasive bacterial infections among young infants aged 0-89 days in a district hospital in Southern Mozambique, found that the most common causes of bacteraemia were *S. aureus*, followed by *GBS*, *S.pneumoniae* and several different gram negative bacilli such as *Klebsiella pneumoniae* (K. pneumoniae) and *E. coli*[107]. By contrast, the Aetiology of Neonatal Sepsis in South Asia (ANISA) study, which investigated the infectious aetiology of young infants with possible serious bacterial infection (PSBI) in Bangladesh, India, and Pakistan found that only 28% of infants had a specific bacterial or viral aetiology identified. The most common pathogens in this large case-control were *RSV*, *Ureaplasma urealyticum* and *E. coli* (ANISA study group, personal communication). In contrast to the above studies of community-acquired sepsis, a study of hospital-onset sepsis in neonates admitted to a large university neonatal intensive care unit in Zambia revealed that over 33% of enrolled neonates experienced one or more episodes of sepsis. Notably, gram-negative organisms predominated with *K. pneumoniae* isolated in 75% of positive blood cultures, with high number

of K. pneumoniae isolates multi-drug resistant, and high sepsis-associated mortality that ranged from 30 to 70%[108].

3.2.3 Intrapartum-related deaths

The process of labour and delivery is a traumatic one and an important but preventable CoD for babies around the world. Intrapartum-related neonatal deaths (previously called "birth asphyxia" deaths) is defined as neonatal deaths of term infants with neonatal encephalopathy or who cannot be resuscitated (or for whom resuscitation is not available) after excluding other lethal causes such as lethal congenital malformations[109]. Almost one quarter of newborn deaths are attributable to intrapartum-related events (637 000 deaths)[110], most of them birth asphyxia, but this figure also includes a smaller group of infants who die from birth injury without hypoxic brain injury—for example, organ rupture[11,109].

The burden of intrapartum-related deaths occur almost entirely in LIC and LMIC, yet coverage of skilled birth attendance, considered a marker of health system access and capacity, is lowest in countries with the greatest neonatal mortality rates, maternal mortality ratios, and stillbirth rates[109].

In addition to this high mortality, such babies with birth asphyxia have a high risk of neurological and developmental deficits that are difficult to predict. Virtually all infants with mild neonatal encephalopathy who are normal at the end of the first week of life will be free of long-term neurological damage. The majority of infants with severe neonatal encephalopathy will die or manifest severe neurological impairment[109].

3.2.4 Congenital abnormalities

Congenital abnormalities (also known as birth defects, congenital disorders or congenital malformations) are defined as structural or functional anomalies that occur during intrauterine life and can be identified prenatally, at birth, or sometimes may only be detected later in infancy, such as hearing defects[110]. An estimated 303000 newborns die in the first 28 days of life every year, worldwide, due to congenital anomalies[110]. Moreover, birth defects cause a significant albeit poorly quantified number of stillbirths[111]. It is estimated that about 94% of severe congenital anomalies occur in LIC and LMIC, likely due to a possible lack of access to sufficient nutritious foods by pregnant women, an increased exposure to agents or factors such as infection and alcohol, consanguinity or poorer access to healthcare and screening[110].

Congenital abnormalities may appear as the result of a single genetic alteration or changes in the transmitted chromosomes, or a variety of other conditions, including infections (i.e. TORCH syndrome or *Zika virus*), nutritional issues (maternal malnutrition) or environmental factors (maternal exposure to pesticides and other chemicals, as well as certain medications, alcohol, tobacco or radiation during pregnancy), but it is usually challenging to identify the exact causes110. The most severe congenital anomalies are chromosomal alterations (as for instance trisomies or other chromosomopathies), heart defects, or neural tube defects, which could lead to death or can contribute to long-term disability, which may have significant impacts on individuals, families, health-care systems, and societies[110].

The current *Zika virus* outbreaks and their association with an increase in microcephaly and other congenital malformations have raised great concern across the world, particularly in the Americas[110] and have contributed to highlight the emerging threat that maternal viral infections may carry for the health of the foetus and newborn[112]. Infections encompassed under the TORCH syndrome are an important contributor of neonatal and infant morbidity and mortality and, although they are an infectious cause, WHO classifies them within the congenital abnormalities[110]. TORCH stands for the following: *Toxoplasma gondii* (*T. gondii*) ; Other: *syphilis*, *hepatitis B (HBV)*, *varicella zoster virus (VZV)*, *HIV*, *parvovirus B19 (B19V)*, *enteroviruses (EV)*, *lymphocytic choriomeningitic virus*; *Rubella virus*; *Cytomegalovirus (CMV) and Herpes simplex virus (HSV)*[113-115].

Perinatally acquired *CMV* infection stands out as the paradigm of the existing infectious causes of congenital abnormalities, particularly in the context of decreasing trends of rubella, measles or toxoplasma-associated congenital syndromes. Congenital *CMV* (cCMV) infection is the most prevalent congenital infection worldwide, varying the prevalence in live born infants from approximately 0.2% to 2% (average 0.65%). *CMV* can be transmitted to an infant during pregnancy (trans placental transmission), during delivery (via contact with infected genital tract secretions), or postnatally (via ingestion of contaminated human milk or direct contact with other body fluids such as urine and saliva)116. Risk factors for congenital *CMV* infection are *HIV* maternal *CMV* seroconversion during pregnancy117 and pre-term delivery118. Higher overall rates of cCMV are found in countries with higher maternal seroprevalence[119-121], leading to an increased chance of reactivation within a host, reinfection of seropositive hosts or primary

infection of seronegative hosts within the population. Prevalence of cCMV may increase up to 10.3% in infants HIV-infected122-126. However, the prevalence of CMV infection have decreased over time among neonates exposed but HIV-uninfected, reaching levels similar to those observed in the general population, following the introduction and increasing use of highly active antiretroviral therapy (HAART) for prevention of MTCT of HIV123,127.

In-utero *CMV* infection is potentially fatal to the foetus and, it is associated with a range of adverse outcomes involving multiple organs such as liver and central nervous system (CNS) and IUGR, especially when maternal infection occurs before 20 weeks gestational age (GA). Among *CMV* infected neonates, 10-15% have systemic disease at birth: fever, respiratory disease, hepatosplenomegaly, hepatitis and jaundice and retardation of psychomotor development. Neurological and sensory sequelae occur in 40%–58% of neonates with symptoms, although 13.5% of children with no symptoms at birth also developed such sequelae, in particular audito-neurological complications, with hearing loss being the most prevalent[119,128-130]. Neonatal mortality associated with symptomatic congenital *CMV* infection during the first year of life is estimated to be over 10%[131]. *HIV*-infected newborns also had a 3-fold higher risk for symptomatic congenital *CMV* infection than uninfected newborns, and higher risk for infant mortality, and among survivors, there is an accelerated progression of CNS disease, especially developmental delay and worsening motor deficit[116,124].

4. The Sustainable Development Goals (SDGs) era

4.1 New targets for 2030

At the end of the era of the MDGs, the international community decided to renew the commitment to the world's children agreeing on a new framework: the Sustainable Development Goals (SDGs). The new target for child mortality is to reduce in all countries U5 children deaths to a maximum of 25 deaths per 1000 LB and neonatal deaths below 12 deaths per 1000 LB[61].

First estimates in the SDGs era show that the total number of U5 deaths dropped to 5.6 million in 2016 and globally 2.6 million newborns died the same year. The U5MR was 41 deaths per 1000 LB in 2016, with disparities across regions: 1 child in 13 dies before his or her fifth birthday in SSA, while in HIC the ratio is 1 in 189. The NMR in 2016 was 19 per 1000 LB.

By comparison, the probability of dying after the first month but before reaching 1 year of age was 12 per 1000 LB, and during the period 1-<5 years of age was 11 per 1000 LB[61]. In SSA, about 1 child in 36 dies in their first month of life, while in HIC the ratio is 1 in 333. The largest number of newborn deaths in 2016 occurred in SA (39%) followed by SSA (38%) and the countries with higher number of neonatal deaths were India, Pakistan, Nigeria, the Democratic Republic of the Congo and Ethiopia (figure 10), mostly reflecting also the crude numbers of deliveries occurring in such hyperpopulated nations[61].

Estimates on CoD in 2016 show that neonatal deaths accounted for 46% of all U5 deaths, increasing their relative contribution to U5 deaths (figure 11)[61]. In that year, main CoD among neonates were preterm birth complications (35%) followed by intrapartum-related events (24%), sepsis or meningitis (15%), congenital abnormalities (11%), pneumonia (6%), diarrhoea (1%) and tetanus (1%)[61]. However, preliminary data on the causes of neonatal death using MITS in South Africa have shown that infections were the immediate CoD in 73% of 80 neonates with prematurity as underlying condition and usually classified as preterm birth complications in global estimates (S. Madhi, personal communication).

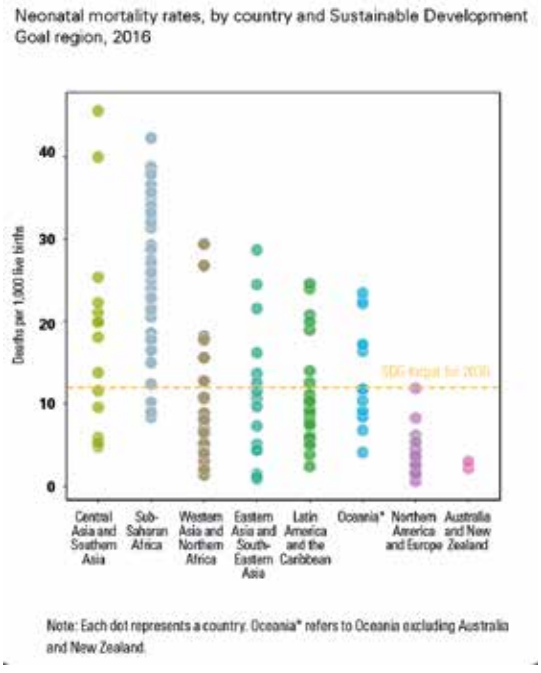

Figure 10. Neonatal mortality rates by country and Sustainable Development Goal region, 2016. Source United Nations Inter-agency for Child Mortality estimation (UN IGME)[61].

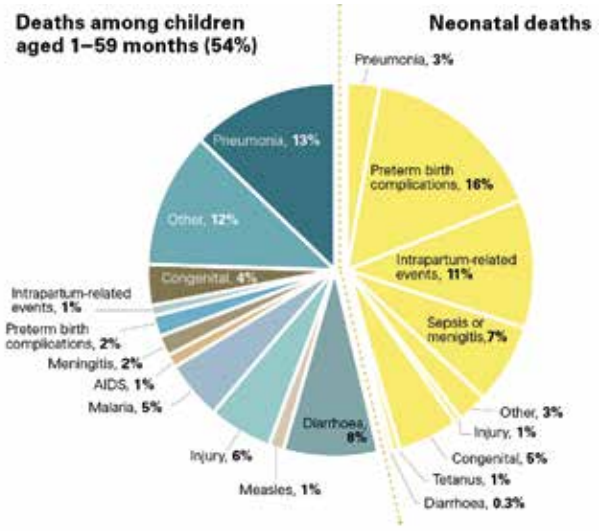

Figure 11. Global distribution of deaths among children under five years old by cause, 2016. Source United Nations Inter-agency for Child Mortality estimation[61].

Drawing on the UN Sustainable Development Goals, the WHO targets for universal civil registration of births and deaths targeting new goals to achieve significant improvements in CRVS reporting by 2020[14]:

- 60% of deaths in per year are continuously notified, registered and certified with key characteristics
- 80% of deaths in hospitals have a reliably cause of death determined and officially certified in real time
- 50% of deaths in communities have probable cause of death determined in real time, and collection systems designed in a representative way.

If current trends on child mortality continue, 60 million children U5 will die between 2017 and 2030, being half of them newborns. Many lives can be saved if the gaps across countries are closed. If every country achieves the SDG target on child survival by 2030, an additional 10 million lives of children U5 will be saved throughout the period 2017–2030 and about half of them will be newborns[61].

4.2 Strategies to reduce under-five child mortality

To save lives in those countries with higher U5MR, it is critical to understand the locally-relevant CoD, and to improve registration of data, as a necessary step to design more efficient preventive and curative strategies.

To address the goals targeting improvement in CVRS reporting is needed to strength surveillances and vital registration systems, especially in those countries with higher U5MR. The use of more sophystified VA methods through tablet computers, mobile communications and diagnostic algorithms has emerged, making it possible to integrate VA into vital registration systems. Despite of their alleged technical and methodological challenges, recent methodological developments suggest that VA is ready for wider national application in routine CRVS systems providing reliable information on causes of death[14].

Innovative approaches such as the newer but less invasive post-mortem sampling methods to investigate CoD (MIA or MITS) have been validated by comparing with CA results and they can identify with significant precision and accuracy the ultimate cause of a child's death, with the additional significant advantage of being much more acceptable than traditional more invasive methods[132,133]. Currently MITS are being investigated as a useful tool for CoD determination in children, including stillbirths and newborns through the Child Health and Mortality Prevention Surveillance Network (CHAMPS)[67,134]. Although such methods can be utilized in resource-constrained settings for CoD investigation and mortality surveillance, understanding the external validity of these approaches and generated estimates is crucial before using them to change preventive strategies[11,135.] Generation of new evidence on causes of death through CHAMPS network should lead to a continuous review of guidelines and interventions on child and neonatal care.

According to last estimates available, most U5 deaths are caused by diseases that are readily preventable or treatable with proven, cost-effective interventions. Infectious diseases and neonatal conditions are responsible for the vast majority of U5 deaths globally[11,61,136].

The implementation of many life-saving interventions targeting various leading causes of U5 deaths have significantly contributed to improve child survival during this time[137], including among others insecticide-treated nets[22,23] artemisinin-based combination therapies[24], the prevention of mother-to-child transmission of HIV[28] and other evidence–based interventions such as highly efficacious vaccines[25,26], oral rehydration therapy for diarrhoeal diseases[31] or antibiotics for pneumonia or neonatal sepsis[53]. Additionally, management of environmental risks, such as water and sanitation, have surely also contributed to reduce child mortality in many countries and they should be maintained or up-scaled globally[138].

However, considering that almost half of U5MR occurred in the neonatal period[11], especially in LIC and LMIC[4,12], financial, international and national endeavours and interventions should be addressed to enhance main causes of neonatal deaths[60,139]. An expert group has reviewed a panel of published interventions and care-based strategies and analysed which of those interventions may effectively improve neonatal and child survival, and what may work but still requires more evidence to assess their potential impact and effectiveness[140]. Among six interventions identified as clearly effective to reduce child mortality, four were found to be particularly effective among neonates: antenatal corticosteroids for preventing neonatal respiratory distress syndrome in preterm infants (>30% of NMR reduction); early initiation of breastfeeding (NMR decreased by 44%); hygienic cord care (NMR reduction of 23%) and kangaroo care for preterm infants which can reduce neonatal deaths by 50%. Other promising interventions to reduce neonatal and infant mortality reviewed by this expert group included: Antenatal care; tetanus immunization in pregnancy; prophylactic antimalarials during pregnancy; induction of labour for prolonged pregnancy; case management of neonatal sepsis, meningitis and pneumonia; prophylactic and therapeutic use of surfactant; continuous positive airway pressure for neonatal resuscitation; and home visits across the continuum of care, with a particular emphasis in a first post-natal visit. The same expert group has estimated, in one of the "Lancet every newborn" series, that available interventions could reduce neonatal deaths related to prematurity by 58%, intrapartum problems by 79%, and infections by 84% among neonates and that approximately 75% of all neonatal deaths could be averted by 2025 if countries were to implement available interventions at a high coverage[141]. These interventions need to be deployed to all and promoted from the very outset. They need to be supported by other activities such as community-based approaches in promotion of care and delivery, community engagement and empowerment to increase women attending antenatal clinics and numbers of facility births, and strengthening of the health system; to ensure a proper antenatal care in these setting with higher burden of NMR.

Other interventions still on early research phase or recently incorporated include: a) the development and implementation of new vaccines to decrease vertically transmitted infectious diseases such as anti-*GBS*, against *RSV* or anti-*CMV* vaccines, to be administered during pregnancy so as to protect through the passive transfer of antibodies their newborns. Recent estimates on *GBS* burden have shown that a vaccine with 80% efficacy

and 90% coverage could prevent 229 000 infant and maternal *GBS* cases, 41 000 stillbirths, and 67 000 infant deaths per year[84] and that it would be logistically easier to implement than other strategies such as IAP[68]; b) WHO guidelines on simple, safe and effective antibiotic regimens for outpatient treatment of clinical severe infections in those newborns for whom access to referral care is challenging or simply not feasible[142]; c) To develop algorithms applied at discharge to identify children with higher risk of dying at home after a hospitalization would facilitate a more evidence-based discharge planning with recommendations on home-based care supported by community health workers[143].

5. Life-threatening conditions for children in Mozambique

Mozambique is a country located in SSA, with ~29 million inhabitants in 2016, classified by the World Bank as a LIC and occupying one of the last ten positions in The Human Development Index[144].

In 2016, the U5MR in Mozambique was 71 per 1000 LB[145] and NMR was 27 per 1000 LB[146]. According to last reported national statistics in 2013, overall prevalence of HIV in the country was 12.3% and 50% of pregnant women were believed to attend antenatal care regularly, where B+ strategy for the prevention of mother-to-child HIV transmission is offered to *HIV*-positive mothers free of charge[147]. About 50% of all births are attended by skilled health staff, and 15.6% of children U5 are underweight[147]. In 2013, main CoD in this age-group included malaria, acute respiratory infections, prematurity, birth asphyxia, diarrhoea, neonatal sepsis, HIV and congenital abnormalities, similar to what has been described in global estimates. However, coverage of cause of death registration is low, with updated estimates unknown[147].

Data from Manhiça, a semi-rural area in Maputo province (Southern Mozambique) where the studies presented in this thesis were conducted, are similar to national estimates regarding U5MR and NMR. The demographic structure of the Manhiça population includes predominance of young people, with nearly half of the population being under the age of 15 years. For *HIV,* however, estimates appear to differ from national estimates, as community based studies conducted among community adults in Manhiça, performed in 2010[148] and 2012[149], reported an *HIV* prevalence of around 40%, with ~30% of the district's pregnant women attending the antenatal clinics at the Manhiça District Hospital (MDH) being positive. A study describing causes of mortality in children <15 years in the Manhiça district using verbal autopsies found that communicable diseases accounted for

74% of the total deaths. Among them, infectious and parasitic diseases (malaria, pneumonia, HIV and diarrhoea) were the most common CoD followed by perinatal disorders. In this study, 54% of deaths occurred at home, out of any health facility[150].

Recent published data on the incidence of invasive bacterial disease among infants aged 0-89 days in the MDH during the last 15 years, reported that the most common causes of bacteraemia were *S. aureus*, *GBS* and *S. pneumoniae*, declining the incidence for all pathogens over time but not for GBS. Incidence of invasive bacterial disease for all microorganisms was 9.2 per 1000 LB and CFR associated was 11.7%[107].

Mozambique still has high U5MR and NMR. If the country aims to achieve SDGs target for child mortality by 2030, it must decrease U5MR and NMR by more than 50% in less than 15 years. Interventions to reduce some of the main CoD such as malaria, acute respiratory infections or diarrhoea have been already implemented in the last years, and a reduction on mortality secondary to these diseases is already occurring and expected to continue. However, implementation of interventions to reduce infectious diseases among young infants and neonates has not seen the same impetus than other interventions primarily targeting older children such as the anti-pneumococcal or rotavirus vaccines or preventive measures against malaria. Reasons for this may include a shortage of reliable estimates of the main cause of neonatal deaths, underestimation or the real burden of NMR since many neonates die at home without death registration, or more generally difficulties in improving the health system so as to guarantee an adequate and well-supervised delivery of interventions during pregnancy or at delivery.

This thesis aims to address issues related with those severe diseases that cause the majority of morbidity and deaths among neonates, infants and children under five and for which no specific interventions have yet been implemented. Most of the studies included this thesis have been conducted in Manhiça, a semi-rural area in southern Mozambique, and a paradigmatic example of a resource-constrained setting.

02 | HYPHOTESIS AND OBJECTIVES

1. Hyphoteses

The hypotheses of this thesis include:

1. Vertically transmitted infectious diseases such as *GBS* or *CMV*, for which no interventions have been yet implemented in the majority of low-income countries, are leading causes of morbidity and death among African infants and children.
2. The prevalence of maternal carriage of *GBS* and *E. coli* in pregnant women in southern Mozambique is comparable to its prevalence in high-income countries, and may be increased among *HIV* positive women.
3. Complications of infectious diseases such as hypoglycaemia are associated to a high mortality risk, which is even greater among newborns.
4. Community deaths among children after a hospitalization are as high as inpatient deaths and that there are specific risk factors that could be identified on admission and help build algorithms to identify children at a higher risk of dying.

2. Objectives

i. General objective

The overarching goal of this research thesis is to explore and better characterize the burden and impact of vertically transmitted infectious diseases among African children for which no interventions have been implemented in low-income countries. Additionally, it aims to determine the attributable mortality associated to hypoglycaemia, and the mortality burden in children following hospitalization in a semi-rural hospital in Southern Mozambique, and identify predictors of mortality following discharge.

ii. Specific objectives

1. To comment on the burden and importance of neonatal mortality globally, and discuss main causes of death among this age group (article I)

2. To provide a comprehensive, systematic literature review and meta-analysis on the global burden of infant invasive *GBS* disease (article II), including:
    a. Incidence of infant *GBS* disease: overall incidence risk, including by stratification by EOD and LOD.
    b. Case fatality rate for EOD and LOD and neonatal disease.
    c. Serotype distribution: prevalence of *GBS* serotypes causing GBS disease among infants under 3 months.

3. To investigate the epidemiology of *GBS* and *E. Coli* infections in Manhiça, Mozambique, in order to better characterize maternal carriage (article III), including:
    a. To estimate the prevalence for maternal carriage of 1) *GBS* (vagina and rectal); and 2) *E. coli* (vaginal and urinary) in women during the third trimester of pregnancy
    b. To estimate differences in *GBS* carriage in pregnancy according to *HIV* status.
    c. To determine the antimicrobial susceptibility of *GBS* and *E. coli* isolates.
    d. To determine the most common circulating *GBS* serotypes in order to estimate potential vaccine coverage by current available *GBS* candidate vaccines.
    e. To assess the cultural context of acceptability of using vagino-rectal swabs for *GBS* and *E. coli* detection by pregnant women in Mozambique.

4. To review congenital infectious diseases in resource-constrained settings (article IV).

5. To assess the prevalence of *cytomegalovirus*, *parvovirus B19* and *enterovirus* vertical transmission in Manhiça, Mozambique, and compare results of screening of congenital *CMV* obtained from two different specimens in a semirural Mozambican maternity (article V).

6. To describe the prevalence and incidence of hypoglycaemia among admitted Mozambican children, distribution among age groups and associated risk factors and case fatality rates (article VI).

7. To determine post-discharge mortality risk among children less than 15 years of age over three different time-periods: 1-30 days, 31-60 days and 61-90 days following any hospital discharge in a semi-rural area from Southern Mozambique, Manhiça (article VII), including:
    a. To identify predictors of mortality following discharge.
    b. To develop models that could efficiently stratify children according to post-discharge mortality risk.
    c. To examine time trends in post-discharge mortality.

03 | MATERIALS AND METHODS

1. Thesis Research Context

This thesis is based on the research work undertaken under the umbrella of the Barcelona Institute for Global Health (ISGlobal)/ Hospital Clinic-Universitat de Barcelona, in Spain. ISGlobal is a research centre, fruit of an innovative alliance between the "la Caixa" Foundation", academic institutions and government bodies, aiming to contribute to the efforts undertaken by the international community to address the most pressing challenges in global health. This thesis work has been directed by Prof. Quique Bassat, with proven experience in epidemiology, cohort studies and clinical trials in Mozambique.

All original research articles included in this thesis (four articles) are based on studies conducted at the Centro de Investigacão em Saude de Manhica (CISM) in Mozambique, with whom ISGlobal has a longstanding partnership. The CISM was created in 1996 as part of a collaborative programme between the Mozambican and Spanish governments through the "Agencia Española de Cooperación Internacional para el desarrollo" (AECID) and the Eduardo Mondlane University of Medicine (Maputo) and the Hospital Clínic of Barcelona, to promote and conduct biomedical research on those diseases with high morbidity and mortality among the local population.

Article II included in this thesis is part of a project called "The Worldwide Burden of Group B Streptococcus for Pregnant Women, Stillbirths and Children" funded by Bill & Melinda Gates Foundation and led by The March Centre of London School of Hygiene and Tropical Medicine (LSHTM).

2. Study Area and Research facilities

The District of Manhiça is located within the Maputo province, at the very southern part of Mozambique (Figure 12). Manhiça, capital of the District, is a semi-rural town set on a plateau that borders the flood plains of the Incomati River. There are two seasons, a hot and rainy one (November–April) and a dry and cooler period during the rest of the year.

The CISM (Figure 13A) runs a demographic surveillance system (DSS) in its study area and a round the clock morbidity surveillance system (MSS) at the Manhiça hospital and other peripheral health posts, through which demographic data, signs, symptoms and diagnoses of all outpatients and inpatients under the age of 15 years are routinely collected.

The MDH admits around 3,000 children under 15 years old annually (Figure 13B). The main causes of admission at paediatric ward of MDH are malaria, pneumonia, diarrhoea, malnutrition, and neonatal pathologies[140] (Figure 13C). HIV prevalence in the area is among the highest in the world[138] however, there is no recent data about HIV in children in the study area. The last national report, published in 2009[177] showed a prevalence of HIV 2.3% in children under 11 months, decreasing between 1 and 5 years (<1%) and being 1-2% in children aged 5-9 years. In 2007, the prevalence of HIV among 834 hospitalized children was 25.7%[178].

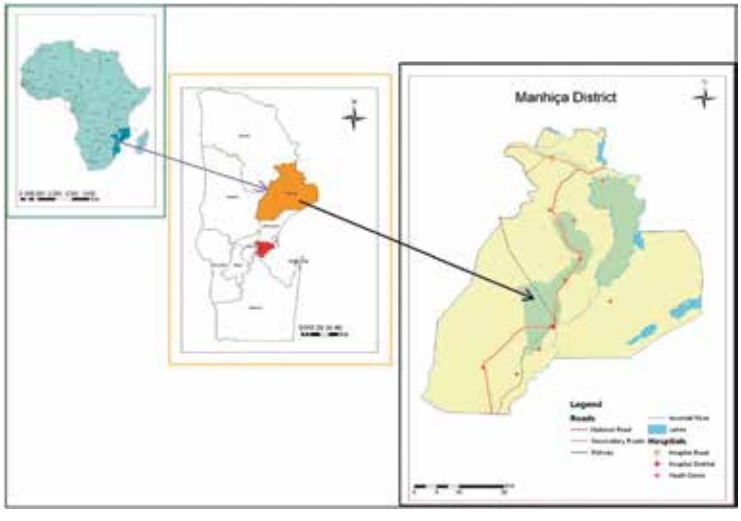

Figure 12. Manhiça study area. Mozambique, Maputo province, Manhiça district and CISM study area.

The CISM has also contributed in recent years to the improvement of antenatal care (ANC), and has standardized consultations to guarantee that all women attending the hospital during their pregnancies are adequately followed and studied within the constraints of the challenging resource-poor environment (Figure 13D). In brief, more than half of the pregnant women in

the area have at least one antenatal consultation (generally in their second or third trimester), where HIV and syphilis screening is routinely offered. Malaria transmission of moderate intensity is perennial with some seasonality and, intermittent preventive treatment during pregnancy (IPTp) for malaria prevention is recommended[179]. An average of 3500-4000 deliveries takes place annually and it has been estimated that around 85% of all deliveries are institutionalized (A. Nhacolo, personal communication) (Figure 13E). A facility exists for pregnant women with risk factors for a complicated delivery to settle by the hospital in attendance of labour, facilitating a supervised delivery ("waiting home").

Manhiça is the paradigm of a poor, resource-constrained rural SSA setting, with a population predominantly young (19% of which less than 5 years of age). In 2016, the NMR and U5MR rates for the study area were similar to the national estimates, 27 per 1000 LB and were 71 per 1000 LB respectively[136]. Latest estimates of HIV prevalence among pregnant women attending the ANC clinic at the MDH are high (~30%)[138]. Studies in this area have also estimated a prevalence of 9% of mother to child transmission at first month of age and of 27% in the first year[155]. In 2013, MDH introduced WHO-recommended Option B+ for the prevention of mother-to-child HIV transmission[180], which is offered to mothers free of charge. No proactive strategies to screen for risk factors of neonatal sepsis or to prevent it are currently implemented in Mozambique.

Over the past decade, the CISM research agenda is focused on the most pressing public health problems in the country such as malaria, HIV/AIDS, tuberculosis, diarrhoeal diseases, pneumonias and maternal and reproductive health, among others, with important impact on public health policies in the country.

A detailed description of CISM and the study area can be found elsewhere[179].

Lab facilities

The CISM's laboratory (Figure 13F) provides infrastructure and diagnostic support to the centre's research projects and healthcare activities performed at the community or at the MDH. The geographical location of the CISM near South Africa allows for regular shipping and maintenance of equipment, so that molecular and immunological experiments can be done onsite. The laboratory works in accordance with Good Clinical Laboratory Practices

(GCLP) and standardized operating procedures (SOP). Study samples are labelled and archived using barcodes and managed by a laboratory information system based on Servolab® Software version 4. The laboratory has extensive sample storage capacity including temperature-monitored -80°C freezers and liquid nitrogen tanks, being the whole centre secured throughout backup power generators. The CISM's laboratory activities comprise: a) Clinical analyses: hematology/biochemistry; b) Microbiology: general bacteriology and parasitology; c) Immunology; d) Molecular biology; e) Tuberculosis; f) Quality assurance & biosafety: this unit ensures that appropriate quality regulations are properly followed.

Figure 13. (A) CISM research center; (B) Manhiça District Hospital (MDH); (C) Paediatric ward.; (D) antenatal care and outpatient wards in MDH; (E) maternity in MDH; (F) laboratory of bacteriology in CISM.

Demographic Surveillance System

The Manhica study area at the time of this thesis covered 500 km^2 (one fifth of the whole Manhica district, see figure 11) ~94000 inhabitants and around 20000 households (HH). Currently, the study area has been expanded covering 2300 km^2 and 183000 inhabitants. All households in this area are geo-positioned using global positioning system (GPS) and all individuals in the DSS receive a Permanent Identification number (Perm-ID) allowing monitoring of longitudinal demographic information, which is collected electronically through three basic procedures: (i) annual household visits

(Figure 14) (ii) maternity and morgue visits to record births and deaths, and (iii) contact with key community informants.

The data collected in the Manhiça DSS comprises HH and individual features, socio-economic status, vital data, migration, health history, cause and date of death, among others. Some studies conducted in this DSS have helped the local health authorities to make decisions in health policies, such as the introduction of some vaccines (*Haemophilus influenzae type b*, *Pneumococcus* and Rotavirus) in Mozambique's expanded programme of immunization.

Figure 14. Picture of a field worker doing the annual household visit

Hospital morbidity surveillance system

Hospital surveillance data are routinely collected for all children less than 15 years old visiting the outpatient clinics in the study area and those admitted to the MDH. Clinical data, including medical history, physical examination, routine laboratory basic investigations, ICD-10 based diagnosis, outcome and medication prescribed are collected on questionnaires on a round the clock basis and reviewed daily by senior medical staff before being entered in specific databases. Figure 15 shows a picture of the data collection at the MDH.

On admission, a finger-prick blood sample is collected to determine PCV and blood glucose concentration. In order to quantify *Plasmodium falciparum*

parasitaemia, thick and thin blood films are prepared and processed. Blood cultures are systematically performed for all children under 2 years and, in older children, in the presence of severe symptoms or according to the admitting clinician's call.

HIV status information is not routinely collected. A HIV rapid test or molecular methods among <18months is performed to those children with suspected HIV infection.

The DSS and MSS are able to link demographic data and clinical data to conduct biomedical research in priority health fields.

Figure 15. Picture of the MDH showing how data collection is performed.

3.Overview of the articles included in the thesis and role of the candidate in each piece of the work

The thesis is presented as a collection of seven articles, three of them already published and two accepted for publication in peer-reviewed international journals, and two manuscripts under review for publication. The author of this thesis has led the authorship of the seven articles included in this thesis being the first author of all of them.

The presented work is based on the following patient cohorts or data bases:

A. **Article I:** *The Golden 28 days of child survival:* commentary.

B. **Article II:** *Global landscaping of Group B Streptococcus infection:*

Paper II was led by the author of this thesis and is part of a project supported by a grant to the LSHTM from the Bill & Melinda Gates Foundation (ref OPP1131158) aiming to examine the incidence of GBS invasive disease among young infants and the associated CFR and serotypes causing GBS disease. The project was submitted for ethical approval to the LSHTM (ref 11966) and approved on 30 November 2016. It is part of a Monographic Peer-reviewed Supplement estimating the burden of GBS disease among pregnant women, stillbirths and infants. The Supplement includes systematic reviews and meta-analyses of maternal colonization[87], maternal GBS disease[156], GBS attributable stillbirths[88] or preterm births[83], use of intrapartum antibiotic prophylaxis[157], risk of GBS newborn disease86, GBS-associated neonatal encephalopathy[158], and impairment after neonatal GBS disease[96]. These are reported individually, and according to international guidelines[159,160]. Together these papers provide data inputs for estimating the worldwide burden of GBS disease among pregnant women, stillbirths and infants[84].

C. **Article III and V**: *Perinatal Group B Streptococcus, Escherichia coli and Pneumocystis jirovecii infections in pregnant women and newborns in Manhiça, Mozambique (PIPAC study):*

A cross-sectional cohort of pregnant women and their offspring enrolled between June 2014 and January 2015 aiming to investigate the epidemiology of GBS, *E. Coli* and *P. jirovecii and other* infections in Manhiça, Mozambique, in order to better characterize maternal carriage, vertical transmission and newborn-related morbidity and mortality. The implementation of the study, screening and recruitment of the study participants were coordinated at the field by the author of this thesis, together with the supervision of data collection and sample processing at the laboratory facilities. Data cleaning and data analysis was also led by the author of this thesis. This study was approved by national institutional review boards at Mozambican Ethics Committee (ref 370/CNBS/13). Written informed consent was obtained from patients prior to participation. This study is part of a grant obtained by Quique Bassat from Instituto de Salud Carlos III (ISCIII) through a program Miguel Servet (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269.

D. **Article IV:** *Congenital and perinatally-acquired infections in resource-constrained settings:* Review.

E. **Article VI:** *Hypoglycaemia in paediatric admissions in a rural hospital in Mozambique (GLYCEC study):*

This is a retrospective analysis of data collected in the context of routine clinical practice through the Manhiça MSS from children younger than 15 years who were admitted to MDH during a 13-year long period (2001-2013). The MSS in place at MDH and its updates over time have been approved by the Mozambican Ethics Committee (ref 017/CNBS/03 and CIBS_CISM/05/13). The analytical plan of this specific analysis was assessed and approved by Manhiça's Internal Scientific committee (ref CCI/27/Feb/2013).

F. **Article VII:** *Post−discharge mortality in children admitted to a rural Mozambican hospital: development of a prediction model to identify children at risk of death.*

This study is also a retrospective analysis of data collected in the context of routine clinical practice through the Manhiça MSS from children younger than 15 years who were admitted to MDH during a 16-year long period (2000-2016) and linked to socio-demographic data collected through the Manhiça DSS. The MSS and its updates over time have been approved by the Mozambican Ethics Committee as stated above (ref 017/CNBS/03 and CIBS_CISM/05/13) and the DSS and its updates over time have been also approved by the National Ethics Committee of Mozambique (ref 174/CNBS/12). The research analysis was assessed and approved by Manhiça's Internal Scientific committee (ref CCI/152/Jan/2016). This study was the summer project of the MSc Epidemiology done by the thesis author at the LSHTM during the course 2015/16 and it was approved by MSc Research Ethics Committee of LSHTM (ref 11204).

During the performance of these studies, the author of this thesis obtained a Pre-doctoral fellow grant of Centro de Reserca en Salut Internacional de Barcelona (CRESIB-ISGLOBAL) (ref.: PhD-2011-11) and a fellowship from the program Rio Hortega of the ISCIII (grant no.: CP13/00260). The printing of this thesis was supported by the PhD student program at ISGlobal. The CISM receives core funding from the Spanish Agency for International Cooperation and Development (AECID). ISGlobal is a member of the CERCA Programme, Generalitat de Catalunya.

04 | RESULTS

ARTICLE 1

The Golden 28 days of child survival

Lola Madrid, Rosauro Varo and Quique Bassat

Under review

The Golden 28 days of child survival

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ABSTRACT

Background: The first month of life is undeniably the most vulnerable period for the child's survival. Despite child mortality has decreased by 56% from 1990 to 2015, the 2/3rds reduction stipulated by Millennium Development Goals (MDGs) 4 has not been achieved.

Main text: Whereas annual decrease of children aged 1-59 months has been 4.7% its equivalent in neonatal mortality (NM) was 3.1%. The targeted MDG 4 would have been possible if neonatal deaths had been declining at a rate achieved by the 1-59 month age group. NM has become an increasing driver of the overall global paediatric mortality, representing an increasing proportion of all annual child deaths. Thus, almost half of children under five die in the first month of life, and most of them, in the first week. The new Sustainable development goals (SDGs) specify an even more ambitious global target by 2030. In order to achieve this target, international community have to tackle main cause of deaths (CoD) among neonates, otherwise, the world will fail to significantly reduce overall child mortality. To improve evidence-based data on CoD is essential to know why children are dying. Innovative techniques such as the post-mortem minimally invasive tissue sampling may provide reliable data and thus contribute to better characterize mortality in this age group. Universal coverage of essential interventions tackling main CoD among neonates has the potential to reduce neonatal deaths by an estimated 71%, benefit women and children after the first month, and reduce stillbirths.

Conclusion: The SDG target aims to reduce neonatal deaths to a maximum of 12 per 1000 live births in every country of the world by 2030. If every country achieves this target, approximately 5 million neonatal lives will be saved throughout the period 2017-2030 and all of the efforts focused in helping neonates go through "the golden first 28 days of life" will have been worthwile.

KEYWORDS

neonatal mortality, neonatal deaths, child mortality, cause of death, sustainable development goals.

BACKGROUND

The first two decades of the 21st century can indisputably be characterized by a global health revolution that has led to massive improvements in health outcomes, particularly in terms of child survival. Indeed, never before in the history of mankind the chances of surviving for any new baby born in this world have been greater, with child mortality becoming anecdotal in many industrialized regions, and the number of child deaths decreasing globally at a fast pace, from over 17 million annual deaths in the 1970's to around 5.6 million in 2016[1,2]. Such unprecedented and impressive reductions are the result of many national and international efforts and coinciding circumstances, but have essentially been catalyzed by the establishment, back in the year 2000, of the Millennium Development Goals (MDG), a set of laudable aims agreed upon by all 191 United Nations countries, designed to combat poverty, hunger, disease, illiteracy, environmental degradation, and discrimination against women[3]. Many countries succeeded in achieving in the period 1990-2015 the 2/3rds reduction in child mortality stipulated by MDG4, but global progress was unequal, and the global target has not been completely achieved[4]. The particular push and drive for child survival provided by the MDGs has now been taken over by the more recent Sustainable Development Goals (SDGs), and in particular by SDG3.2[5], which specifies an even more ambitious global target, to be achieved by the year 2030, and which should be seen as a new opportunity to save millions of lives.

MAIN TEXT

Such applauded decreases in child mortality achieved in the last decades offer significant nuances. From a geographical and socio-economic point of view, and despite the fact that such falls have been confirmed in all areas of the world, reductions have been more modest in low- or middle-income countries (LMIC) and in particular in Sub-Saharan Africa (SSA). It is no coincidence that up to 99% of all child deaths are now circumscribed to these settings[6,7,] a strident reminder of the many inequities driving global health. From an age category point of view, neonatal deaths, those defined as occurring within the first 28 days of life, have also decreased, but at a much slower rate, estimated at around 3.1% annually, from the 30 deaths per 1000 live births in 2000 to 19 in 2016[1,2,8]. Thus, neonatal mortality has become an increasing driver of the overall global paediatric mortality, representing an increasing proportion of all annual child deaths (37.4% in 1990, 46% or an equivalent of 2.6 Million deaths in 2016[1,2]). Decreases in

neonatal mortality rates (NMR) have also shown similar regional variations, with reductions ranging from ~80% in eastern Asia, or ~50% in southern Asia (SEA) to ~40% in SSA in the same period[8]. The highest NMR are found in SSA (27.7 per 1000 Live births (LB) and in SEA (27.6 per 1000 LB), multiplying by 10 fold those documented in Europe (2.9 per 1000 LB)7,8. In the absence of changes in the current declining rates, it will be over a century before a newborn from SSA has the same survival probability as one born in Europe[7].

The first month of life is undeniably the most vulnerable period for the child's survival. Within these first four weeks, the risk is greatest at the very beginning of life, with up to three-quarters of all deaths occurring in the first seven days, and half of those in the first 24h[1,8,9]. Surviving therefore through childbirth and the "golden first 28 days" appears therefore as a key milestone required to thrive and lead a healthy subsequent life. But, what makes the neonatal period such a critical moment? Why should a time period accounting for as little as 0.1% of the entire length of an average life (70 years), define more significantly the likelihood of surviving than any other time? There are multiple reasons that can account for this, but most of them gravitate around two premises: 1) The vicious circle established between poverty and disease, which appears very challenging to break and is particularly noticeable in this vulnerable period; and 2) the global disparities existing in terms of the quality of and access to health care[10], seriously jeopardized in those regions with less resources. If you add to the aforementioned fragile and weak health systems a myriad of diseases, particularly prevalent in those impoverished areas, then it is not difficult to understand why certain nations struggle to improve their health indicators.

More specifically, a healthy birth requires a previous healthy intrauterine development. For this, the pregnant mother needs to remain as healthy as possible, and a series of factors need to be controlled during the entire duration of gestation, and the puerperium. In rich countries, this is guaranteed by the constant control of the pregnancy, and the implementation of a series of preventive and therapeutic strategies designed to keep the dyad mother-child healthy. Comprehensive antenatal consultations are also capable of triggering actions that can accelerate an agile delivery should this become necessary. In poorer regions, antenatal care (ANC) consultations are increasing, although coverage rates are still far from reaching the current World Health Organization (WHO) recommendation

of a minimum of four ANC visits[11]; and more importantly, the quality and availability of the health care interventions provided remains suboptimal. Similar challenges face the critical moment of childbirth, with coverage rates for deliveries attended by a skilled professional remaining insufficiently high, at a median of 65% during the period 2009- 2014[12]. Solving the maternal part of the equation responsible for neonatal outcomes requires starting by ensuring that maternal mortality rates also continue to globally decrease[13], in addition to heavily investing in improving the primary health care bottlenecks[14], and progressively implementing and scaling-up strategies that have already shown their cost-effectiveness in other settings of the world, or other innovative measures[14,15]. Not an easy task, or at least not one with immediate results. Such challenges are particularly blatant also in the context of stillbirths, the neglected victims of poverty, accounting for almost identical numbers of annual deaths to those occurring among newborns[16], and whose oblivion is finally starting to be adequately addressed in the global health agenda[6,17].

From the newborn's point of view, similar challenges can be expected in LMICs in relation to access to care, or quality of the preventive or therapeutic strategies available in country, leading to worsened health outcomes. However, the demographic explosion currently seen in SSA implies that this continent has become the principal birthplace of the world. Indeed, of the circa 130 million new babies born every year in the world, over 30% are already born in SSA, and this trend is expected to increase, with more than 1 in 3 children in the world expected to live in the continent by 2050[18]. Coverage rates of well-known and effective interventions to improve the care and health of the newborns are still lagging in these settings, providing much room for improvement[7,12,17]. A simple but rather paradigmatic example has to do with the coverage of post-natal visits for babies, a clear WHO recommendation[19] that is only followed in a median of 28% of the births, globally[12]. How can you prevent, detect or treat disease, if you are missing nearly three quarters of all your target population? This is, indeed, the most neglected period for the provision of quality care[19].

A more philosophical issue with very significant practical implications also hinders our current understanding on how to count, prevent and treat those conditions prematurely killing newborns, and is linked to our fundamentally limited knowledge of what is really killing them. In addition to those "invisible" deaths[20] occurring at the community level outside the

health system, and in the absence of functioning vital registration systems, there is a profound lack of knowledge of the real underlying causes for these deaths. According to the last global estimates in 2016, leading causes of death among newborns included preterm birth complications (35%), intrapartum-related events (24%), infectious diseases including sepsis/ meningitis, pneumonia and tetanus (22%) and congenital disorders (11%)[2]. However, models utilized to build estimates for LMIC, where the vast majority of preventable deaths concentrate, have shown many flaws, and discrepancies in cause-attributable disease figures[21] have unraveled the shortfall of current methods, predominantly based on data derived from unreliable or poorly specific sources, including verbal autopsy or clinical records. The paucity of evidence-based data, and the obvious limitations of currently used methodologies to infer cause of death (CoD) in resource-constrained settings, warrant caution when interpreting current CoD estimates, at least if those are to be used for rational health planning and prioritisation decisions[22]. Innovation is key to overcome such limitations, and in this respect, more robust methodologies, such as the minimally invasive tissue sampling (MITS) post-mortem sampling techniques[23]. This technique has been validated against the reference complete pathological autopsy as a reliable CoD investigation tool in all age groups, -including newborns[24]-, and may appear as useful alternatives that can provide actionable and reliable data and thus contribute to better characterize mortality in this age group, and the necessary measures to specifically address it. Importantly, such methods are currently being implemented in sentinel sites across SSA and Asia, for perinatal and paediatric mortality surveillance through the Child Health and Mortality Prevention Surveillance Network (CHAMPS)[25,26]. Dissemination on an ongoing basis of the results of this ambitious and long-lasting project will surely contribute to better neonatal survival practices.

Irrespective of the said caveats regarding the precision and robustness of current estimates, infectious diseases are well-characterized contributors in LMIC to morbidity and mortality in the neonatal period[1,2,27]. In the newborn, some of these infections can be acquired in the community soon after birth, although the majority result from the vertical transmission of microorganisms from mother to child, either during gestation or at delivery, in particular bacteria, which are normal commensals or pathogens of the mother's genitourinary and gastrointestinal tracts. Important infectious clinical syndromes causing neonatal death include sepsis[28], meningitis

and pneumonia. The microorganisms more frequently involved in serious infections in children younger than 3 months in LMIC include, among others, group B Streptococcus (GBS)[29], Escherichia coli (E. coli), Listeria monocytogenes, Staphylococcus aureus, other gram-negative bacteria, Haemophilus influenzae type B, Streptococcus pneumoniae, respiratory syncytial virus (RSV), Neisseria meningitides and Bordetella pertussis. However, in the context of the Human immunodeficiency virus (HIV) pandemic, many other microorganisms (including viruses, parasites or fungi) have emerged as important pathogens for the newborn. This information is relevant because for the majority of those neonatal infections, highly effective preventive or therapeutic strategies are available, and as a result of their implementation their burden in neonates from industrialized countries has been significantly reduced. As an example, GBS and E. coli, two well-known leading causes of neonatal morbidity and mortality, are vertically transmitted and particularly associated with early and late-onset neonatal sepsis, preterm birth and very-low-birth-weight delivery[30,31]. Overall case fatality rates associated to GBS in a global systematic review were 8.4%, peaking in SSA at 19%[32], precisely the place where highly effective prevention strategies such as intrapartum antibiotic prophylaxis (IAP) have not been implemented due to the scarcity of laboratory support and the fragility of the health systems[30]. E. coli is considered the most frequent cause of invasive bacterial infection in preterm infants and to date, no prevention measures have been developed[33]. Other infectious diseases also considered as important contributors to neonatal mortality include infections encompassed under the TORCH syndrome. Among them, congenital cytomegalovirus (CMV) infection is the most prevalent, but also remains majorly neglected in both the developed and developing world[34], with its real burden and impact being largely unknown[35]. An interesting approach to prevent many of these neonatal infections now includes maternal vaccination during pregnancy[36], under the assumption that maternal transfer of antibodies to the newborn will be more feasible, effective and rapidly protective than waiting for the generation of neonatal immune responses to vaccines administered directly to them. This “vertical vaccination” strategy has already been successfully implemented for tetanus and pertussis control, and is currently being explored for several other pathogens, including GBS, RSV, Flu and S. pneumoniae[37].

Other major well-characterized non-infectious causes of neonatal death include preterm-birth complications, intrapartum–related complications and congenital abnormalities. Births that follow spontaneous preterm labour usually

are the consequence of specific maternal factors, including pregnancy specific complications (hypertension, diabetes, malnutrition), infections, and other non-preventable causes[38]. Some of these causes, as previously mentioned, can be proactively screened for during ANCs clinics and easily managed. Intrapartum-related complications, often leading to life-threatening perinatal asphyxia, are however more difficult to prevent, and their management is highly dependent on the availability of skilled staff attending the delivery[39]. Congenital abnormalities are much harder to prevent, particularly in the absence of early ultrasound screening for pregnancies, a strategy poorly implemented and often unavailable in LMICs. Such congenital abnormalities, however, may be preventable in some cases (for instance by providing folic acid supplementation during pregnancy), and may occur more frequently in those countries with high levels of consanguineous partnerships[39]. Finally, other well-known but moderately neglected (likely due to their multifactorial aetiology) causes of neonatal mortality include metabolic disturbances such as hypoglycaemia[40]. Again, limitations in the availability or usage of cheap diagnostic tools for its diagnosis, hinders the adequate recognition of simple complications that in the absence of a rapid detection and correction become rapidly lethal.

CONCLUSIONS

The specific neonatal mortality SDG target aims to reduce NMRs to a maximum of 12 per 1000 live births in every country of the world by 2030[5]. The SDGs offer also an international collaborative platform of international consensus to advance together towards the global achievement of these goals. The wider ambitions of the SDGs in comparison to the MDGs cannot dilute the importance of health and child survival in these new objectives. Importantly, in the absence of an accelerated progress in neonatal mortality, the world will fail to significantly reduce overall child mortality. Universal coverage of essential interventions has the potential to reduce neonatal deaths by an estimated 71%, benefit women and children after the first month, and reduce stillbirths[14]. Particular support, attention and stewardship to LMICs, which have the greatest needs -but also the tiniest resources- will be critical so as to enhance the uptake and scale-up of these interventions, in an equitable manner. If every country achieves the SDG target for neonatal survival by 2030, approximately 5 million neonatal lives will be saved throughout the period 2017–20308 and all of the efforts concentrated in helping newborns go through the arid and sinuous “golden first 28 days of life” will have been worthwile.

REFERENCES

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; 388(10063): 3027-35.

2. United Nations. Levels & Trends Child Mortality. Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation. *Report* 2017. Available at: http://www.childmortality.org/index.php?r=site/index ; (accessed Nov 11, 2017). New York, 2017.

3. World Health Organization. Millennium Development Goals. Available at: http://www.who.int/topics/millennium_development_goals/about/en/ (Accessed 06 Dec 2017). 2000.

4. Denno DM, Paul SL. Child Health and Survival in a Changing World. *Pediatr Clin North Am* 2017; 64(4): 735-54.

5. United Nations. Sustainable Development Goals. New York: United Nations, 2015, (accessed Oct 11, 2017). Available at: http://www.un.org/sustainabledevelopment/summit 2015.

6. Global Burden of Disease Child Mortality Collaborators. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388(10053): 1725-74.

7. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. *Lancet* 2014; 384(9938): 189-205.

8. UNICEF. UNICEF Data: Monitoring the Situation of Children and Women. Available at: https://data.unicef.org/topic/child-survival/neonatal-mortality/ (Accessed: 24th October 2017). 2017.

9. Oza S, Cousens SN, Lawn JE. Estimation of daily risk of neonatal death, including the day of birth, in 186 countries in 2013: a vital-registration and modelling-based study. *Lancet Glob Health* 2014; 2(11): e635-44.

10. Healthcare Access and Quality Index based on mortality from causes amenable to personal health care in 195 countries and territories, 1990-2015: a novel analysis from the Global Burden of Disease Study 2015. *Lancet* 2017; 390(10091): 231-66.

11. World Health Organization. WHO recommendations on antenatal care for a positive pregnancy experience. Geneva; 2016.

12. Victora CG, Requejo JH, Barros AJ, et al. Countdown to 2015: a decade of tracking progress for maternal, newborn, and child survival. *Lancet* 2016; 387(10032): 2049-59.

13. Kassebaum NJ, Bertozzi-Villa A, Coggeshall MS, et al. Global, regional, and national levels and causes of maternal mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 2014; 384(9947): 980-1004.

14. Dickson KE, Simen-Kapeu A, Kinney MV, et al. Every Newborn: health-systems bottlenecks and strategies to accelerate scale-up in countries. *Lancet* 2014; 384(9941): 438-54.
15. Moxon SG, Ruysen H, Kerber KJ, et al. Count every newborn; a measurement improvement roadmap for coverage data. BMC *Pregnancy Childbirth* 2015; 15 Suppl 2: S8.
16. World Health Organization. Making every baby count: audit and review of stillbirths and neonatal deaths. Geneva; 2016.
17. Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? *Lancet* 2014; 384 (9940): 347-70.
18. UNICEF. Brochure: CHILDREN IN AFRICA. Key statistics on child survival, protection and development. Accessed Dec 06, 2017. (Available at: https://data.unicef.org/wp-content/uploads/2015/12/Children-in-Africa-Brochure-Nov-23-HR_245.pdf). 2015.
19. World Health Organization. WHO recommendations on Postnatal care of the mother and newborn. Geneva; 2013.
20. Setel PW, Macfarlane SB, Szreter S, et al. A scandal of invisibility: making everyone count by counting everyone. *Lancet* 2007; 370(9598): 1569-77.
21. Liu L, Black RE, Cousens S, Mathers C, Lawn JE, Hogan DR. Causes of child death: comparison of MCEE and GBD 2013 estimates. *Lancet* 2015; 385(9986): 2461-2.
22. Byass P. Who needs cause-of-death data? PLoS Med 2007; 4(11): e333.
23. Bassat Q. Minimally Invasive Autopsy: Welcoming a New Tool for Cause of Death Investigation in Children in Resource-constrained Countries.
J Trop Pediatr 2017.
24. Menendez C, Castillo P, Martinez MJ, et al. Validity of a minimally invasive autopsy for cause of death determination in stillborn babies and neonates in Mozambique: An observational study. *PLoS Med* 2017; 14(6): e1002318.
25. Farag TH, Koplan JP, Breiman RF, et al. Precisely Tracking Childhood Death. *Am J Trop Med Hyg* 2017; 97(1): 3-5.
26. Foundation. TBMG. The Bill & Melinda Gates Foundation to fund disease surveillance network in Africa and Asia to prevent childhood mortality and help prepare for the next epidemic. Seattle, USA. Available at: http://www.gatesfoundation.org/Media-Center/Press-Releases/2015/05/Child-Health-and-Mortality-Prevention-Surveillance-Network; (accessed Oct 23, 2017). 2015.
27. Global Burden of Disease Child Mortality Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; 388(10053): 1459-544.

28. Shane AL, Sanchez PJ, Stoll BJ. Neonatal sepsis. *Lancet* 2017; 390(10104): 1770-80.
29. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clin Infect Dis* 2017; 65(suppl_2): S200-S19.
30. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet* 2012; 379(9815): 547-56.
31. Krohn MA, Thwin SS, Rabe LK, Brown Z, Hillier SL. Vaginal colonization by Escherichia coli as a risk factor for very low birth weight delivery and other perinatal complications. *J Infect Dis* 1997; 175(3): 606-10.
32. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; 65(suppl_2): S160-S72.
33. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. *Pediatrics* 2011; 127(5): 817-26.
34. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. *Clin Infect Dis* 2009; 49(4): 522-8.
35. Lanzieri TM, Dollard SC, Bialek SR, Grosse SD. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. Int J Infect Dis 2014; 22: 44-8.
36. Bassat Q. Maternal immunization: an intelligent solution to reduce the hidden burden of group B streptococcus perinatal disease. *J Trop Pediatr* 2013; 59(5): 333-7.
37. Lassi ZS, Middleton PF, Crowther C, Bhutta ZA. Interventions to Improve Neonatal Health and Later Survival: An Overview of Systematic Reviews. *EBioMedicine* 2015; 2(8): 985-1000.
38. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; 371(9606): 75-84.
39. Garces AL, McClure EM, Perez W, et al. The Global Network Neonatal Cause of Death algorithm for low-resource settings. *Acta Paediatr* 2017; 106(6): 904-11.
40. Madrid L, Acacio S, Nhampossa T, et al. Hypoglycemia and Risk Factors for Death in 13 Years of Pediatric Admissions in Mozambique. *Am J Trop Med Hyg* 2016; 94(1): 218-26.

ARTICLE 2

Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses

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SUPPLEMENT ARTICLE

Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses

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Background. Group B *Streptococcus* (GBS) remains a leading cause of neonatal sepsis in high-income contexts, despite declines due to intrapartum antibiotic prophylaxis (IAP). Recent evidence suggests higher incidence in Africa, where IAP is rare. We investigated the global incidence of infant invasive GBS disease and the associated serotypes, updating previous estimates.

Methods. We conducted systematic literature reviews (PubMed/Medline, Embase, Latin American and Caribbean Health Sciences Literature [LILACS], World Health Organization Library Information System [WHOLIS], and Scopus) and sought unpublished data regarding invasive GBS disease in infants aged 0–89 days. We conducted random-effects meta-analyses of incidence, case fatality risk (CFR), and serotype prevalence.

Results. We identified 135 studies with data on incidence (n = 90), CFR (n = 64), or serotype (n = 45). The pooled incidence of invasive GBS disease in infants was 0.49 per 1000 live births (95% confidence interval [CI], .43–.56), and was highest in Africa (1.12) and lowest in Asia (0.30). Early-onset disease incidence was 0.41 (95% CI, .36–.47); late-onset disease incidence was 0.26 (95% CI, .21–.30). CFR was 8.4% (95% CI, 6.6%–10.2%). Serotype III (61.5%) dominated, with 97% of cases caused by serotypes Ia, Ib, II, III, and V.

Conclusions. The incidence of infant GBS disease remains high in some regions, particularly Africa. We likely underestimated incidence in some contexts, due to limitations in case ascertainment and specimen collection and processing. Burden in Asia requires further investigation.

Keywords. group B *Streptococcus*; early onset; late onset; estimate; case fatality risk.

Group B *Streptococcus* (GBS; *Streptococcus agalactiae)* is a leading infectious cause of neonatal morbidity and mortality, well described in high-income contexts (HICs) [1–8], but less well studied in low- to middle-income contexts (LMICs) and low-income contexts (LICs) [9]. A systematic review in 2012 [9], reported an overall incidence of invasive GBS disease among infants of 0.53 per 1000 live births (95% confidence interval [CI], .41–.62), with the highest incidence in Africa (1.21 per 1000 live births), followed by the Americas (0.67 per 1000 live births) and the lowest incidence in the Western Pacific (0.15 per 1000 live births) and Southeast Asia (0.016 per 1000 live births). Although data, especially from LICs, were limited, case fatality risks (CFRs) were higher in Africa (22%) compared with the Americas (11%) or Europe (7%) [9].

Understanding the global burden of GBS disease in young infants (0–89 days), including neonates (0–27 days), is important to guide public health decision making on interventions. Many HICs have implemented intrapartum antibiotic prophylaxis (IAP), aiming to reduce early-onset GBS disease (EOGBS; days 0–6) for women with rectovaginal GBS colonization detected through microbiological screening or with clinical risk

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factors [10, 11]. However, this strategy will not reduce late-onset infant GBS disease (LOGBS; onset on days 7–89 of life), and in LMICs and LICs, where there are more home deliveries and women present later for delivery, IAP may be less feasible and effective than other potential strategies for prevention, such as a maternal GBS vaccine.

This article therefore aims to examine the incidence of invasive GBS disease among young infants and the associated CFR and serotypes causing GBS invasive disease (Figure 1). It is part of a supplement estimating the burden of GBS disease among pregnant women, stillbirths, and infants [12]. The supplement includes systematic reviews and meta-analyses on GBS colonization, and adverse outcomes associated with GBS around birth [10, 13–19], which provide data inputs for estimating the worldwide burden of GBS [20].

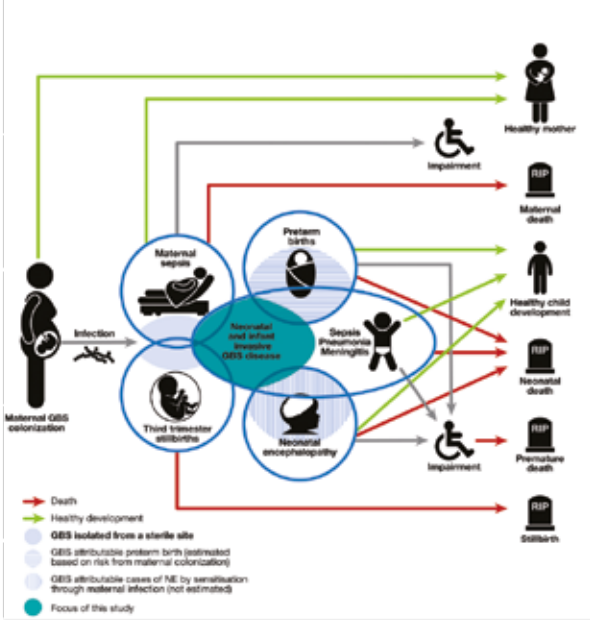
OBJECTIVES

1. To provide a comprehensive, systematic literature review and meta-analyses on the burden of infant invasive GBS disease to include:
   a. Incidence of infant GBS disease: overall incidence risk, including stratification by EOGBS and LOGBS.
   b. CFR for EOGBS and LOGBS (7–89 days) and neonatal disease (7–27 days).
   c. Serotype distribution: prevalence of GBS serotypes causing GBS disease among infants.
2. To generate parameters to be used as data inputs in a compartmental model estimating the burden of GBS in pregnancy for women, stillbirth, and infants; including
   a. EOGBS to LOGBS ratio.
   b. Clinical syndrome (proportion of neonatal disease that was meningitis or sepsis).
3. To evaluate data gaps and recommend improvements for the data regarding GBS disease in young infants.

METHODS

This article is part of a protocol entitled "Systematic estimates of the global burden of GBS in pregnant women, stillbirths and infants," submitted for ethical approval to the London School of Hygiene & Tropical Medicine (reference number 11966) and approved on 30 November 2016. The general methods are described elsewhere [12]; here we present details specific to estimates related to the incidence of invasive GBS disease among infants.

We included studies that described incidence risk, deaths, or serotypes of bacterial isolates among infants aged 0–89 days with invasive GBS disease. Eligible studies were those reporting data published or unpublished between 1 January 2000 and 31 January 2017, limited to humans and with no language



Parameter	Case definitions used for estimates	Study
Group B Streptococcus maternal colonization	Isolation by culture of GBS from either the vagina (high or low), rectum or peri-anal region at any time during pregnancy	Russell et al. [13]
Maternal GBS disease	Laboratory isolation of GBS from sterile site in pregnant or postpartum woman (up to 42 days postpartum), with clinical signs of sepsis	Hall et al. [14]
Stillbirth GBS invasive disease	Birth of a fetus weighting >1000g and/ or ≥28 weeks' gestation age with no signs of life and evidence of GBS invasive disease from a normally sterile site such as fetal blood, lung aspirate or cerebrospinal fluid	Seale et al. [15]
Neonatal and infant GBS invasive disease	Laboratory isolation of Streptococcus agalactiae from a normally sterile site in an infant aged 0 to 89 days with signs of clinical disease, including meningitis, sepsis or bacteremic pneumonia	
Neonatal encephalopathy with invasive GBS disease	Laboratory isolation of GBS from a sterile site (blood, cerebrospinal fluid analysis or on a post mortem sample) in cases of neonatal encephalopathy, hypoxic-ischaemic encephalopathy and intrapartum-related death	Tann et al. [18]
Neurodevelopment impairment in children after GBS invasive disease	Cognitive and/or motor, vision or hearing impairment in survivors of invasive infant GBS disease isolated from a normally sterile site	Kohli-Lynch et al. [19]
Preterm birth associated with GBS maternal colonization	Delivery prior to completion of 37 weeks' gestation from mother with maternal GBS colonization isolated from vaginal, cervical and/or rectal swabs	Bianchi Jassir et al. [16]

Figure 1. Infant group B streptococcal (GBS) disease in disease schema for GBS, as described by Lawn et al [12].

restrictions. We identified data through systematic review of the published literature and through an investigator group that sought unpublished data from clinicians, researchers, and relevant professional institutions worldwide.

Definitions

Invasive GBS disease was defined as laboratory isolation of *S. agalactiae* from any normally sterile site using conventional microbiological methods together with any signs of clinical disease. EOGBS was defined as invasive GBS disease in infants aged 0–6 days after birth and LOGBS in infants 7–89 days after birth. Incidence risk was defined as cases per 1000 live births and CFR as number of deaths in GBS cases divided by total GBS cases.

Search Strategy

We undertook systematic literature searches of PubMed/Medline, Embase, Literature in the Health Sciences in Latin America and the Caribbean (LILACS), the World Health Organization Library Information System (WHOLIS), and Scopus databases using the search terms ("*Streptococcus agalactiae*" [Medical subject headings (MeSH)] OR "*Streptococcus* Group B" OR "Group B streptococcal") AND "infant," "outcome," "death," "mortality," "case AND fatality AND rate." We limited searches to humans and publications from 1 January 2000 to 31 January 2017 (see Supplementary Table 1 for the full list of search terms). For consistency, we used the same search terms as a previous systematic review [9]. We did not apply date or language restrictions; texts were translated to English when published in other languages. An additional search for reports with serotype data was performed, using the search terms ("*Streptococcus agalactiae serotype*" [MeSH] OR "*Streptococcus* Group B serotype" OR "Group B streptococcal serotype") using the same limits above. We used snowball searches of article reference lists including reviews to identify additional studies

One investigator performed the database search, screened for duplicates, and screened titles and abstracts to assess eligibility for inclusion. Two independent investigators (L. M. and M. K. L.) assessed the full-length articles associated with selected abstracts to confirm eligibility and extract data. Where there was discrepancy between the 2 reviewers, a third investigator (A. S.) made the final decision.

Study Selection

We included studies with original data on GBS disease in infants who were aged 0–89 days at onset of infection episode, with clinical specimens obtained from a sterile site, which had a population denominator (total live births). We excluded studies focusing on very high-risk groups (such as only human immunodeficiency virus [HIV]–infected infants or only preterm infants), where data were not representative of live births in the population. Where countries had multiple or duplicated publications or systematically collected surveillance data, we included the most recent data. Studies reporting GBS disease in infants aged 0–90 days that did not specify age at onset for the individual cases were included with the 0–89 day studies as the probability of a case on day 90 is negligible. For full details of inclusion and exclusion criteria, see Supplementary Table 2.

Data Abstraction

We used a standardized data abstraction tool to capture information on the study design (prospective or retrospective), setting (health facility or not), use of IAP, timing of clinical disease (onset in the first 24–48 hours, EOGBS, and LOGBS), outcomes (survived or died), sample type (cerebrospinal fluid, blood, or other sterile site) and GBS serotype. For facility-based studies limited to babies born at the facility, facility live births was used as denominator. Where studies included inborn and outborn babies, a population denominator of all live births in the catchment area of the health facility was used. Data on study location were also abstracted including country and town. These data were imported into Stata version 14 software.

Analysis

We used random-effects meta-analyses to estimate overall infant disease incidence, EOGBS and LOGBS incidence, the EOGBS to LOGBS incidence ratio, and CFRs using the DerSimonian and Laird method [21]. In addition to worldwide estimates, estimates by United Nations regions and/or subregions were obtained when sufficient data were available.

To assess bias, we performed the following sensitivity analyses:

1. Invasive disease:
   a. Infant invasive disease limited to facility-based studies where denominator was facility births.
   b. EOGBS estimates limited to studies including data for days 0–6 after birth.
   c. LOGBS estimates limited to studies including data for days 7–89 after birth.
   d. Late-onset neonatal incidence limited to studies with data for days 7–27 after birth.
2. The ratio of early-onset disease to late-onset disease, including only studies considered to be less subject to case finding bias resulting from low access to care, nonsystematic sampling, or suboptimal laboratory detection methods [22–25] as considered by the expert advisory group.

RESULTS

Literature Search and Study Selection

We identified 7535 articles for consideration from database searches, 318 additional records from expert groups in neonatal care and reference lists, and 7 datasets from an investigator group [22, 23, 26] (Araujo da Silva et al. unpublished, Dhaded et

al. unpublished, Saha et al. unpublished, Sigaúque et al. unpublished). One hundred thirty-five articles (reporting data from 57 countries) met our inclusion criteria (search strategy of study selection in Figure 2). Of these, 90 reported incidence [22–96], (EOGBS: 74 studies; LOGBS: 33 studies), 64 reported CFR [22–27, 30, 34, 37, 38, 41–43, 46, 49–57, 59, 61, 63, 68, 70, 71, 73–75, 78–81, 84, 89, 90, 92, 95–111], and 45 reported serotype data [22–25, 31, 42, 47, 50, 55, 61, 63, 66, 70, 73, 78, 81, 84, 112–138]. (The full list of articles included in this review is available in Supplementary Table 3.) Articles excluded because more recent data from the same population were available are shown in Supplementary Table 4 and Supplementary Figure 1. Compared to the previously published global GBS invasive disease estimates [9], we included 61 additional studies: 34 reporting incidence, 35 CFR, and 26 serotype (Supplementary Figure 2).

Study Characteristics

There were more data from HICs (77 studies) compared to LMICs (18 studies), of which 12 were from Africa (11 in sub-Saharan Africa, 1 in North Africa). Data inputs are illustrated in Figure 3A and 3B. Data inputs of the previous systematic review [9] are shown in Supplementary Figures 3*A* and 3*B*. Most studies (109/135) were facility based and information about IAP use was available from 116 of 135 studies (Table 1). Seventy-six studies reported any use of IAP: 27 of 76 (35.5%) were based on screening, 14 of 76 (18.4%) were based on a risk factor algorithm, and 35 of 76 (46.1%) did not specify a strategy. Of those studies reporting incidence, 58 of 90 (64.4%) reported use of any IAP; this was highest in developed countries (46/58 [79.3%]) and lowest in sub-Saharan Africa (3/11 [27.3%]) (Supplementary Figure 4). Of 74 studies that reported EOGBS, 49 (67.1%) reported IAP use and approximately one-third of articles (24/74 [32.4%], including 6 studies from LICs and LMICs) reported information about age at onset of EOGBS. Serotype was available in studies from 25 countries (developed countries, 16; Central and South America, 4; Southern and Eastern Africa, 3; Eastern Asia, 2). We were unable to abstract data on laboratory methods used, maternal risk factors, and weight or gestational age at birth of neonates.

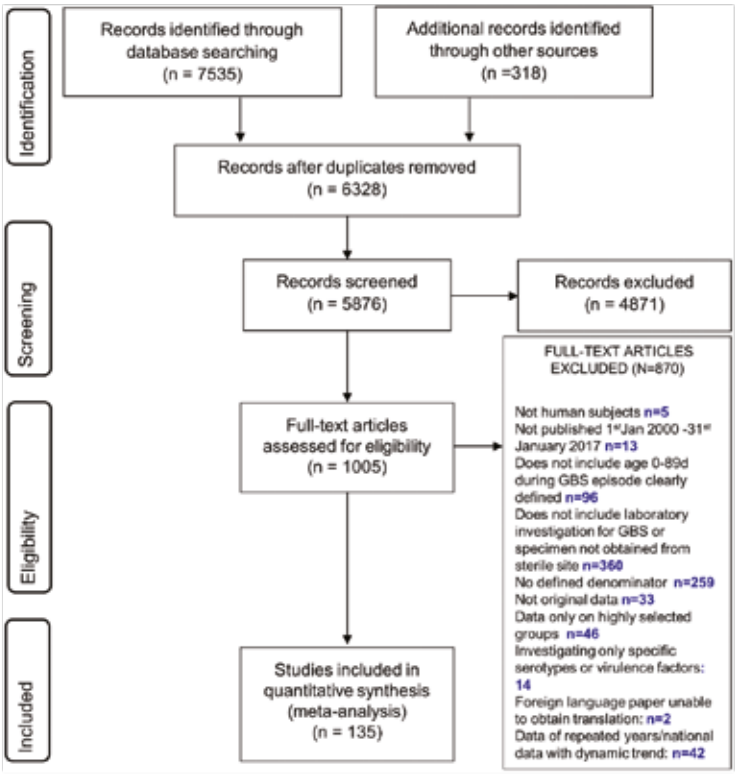


Figure 2. Search strategy and process of study selection. Abbreviation: GBS, group B *Streptococcus*.

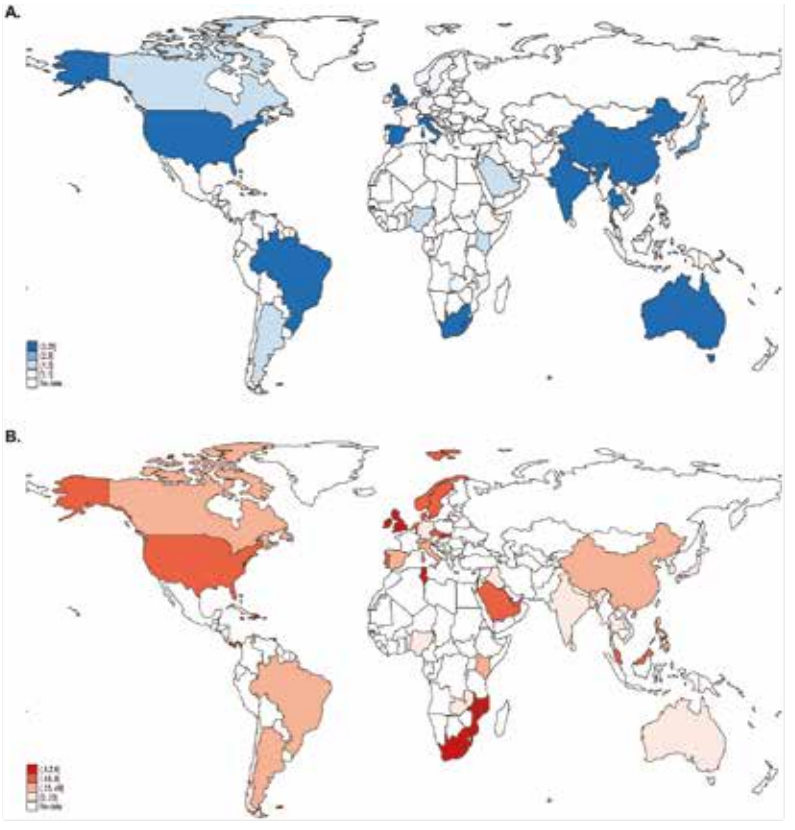


Figure 3. Worldwide distribution of data inputs. *A*, Map illustrating number of studies by country reporting incidence of group B streptococcal (GBS) invasive disease. *B*, Map illustrating overall incidence of GBS disease among infants by country included in the meta-analyses. Borders of countries/territories in map do not imply any political statement.

Incidence Risk of Group B *Streptococcus* Disease

There were 6199 infants with invasive GBS disease among 13 300 000 live births in 53 countries. The incidence risk (per 1000 live births) for infant GBS disease was 0.49 (95% CI, .43–.56) overall, being 1.12 in Africa, 0.49 in Latin America and the Caribbean, 0.46 in developed countries, and the lowest in Asia, 0.30. Incidence was highest in Southern Africa (2.00 [95% CI, .74–3.26]) and lowest in Southeast Asia (0.21 [95% CI, .09–.32]; meta-analysis in Figure 4). There were 3664 cases of EOGBS from 9 866 793 live births. Incidence risk (per 1000 live births) of EOGBS worldwide was 0.41 (95% CI, .36–.47) and ranged from 0.32 (95% CI, .22–.41) in Asia to 0.71 (95% CI, .24–1.18) in Africa. The Caribbean had the highest incidence risk of EOGBS (1.47), followed by Southern Africa (1.07) and South Asia the lowest (0.20) (Supplementary Figure 5). Among EOGBS cases, 68% (95% CI, 57%–79%) developed symptoms in the 24 hours after birth, being higher in HIC (74% [95% CI, 58%–89%]) compared with LICs (31% [95% CI, −20% and 82%]); meta-analysis included as Supplementary Figure 6). There were 2003 cases of LOGBS among 8 975 899 live births. Incidence risk of LOGBS worldwide was 0.26 (95% CI, .21–.30), ranging from 0.04 (95% CI, −.02 to .09) in Asia to 0.65 (95% CI, .25–1.05) in Africa. Southern Africa had the highest incidence risk of LOGBS (0.93), and South America, Western Africa, and Southeastern Asia had the lowest (0.0, 0.0, and 0.03, respectively, based on the single study captured from each of these regions; Supplementary Figure 7).

Case Fatality Risk

There were 570 deaths among 6501 infant cases. The overall CFR was 8.4% (95% CI, 6.6%–10.2%). CFR in Africa (18.9% [95% CI, 13.7%–24.0%]) was 4 times higher than in developed countries (4.7% [95% CI, 3.3%–6.1%]) (meta-analysis in Supplementary Figure 8). EOGBS CFR was 10.0% (95% CI, 7.0%–12.0%) ranging from 5.0% (95% CI, 4.0%–7.0%) in developed countries to

Table 1. Characteristics of Included Studies Investigating Invasive Group B Streptococcal Disease in Infants

Characteristic	Total (135 Articles)	Incidence (90 Articles)	CFR (64 Articles)	Serotypes (47 Articles)
United Nations subregion				
Developed countries	58 (43.0)	32 (35.6)	28 (43.8)	32 (68.1)
Central America	2 (1.5)	2 (2.2)	1 (1.6)	1 (2.1)
Caribbean	6 (4.4)	5 (5.6)	4 (6.2)	0 (0.0)
South America	15 (11.1)	9 (10.0)	8 (12.5)	4 (8.5)
Northern Africa	1 (0.7)	1 (1.1)	0 (0.0)	0 (0.0)
Eastern Africa	5 (3.7)	4 (4.4)	4 (6.2)	2 (4.3)
Western Africa	3 (2.2)	3 (3.4)	1 (1.6)	0 (0.0)
Southern Africa	3 (2.2)	3 (2.3)	3 (4.7)	2 (4.3)
Eastern Asia	17 (12.6)	7 (7.8)	8 (12.5)	5 (10.6)
Western Asia	8 (5.9)	7 (7.8)	2 (3.1)	0 (0.0)
Southern Asia	7 (5.2)	7 (7.8)	3 (4.7)	1 (2.1)
Southeastern Asia	10 (7.4)	10 (11.1)	2 (3.1)	0 (0.0)
Study design				
Prospective	53 (39.3)	46 (51.1)	26 (40.6)	12 (25.5)
Retrospective	82 (60.7)	44 (48.9)	38 (59.3)	35 (74.5)
Population/facility-based study[a]				
Population-based	24 (18.8)	18 (20.0)	13 (20.3)	35 (76.1)
Facility based	109 (81.2)	71 (78.9)	50 (78.1)	11 (23.9)
Reporting period				
Full period (0–89 d)[b]	10 (7.4)	10 (11.1)	10 (15.6)	6 (12.7)
Full EOGBS period (0–6 d)[c]	42 (31.1)	42 (46.7)	30 (46.9)	13 (27.7)
Full LOGBS period (7–89 d)[d]	11 (8.1)	11 (12.2)	11 (17.2)	5 (10.6)
Specimen type				
Blood only	27 (20.0)	19 (21.8)	12 (18.8)	3 (6.4)
CSF only	5 (3.7)	2 (2.3)	2 (3.1)	2 (4.3)
Blood and CSF	75 (55.6)	53 (58.9)	36 (56.3)	27 (57.5)
All sterile sites	25 (18.5)	14 (15.6)	14 (21.9)	15 (31.9)
IAP				
Any IAP used	76 (65.5)	58 (69.9)	41 (70.7)	21 (43.8)
No IAP	40 (34.5)	25 (30.1)	17 (29.3)	27 (56.2)
Rural/urban				
Rural	2 (1.5)	2 (2.2)	1 (1.6)	1 (2.1)
Urban	69 (51.1)	46 (51.1)	33 (51.6)	21 (44.7)
Semirural	2 (1.5)	2 (2.2)	2 (3.1)	2 (4.3)
Mixed	30 (22.2)	22 (24.4)	15 (23.4)	11 (23.4)
Not described	32 (23.7)	18 (20.0)	13 (20.3)	12 (25.5)

Data are presented as No. (%).

Abbreviations: CFR, case fatality risk; CSF, cerebrospinal fluid; EOGBS, early-onset group B *Streptococcus*; IAP, intrapartum antibiotic prophylaxis; LOGBS, late-onset group B *Streptococcus*.

[a]Two missing values for population/facility-based and 19 missing values for IAP use.

[b]Studies reporting incidence among infants for the whole period aged 0-89 days among all studies.

[c]Studies reporting EOGBS cases among infants for the whole period aged (0-6 days) among studies reporting EOGBS in each category.

[d]Studies reporting LOGBS cases among infants for the whole period (7–89 days) among studies reporting EOGBS in each category.

27.0% (95% CI, 17.0%–37.0%) in Africa. LOGBS CFR was 7.0% (95% CI, 4.0%–9.0%) and, consistently with overall and EOGBS CFR, was lowest in developed countries (4.0% [95% CI, 3.0%–6.0%]) and highest in Africa (12.0% [95% CI, 5.0%–19.0%]) (meta-analysis in Supplementary Figures 9 and 10, respectively).

Serotype Distribution

A total of 6500 bacterial isolates were included in the meta-analysis of serotype prevalence (data inputs are illustrated in Supplementary Figure 11). Five serotypes (Ia, Ib, II, III, and V) accounted for 97% of invasive isolates in all regions with serotype data (Figure 5). Serotype III was the most prevalent serotype across the United Nations subregions, although it was lower in South America (34%) compared with other subregions. Nearly half (47%) of EOGBS cases and 73.0% of LOGBS cases were caused by serotype III. Serotype Ia, Ib, and V were frequently isolated in EOGBS (22.8%, 8.0%, and 10.6%, respectively) and LOGBS (14.2%, 5.3%, and 4.0%) (Supplementary Figure 12).

Early-Onset to Late-Onset Group B Streptococcus Disease Ratio

The overall ratio of EOGBS to LOGBS disease was 1.72 (95% CI, 1.35–2.21). The highest ratio was in Asia (5.99 [95% CI,

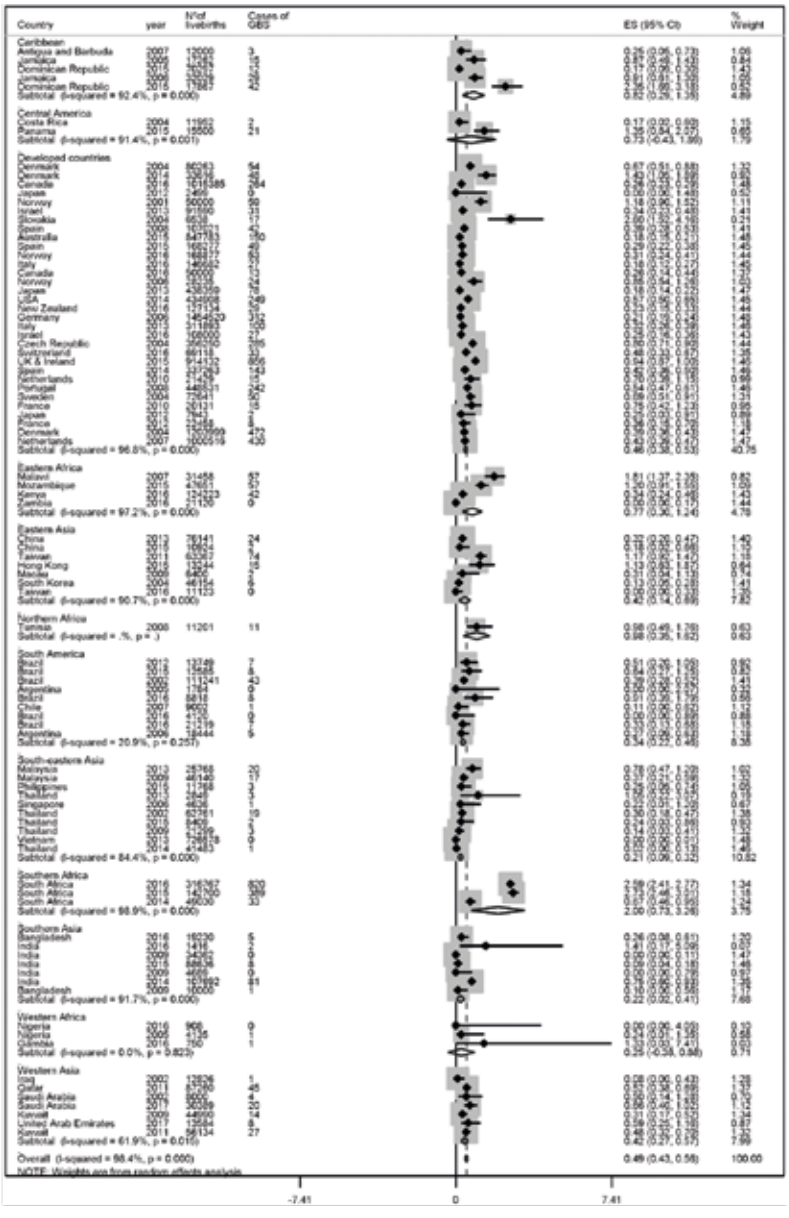


Figure 4. Pooled estimated incidence risk per 1000 live births of overall infant invasive group B streptococcal disease. Abbreviations: CI, confidence interval; ES, effect size; GBS, group B *Streptococcus*.

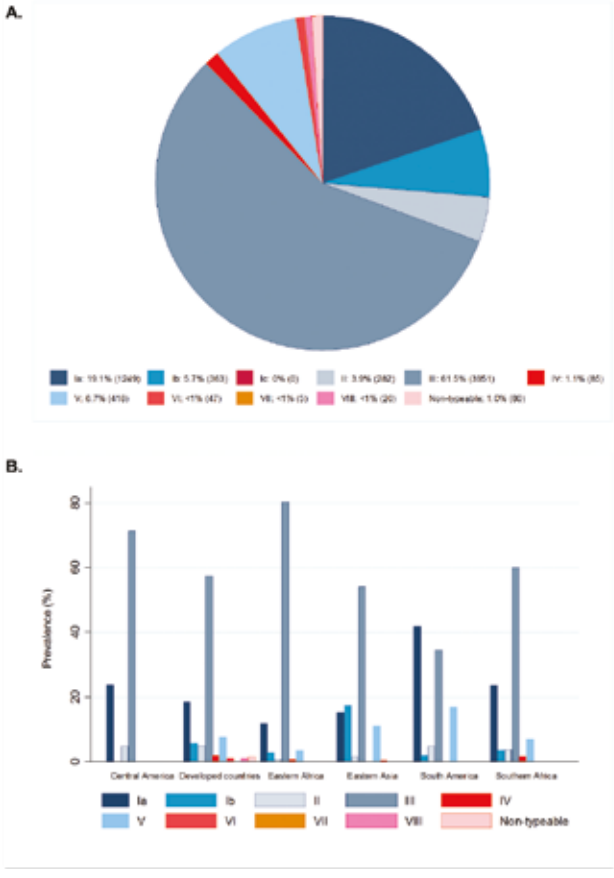


Figure 5. Global distribution of group B *Streptococcus* (GBS) serotypes in invasive disease in young infants (N = 6500 isolates). *A*, Prevalence of GBS serotypes presented as percentage (number of cases). *B*, Distribution of GBS serotypes by region. Serotypes included in a pentavalent vaccine are shown in blue and those not included are shown in red.

2.40–14.92]) and lowest in Africa (1.02 [95% CI, .82–1.28]). The ratio in developed countries was similar to the overall ratio, at 1.82 (95% CI, 1.29–2.57) (meta-analysis as Supplementary Figure 13).

Clinical Syndrome (Sepsis or Meningitis)

Twenty-three percent of all GBS invasive cases (95% CI, 14%–32%) were meningitis. Among EOGBS cases, 78% (95% CI, 67%–88%) had sepsis and 16% (95% CI, 8%–25%) had meningitis (meta-analysis of meningitis cases among EOGBS cases in Supplementary Figure 14). The meningitis/sepsis ratio was 0.18 (95% CI, .13–.25). Among LOGBS cases, there was a lower percentage of sepsis; 53% (95% CI, 43%–62%) had sepsis and 43% (95% CI, 34%–51%) had meningitis (meta-analysis of meningitis cases among LOGBS cases in Supplementary Figure 15). The meningitis:sepsis ratio was 0.78 (95% CI, .55–1.10).

Sensitivity Analyses to Assess Bias

Among facility-based studies with facility births denominator (n = 71), the incidence among infants 0–89 days of age was slightly higher than the main analysis (0.53 [95% CI, .44–.61]). The highest incidence was in Southern Africa (2.00 [95% CI, .73–3.26]) and the lowest in Southeastern Asia (0.21 [95% CI, .09–.32]) (Supplementary Figure 16). EOGBS incidence was 0.43 (95% CI, .35–.50) per 1000 live births (Supplementary Figure 17) and LOGBS incidence was 0.31 (95% CI, .24–.38) per 1000 live births (Supplementary Figure 18).

When we limited estimates of EOGBS to studies with reported data for days 0–6 of life (42/74 studies with EOGBS data reported), EOGBS incidence (0.42 [95% CI, .35–.49]) was similar to the main analysis (meta-analyses in Supplementary Figure 19).

When we limited LOGBS estimates to studies with complete data for days 7–89 after birth (11/33 studies), the incidence estimate was 0.40 per 1000 live births (95% CI, .27–.53), higher than the main analysis (meta-analysis in Supplementary Figure 20). Including only studies with data for days 7–27 after birth (3/32 studies), the incidence was 0.16 per 1000 live births (95% CI, .08–.24) (meta-analysis in Supplementary Figure 21).

When we limited estimates of the EOGBS to LOGBS ratio to studies considered to be less subject to bias (14 studies [22–25, 31, 42, 52, 56, 62, 63, 73, 81]), the estimated ratio was 1.11 (95% CI, .96–1.30) and, unlike the main analysis, was similar across geographic regions (meta-analysis in Supplementary Figure 13).

DISCUSSION

Our comprehensive review and meta-analyses represent an important update to the previous global invasive infant GBS disease burden estimates [9], and most notably include new data from LMICs (18 new studies from 10 LICs and LMICs). Infant invasive GBS disease incidence and case fatality is high in every world region, yet likely considerably underestimated in settings with limited access to care and diagnostics as <10% of neonates with suspected serious infection have a positive blood culture [139, 140]. The overall estimated incidence of infant GBS disease, 0.49 per 1000 live births, is slightly lower than the previous estimate of 0.53 per 1000 live births (95% CI, .41–.62) [9]. While fewer studies in this review reported IAP use compared to the previous review (66% vs 77.0%), more weight (>50%) was applied to the data from Europe and the Americas where IAP is in use. The reduction in overall incidence is likely driven by lower incidence of invasive infant GBS disease in the Americas (0.43/1000 live births here vs 0.67/1000 live births in the previous review), and Europe (0.53 vs 0.57/1000 live births). This difference, especially in the United States where infant GBS rates declined notably during the study period, reflects the use of more recent data in our analysis.

Similarly, the incidence of invasive infant GBS disease in Africa (1.12/1000 live births) was also slightly lower than previously reported, although >2 times higher than in developed countries (0.46/1000 live births). This is the result of broader incidence data from Africa, including large studies in South Africa [24, 42, 96] Mozambique "(Sigaúque et al, Unpublished data)", and Gambia [68] reporting a high incidence of invasive GBS disease, in contrast to studies in Nigeria [28, 77] and Zambia [62] reporting a very low incidence. Our point estimate for EOGBS incidence for Africa was higher compared to that reported in the most recent worldwide review [9], although LOGBS incidence for the same region was similar in both reviews [9]. There are many possible reasons for the increase in EOGBS incidence in Africa, which could be due to true emergence, increases in comorbidities such as HIV [42], or improved data collection to detect early disease. This high incidence is important in terms of total burden, as CFRs in Africa were also 4 times higher than in developed countries (18.9% and 4.7%, respectively); thus the greatest burden of cases, and deaths, is in Africa. However, our data are limited to few African studies mostly in Southern and Eastern Africa.

There are other important regional differences. The incidence of infant GBS disease was strikingly low in Asia at 0.31 per 1000 live births, with the lowest incidence in Southeast Asia (0.21/1000 live births). This may reflect a true regional difference, which could be related to differences in lower overall prevalence of maternal colonization and/or lower prevalence of serotype III [13], which is more commonly associated with the most virulent clone, clonal complex 17. Some of the difference may also be due to incomplete case ascertainment, being in Asia more challenging as they have more home births than Africa. For the earliest-onset cases (<24 hours of birth), differences in access to care and rapid and high case fatality can reduce case ascertainment. Cerebrospinal fluid sampling is infrequently performed in many parts of this region and that would reduce the apparent incidence of LOGBS disease, which is more frequently associated with meningitis. However, the lack of late-onset cases in this region does not fully align with those reasons and also suggests there may be more at play, potentially related to strain differences, level of natural acquired protective maternal antibody, or other host, environmental, or behavioral factors that may affect disease burden.

Difficulties in case ascertainment in LICs likely contribute to the higher incidences observed when the analysis was limited to facility-based studies, particularly in Africa. Studies in contexts where access to care, particularly for home deliveries, is difficult are likely to underestimate EOGBS disease incidence, due to the preponderance of cases with onset on day zero, which can be as high as 90% in studies with high-quality ascertainment but was 68% among the studies we included where this information could be extracted. Late-onset disease is likely underestimated too, due to studies that did not capture cases for the full 7–89 days; the sensitivity analysis showed the incidence of late onset disease to be almost twice as high (0.40 vs 0.26) when only studies with data for days 7–89 were included. This may result in an underestimation of the burden of GBS meningitis in particular, a significant concern given the morbidity associated with this condition.

Differences in the early- to late-onset disease ratios in different regions in the main analysis may also reflect (and reveal) biases in the data. Asia had the highest ratio of EOGBS disease to LOGBS disease, with the lowest in Africa (5.99 vs 1.02). It is possible that Asia has less LOGBS disease, consistent with the lower prevalence of maternal colonization with serotype III, a serotype commonly associated with LOGBS disease. Interestingly, when limiting the EOGBS:LOGBS ratio to high-quality studies, the estimate was similar across regions and lower than the overall EOGBS:LOGBS ratio. This is likely to be influenced by those

Table 2. Key Findings and Implications

What's new about this?
- Most comprehensive review to date of published data, and supplemented by unpublished data, including more data especially from LICs and LMICs with total of 18 studies from 10 LICs and LMICs.
- Extensive attempts to standardize inputs and to assess biases through a set of sensitivity analyses.

What was the main finding?
- GBS is an important cause of invasive disease among infants worldwide, despite widespread use of intrapartum antibiotic prophylaxis in many countries in developed regions.
- Highest incidence is in Africa; the lowest incidence is in Asia and not fully explained by these data.

How can the data be improved?
- Lower incidence in Asia may be partially explained by lower maternal colonization rates and less-virulent serotypes, but more data are required to better understand regional differences.
- Improved reporting of studies to better understand the biases in the data reported, for example, if low case ascertainment in the first 24 hours after birth, may be reducing reported GBS incidence rates, and if this occurs more frequently in some regions.

What does it mean for policy and programs?
- Prevention strategies are needed in all settings and particularly in the highest-burden settings (in Africa)
- Higher proportion of meningitis is among LOGBS cases, for which there are currently no preventive strategies.
- A pentavalent vaccine (serotypes Ia/Ib/II/III/V) would cover the GBS serotypes causing almost all (96%) invasive disease in infants.

Abbreviations: GBS, group B *Streptococcus*; LIC, low-income context; LMIC, low- to middle-income context; LOGBS, late-onset group B *Streptococcus*.

countries with widespread IAP as well as South Africa, which may have a uniquely low ratio due to the high prevalence of maternal HIV infection, which predisposes to late-onset disease. However, no study from Asia was included in this analysis.

In terms of serotypes causing invasive disease, serotype III accounted for over half of all disease-causing isolates followed by serotypes Ia, V, and II, consistent with previous work [9, 141]. Disease-causing serotypes were similar in prevalence across different regions, with some slight variations. The lowest prevalence of serotype III (where data were available) was found in South America and Southeast Asia, 2 of the regions with the lowest prevalence of serotype III among colonized pregnant women [13]. Serotype distribution was similar to that reported in the previous review [9], suggesting stability over time.

Our findings suggest GBS disease is an important cause of infant disease, despite the limitations in the data and uncertainties about the low incidence in Asia. In Africa, where the incidence is highest, the CFR is also highest, suggesting this is the region where prevention strategies are most critical to introduce. Existing preventive strategies using IAP are not usually available in low-income contexts and, with a higher number of home deliveries and late presentation to health facilities for delivery, IAP may be more difficult to implement. Maternal vaccination offers an alternative strategy, and the data we have suggest that a pentavalent conjugate vaccine (including Ia/Ib/II/III/V) would cover almost all disease-causing serotypes (96%) in young infants worldwide (Table 2).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The concept of the estimates and the technical oversight of the series were led by J. E. L. and A. C. S. The reviews, analyses, and first draft of the manuscript were undertaken by L. M. with A. C. S. and S. S. Other specific contributions were made by M. K. L., K. M. E., J. E. L., P. T. H., S. A. M. The GBS Estimates Expert Advisory Group (C. J. B., L. B., C. C., M. G. G., M. I., K. L. D., C. E. R., S. K. S., A. S.-t. M., J. V.) contributed to the conceptual process throughout, notably on the disease schema and data inputs. The Infant GBS Invasive Disease Investigator Group (R. A., A. R. A. d. S., Q. B., J. A. B., Z. B., S. D., E. G., H. M., K. L. D., C. O. S., S. Sri., H. S., D. N. B. S. S., B. S., G. T., P. V. K.) input data for the analyses. All the authors reviewed and gave input to the manuscript.

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References

1. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. MMWR Recomm Rep **1996**; 45:1–24.
2. Centers for Disease Control and Prevention. Perinatal group B streptococcal disease after universal screening recommendations—United States, 2003–2005. MMWR Morb Mortal Wkly Rep **2007**; 56:701–5.
3. López Sastre JB, Fernández Colomer B, Coto Cotallo GD, Ramos Aparicio A; Grupo de Hospitales Castrillo. Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatr **2005**; 94:451–7.
4. Melin P, Schmitz M, De Mol P, Foidart JM, Rigo J. Group B *Streptococcus*, primary cause of life-threatening infections in infants. Epidemiology and prevention strategy [in French]. Rev Med Liege **1999**; 54:460–7.
5. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, MMWR Recomm Rep **2002**; 51:1–22.
6. Schrag SJ, Zell ER, Lynfield R, et al; Active Bacterial Core Surveillance Team. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N Engl J Med **2002**; 347:233–9.
7. Schuchat A. Group B *Streptococcus*. Lancet **1999**; 353:51–6.
8. Stoll BJ, Hansen NI, Sánchez PJ, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. Pediatrics **2011**; 127:817–26.
9. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet **2012**; 379:547–56.
10. Le Doare K, O'Driscoll M, Turner K, et al. Intrapartum antibiotic chemoprophylaxis policies for the prevention of group B streptococcal disease worldwide: systematic review. Clin Infect Dis **2017**; 65(suppl 2):S143–51.
11. Caffrey Osvald E, Prentice P. NICE clinical guideline: antibiotics for the prevention and treatment of early-onset neonatal infection. Arch Dis Child Educ Pract Ed **2014**; 99:98–100.
12. Lawn JE, Bianchi-Jassir F, Russell N, et al. Group B streptococcal disease worldwide for pregnant women, stillbirths, and children: why, what, and how to undertake estimates? Clin Infect Dis **2017**; 65(suppl 2):S89–100.
13. Russell N, Seale AC, O'Driscoll M, et al. Maternal colonization with group B *Streptococcus* and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S100–11.
14. Hall J, Hack Adams N, Bartlett L, et al. Maternal disease with group B *Streptococcus* and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S112–24.
15. Seale AC, Blencowe H, Bianchi-Jassir F, et al. Stillbirth with group B streptococcal disease worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S125–32.
16. Bianchi-Jassir F, Seale AC, Kohli-Lynch M, et al. Preterm birth associated with group B *Streptococcus* maternal colonization worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S133–42.
17. Russell N, Seale AC, O'Sullivan C, et al. Risk of early-onset neonatal group B streptococcal disease with maternal colonization worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S152–9.
18. Tann CJ, Martinello K, Sadoo S, et al. Neonatal encephalopathy with group B streptococcal disease worldwide: systematic review, investigator group datasets, and meta-analysis. Clin Infect Dis **2017**; 65(suppl 2):S173–89.
19. Kohli-Lynch M, Russell N, Seale AC, et al. Neurodevelopmental impairment in children after group B streptococcal disease worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S190–9.
20. Seale AC, Bianchi-Jassir F, Russell N, et al. Estimates of the burden of group B streptococcal disease worldwide for pregnant women, stillbirths and children. Clin Infect Dis **2017**; 65(suppl 2):S200–19.
21. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials **1986**; 7:177–88.
22. O'Sullivan C, Lamagni T, Efstratiou A, et al. Group B Streptococcal (GBS) disease in UK and Irish infants younger than 90 days, 2014–2015. Archives of Disease in Childhood **2016**; 101(suppl 1):A2.
23. Centers for Disease Control and Prevention (CDC). Active Bacterial Core Surveillance (ABCs) report. Streptococcus. Atlanta, GA: CDC, **2014**.
24. Dangor Z, Cutland CL, Izu A, et al. Temporal changes in invasive group B *Streptococcus* serotypes: implications for vaccine development. PLoS One **2016**; 11:e0169101.
25. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with *Streptococcus agalactiae* and associated stillbirth and neonatal disease in coastal Kenya. Nat Microbiol **2016**; 1:16067.
26. Investigators of the Delhi Neonatal Infection Study (DeNIS) collaboration. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. Lancet Glob Health **2016**; 4:e752–60.
27. Abdelmaaboud M, Mohammed AF. Universal screening vs. risk-based strategy for prevention of early-onset neonatal group-B streptococcal disease. J Trop Pediatr **2011**; 57:444–50.
28. Akindolire AE, Tongo O, Dada-Adegbola H, Akinyinka O. Etiology of early onset septicemia among neonates at the University College Hospital, Ibadan, Nigeria. J Infect Dev Ctries **2016**; 10:1338–44.
29. Al-Taiar A, Hammoud MS, Cuiqing L, et al. Neonatal infections in China, Malaysia, Hong Kong and Thailand. Arch Dis Child Fetal Neonatal Ed **2013**; 98: F249–55.
30. Al-Taiar A, Hammoud MS, Thalib L, Isaacs D. Pattern and etiology of culture-proven early-onset neonatal sepsis: a five-year prospective study. Int J Infect Dis **2011**; 15:e631–4.
31. Alhhazmi A, Hurteau D, Tyrrell GJ. Epidemiology of invasive group B streptococcal disease in Alberta, Canada, from 2003 to 2013. J Clin Microbiol **2016**; 54:1774–81.
32. Al-Zwaini EJ. Neonatal septicaemia in the neonatal care unit. Al-Anbar governorate, Iraq. East Mediterr Health J **2002**; 8:509–14.
33. Andersen J, Christensen R, Hertel J. Clinical features and epidemiology of septicaemia and meningitis in neonates due to *Streptococcus agalactiae* in Copenhagen County, Denmark: a 10 year survey from 1992 to 2001. Acta Paediatr **2004**; 93:1334–9.
34. Barbosa NG, Dos Reis H, Mantese OC, Muni-Pinhata MM, Abdallah VO, Gontijo Filho PP. Early-onset neonatal sepsis by group B *Streptococcus* in a Brazilian public hospital. Braz J Infect Dis **2016**; 20:647–8.
35. Bell Y, Barton M, Thame M, Nicholson A, Trotman H. Neonatal sepsis in Jamaican neonates. Ann Trop Paediatr **2005**; 25:293–6.
36. Ben Hamida Nouaili E, Harouni M, Chaouachi S, Sfar R, Marrakchi Z. [Early-onset neonatal bacterial infections: a retrospective series of 144 cases (in French)]. Tunis Med **2008**; 86:136–9.
37. Berardi A, Baronsi L, Bacchi Reggiani ML, et al; GBS Prevention Working Group Emilia-Romagna. The burden of early-onset sepsis in Emilia-Romagna (Italy): a 4-year, population-based study. J Matern Fetal Neonatal Med **2016**; 29:3126–31.
38. Berardi A, Rossi C, Lugli L, et al. Group B *Streptococcus* late-onset disease: 2003–2010. Pediatrics **2013**; 131:e361–8.
39. Bromiker R, Ernest N, Meir MB, et al. Correlation of bacterial type and antibiotic sensitivity with maternal antibiotic exposure in early-onset neonatal sepsis. Neonatology **2013**; 103:48–53.
40. Bulkowstein S, Ben-Shimol S, Givon-Lavi N, Melamed R, Shany E, Greenberg D. Comparison of early onset sepsis and community-acquired late onset sepsis in infants less than 3 months of age. BMC Pediatr **2016**; 16:82.
41. Carbonell-Estrany X, Figueras-Aloy J, Salcedo-Abizanda S, de la Rosa-Fraile M. Probable early-onset group B streptococcal neonatal sepsis: a serious clinical condition related to intrauterine infection. Arch Dis Child Fetal Neonatal Ed **2008**; 93:F85–9.
42. Cutland CL, Schrag SJ, Thigpen MC, et al. Increased risk for group B *Streptococcus* sepsis in young infants exposed to HIV, Soweto, South Africa, 2004–2008. Emerg Infect Dis **2015**; 21:638–45.
43. Darlow BA, Voss L, Lennon DR, Grimwood K. Early-onset neonatal group B *Streptococcus* sepsis following national risk-based prevention guidelines. Aust N Z J Obstet Gynaecol **2016**; 56:69–74.

44. Darmstadt GL, Saha SK, Choi Y, et al; Bangladesh Projahnno-2 (Mirzapur) Study Group. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: an observational study. J Infect Dis **2009**; 200:906–15.
45. Delgado Picado E, Saenz-Sanchez C, Calderon Zuniga A. Rate of colonization of streptococcus agalactiae in pregnant women and neonates, Women's Hospital Dr Adolfo Carit Eva. Rev Costarric Cienc Méd **2004**; 25:25–32.
46. Didier C, Streicher MP, Chognot D, et al. Late-onset neonatal infections: incidences and pathogens in the era of antenatal antibiotics. Eur J Pediatr **2012**; 171:681–7.
47. Ekelund K, Konradsen HB. Invasive group B streptococcal disease in infants: a 19-year nationwide study. Serotype distribution, incidence and recurrent infection. Epidemiol Infect **2004**; 132:1083–90.
48. El-Said MF, Bessisso MS, Janahi MA, Haboob LH, El-Shafie SS. Epidemiology of neonatal meningitis in Qatar. Saudi Med J **2002**; 23:789–92.
49. Evangelista ML, Freitas FT. Group B *Streptococcus* neonatal infection in an intensive care unit in Brazil: high fatality and missed opportunities for antibiotic prophylaxis. Braz J Infect Dis **2015**; 19:98–9.
50. Fiolo K, Zanardi CL, Salvadego M, et al. Infection rate and *Streptococcus agalactiae* serotypes in samples of infected neonates in the city of Campinas (São Paulo), Brazil. Rev Bras Ginecol Obstet **2012**; 34:544–9.
51. Fjalstad JW, Stensvold HJ, Bergseng H, et al. Early-onset sepsis and antibiotic exposure in term infants: a nationwide population-based study in Norway. Pediatr Infect Dis J **2016**; 35:1–6.
52. Fluegge K, Siedler A, Heinrich B, et al; German Pediatric Surveillance Unit Study Group. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. Pediatrics **2006**; 117:e1139–45.
53. Freitas FT, Romero GA. Early-onset neonatal sepsis and the implementation of group B *Streptococcus* prophylaxis in a Brazilian maternity hospital: a descriptive study. Braz J Infect Dis **2017**; 21:92–7.
54. Giannoni E, Berger C, Stocker M, et al; Swiss Pediatric Sepsis Study Group. Incidence and outcome of group B streptococcal sepsis in infants in Switzerland. Pediatr Infect Dis J **2016**; 35:222–4.
55. Gimenez M, Sanfeliu I, Sierra M, et al. Group B streptococcal early-onset neonatal sepsis in the area of Barcelona (2004–2010). Analysis of missed opportunities for prevention [in Spanish]. Enferm Infecc Microbiol Clin **2015**; 33:446–50.
56. Gray KJ, Bennett SL, French N, Phiri AJ, Graham SM. Invasive group B streptococcal infection in infants, Malawi. Emerg Infect Dis **2007**; 13:223–9.
57. Hajdu A, Blystad H, Haiby EA, Klouman E, Schimmer B, Nygård K. Unexpected increase in case fatality of invasive group B streptococcal infections in infants in Norway, January-July 2006. Euro Surveill **2006**; 11:E060727.2.
58. Hammond MS, Al-Taiar A, Al-Abdi SY, et al. Culture-proven early-onset neonatal sepsis in Arab states in the Gulf region: two-year prospective study. Int J Infect Dis **2017**; 55:11–5.
59. Hasselvedt V, et.al. Systemic streptococcal group B disease in Norway—an increasing health problem. Euro Surveill **2001**; 5:2086.
60. Lea J. Screening of hemolytical *Streptococcus* of group B in pregnancy and prevention of infection in newborns. Ceska Gynekologie **2004**; 69:91–4.
61. Juncosa-Morros T, Guardia-Llobet C, Bosch-Mestres J, et al. *Streptococcus agalactiae* late-onset neonatal infections in Barcelona (1996–2010) [in Spanish]. Enferm Infecc Microbiol Clin **2014**; 32:574–8.
62. Kalwe M, Tembo J, Chilukuta L, et al. Etiology, antibiotic resistance and risk factors for neonatal sepsis in a large referral center in Zambia. Pediatr Infect Dis J **2016**; 35:e191–8.
63. Ko DW, Zurynski Y, Gilbert GL; GBS Study Group. Group B streptococcal disease and genotypes in Australian infants. J Paediatr Child Health **2015**; 51:808–14.
64. Kim JS, Jang YT, Kim JD, et al. Incidence of *Haemophilus influenzae* type B and other invasive diseases in South Korean children. Vaccine **2004**; 22:3952–62.
65. Kruse AY, Thieu Chuong do H, Phuong CN, et al. Neonatal bloodstream infections in a pediatric hospital in Vietnam: a cohort study. J Trop Pediatr **2013**; 59:483–8.
66. Kuhn P, Dheu C, Bolender C, et al. Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. Paediatr Perinat Epidemiol **2010**; 24:479–87.
67. Larcher JS, Capellino F, De Giusto R, et al. Group B streptococcus colonization during pregnancy and prevention of early onset of disease [in Spanish]. Medicina (B Aires) **2005**; 65:201–6.
68. Le Doare K, Jarju S, Darboe S, et al. Risk factors for group B *Streptococcus* colonisation and disease in Gambian women and their infants. J Infect **2016**; 72:283–94.
69. Li YP, Kuok CM, Lin SY, Hsieh WS, Shyu MK. Group B *Streptococcus* antimicrobial resistance in neonates born to group B *Streptococcus*-colonized mothers: single-center survey. J Obstet Gynaecol Res **2016**; 42:1471–5.
70. Liu H, Zeng H, Wang W, et al. Estimating the burden of invasive group B streptococcal disease in young infants in southern mainland China: an observational study. Int J Clin Exp Med **2015**; 8:13699–707.
71. Lomuto C, Queiruga M, Nigri C, Fatur D, Messina A, Santos N. Approach to the epidemiologic situation in Argentina of the perinatal infection by beta hemolytic streptococcus of the B group (EGB). Rev Hosp Matern Infant Ramon Sarda **2006**; 25:13–9.
72. Martin TC, Adamson J, Dickson T, DiGiantomasso E, Nesbitt C. Does group B streptococcal infection contribute significantly to neonatal sepsis in Antigua and Barbuda? West Indian Med J **2007**; 56:498–501.
73. Matsubara K, Hoshina K, Suzuki Y, Matsubara K, Hoshina K, Suzuki Y. Early-onset and late-onset group B streptococcal disease in Japan: a nationwide surveillance study, 2004–2010. Int J Infect Dis **2013**; 17:e379–84.
74. Miyata A, Takahashi H, Kubo T, et al. Early-onset group B streptococcal disease following culture-based screening in Japan: a single center study. J Obstet Gynaecol Res **2012**; 38:1052–6.
75. Neto MT. Group B streptococcal disease in Portuguese infants younger than 90 days. Arch Dis Child Fetal Neonatal Ed **2008**; 93:F90–3.
76. Nidhraje K, Amutha C, Roy J. Early neonatal streptococcal infection. Indian J Pediatr **2006**; 73:573–6.
77. Ojukwu JU, Aboeyi LE, Ugwu J, . Neonatal septicemia in high risk babies in south-eastern Nigeria. J Perinat Med **2005**; 34:166–72.
78. Persson E, Berg S, Trollfors B, et al. Serotypes and clinical manifestations of invasive group B streptococcal infections in western Sweden 1998–2001. Clin Microbiol Infect **2004**; 10:791–6.
79. Petersen KB, Johansen HK, Rosthoj S, Krebs L, Pinborg A, Hedegaard M. Increasing prevalence of group B streptococcal infection among pregnant women. Dan Med J **2014**; 61:A4908.
80. Poliquin V, Ceben E, Poliquin PG, Schneider C, Menticeoglou S. Ongoing cases of early-onset group B streptococcal disease in the era of screening and prophylaxis. J Obstet Gynaecol Can **2016**; 38:926–9.
81. Rivera L, Sáez-Llorens X, Feris-Iglesias J, et al. Incidence and serotype distribution of invasive group B streptococcal disease in young infants: a multi-country observational study. BMC Pediatr **2015**; 15:143.
82. Sakata H. Evaluation of intrapartum antibiotic prophylaxis for the prevention of early-onset group B streptococcal infection. J Infect Chemother **2012**; 18:853–7.
83. Sridhar S, Grace R, Nithya PJ, et al. Group B streptococcal infection in a tertiary hospital in India—1998–2010. Pediatr Infect Dis **2014**; 33:1091–2.
84. Straková L, Motlová J. Active surveillance of early onset disease due to group B streptococci in newborns. Indian J Med Res **2004**; 119(suppl):205–7.
85. Sundaram V, Kumar P, Dutta S, et al. Blood culture confirmed bacterial sepsis in neonates in a North Indian tertiary care center: changes over the last decade. Jpn J Infect Dis **2009**; 62:46–50.
86. Tapia IJ, Reichhard TC, Sakdias RM, et al. Neonatal sepsis in the era of antenatal antibiotic prophylaxis. Rev Chilena Infectol **2007**; 24:111–6.
87. Thatrimontrichai A, Chanvitan P, Janjindamai W, Dissaneevate S, Jefferies A, Shah V. Trends in neonatal sepsis in a neonatal intensive care unit in Thailand before and after construction of a new facility. Asian Biomedicine **2014**; 8:771–8.
88. Tiskumara R, Fakharee SH, Liu CQ, et al. Neonatal infections in Asia. Arch Dis Child Fetal Neonatal Ed **2009**; 94:F144–8.
89. Trijbels-Smeulders M, de Jonge GA, Pasker-de Jong PC, et al. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. Arch Dis Child Fetal Neonatal Ed **2007**; 92:F271–6.
90. Trotman H, Bell Y. Neonatal group B streptococcal infection at the University Hospital of the West Indies, Jamaica: a 10-year experience. Ann Trop Paediatr **2006**; 26:53–7.
91. Vaciloto E, Richtmann R, de Paula Fiod Costa H, Kusano EJ, de Almeida MF, Amaro ER. A survey of the incidence of neonatal sepsis by group B *Streptococcus* during a decade in a Brazilian maternity hospital. Braz J Infect Dis **2002**; 6:55–62.
92. Villanueva-Uy ME, Wongsiridej P, Sangtawesin V, et al. The burden of invasive neonatal group B streptococcal (GBS) disease in Thailand and the Philippines. Southeast Asian J Trop Med Public Health **2015**; 46:728–37.
93. Yossuck P, Preedisripipat K. Neonatal group B streptococcal infection: incidence and clinical manifestation in Siriraj Hospital. J Med Assoc Thai **2002**; 85(suppl 2):S479–87.
94. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. Neonatology **2010**; 97:22–8.
95. Yu HW, Lin HC, Yang PH, et al. Group B streptococcal infection in Taiwan: maternal colonization and neonatal infection. Pediatr Neonatol **2011**; 52:100–5.
96. Frigati L, van der Merwe JL, Harvey J, Rabie H, Theron G, Cotton MF. A retrospective review of group B streptococcal infection in the Metro East area of the Western Cape province: 2010 to 2011. S Afr J Infect Dis **2014**; 29:33–6.
97. Bartlett AW, Smith B, George CR, et al. Epidemiology of late and very late onset group B streptococcal disease: fifteen-year experience from two Australian tertiary pediatric facilities. Pediatr Infect Dis J **2017**; 36:20–4.

98. Cantoni L, Ronfani L, Da Riol R, et al. Physical examination instead of laboratory tests for most infants born to mothers colonized with group B *Streptococcus*: support for the Centers for Disease Control and Prevention's 2010 recommendations. J Pediatr **2013**; 163: 568–73.
99. Chang CJ, Chang WN, Huang LT, et al. Neonatal bacterial meningitis in southern Taiwan. Pediatr Neurol **2003**; 29:288–94.
100. Cho HK, Lee H, Kang JH, et al. The causative organisms of bacterial meningitis in Korean children in 1996–2005. J Korean Med Sci **2010**; 25:895–9.
101. Costa N, Carvalho M, Moura S, Júnior SC. Beta-hemolytic streptococcus in pregnant women and their newborn infants: a critical analysis of the protocol used at Fernandes Figueira Institute, Oswaldo Cruz Foundation, in Brazil. Rev Paul Pediatr **2010**; 28.
102. Díaz-Álvarez M, Claver Isás D, Pérez-Amarillo J. Infections due to *Streptococcus agalactiae* at an open neonatology service. Rev Cubana Pediatr **2008**; 80.
103. Hashavya S, Benenson S, Ergaz-Shaltiel Z, Bar-Oz B, Averbuch D, Eventov-Friedman S. The use of blood counts and blood cultures to screen neonates born to partially treated group B *Streptococcus*-carrier mothers for early-onset sepsis: is it justified? Pediatr Infect Dis J **2011**; 30:840–3.
104. Jiang JH, Chiu NC, Huang FY, et al. Neonatal sepsis in the neonatal intensive care unit: characteristics of early versus late onset. J Microbiol Immunol Infect **2004**; 37:301–6.
105. Mendoza OM, Arias M, Mendoza L. Sepsis neonatal Group B *Streptococcus* and gram negative bacteria: prevalence and risk of meningitis. Rev Chil Pediatr **2013**; 84.
106. Milledge J, Calis JC, Graham SM, et al. Aetiology of neonatal sepsis in Blantyre, Malawi: 1996–2001. Ann Trop Paediatr **2005**; 25:101–10.
107. Ovalle A, Levancini M. Urinary tract infections in pregnancy. Curr Opin Urol **2001**; 11:55–9.
108. Park KH, Kim KH, Kang JH, et al. Current status and clinical presentations of invasive neonatal group B streptococcal infections in Korea. Pediatr Int **2010**; 53:236–9.
109. Reinheimer C, Kempf VA, Wittekindt BE, et al. Group B *Streptococcus* infections in neonates admitted to a German NICU: emphasis on screening and adherence to pre-analytical recommendations. Early Hum Dev **2016**; 103:37–41.
110. Sakata H, Sakata H. Pediatric invasive streptococcal infection in northern and eastern regions of Hokkaido, Japan from 2010 to 2012. Pediatr Int **2014**; 56:360–3.
111. Zeng SJ, Tang XS, Zhao WL, Qiu HX, Wang H, Feng ZC. Clinical analysis of cases of neonatal *Streptococcus agalactiae* sepsis. Genet Mol Res **2016**; 15.
112. Almeida A, Villain A, Joubrel C, et al. Whole-genome comparison uncovers genomic mutations between group B streptococci sampled from infected newborns and their mothers. J Bacteriol **2015**; 197:3354–66.
113. Matsubara K, Yamamoto G. Invasive group B streptococcal infections in a tertiary care hospital between 1998 and 2007 in Japan. Int J Infect Dis **2009**; 13:679–84.
114. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis **2014**; 14:1083–9.
115. Bidet P, Brahimi N, Chalas C, Aujard Y, Bingen E. Molecular characterization of serotype III group B-*Streptococcus* isolates causing neonatal meningitis. J Infect Dis **2003**; 188:1132–7.
116. Brzychczy-Wloch M, Gosiewski T, Bulanda M. Multilocus sequence types of invasive and colonizing neonatal group B streptococci in Poland. Med Princ Pract **2014**; 23:323–30.
117. Campisi E, Rosini R, Ji W, et al. Genomic analysis reveals multi-drug resistance clusters in group B *Streptococcus* CC17 hypervirulent isolates causing neonatal invasive disease in southern mainland China. Front Microbiol **2016**; 7:1265.
118. Chang B, Wada A, Hosoya M, et al; Japanese Invasive Disease Study Group. Characteristics of group B *Streptococcus* isolated from infants with invasive infections: a population-based study in Japan. Jpn J Infect Dis **2014**; 67:356–60.
119. Davies HD, Raj S, Adair C, Robinson J, McGeer A; Alberta GBS Study Group. Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation. Pediatr Infect Dis J **2001**; 20:879–84.
120. Davies HD, Jones N, Whittam TS, Elsayed S, Bisharat N, Baker CJ. Multilocus sequence typing of serotype III group B *Streptococcus* and correlation with pathogenic potential. J Infect Dis **2004**; 189:1097–102.
121. Figueira-Coelho J, Ramirez M, Salgado MJ, Melo-Cristino J. *Streptococcus agalactiae* in a large Portuguese teaching hospital: antimicrobial susceptibility, serotype distribution, and clonal analysis of macrolide-resistant isolates. Microb Drug Resist **2004**; 10:31–6.
122. Fluegge K, Supper S, Siedler A, Berner R. Serotype distribution of invasive group B streptococcal isolates in infants: results from a nationwide active laboratory surveillance study over 2 years in Germany. Clin Infect Dis **2005**; 40:760–3.
123. Fluegge K, Wons J, Spellerberg B, et al. Genetic differences between invasive and noninvasive neonatal group B streptococcal isolates. Pediatr Infect Dis J **2011**; 30:1027–31.
124. Hoshina K, Suzuki Y, Nishida H, et al. Trend of neonatal group B streptococcal infection during the last 15 years. Pediatr Int **2002**; 44:641–6.
125. Imperi M, Gherardi G, Berardi A, et al. Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clin Microbiol Infect **2011**; 17:1834–9.
126. Ip M, Ang I, Fung K, Liyanapathirana V, Luo MJ, Lai R. Hypervirulent clone of group B *Streptococcus* serotype III sequence type 283, Hong Kong, 1993–2012. Emerg Infect Dis **2016**; 22:1800–3.
127. Joubrel C, Tazi A, Six A, et al. Group B *Streptococcus* neonatal invasive infections, France 2007–2012. Clin Microbiol Infect **2015**; 21:910–6.
128. Martínez MA, Ovalle A, Durán C, et al. Serotypes and antimicrobial susceptibility of Streptococcus agalactiae [in Spanish]. Rev Med Chil **2004**; 132:549–55.
129. Martins ER, Pessanha MA, Ramirez M, Melo-Cristino J; Portuguese Group for the Study of Streptococcal Infections. Analysis of group B streptococcal isolates from infants and pregnant women in Portugal revealing two lineages with enhanced invasiveness. J Clin Microbiol **2007**; 45:3224–9.
130. Morozumi M, Wajima T, Kuwata Y, et al. Associations between capsular serotype, multilocus sequence type, and macrolide resistance in *Streptococcus agalactiae* isolates from Japanese infants with invasive infections. Epidemiol Infect **2014**; 142:812–9.
131. Óladóttir GL, Erlendsdóttir H, Pálsson G, Björnsdóttir ES, Kristinsson KG, Haraldsson Á. Increasing incidence of late-onset neonatal invasive group B streptococcal infections in Iceland. Pediatr Infect Dis J **2011**; 30:661–3.
132. Six A, Firon A, Plainvert C, et al. Molecular characterization of nonhemolytic and nonpigmented group B streptococci responsible for human invasive infections. J Clin Microbiol **2016**; 54:75–82.
133. Souza VC, Kegele FC, Souza SR, Neves FP, de Paula GR, Barros RR. Antimicrobial susceptibility and genetic diversity of *Streptococcus agalactiae* recovered from newborns and pregnant women in Brazil. Scand J Infect Dis **2013**; 45:780–5.
134. Teatero S, McGeer A, Low DE, et al. Characterization of invasive group B *Streptococcus* strains from the greater Toronto area, Canada. J Clin Microbiol **2014**; 52:1441–7.
135. Trijbels-Smeulders MA, Kimpen JL, Kollée LA, et al. Serotypes, genotypes, and antibiotic susceptibility profiles of group B streptococci causing neonatal sepsis and meningitis before and after introduction of antibiotic prophylaxis. Pediatr Infect Dis J **2006**; 25:945–8.
136. Wang P, Ma Z, Tong L, et al. Serotype distribution, antimicrobial resistance, and molecular characterization of invasive group B *Streptococcus* isolates recovered from Chinese neonates. Int J Infect Dis **2015**; 37:e115–e8.
137. Yoon IA, Jo DS, Cho EY, Choi EH, Lee HJ, Lee H. Clinical significance of serotype V among infants with invasive group B streptococcal infections in South Korea. Int J Infect Dis **2015**; 38:136–40.
138. Zhao Z, Kong F, Zeng X, Gidding HF, Morgan J, Gilbert GL. Distribution of genotypes and antibiotic resistance genes among invasive *Streptococcus agalactiae* (group B *Streptococcus*) isolates from Australasian patients belonging to different age groups. Clin Microbiol Infect **2008**; 14:260–7.
139. Cutland CL, Schrag SJ, Zell ER, et al; PoPS Trial Team. Maternal HIV infection and vertical transmission of pathogenic bacteria. Pediatrics **2012**; 130: e581–90.
140. Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. Lancet **2005**; 365:1175–88.
141. Sinha A, Russell LB, Tomczyk S, et al; GBS Vaccine Cost-Effectiveness Analysis in Sub-Saharan Africa Working Group. Disease burden of group B *Streptococcus* among infants in sub-Saharan Africa: a systematic literature review and meta-analysis. Pediatr Infect Dis J **2016**; 35:933–42.

Supplementary information

Supplementary Table S1: Search terms

Infant
Outcome
Death
Mortality
Case AND Fatality AND rate
Death [MeSH Terms]
Mortality [MeSH Terms]
Case fatality rate [MeSH Terms]
AND
Streptococcus
Streptococcal
Streptococci AND (Group AND B) or agalactiae
Streptococcus agalactiae [MeSH Terms]
AND
Streptococcus serotype
Streptococcal serotype
Streptococcus agalactiae erotype[MeSH Terms]

Supplementary Table S2: Inclusion and exclusion criteria

	Inclusion criteria	**Exclusion criteria**
Population	Invasive GBS disease in infants aged 0-89 days at onset of infection	Studies containing only information on very high-risk groups
Laboratory	GBS confirmed by blood / CSF culture.	
Search	No language restrictions	Foreign language papers where it was not possible to obtain English translation
Article type	Study reporting more recent data from country with dynamic trend	Case reports, case series, reviews. Studies from the same country reporting repeated years or with dynamic trend over time

Supplementary Table S3: Characteristics of studies included and data type extracted for invasive infant GBS disease

Author	Country	Year	Year data collection	Study type	Incidence	Case fatality risk	Serotype	IAP use	Included in previous review[1]
Andersen[2]	Denmark	2004	1992-2001	Community-based	Yes			Yes	Yes
Bell[3]	Jamaica	2005	1995-2000	Facility-based	Yes			No	Yes
Carbonell-Estrany[4]	Spain	2008	2004-2005	Facility-based	Yes	Yes		Yes	Yes
Chang[5]	Taiwan	2003	1986-2001	Facility-based		Yes	Yes	Yes	Yes
Davies[6]	Canada	2001	1993-1999	Community-based			Yes	Yes	Yes
Ekelund[7]	Denmark	2004	1984-2002	Facility-based	Yes		Yes	Yes	Yes
Fluegge[8]	Germany	2006	2001-2003	Community-based	Yes	Yes		Yes	Yes
Hasseltvedt[9]	Norway	2001	2000	Community-based	Yes	Yes		Yes	Yes
Janek[10]	Slovakia	2004	2000-2003	Facility-based	Yes			Yes	Yes
Jiang[11]	Taiwan	2004	1992-2001	Facility-based		Yes		No	Yes
Kim[12]	South Korea	2004	1999-2001	Community-based	Yes			No	Yes
Neto[13]	Portugal	2008	2001-2005	Community-based	Yes	Yes		Yes	Yes
Niduvaje[14]	Singapore	2006	1999-2000	Facility-based	Yes			Yes	Yes
Ben Hamida[15]	Tunisia	2008	2001-2003	Facility-based	Yes			No	Yes
Trijbels-Smeulders[16]	Netherlands	2007	1997-2001	Facility-based	Yes	Yes		Yes	Yes
Trotman[17]	Jamaica	2006	1991-2000	Facility-based	Yes	Yes		No	Yes
Yossuck[18]	Thailand	2002	1996-2001	Facility-based	Yes			No	Yes
Hoshina[19]	Japan	2002	1983-1997	Facility-based			Yes	No	Yes
Gray[20]	Malawi	2007	2004-2005	Facility-based	Yes	Yes		No	Yes
Figueira-Coelho[21]	Portugal	2004	1999-2000	Facility-based			Yes	Yes	Yes
Hajdu[22]	Norway	2006	2006	UNK	Yes	Yes		Yes	Yes
Vaciloto[23]	Brazil	2002	1991-2000	Facility-based	Yes			No	Yes
Strakova[24]	Czech Republic	2004	2001-2002	Facility-based	Yes	Yes	Yes	No	Yes
Park[25]	South Korea	2010	1996-2005	Facility-based		Yes		No	Yes
Matsubara[26]	Japan	2009	1998-2007	Facility-based			Yes	No	Yes
Darmstadt[27]	Bangladesh	2009	2004-2006	Community-based	Yes			Yes	Yes
Al-Zwaini[28]	Iraq	2002	2000-2001	Facility-based	Yes			No	Yes
Sundaram[29]	India	2009	1995-2006	Facility-based	Yes			No	Yes
Martin[30]	Antigua and Barbuda	2007	1994-2002	Facility-based	Yes			Yes	Yes
Cho[31]	South Korea	2010	1996-2005	Facility-based		Yes		No	Yes
Van den Hoogen[32]	Netherlands	2010	1978-2006	Facility-based	Yes			Yes	Yes
Kuhn[33]	France	2010	2003-2004	Community-based	Yes		Yes	Yes	Yes
Milledge[34]	Malawi	2005	1996-2001	Facility-based		Yes		No	Yes
Zhao[35]	Australia	2008	1994-2005	Facility-based			Yes	Yes	Yes
Martins[36]	Portugal	2007	2000-2004	UNK			Yes	Yes	Yes
Trijbels-Smeulders[37]	Netherlands	2006	1997-1999	Facility-based			Yes	Yes	Yes
Fluegge[38]	Germany	2005	2001-2003	Facility-based			Yes	Yes	Yes
Persson[39]	Sweden	2004	1998-2001	Facility-based	Yes	Yes	Yes	Yes	Yes
Davies[40]	Canada	2004	1995-1999	Community-based			Yes	Yes	Yes

Bidet[41]	France	2003	1990-2002	Facility-based			Yes	No	Yes
Lopardo[42]	Argentina	2003	1998-1999	Facility-based			Yes	No	Yes
El-Said[43]	Saudi Arabia	2002	1998-2000	Facility-based	Yes			No	Yes
Ojukwu[44]	Nigeria	2005	2002-2003	Facility-based	Yes			No	Yes
Tiskumara[45]	India	2009	2008-2009	Facility-based	Yes			Yes	Yes
Tiskumara[45]	Kuwait	2009	2006-2009	Facility-based	Yes			Yes	Yes
Tiskumara[45]	Macau	2009	2006-2008	Facility-based	Yes			Yes	Yes
Tiskumara[45]	Malaysia	2009	2006-2009	Facility-based	Yes			Yes	Yes
Tiskumara[45]	Thailand	2009	2007-2009	Facility-based	Yes			Yes	Yes
Abdelmaaboud[46]	Qatar	2011	2003-2009	Facility-based	Yes	Yes	Yes	Yes	No
Al-Taiar[47]	China	2013	2006-2009	Facility-based	Yes			UNK	No
Al-Taiar[47]	Malasya	2013	2006-2009	Facility-based	Yes			UNK	No
Al-Taiar[47]	Thailand	2013	2006-2009	Facility-based	Yes			UNK	No
Al-Taiar[47]	Kuwait	2011	2005-2009	Facility-based	Yes	Yes		Yes	No
Bekker[48]	Netherlands	2014	1987-2011	Facility-based			Yes	Yes	No
Berardi[49]	Italy	2013	2003-2010	Community-based	Yes	Yes		Yes	No
Bromiker[50]	Israel	2013	1997-2007	Facility-based	Yes			Yes	No
Chang[51]	Japan	2014	2007-2012	Community-based			Yes	Yes	No
Cutland[52]	South Africa	2015	2004-2008	Facility-based	Yes	Yes		Yes	No
Cantoni[53]	Italy	2013	2004-2006	Community-based		Yes		Yes	No
Didier[54]	France	2012	2007	Community-based	Yes	Yes		Yes	No
Evangelista[55]	Brazil	2015	2012-2013	Facility-based	Yes	Yes		No	No
Fiolo[56]	Brazil	2012	2007-2011	Facility-based	Yes	Yes	Yes	Yes	No
Giannoni[57]	Switzerland	2016	2011-2015	Facility-based	Yes	Yes		Yes	No
Giménez[58]	Spain	2015	2004-2010	Facility-based	Yes	Yes	Yes	Yes	No
Hashavya[59]	Israel	2011	2005-2009	Facility-based		Yes		Yes	No
Juncosa-Morros[60]	Spain	2014	1996-2010	Facility-based	Yes	Yes		Yes	No
Kruse[61]	Vietnam	2013	2009-2010	Facility-based	Yes			No	No
Liu[62]	China	2015	2013-2014	Facility-based	Yes		Yes	No	No
Matsubara[63]	Japan	2013	2004-2010	Facility-based	Yes	Yes	Yes	Yes	No
Miyata[64]	Japan	2012	2002-2009	Facility-based	Yes	Yes		Yes	No
Oladottir[65]	Iceland	2011	1975-2006	Facility-based			Yes	UNK	No
Petersen[66]	Denmark	2014	2002-2010	Facility-based	Yes			Yes	No
Sakata[67]	Japan	2014	2010-2012	Facility-based		Yes		UNK	No
Sridhar[68]	India	2014	1998-2010	Facility-based	Yes			Yes	No
Yu[69]	Taiwan	2011	2001-2005	Facility-based	Yes	Yes		Yes	No
Thatrimontrichai[70]	Thailand	2014	1995-2010	Facility-based	Yes			UNK	No
Ko Danny[71]	Australia	2015	2005-2008	Community-based	Yes	Yes	Yes	Yes	No
Morozumi[72]	Japan	2014	2006-2011	Community-based			Yes	Yes	No
Almeida[73]	France	2015	2008-2012	Facility-based			Yes	No	No
Brzychczy-Wloch[74]	Poland	2014	2006-2010	Facility-based			Yes	UNK	No
Fluegge[75]	Germany	2011	2001-2003	Facility-based	Yes		Yes	UNK	No
Imperi[76]	Italy	2011	2005-2008	Facility-based	Yes		Yes	UNK	No
Joubrel[77]	France	2015	2007-2012	Facility-based	Yes			Yes	No
Six[78]	France	2016	2006-2013	Facility-based			Yes	Yes	No
Souza[79]	Brazil	2013	2008-2010	Facility-based			Yes	UNK	No

Teatero[80]	Canada	2014	2009-2012	Community-based			Yes	Yes	No
Yoon[81]	Korea	2015	1995-2004	Facility-based			Yes	No	No
Wang[82]	China	2015	2008-2013	Facility-based			Yes	UNK	No
Sakata[83]	Japan	2012	2009-2011	Facility-based	Yes			Yes	No
Rivera[84]	Panama	2015	UNK	Facility-based	Yes	Yes	Yes	Yes	No
Villanueva-Uy[85]	Thailand	2015	UNK	Facility-based	Yes	Yes		UNK	No
Larcher[86]	Argentina	2005	2001-2002	Facility-based	Yes			Yes	No
Sigauque[87]	Mozambique	2015	2001-2015	Facility-based	Yes	Yes	Yes	No	No
Vinod[88]	India	2016	2011-2015	Facility-based	Yes	Yes		Yes	No
Le Doare[89]	Gambia	2016	2014	Facility-based	Yes	Yes		No	No
Saha[90]	Bangladesh	2016	2012-2013	Facility-based	Yes	Yes		No	No
Araujo da Silva[91]	Brazil	2016	2015-2016	Facility-based	Yes			Yes	No
Dhaded[92]	India	2016	2014-2015	Facility-based	Yes	Yes		Yes	No
Dangor[93]	South Africa	2016	2005-2014	Facility-based	Yes	Yes	Yes	Yes	No
Rivera[84]	Dominican Republic	2015	UNK	Facility-based	Yes	Yes		Yes	No
Rivera[84]	Hong Kong	2015	UNK	Facility-based	Yes	Yes		Yes	No
Rivera[84]	Dominican Republic	2015	UNK	Facility-based	Yes	Yes		Yes	No
Villanueva-Uy[85]	Philippines	2015	UNK	Facility-based	Yes	Yes		UNK	No
CDC[94]	United States	2014	2014	Community-based	Yes	Yes	Yes	Yes	No
O´Sullivan[95]	United Kingdom	2015	2014-2015	Community-based	Yes	Yes	Yes	Yes	No
Seale [96]	Kenya	2016	1998-2013	Community-based	Yes	Yes	Yes	No	No
Alhhazmi[97]	Canada	2016	2003-2013	Community-based	Yes		Yes	Yes	No
Barbosa[98]	Brazil	2016	2008-2011	Facility-based	Yes	Yes		No	No
Bartlett[99]	Australia	2017	2000-2015	Facility-based		Yes		Yes	No
Berardi[100]	Italy	2016	2009-2012	Community-based	Yes	Yes		Yes	No
Bulkowstein[101]	Israel	2016	2007-2013	Community-based	Yes			No	No
Campisi[102]	China	2016	2013-2014	Facility-based			Yes	UNK	No
Darlow[103]	Australia	2016	2009-2011	Community-based	Yes	Yes		Yes	No
Fjalstad[104]	Norway	2016	2009-2011	Community-based	Yes	Yes		UNK	No
Freitas[105]	Brazil	2016	2012-2015	Facility-based	Yes	Yes		Yes	No
Ip[106]	Hong Kong	2016	1993-2012	Facility-based			Yes	UNK	No
Hammoud[107]	Kuwait	2017	2013-2015	Facility-based	Yes			Yes	No
Hammoud[107]	United Arab Emirates	2017	2013-2015	Facility-based	Yes			Yes	No
Hammoud[107]	Saudi Arabia	2017	2013-2015	Facility-based	Yes			Yes	No
Kang[108]	South Korea	2017	1995-2015	Facility-based		Yes		No	No
Li YP[109]	Taiwan	2016	2006-2013	Facility-based	Yes	Yes		Yes	No
Lomuto[110]	Argentina	2006	2002-2005	Facility-based	Yes	Yes		Yes	No
Mendoza[111]	Colombia	2013	2005-2012	Facility-based		Yes		UNK	No
Martinez[112]	Chile	2004	1998-2002	Facility-based			Yes	UNK	No
Poliquin[113]	Canada	2016	2008-2013	Facility-based	Yes	Yes		Yes	No
Reinheimer[114]	Germany	2016	2010-2016	Facility-based		Yes		Yes	No
Zeng[115]	China	2016	2012-2014	Facility-based		Yes		UNK	No
Tapia[116]	Chile	2007	2001-2004	Facility-based	Yes			Yes	No
Kabwe[117]	Zambia	2016	2013-2014	Facility-based	Yes			No	No
Akindolire[118]	Nigeria	2016	2104	Facility-based	Yes			No	No

Ovalle[119]	Chile	2002	1990-2001	Facility-based		Yes	Yes	No
Delgado-Picado[120]	Costa Rica	2004	2002-2004	Facility-based	Yes		No	No
Diaz Alvarez[121]	Cuba	2008	1992-2007	Facility-based		Yes	No	No
Costa[122]	Brazil	2010	2003-2006	Facility-based		Yes	Yes	No
Frigarti[123]	South Africa	2014	2010-2011	Facility-based	Yes	Yes	Yes	No

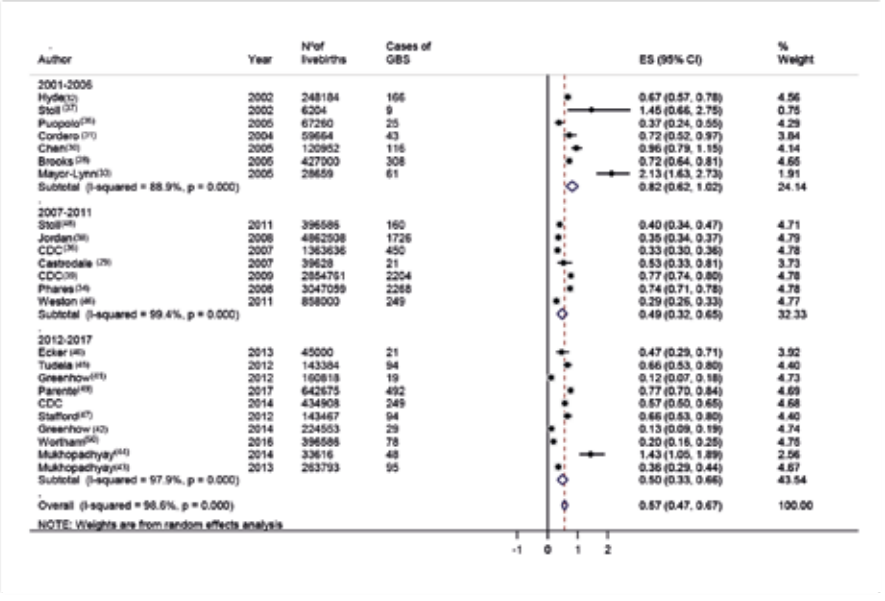
CFR: case fatality risk. IAP: intrapartum antibiotic prophylaxis. UNK: unknown (information not available).

Supplementary Table S4: Studies excluded (repeated data or more recent data available)

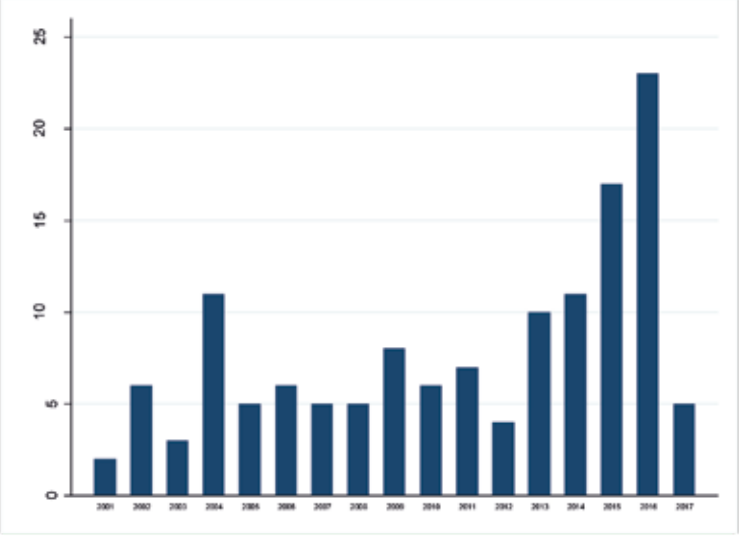
Author	Country	Year	Year data collection	Study type
Angstetra[124]	Australia	2007	1994-2006	Facility-based
Ireland[125]	Australia	2014	2002-2011	Facility-based
Daley[126]	Australia & NewZealand	2004	1992-2001	Facility-based
May[127]	Australia & NewZealand	2005	1992-2002	Facility-based
Meehan[128]	Ireland	2014	2007-2011	Facility-based
Berardi[129]	Italy	2007	2003-2005	Community- based
Berardi[130]	Italy	2011	2003-2010	Community- based
Berardi [131]	Italy	2013	2003-2011	Community- based
Berkley[132]	Kenya	2005	1998-2002	Facility-based
Sigauque [133]	Mozambique	2009	2001-2006	Facility-based
Eastwood[134]	Northern Ireland	2015	2008-2010	Facility-based
Madhi[135]	South Africa	2003	1997-1999	Facility-based
Cutland [136]	South Africa	2009	2004-2007	Facility-based
Cutland [137]	South Africa	2012	2004-2007	Unknown
Dangor[138]	South Africa	2015	2012-2014	Facility-based
Schrag[139]	South Africa	2012	2004-2007	Facility-based
Andreu[140]	Spain	2003	1994-2001	Facility-based
Lopez Sastre[141]	Spain	2005	2000-2001	Facility-based
Martins[142]	Spain	2011	1994-2009	Facility-based
Vergnano[143]	United Kingdom	2010	2006-2008	Facility-based
Lamagni[144]	United Kingdom	2013	1991-2010	Facility-based
Weisner[145]	United Kingdom	2004	2000-2001	Community- based
Meehan[146]	United Kingdom	2015	2011-2013	Facility-based
Oddie[147]	United Kingdom	2002	1998-2000	Facility-based
Okike[148]	United Kingdom	2014	2010-2011	Community- based
Heath[149]	United Kingdom & Ireland	2004	2000-2001	Community- based
Brooks[150]	United States	2005	1996-2004	Community- based
Castrodale[151]	United States	2007	2000-2004	Community- based
Chen[152]	United States	2005	1990-2002	Facility-based
Cordero[153]	United States	2004	1986-2002	Facility-based
Hyde[154]	United States	2002	1998-2000	Community- based
Mayor-Lynn[155]	United States	2005	1998-2002	Facility-based
Phares[156]	United States	2008	1999-2005	Community- based
Puopolo[157]	United States	2005	1997-2003	Facility-based
CDC[158]	United States	2007	2003-2005	Community- based

Stoll[159]	United States	2002	1998-2000	Facility-based
Jordan[160]	United States	2008	1990-2005	Community- based
CDC[161]	United States	2009	2000-2006	Community- based
Ecker [162]	United States	2013	1990-2007	Facility-based
Greenhow[163]	United States	2012	2005-2009	Community- based
Greenhow[164]	United States	2014	2005-2011	Community- based
Mukhopadhyay[165]	United States	2013	2008-2009	Facility-based
Mukhopadhyay[166]	United States	2014	2009-2012	Facility-based
Tudela[167]	United States	2012	2000-2008	Facility-based
Weston[168]	United States	2011	2005-2008	Community- based
Stafford[169]	United States	2012	2000-2008	Facility-based
Stoll[170]	United States	2011	2006-2009	Facility-based
Parente[171]	United States	2017	2002-2012	Community- based
Wortham[172]	United States	2016	2006-2009	Facility-based

Supplementary Figure S1: Incidence of invasive infant GBS disease in studies from United States, 2002-2017, grouped in year periods



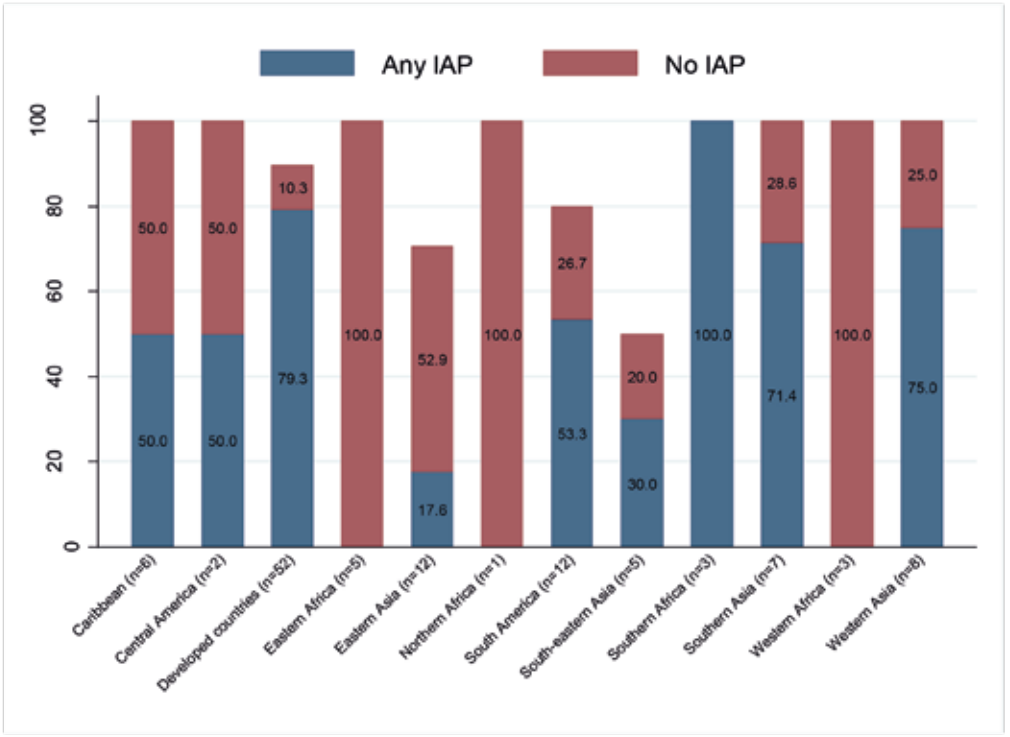
Supplementary Figure S2: Publication years of included studies on incidence of invasive infant GBS disease



Supplementary Figure S3A. Map illustrating number of studies by country reporting incidence of GBS invasive disease in the previous systematic review by Edmond[1].

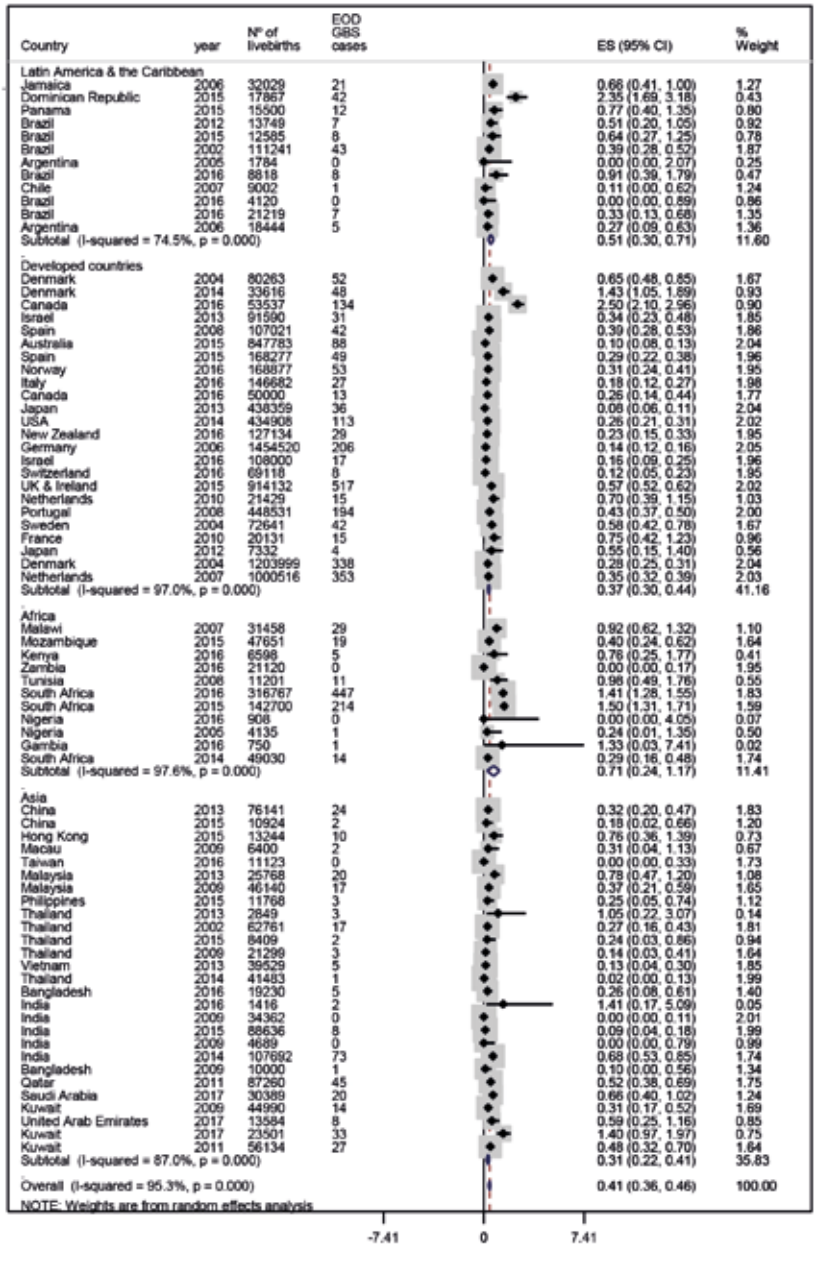
Supplementary Figure S3B. Map illustrating overall incidence of GBS disease among infants aged 0-89 days by country included in the previous meta-analysis by Edmond[1].

Supplementary Figure S4. Regional distribution of included studies assessing incidence of infant GBS disease, and use of intrapartum antibiotic prophylaxis, 2000-2017. *

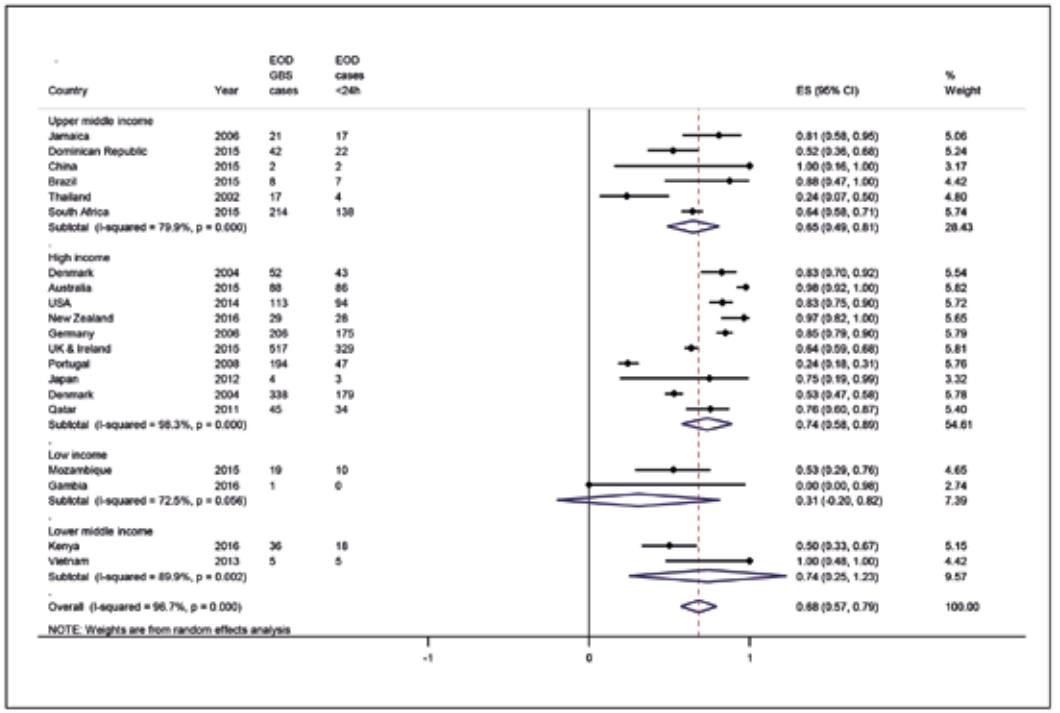


* Information about intrapartum antibiotic prophylaxis (IAP) was available for 116 of 135 studies included in the meta-analysis. Not available information for 6 developed countries, 5 countries from Eastern Asia, 3 countries from South America and 5 countries form South-eastern Asia.

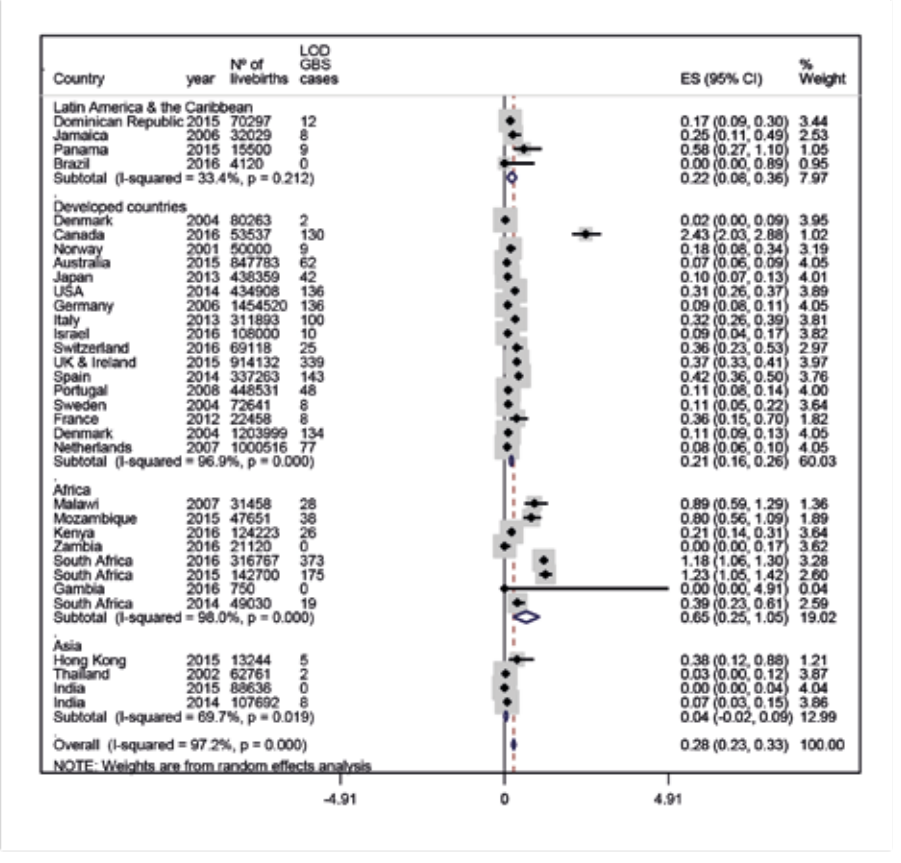
Supplementary Figure S5: Incidence risk of early-onset GBS disease worldwide



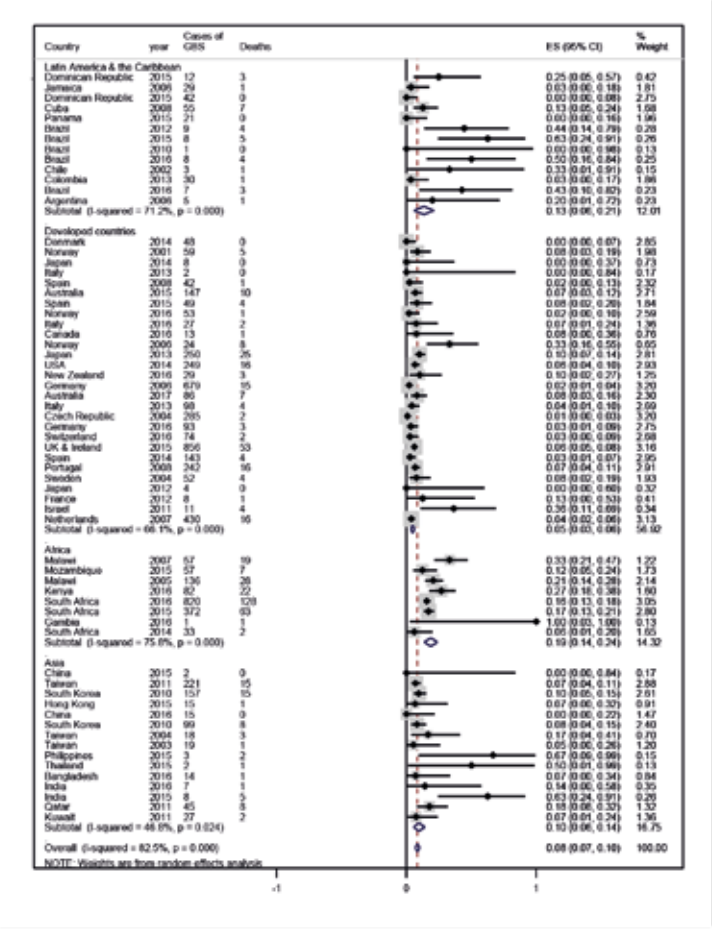
Supplementary Figure S6: Percentage of early-onset GBS disease cases in the first 24 hours after birth, of all early-onset (days 0-6) cases.



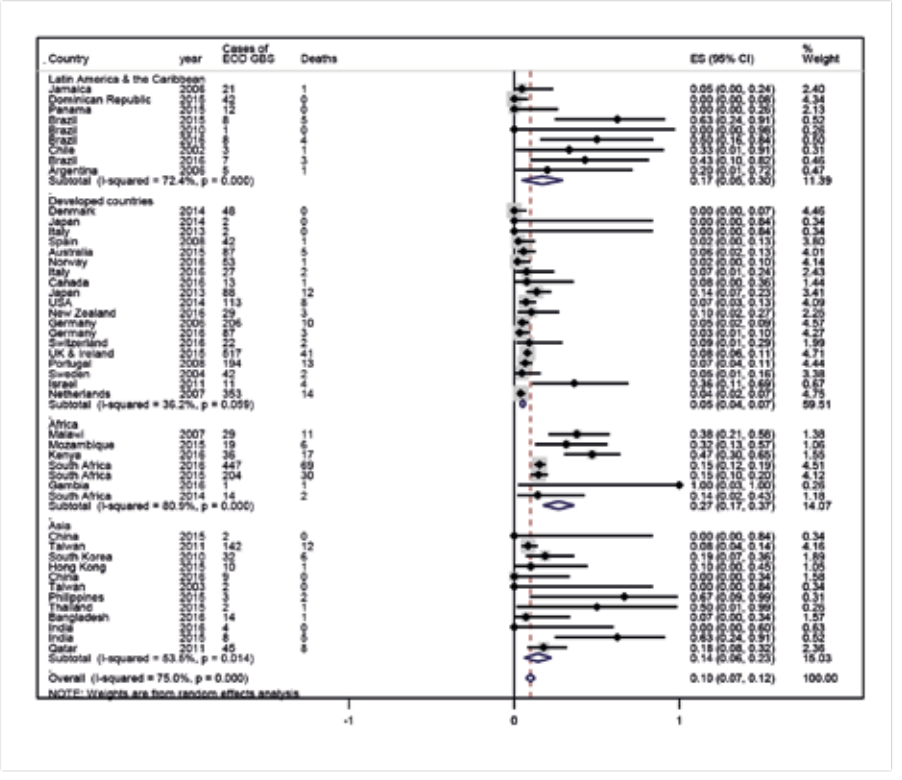
Supplementary Figure S7: Incidence risk of late-onset GBS disease worldwide by region



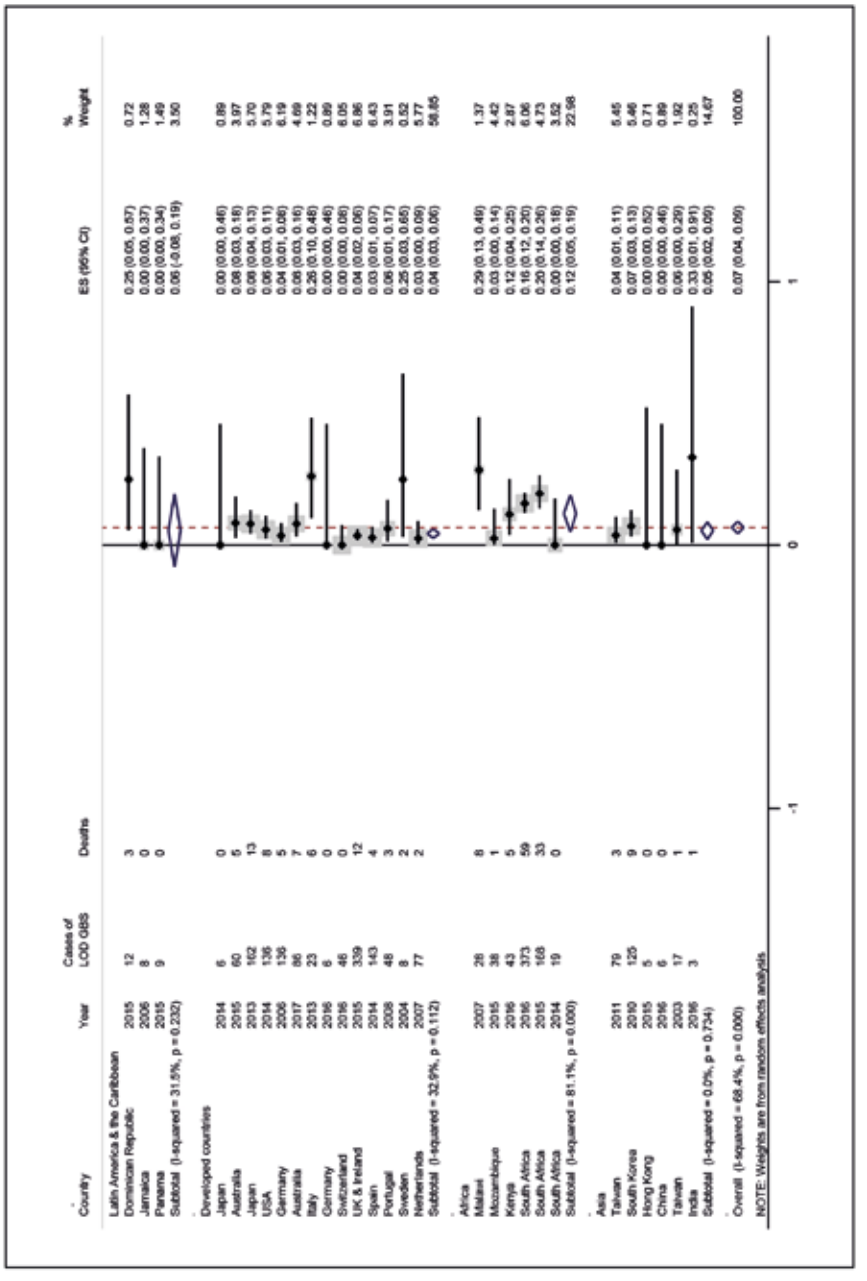
Supplementary Figure S8: Case fatality risk of GBS disease in infants aged 0-89 days worldwide by region

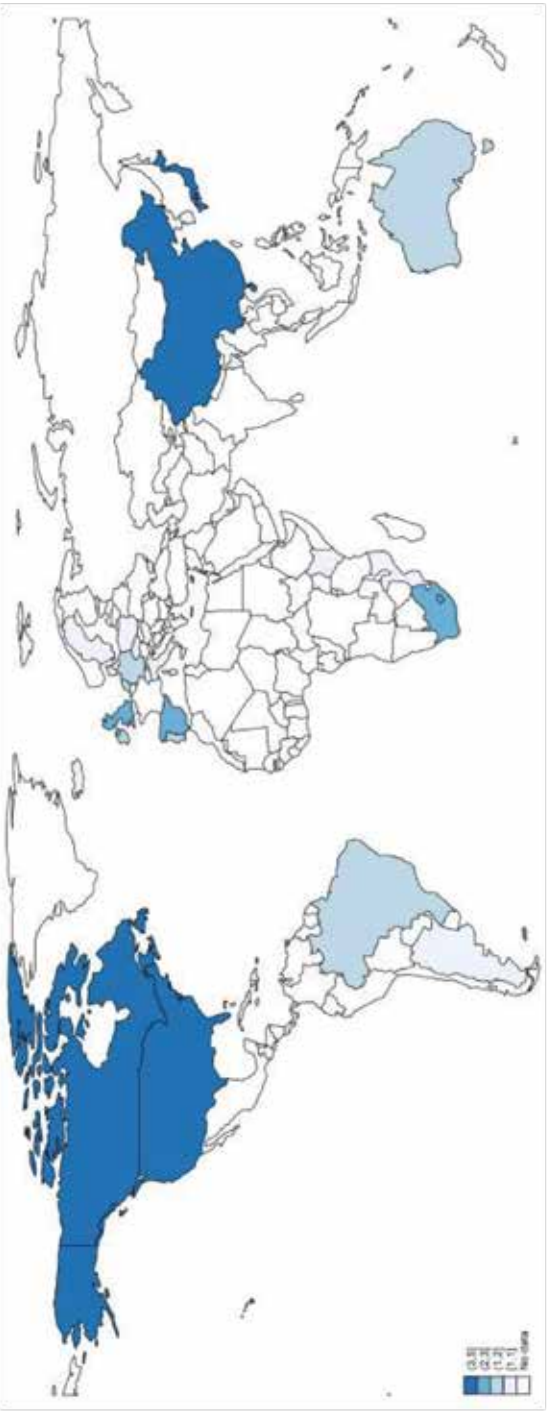


Supplementary Figure S9: Case fatality risk of early-onset GBS disease by region.



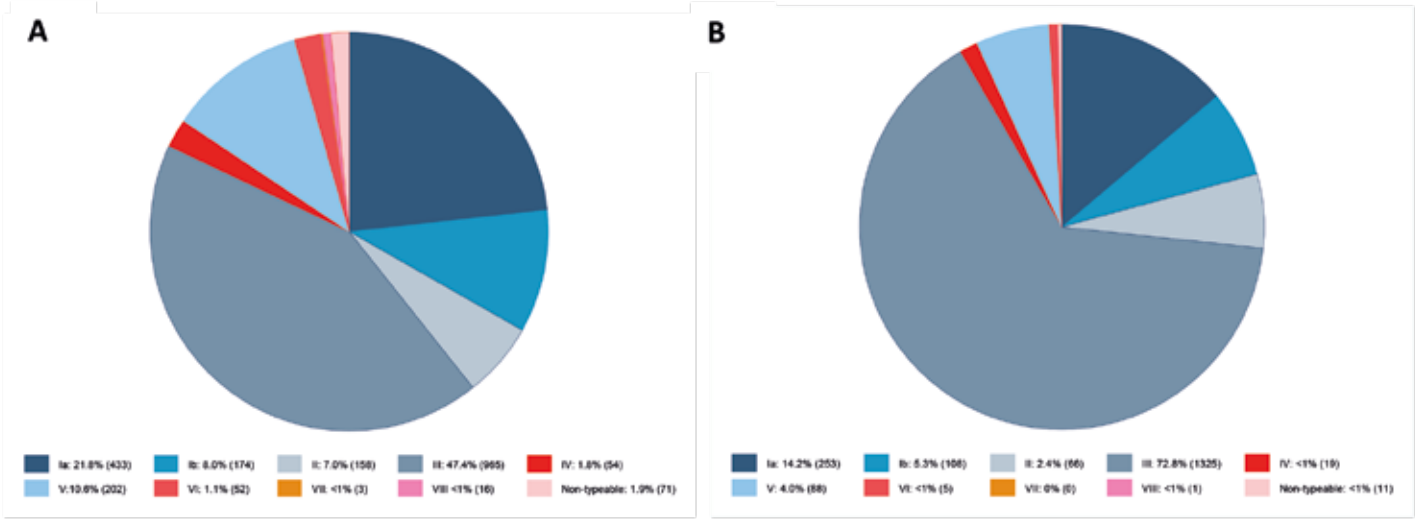
Supplementary Figure S10: Case fatality risk of late-onset GBS disease by region.





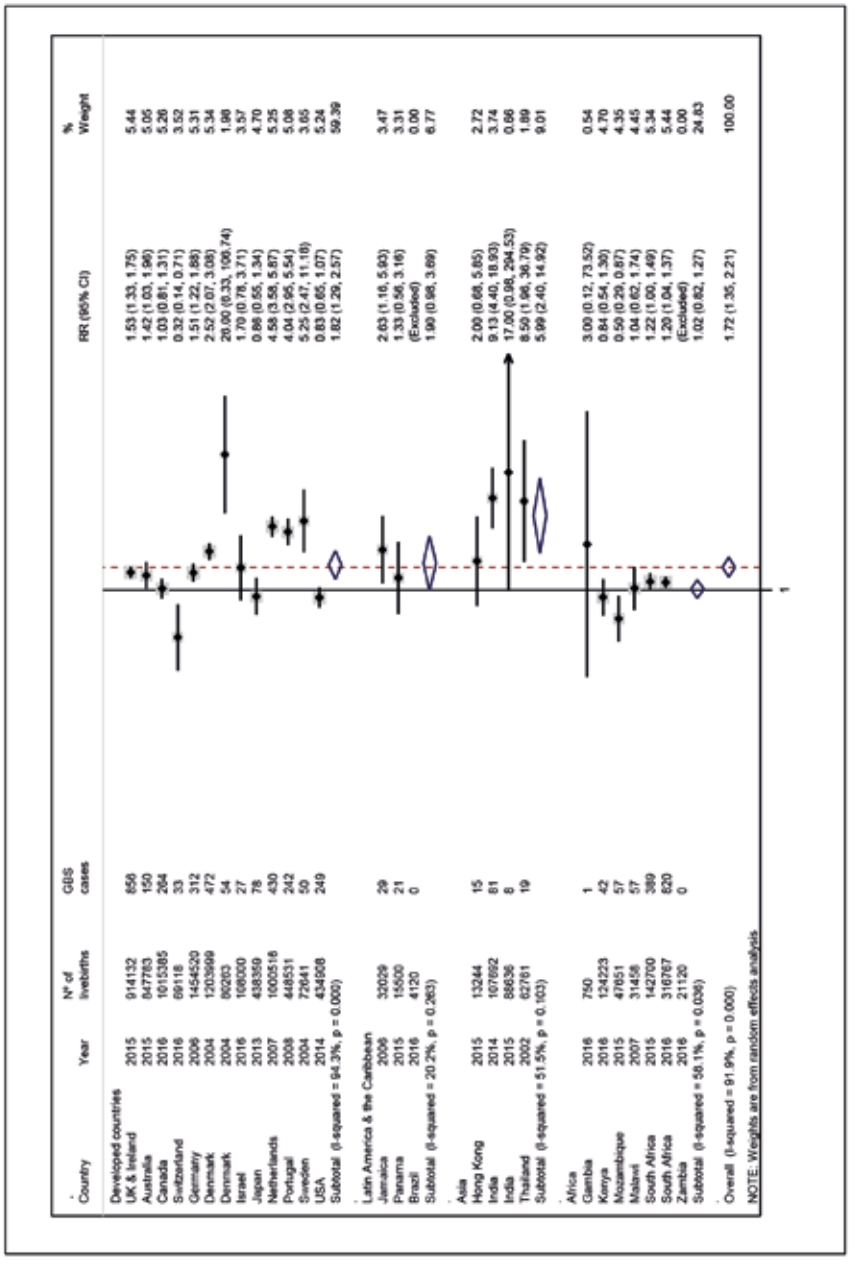
Supplementary Figure S11: Map of included studies reporting serotype data for infant GBS disease

Supplementary Figure S12: Distribution of GBS serotypes for A) early onset GBS disease and B) late onset GBS disease

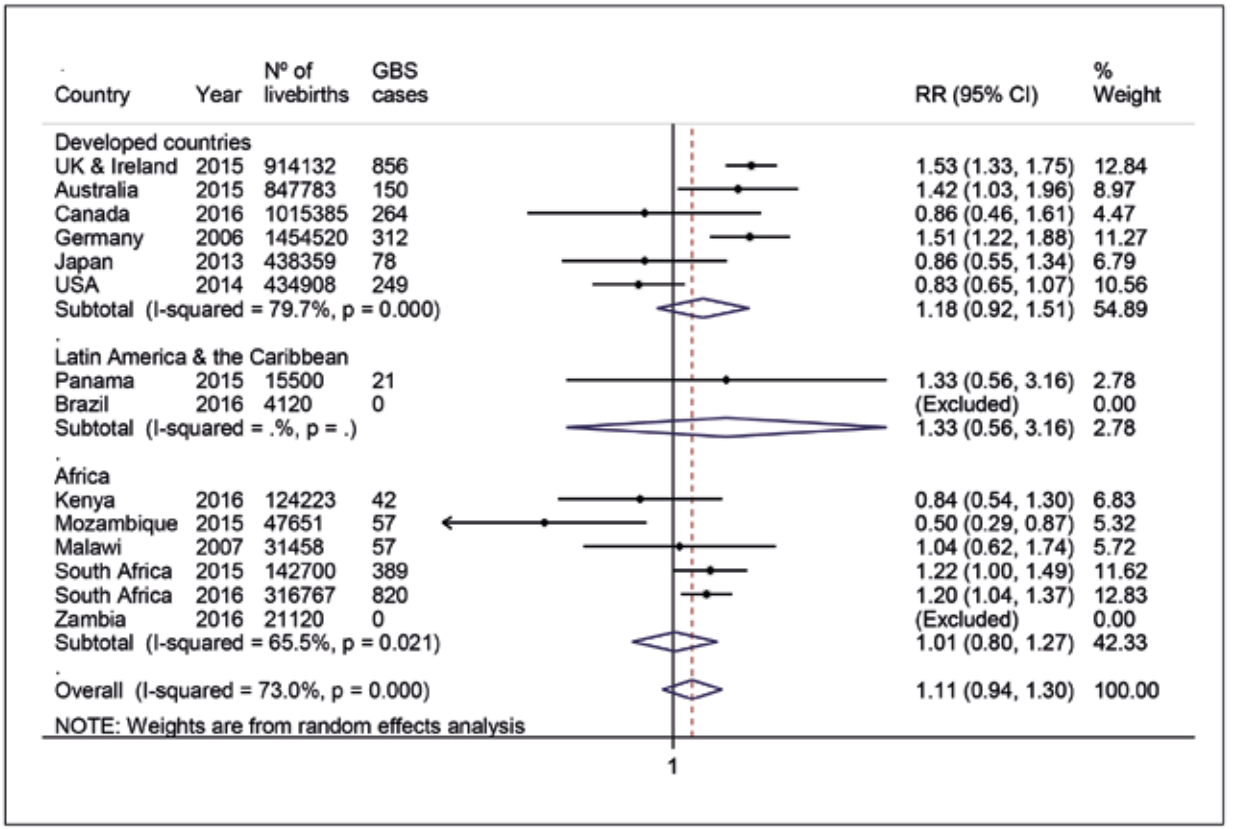


*Figures presenting prevalence of serotype in % (n).

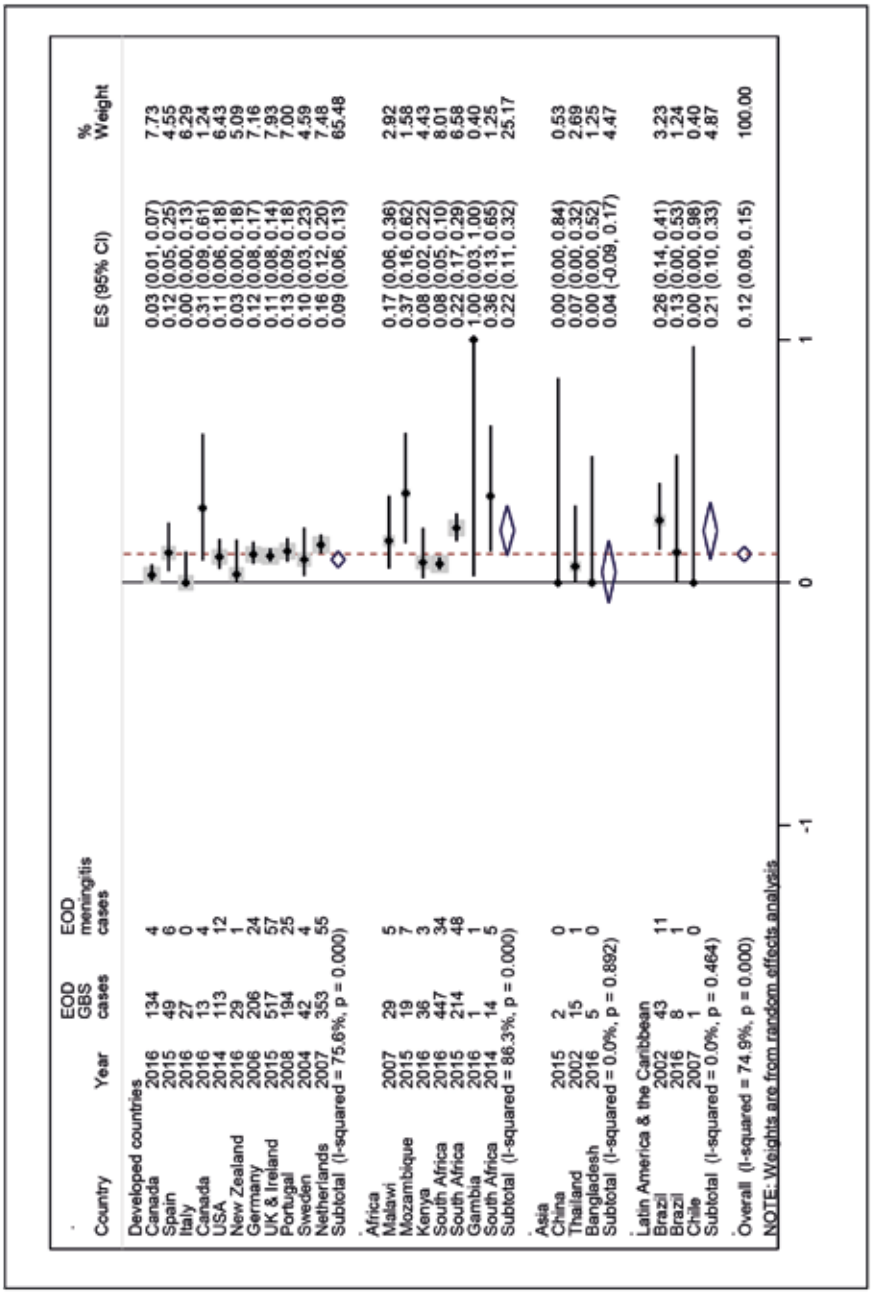
Supplementary Figure S13A: Ratio of early to late-onset GBS disease cases among infant GBS disease cases



Supplementary Figure S13B: Early to late-onset ratio for infant invasive GBS disease in high quality studies.

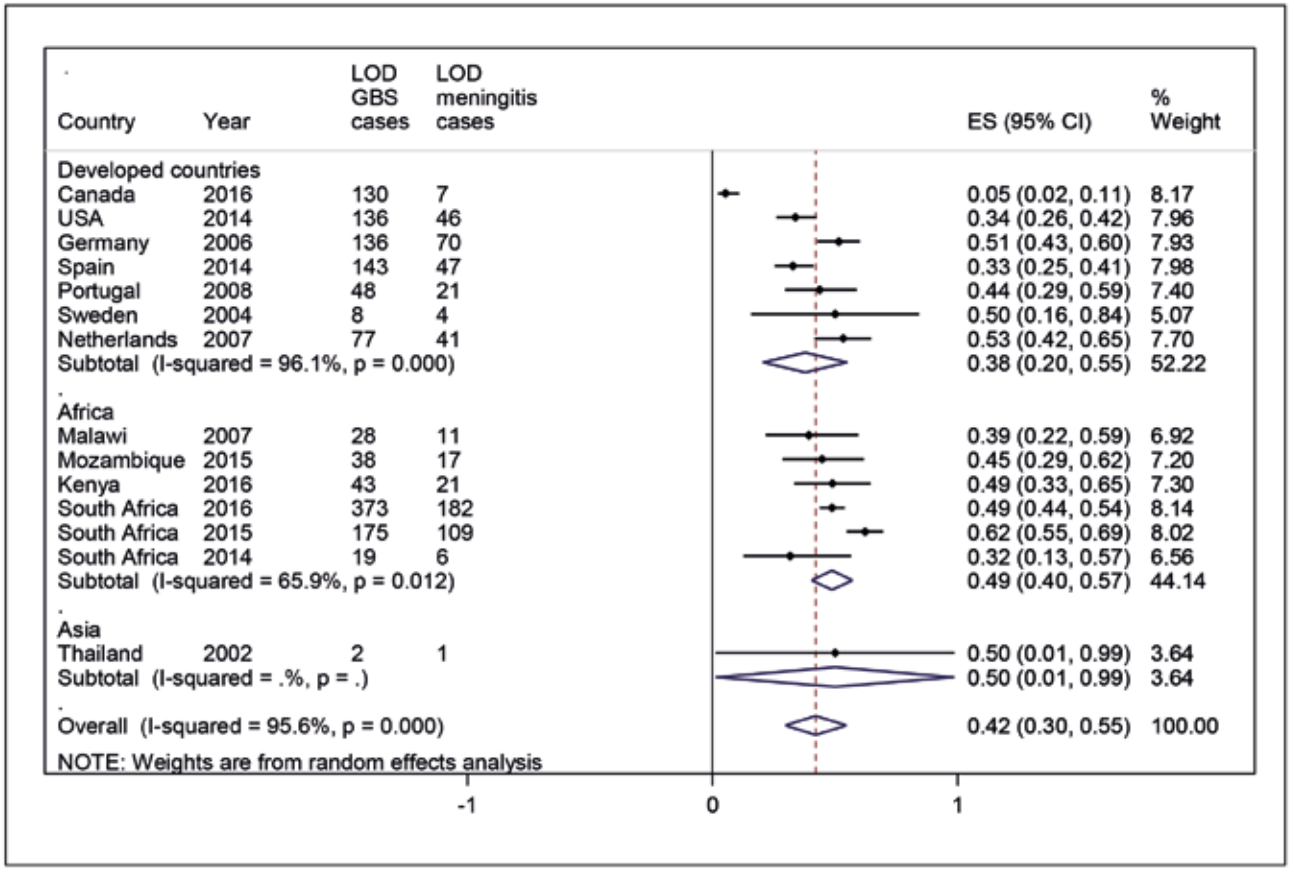


*Analysis was restricted to those studies reporting early to late-onset ratio between 0.5 and 1.5 based on high quality studies in high, lower-middle and upper-middle income countries[93-96].

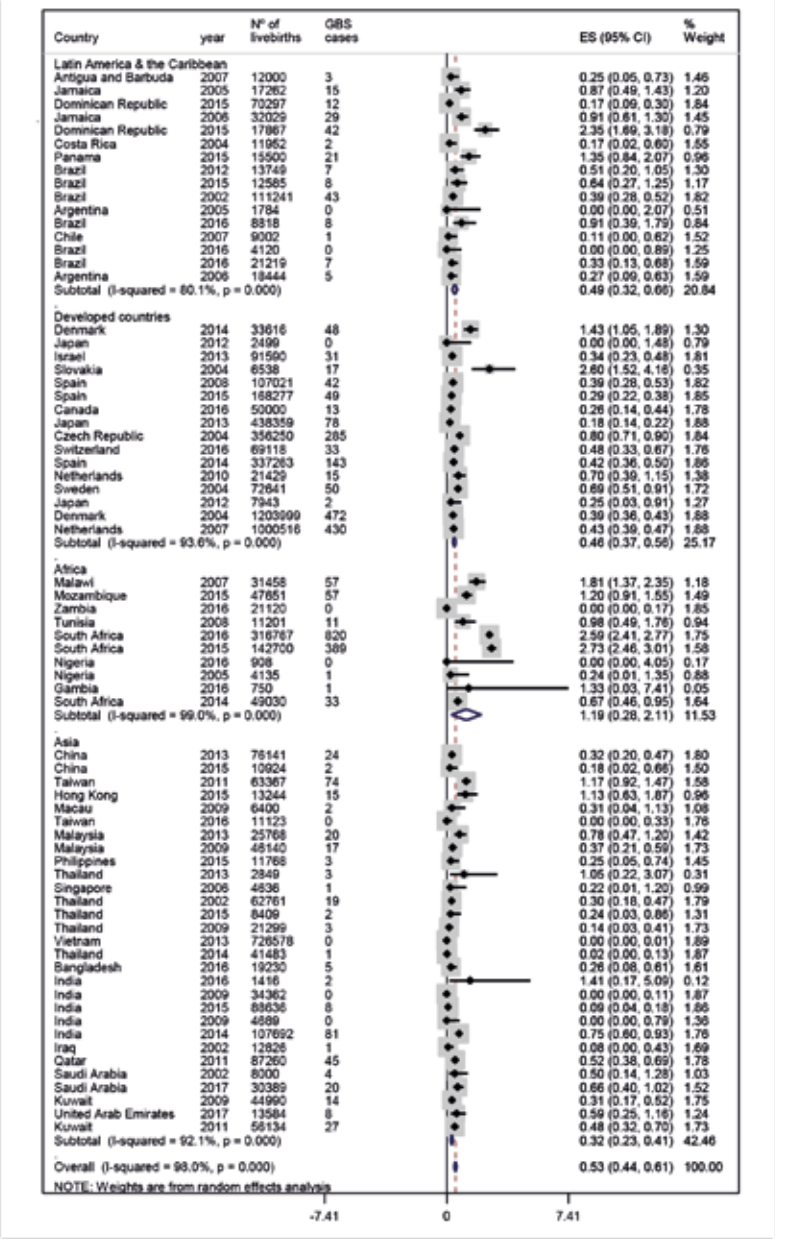


Supplementary Figure S14: Meningitis cases among early-onset GBS cases.

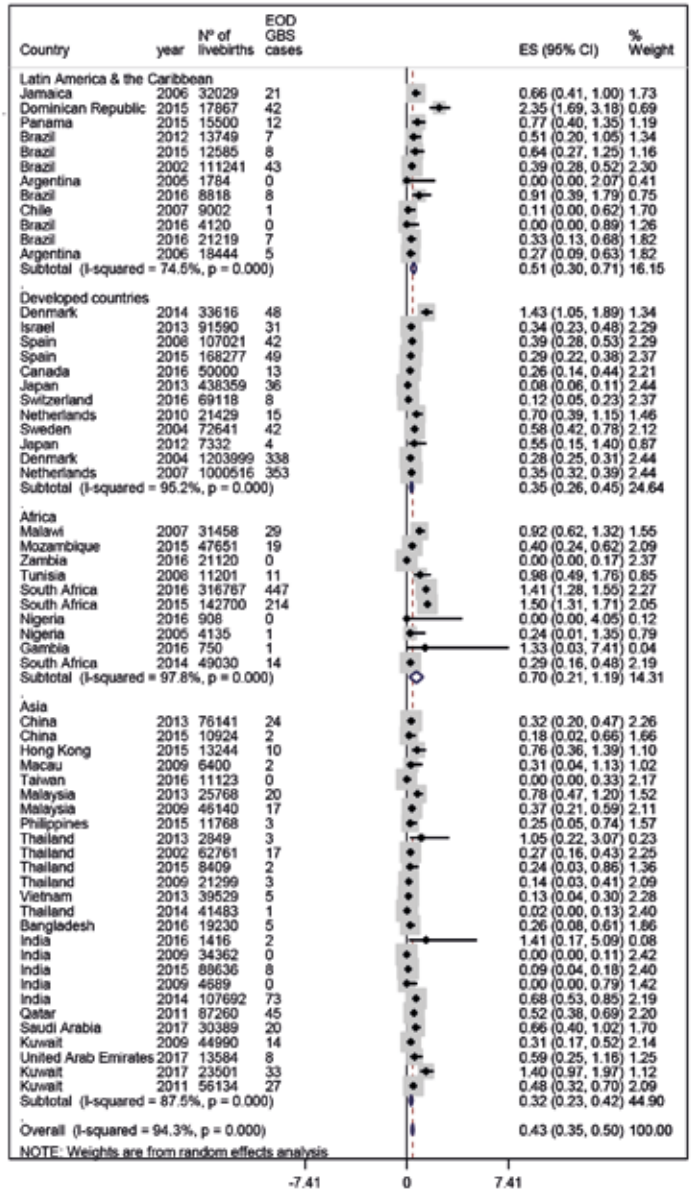
Supplementary Figure S15: Meningitis cases among late-onset GBS cases



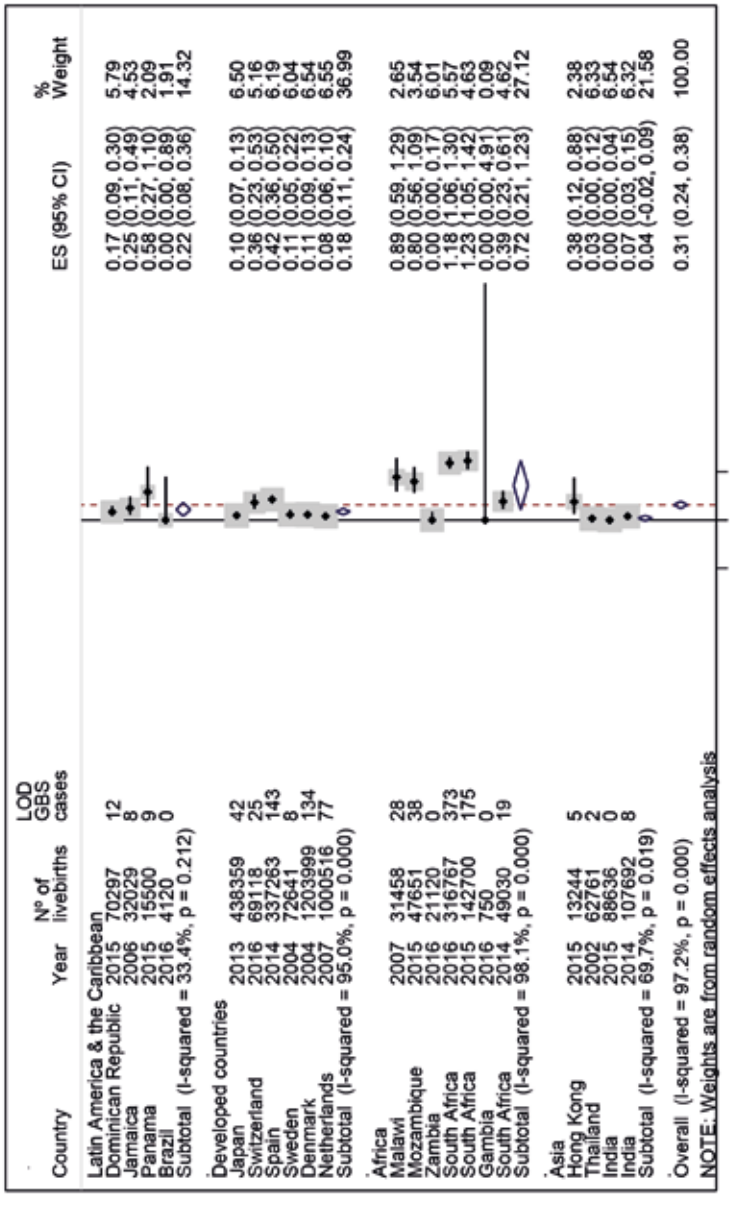
Supplementary Figure S16: Incidence of GBS disease among infants aged 0-89 days in facility-based studies by region.



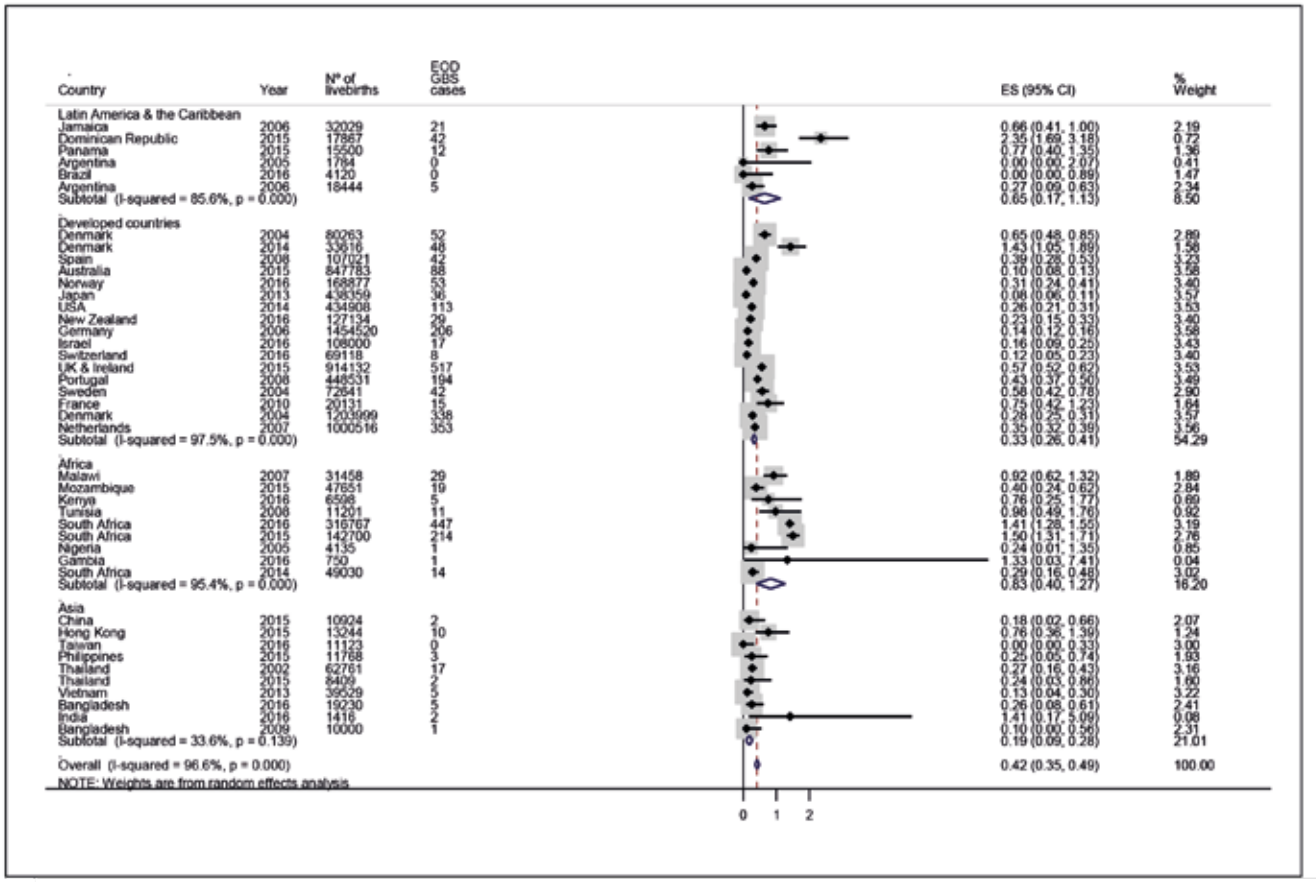
Supplementary Figure S17: Incidence of early-onset GBS disease among infants in facility-based studies by region



Supplementary Figure S18: Incidence of late-onset GBS disease among infants in facility-based studies by region

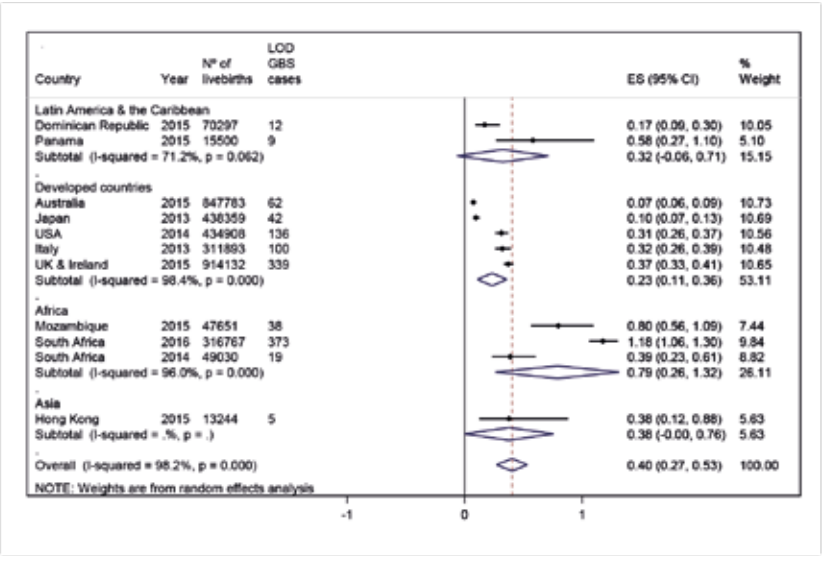


Supplementary Figure S19: Incidence of early-onset of GBS disease among infants aged 0-6 days by region*



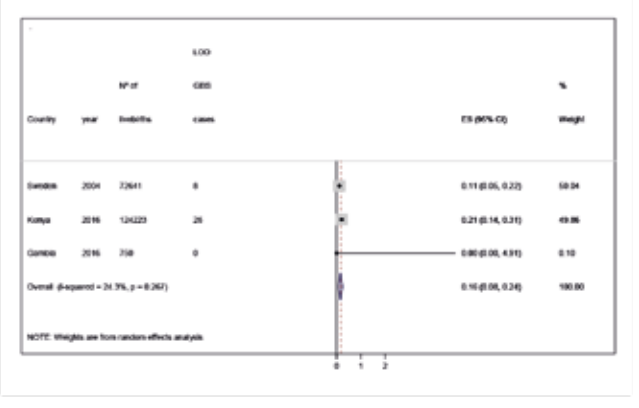
*Analysis was restricted to those studies reporting GBS early-onset incidence for complete period (infants aged 0-6 days).

Supplementary Figure S20: Incidence of late-onset of GBS disease among infants aged 7-89 days by regions*



*Analysis was restricted to those studies reporting GBS late-onset incidence for complete period (infants aged 7-89 days).

Supplementary Figure S21: Incidence of late-onset of GBS disease among infants aged 7-27 days by country



*Analysis was restricted to those studies reporting GBS late-onset incidence in infants aged 7-27 days

REFERENCES

1. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet **2012**; 379(9815): 547-56.
2. Andersen J, Christensen R, Hertel J. Clinical features and epidemiology of septicaemia and meningitis in neonates due to Streptococcus agalactiae in Copenhagen County, Denmark: a 10 year survey from 1992 to 2001. Acta Paediatr **2004**; 93(10): 1334-9.
3. Bell Y ea. Neonatal sepsis in Jamaican neonates. Ann Trop Paediatr **2005**; 25(4): 293-6.
4. Carbonell-Estrany X, Figueras-Aloy J, Salcedo-Abizanda S, de la Rosa-Fraile M. Probable early-onset group B streptococcal neonatal sepsis: a serious clinical condition related to intrauterine infection. Arch Dis Child Fetal Neonatal Ed **2008**; 93(2): F85-9.
5. Chang CJ, Chang WN, Huang LT, et al. Neonatal bacterial meningitis in southern Taiwan. Pediatr Neurol **2003**; 29(4): 288-94.
6. Davies HD, Raj S, Adair C, Robinson J, McGeer A. Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation. Pediatr Infect Dis J **2001**; 20(9): 879-84.
7. Ekelund K, Konradsen HB. Invasive group B streptococcal disease in infants: a 19-year nationwide study. Serotype distribution, incidence and recurrent infection. Epidemiol Infect **2004**; 132(6): 1083-90.
8. Fluegge K, Siedler A, Heinrich B, et al. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. Pediatrics **2006**; 117(6): e1139-45.
9. Hasseltvedt V, et.al. Systemic streptococcal group B disease in Norway-an increasing health problem. Euro Surveill **2001**; 5(40): 2086.
10. Janek Lea. Screening of Hemolytical Streptococcus of Group B in Pregnancy and Prevention of Infection in Newborns. Ceska Gynekologie **2004**; 69(2): 91-4.
11. Jiang JH, Chiu NC, Huang FY, et al. Neonatal sepsis in the neonatal intensive care unit: characteristics of early versus late onset. J Microbiol Immunol Infect **2004**; 37(5): 301-6.
12. Kim JS, Jang YT, Kim JD, et al. Incidence of Haemophilus influenzae type b and other invasive diseases in South Korean children. Vaccine **2004**; 22(29-30): 3952-62.
13. Neto MT. Group B streptococcal disease in Portuguese infants younger than 90 days. Arch Dis Child Fetal Neonatal Ed **2008**; 93(2): F90-3.
14. Niduvaje K, Amutha C, Roy J. Early neonatal streptococcal infection. Indian J Pediatr **2006**; 73(7): 573-6.
15. Ben Hamida Nouaili E, Harouni M, Chaouachi S, Sfar R, Marrakchi Z. [Early-onset neonatal bacterial infections: a retrospective series of 144 cases]. Tunis Med **2008**; 86(2): 136-9.
16. Trijbels-Smeulders M, de Jonge GA, Pasker-de Jong PC, et al. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. Arch Dis Child Fetal Neonatal Ed **2007**; 92(4): F271-6.
17. Trotman H, Bell Y. Neonatal group B streptococcal infection at the University Hospital of the West Indies, Jamaica: a 10-year experience. Ann Trop Paediatr **2006**; 26(1): 53-7.
18. Yossuck P, Preedisripipat K. Neonatal group B streptococcal infection: incidence and clinical manifestation in Siriraj Hospital. J Med Assoc Thai **2002**; 85 Suppl 2: S479-87.
19. Hoshina K ea. Trend of neonatal group B streptococcal infection during the last 15 years. Pediatr Int **2002**; 44(6): 641-6.
20. Gray KJ, Bennett SL, French N, Phiri AJ, Graham SM. Invasive group B streptococcal infection in infants, Malawi. Emerg Infect Dis **2007**; 13(2): 223-9.
21. Figueira-Coelho J, Ramirez M, Salgado MJ, Melo-Cristino J. Streptococcus agalactiae in a large Portuguese teaching hospital: antimicrobial susceptibility, serotype distribution, and clonal analysis of macrolide-resistant isolates. Microb Drug Resist **2004**; 10(1): 31-6.
22. Hajdu A, Blystad H, Hoiby EA, Klouman E, Schimmer B, Nygard K. Unexpected increase in case fatality of invasive group B streptococcal infections in infants in Norway, January-July 2006. Euro Surveill **2006**; 11(7): E060727 2.
23. Vaciloto E, et.al. A survey of the Incidence of Neonatal Sepsis by Group B Streptococcus During a Decade in a Brazilian Maternity Hospital. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases **2002**; 6(2): 55-62.
24. Strakova L, Motlova J. Active surveillance of early onset disease due to group to group B streptococci in newborns. Indian J Med Res **2004**; 119: 205-7.
25. Park KH, Kim KH, Kang JH, et al. Current status and clinical presentations of invasive neonatal Group B streptococcal infections in Korea. Pediatr Int **2010**; 53(2): 236-9.
26. Matsubara K, Yamamoto G. Invasive group B streptococcal infections in a tertiary care hospital between 1998 and 2007 in Japan. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **2009**; 13(6): 679-84.

27. Darmstadt GL, Saha SK, Choi Y, et al. Population-based incidence and etiology of community-acquired neonatal bacteremia in Mirzapur, Bangladesh: an observational study. J Infect Dis **2009**; 200(6): 906-15.
28. Al-Zwaini EJ. Neonatal septicaemia in the neonatal care unit, Al-Anbar governorate, Iraq. East Mediterr Health J **2002**; 8(4-5): 509-14.
29. Sundaram V, Kumar P, Dutta S, et al. Blood culture confirmed bacterial sepsis in neonates in a North Indian tertiary care center: changes over the last decade. Jpn J Infect Dis **2009**; 62(1): 46-50.
30. Martin TC, Adamson J, Dickson T, DiGiantomasso E, Nesbitt C. Does group B streptococcal infection contribute significantly to neonatal sepsis in Antigua and Barbuda? West Indian Med J **2007**; 56(6): 498-501.
31. Cho HK, Lee H, Kang JH, et al. The causative organisms of bacterial meningitis in Korean children in 1996-2005. J Korean Med Sci **2010**; 25(6): 895-9.
32. van den Hoogen A, Gerards LJ, Verboon-Maciolek MA, Fleer A, Krediet TG. Long-term trends in the epidemiology of neonatal sepsis and antibiotic susceptibility of causative agents. Neonatology **2009**; 97(1): 22-8.
33. Kuhn P, Dheu C, Bolender C, et al. Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. Paediatr Perinat Epidemiol **2010**; 24(5): 479-87.
34. Milledge J, et.al. Aetiology of neonatal sepsis in Blantyre, Malawi: 1996-2001. Ann Trop Paediatr **2005**; 25(2): 101-10.
35. Zhao Z, Kong F, Zeng X, Gidding HF, Morgan J, Gilbert GL. Distribution of genotypes and antibiotic resistance genes among invasive Streptococcus agalactiae (group B streptococcus) isolates from Australasian patients belonging to different age groups. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases **2008**; 14(3): 260-7.
36. Martins ER, Pessanha MA, Ramirez M, Melo-Cristino J. Analysis of group B streptococcal isolates from infants and pregnant women in Portugal revealing two lineages with enhanced invasiveness. J Clin Microbiol **2007**; 45(10): 3224-9.
37. Trijbels-Smeulders MA, Kimpen JL, Kollee LA, et al. Serotypes, genotypes, and antibiotic susceptibility profiles of group B streptococci causing neonatal sepsis and meningitis before and after introduction of antibiotic prophylaxis. Pediatr Infect Dis J **2006**; 25(10): 945-8.
38. Fluegge K, Supper S, Siedler A, Berner R. Serotype distribution of invasive group B streptococcal isolates in infants: results from a nationwide active laboratory surveillance study over 2 years in Germany. Clin Infect Dis **2005**; 40(5): 760-3.
39. Persson E, Berg S, Trollfors B, et al. Serotypes and clinical manifestations of invasive group B streptococcal infections in western Sweden 1998-2001. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases **2004**; 10(9): 791-6.
40. Davies HD, Jones N, Whittam TS, Elsayed S, Bisharat N, Baker CJ. Multilocus sequence typing of serotype III group B streptococcus and correlation with pathogenic potential. J Infect Dis **2004**; 189(6): 1097-102.
41. Bidet P, Brahimi N, Chalas C, Aujard Y, Bingen E. Molecular characterization of serotype III group B-streptococcus isolates causing neonatal meningitis. J Infect Dis **2003**; 188(8): 1132-7.
42. Lopardo HA, Vidal P, Jeric P, et al. Six-month multicenter study on invasive infections due to group B streptococci in Argentina. J Clin Microbiol **2003**; 41(10): 4688-94.
43. El-Said Mf, et.al. Epidemiology of neonatal meningitis in Qatar. Saudi Med J **2002**; 23(7): 789-92.
44. Ojukwu JU, et.al. Neonatal septicemia in high risk babies in South-Eastern Nigeria. JPerinatMed **2005**; 34: 166-72.
45. Tiskumara R, Fakharee SH, Liu CQ, et al. Neonatal infections in Asia. Arch Dis Child Fetal Neonatal Ed **2009**; 94(2): F144-8.
46. Abdelmaaboud M, Mohammed AF, Abdelmaaboud M, Mohammed AF. Universal screening vs. risk-based strategy for prevention of early-onset neonatal Group-B streptococcal disease. Journal of Tropical Pediatrics **2011**; 57(6): 444-50.
47. Al-Taiar A, Hammoud MS, Cuiqing L, et al. Neonatal infections in China, Malaysia, Hong Kong and Thailand. Archives of Disease in Childhood Fetal & Neonatal Edition **2013**; 98(3): F249-55.
48. Bekker V, Bijlsma MW, van de Beek D, et al. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. The Lancet Infectious Diseases **2014**; 14(11): 1083-9.
49. Berardi A, Rossi C, Lugli L, et al. Group B streptococcus late-onset disease: 2003-2010. Pediatrics **2013**; 131(2): e361-8.
50. Bromiker R, Ernest N, Meir MB, et al. Correlation of bacterial type and antibiotic sensitivity with maternal antibiotic exposure in early-onset neonatal sepsis.[Erratum appears in Neonatology. 2013;103(1):53]. Neonatology **2013**; 103(1): 48-53.
51. Chang B, Wada A, Hosoya M, et al. Characteristics of group B Streptococcus isolated from infants with invasive infections: a population-based study in Japan. Japanese Journal of Infectious Diseases **2014**; 67(5): 356-60.

52. Cutland CL, Schrag SJ, Thigpen MC, et al. Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1). Emerging Infectious Diseases **2015**; 21(4): 638-45.
53. Cantoni L, Ronfani L, Da Riol R, et al. Physical examination instead of laboratory tests for most infants born to mothers colonized with group B Streptococcus: support for the Centers for Disease Control and Prevention's 2010 recommendations. Journal of Pediatrics **2013**; 163(2): 568-73.
54. Didier C, Streicher MP, Chognot D, et al. Late-onset neonatal infections: incidences and pathogens in the era of antenatal antibiotics. European Journal of Pediatrics **2012**; 171(4): 681-7.
55. Evangelista ML, Freitas FT, Evangelista MLB, Freitas FTdM. Group B streptococcus neonatal infection in an intensive care unit in Brazil: high fatality and missed opportunities for antibiotic prophylaxis. Brazilian Journal of Infectious Diseases **2015**; 19(1): 98-9.
56. Fiolo K, Zanardi CE, Salvadego M, et al. [Infection rate and Streptococcus agalactiae serotypes in samples of infected neonates in the city of Campinas (Sao Paulo), Brazil]. Revista Brasileira de Ginecologia e Obstetricia **2012**; 34(12): 544-9.
57. Giannoni E, Berger C, Stocker M, et al. Incidence and Outcome of Group B Streptococcal Sepsis in Infants in Switzerland. Pediatric Infectious Disease Journal **2016**; 35 (2): 222-4.
58. Gimenez M, Sanfeliu I, Sierra M, et al. Group B streptococcal early-onset neonatal sepsis in the area of Barcelona (2004-2010). Analysis of missed opportunities for prevention. [Spanish]. Enfermedades Infecciosas y Microbiologia Clinica Monografias **2015**; 33 (7): 446-50.
59. Hashavya S, Benenson S, Ergaz-Shaltiel Z, et al. The use of blood counts and blood cultures to screen neonates born to partially treated group B Streptococcus-carrier mothers for early-onset sepsis: is it justified? Pediatric Infectious Disease Journal **2011**; 30(10): 840-3.
60. Juncosa-Morros T, Guardia-Llobet C, Bosch-Mestres J, et al. Streptococcus agalactiae late-onset neonatal infections in Barcelona (1996-2010). [Spanish]. Enfermedades Infecciosas y Microbiologia Clinica Monografias **2014**; 32 (9): 574-8.
61. Kruse AY, Thieu Chuong do H, Phuong CN, et al. Neonatal bloodstream infections in a pediatric hospital in Vietnam: a cohort study. Journal of Tropical Pediatrics **2013**; 59(6): 483-8.
62. Liu H, Zeng H, Wang W, et al. Estimating the burden of invasive Group B Streptococcal disease in young infants in southern mainland China: An observational study. International Journal of Clinical and Experimental Medicine **2015**; 8 (8): 13699-707.
63. Matsubara K, Hoshina K, Suzuki Y, Matsubara K, Hoshina K, Suzuki Y. Early-onset and late-onset group B streptococcal disease in Japan: a nationwide surveillance study, 2004-2010. International Journal of Infectious Diseases **2013**; 17(6): e379-84.
64. Miyata A, Takahashi H, Kubo T, et al. Early-onset group B streptococcal disease following culture-based screening in Japan: A single center study. Journal of Obstetrics and Gynaecology Research **2012**; 38 (8): 1052-6.
65. Oladottir GL, Erlendsdottir H, Palsson G, et al. Increasing incidence of late-onset neonatal invasive group B streptococcal infections in Iceland. Pediatric Infectious Disease Journal **2011**; 30(8): 661-3.
66. Petersen KB, Johansen HK, Rosthoj S, Krebs L, Pinborg A, Hedegaard M. Increasing prevalence of group B streptococcal infection among pregnant women. Danish Medical Journal **2014**; 61(9): 2014.
67. Sakata H, Sakata H. Pediatric invasive streptococcal infection in northern and eastern regions of Hokkaido, Japan from 2010 to 2012. Pediatrics International **2014**; 56(3): 360-3.
68. Sridhar S, Grace R, Nithya PJ, et al. Group B streptococcal infection in a tertiary hospital in India--1998-2010. Pediatric Infectious Disease Journal **2014**; 33(10): 1091-2.
69. Yu HW, Lin HC, Yang PH, et al. Group B streptococcal infection in Taiwan: maternal colonization and neonatal infection. Pediatrics & Neonatology **2011**; 52(4): 190-5.
70. Thatrimontrichai A, Chanvitan P, Janjindamai W, Dissaneevate S, Jefferies A, Shah V. Trends in neonatal sepsis in a neonatal intensive care unit in Thailand before and after construction of a new facility. Asian Biomedicine **2014**; 8 (6): 771-8.
71. Ko DW, Zurynski Y, Gilbert GL. Group B streptococcal disease and genotypes in Australian infants. J Paediatr Child Health **2015**; 51(8): 808-14.
72. Morozumi M, Wajima T, Kuwata Y, et al. Associations between capsular serotype, multilocus sequence type, and macrolide resistance in Streptococcus agalactiae isolates from Japanese infants with invasive infections. Epidemiology & Infection **2014**; 142(4): 812-9.
73. Almeida A, Villain A, Joubrel C, et al. Whole-Genome Comparison Uncovers Genomic Mutations between Group B Streptococci Sampled from Infected Newborns and Their Mothers. Journal of Bacteriology **2015**; 197(20): 3354-66.
74. Brzychczy-Wloch M, Gosiewski T, Bulanda M, Brzychczy-Wloch M, Gosiewski T, Bulanda M. Multilocus sequence types of invasive and colonizing neonatal group B streptococci in Poland. Medical Principles & Practice **2014**; 23(4): 323-30.

75. Fluegge K, Wons J, Spellerberg B, et al. Genetic differences between invasive and noninvasive neonatal group B streptococcal isolates. Pediatric Infectious Disease Journal **2011**; 30(12): 1027-31.
76. Imperi M, Gherardi G, Berardi A, et al. Invasive neonatal GBS infections from an area-based surveillance study in Italy. Clinical Microbiology & Infection **2011**; 17(12): 1834-9.
77. Joubrel C, Tazi A, Six A, et al. Group B streptococcus neonatal invasive infections, France 2007-2012. Clinical Microbiology and Infection **2015**; 21 (10): 910-6.
78. Six A, Firon A, Plainvert C, et al. Molecular characterization of nonhemolytic and nonpigmented group b streptococci responsible for human invasive infections. Journal of Clinical Microbiology **2016**; 54 (1): 75-82.
79. Souza VC, Kegele FC, Souza SR, et al. Antimicrobial susceptibility and genetic diversity of Streptococcus agalactiae recovered from newborns and pregnant women in Brazil. Scandinavian Journal of Infectious Diseases **2013**; 45(10): 780-5.
80. Teatero S, McGeer A, Low DE, et al. Characterization of invasive group B streptococcus strains from the greater Toronto area, Canada. Journal of Clinical Microbiology **2014**; 52(5): 1441-7.
81. Yoon IA, Jo DS, Cho EY, Choi EH, Lee HJ, Lee H. Clinical significance of serotype V among infants with invasive group B streptococcal infections in South Korea. International Journal of Infectious Diseases **2015**; 38: 136-40.
82. Wang P, Ma Z, Tong J, et al. Serotype distribution, antimicrobial resistance, and molecular characterization of invasive group B Streptococcus isolates recovered from Chinese neonates. International Journal of Infectious Diseases **2015**; 37: e115-e8.
83. Sakata H, Sakata H. Evaluation of intrapartum antibiotic prophylaxis for the prevention of early-onset group B streptococcal infection. Journal of Infection & Chemotherapy **2012**; 18(6): 853-7.
84. Rivera L, Saez-Llorens X, Feris-Iglesias J, et al. Incidence and serotype distribution of invasive group B streptococcal disease in young infants: a multi-country observational study. BMC Pediatr **2015**; 15: 143.
85. Villanueva-Uy ME, Wongsiridej P, Sangtawesin V, et al. THE BURDEN OF INVASIVE NEONATAL GROUP B STREPTOCOCCAL (GBS) DISEASE IN THAILAND AND THE PHILIPPINES. Southeast Asian J Trop Med Public Health **2015**; 46(4): 728-37.
86. Larcher JS, Capellino F, De Giusto R, et al. [Group B streptococcus colonization during pregnancy and prevention of early onset of disease]. Medicina (B Aires) **2005**; 65(3): 201-6.
87. Sigaúque B BQ, Vubil D, Mandomando I, Menendez C, Alonso PL. Invasive bacterial infection among admitted neonates, in Manhiça District Hospital, south of Mozambique. Manhiça, Maputo, Mozambique: Centro de Investigaçao em Saúde de Manhiça., **2011**.
88. Paul VK Agarwal R. Characterisation and antimicrobial resistance of sepsis pathogens in neonates born in tertiary care centres in Delhi, India: a cohort study. Lancet Glob Health **2016**; 4(10): e752-60.
89. Le Doare K, Jarju S, Darboe S, et al. Risk factors for Group B Streptococcus colonisation and disease in Gambian women and their infants. J Infect **2016**; 72(3): 283-94.
90. Saha SK et al. Unpublished. Tangail, Bangladesh, **2016**.
91. Araujo da Silva AR et al. Unpublished. Rio de Janeiro, Brazil, **2016**.
92. Dhaded SM et al. Unpublished. Karnataka South India: J N Medical College and KLE Dr P K Hospital Belagavi, **2016**.
93. Dangor Z, Cutland CL, Izu A, et al. Temporal Changes in Invasive Group B Streptococcus Serotypes: Implications for Vaccine Development. PLoS One **2016**; 11(12): e0169101.
94. Centers for Disease Control and Prevention (CDC). Active Bacterial Core Surveillance (ABCs) Report. . In: Streptococcus. EIPNGB, Vol. 2016, **2014**.
95. O'Sullivan CP LT, Patel D, Efstratiou A , Cunney R , Ladhani S , Reynolds A , Campbell R , Doherty L , Davies E , Jones C , Heath P.T. . Group B Streptococcal (GBS) disease in UK and Irish infants younger than 90 days, 2014-2015. **2017**.
96. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. Nature microbiology **2016**; 1(7): 16067.
97. Alhhazmi A, Hurteau D, Tyrrell GJ. Epidemiology of Invasive Group B Streptococcal Disease in Alberta, Canada, from 2003 to 2013. J Clin Microbiol **2016**; 54(7): 1774-81.
98. Barbosa NG, Dos Reis H, Mantese OC, Mussi-Pinhata MM, Abdallah VO, Gontijo Filho PP. Early-onset neonatal sepsis by Group B Streptococcus in a Brazilian public hospital. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases **2016**; 20(6): 647-8.
99. Bartlett AW, Smith B, George CR, et al. Epidemiology of Late and Very Late Onset Group B Streptococcal Disease: Fifteen-Year Experience From Two Australian Tertiary Pediatric Facilities. Pediatr Infect Dis J **2017**; 36(1): 20-4.
100. Berardi A, Baroni L, Bacchi Reggiani ML, et al. The burden of early-onset sepsis in Emilia-Romagna (Italy): a 4-year, population-based study. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet **2016**; 29(19): 3126-31.

101. Bulkowstein S, Ben-Shimol S, Givon-Lavi N, Melamed R, Shany E, Greenberg D. Comparison of early onset sepsis and community-acquired late onset sepsis in infants less than 3 months of age. BMC Pediatr **2016**; 16: 82.
102. Campisi E, Rosini R, Ji W, et al. Genomic Analysis Reveals Multi-Drug Resistance Clusters in Group B Streptococcus CC17 Hypervirulent Isolates Causing Neonatal Invasive Disease in Southern Mainland China. Frontiers in microbiology **2016**; 7: 1265.
103. Darlow BA, Voss L, Lennon DR, Grimwood K. Early-onset neonatal group B streptococcus sepsis following national risk-based prevention guidelines. The Australian & New Zealand journal of obstetrics & gynaecology **2016**; 56(1): 69-74.
104. Fjalstad JW, Stensvold HJ, Bergseng H, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. Pediatr Infect Dis J **2016**; 35(1): 1-6.
105. Freitas FT, Romero GA. Early-onset neonatal sepsis and the implementation of group B streptococcus prophylaxis in a Brazilian maternity hospital: a descriptive study. The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases **2017**; 21(1): 92-7.
106. Ip M, Ang I, Fung K, Liyanapathirana V, Luo MJ, Lai R. Hypervirulent Clone of Group B Streptococcus Serotype III Sequence Type 283, Hong Kong, 1993-2012. Emerg Infect Dis **2016**; 22(10): 1800-3.
107. Hammoud MS, Al-Taiar A, Al-Abdi SY, et al. Culture-proven early-onset neonatal sepsis in Arab states in the Gulf region: two-year prospective study. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases **2017**; 55: 11-5.
108. Kang HM, Lee HJ, Lee H, et al. Genotype Characterization of Group B Streptococcus Isolated from Infants with Invasive Diseases in South Korea. Pediatr Infect Dis J **2017**.
109. Li YP, Kuok CM, Lin SY, Hsieh WS, Shyu MK. Group B streptococcus antimicrobial resistance in neonates born to group B streptococcus-colonized mothers: Single-center survey. The journal of obstetrics and gynaecology research **2016**; 42(11): 1471-5.
110. Lomuto CQ, Marta; Nigri, Carolina; Fatur, Daniel; Messina, Analia; Santos, Norberto. [Approach to the epidemiologic situation in Argentina of the perinatal infection by beta hemolytic streptococcus of the B group (EGB)]. Rev Hosp Matern Infant Ramon Sarda **2006**; 25(1): 13-9.
111. Mendoza OM, Arias M, Mendoza L,. [Sepsis neonatal Group B Streptococcus and gram negative bacteria: prevalence and risk of meningitis]. Rev chil pediatr **2013**; 84(5).
112. Martinez MA, Ovalle A, Duran C, et al. [Serotypes and antimicrobial susceptibility of Streptococcus agalactiae]. Revista medica de Chile **2004**; 132(5): 549-55.
113. Poliquin V, Cohen E, Poliquin PG, Schneider C, Menticoglou S. Ongoing Cases of Early-Onset Group B Streptococcal Disease in the Era of Screening and Prophylaxis. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC **2016**; 38(10): 926-9.
114. Reinheimer C, Kempf VA, Wittekindt BE, et al. Group B streptococcus infections in neonates admitted to a German NICU: Emphasis on screening and adherence to pre-analytical recommendations. Early Hum Dev **2016**; 103: 37-41.
115. Zeng SJ, Tang XS, Zhao WL, Qiu HX, Wang H, Feng ZC. Clinical analysis of cases of neonatal Streptococcus agalactiae sepsis. Genetics and molecular research : GMR **2016**; 15(2).
116. Tapia IJ, Reichhard TC, Saldias RM, et al. [Neonatal sepsis in the era of antenatal antibiotic prophylaxis]. Revista chilena de infectologia : organo oficial de la Sociedad Chilena de Infectologia **2007**; 24(2): 111-6.
117. Kabwe M, Tembo J, Chilukutu L, et al. Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia. Pediatr Infect Dis J **2016**; 35(7): e191-8.
118. Akindolire AE, Tongo O, Dada-Adegbola H, Akinyinka O. Etiology of early onset septicemia among neonates at the University College Hospital, Ibadan, Nigeria. J Infect Dev Ctries **2016**; 10(12): 1338-44.
119. Ovalle A, Levancini M. Urinary tract infections in pregnancy. Curr Opin Urol **2001**; 11(1): 55-9.
120. Delgado Picado E; Saenz-Sanchez C; Calderon Zumiga A. [Rate of Colonization of Streptococcus Agalactiae in Pregnant Women and Neonates, Women's Hospital Dr Adolfo Carit Eva]. Rev costarric cienc méd **2004**; 25(1): 25-32.
121. Díaz-Álvarez M CD, Pérez-Amarillo J. [Infections due to Streptococcus agalactiae at an open neonatology service]. Rev Cubana Pediatr **2008**; 80(4).
122. Costa N; Carvalho M; Moura S; Júnior SC. [Beta-hemolytic streptococcus in pregnant women and their newborn infants: a critical analysis of the protocol used at Fernandes Figueira Institute, Oswaldo Cruz Foundation, in Brazil]. Rev paul pediatr **2010**; 28(2).
123. Frigati L, van der Merwe JL, Harvey J, Rabie H, Theron G, Cotton MF. A retrospective review of group B streptococcal infection in the Metro East area of the Western Cape province: 2010 to 2011. Southern African Journal of Infectious Diseases **2014**; 29(1): 33-6.
124. Angstetra D, Ferguson J, Giles WB. Institution of universal screening for Group B streptococcus (GBS) from a risk management protocol results in reduction of early-onset GBS disease in a tertiary obstetric unit. The Australian & New Zealand journal of obstetrics & gynaecology **2007**; 47(5): 378-82.

125. Ireland S, Larkins S, Kandasamy Y, Ireland S, Larkins S, Kandasamy Y. Group B streptococcal infection in the first 90 days of life in North Queensland. Australian & New Zealand Journal of Obstetrics & Gynaecology **2014**; 54(2): 146-51.
126. Daley AJ, Isaacs D. Ten-year study on the effect of intrapartum antibiotic prophylaxis on early onset group B streptococcal and Escherichia coli neonatal sepsis in Australasia. Pediatr Infect Dis J **2004**; 23(7): 630-4.
127. May M, Daley AJ, Donath S, Isaacs D. Early onset neonatal meningitis in Australia and New Zealand, 1992-2002. Arch Dis Child Fetal Neonatal Ed **2005**; 90(4): F324-7.
128. Meehan M, Cunney R, Cafferkey M, Meehan M, Cunney R, Cafferkey M. Molecular epidemiology of group B streptococci in Ireland reveals a diverse population with evidence of capsular switching. European Journal of Clinical Microbiology & Infectious Diseases **2014**; 33(7): 1155-62.
129. Berardi A, Lugli L, Baronciani D, et al. Group B streptococcal infections in a northern region of Italy. Pediatrics **2007**; 120(3): e487-93.
130. Berardi A, Lugli L, Rossi C, et al. Intrapartum antibiotic prophylaxis failure and group-B streptococcus early-onset disease. Journal of Maternal-Fetal & Neonatal Medicine **2011**; 24(10): 1221-4.
131. Berardi A, Lugli L, Rossi C, et al. Impact of perinatal practices for early-onset group B Streptococcal disease prevention. Pediatr Infect Dis J **2013**; 32(7): e265-71.
132. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. N Engl J Med **2005**; 352(1): 39-47.
133. Sigauque B, Roca A, Mandomando I, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J **2009**; 28(2): 108-13.
134. Eastwood KA, Craig S, Sidhu H, et al. Prevention of early-onset Group B Streptococcal disease - the Northern Ireland experience. BJOG: An International Journal of Obstetrics & Gynaecology **2015**; 122(3): 361-7.
135. Madhi SA, Radebe K, Crewe-Brown H, et al. High burden of invasive Streptococcus agalactiae disease in South African infants. Ann Trop Paediatr **2003**; 23(1): 15-23.
136. Cutland CL, Madhi SA, Zell ER, et al. Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial. Lancet **2009**; 374(9705): 1909-16.
137. Cutland CL, Schrag SJ, Zell ER, et al. Maternal HIV infection and vertical transmission of pathogenic bacteria. Pediatrics **2012**; 130(3): e581-90.
138. Dangor Z, Lala SG, Cutland CL, et al. Burden of invasive group B Streptococcus disease and early neurological sequelae in South African infants. PLoS ONE **2015**; 10 (4) (no pagination)(e0123014).
139. Schrag SJ, Cutland CL, Zell ER, et al. Risk factors for neonatal sepsis and perinatal death among infants enrolled in the prevention of perinatal sepsis trial, Soweto, South Africa. Pediatric Infectious Disease Journal **2012**; 31 (8): 821-6.
140. Andreu A, Sanfeliu I, Vinas L, et al. [Decreasing incidence of perinatal group B streptococcal disease (Barcelona 1994-2002). Relation with hospital prevention policies]. Enferm Infecc Microbiol Clin **2003**; 21(4): 174-9.
141. Lopez Sastre JB, Fernandez Colomer B, Coto Cotallo GD, Ramos Aparicio A. Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatr **2005**; 94(4): 451-7.
142. Martins ER, Andreu A, Correia P, et al. Group B streptococci causing neonatal infections in barcelona are a stable clonal population: 18-year surveillance. Journal of Clinical Microbiology **2011**; 49(8): 2911-8.
143. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. Arch Dis Child Fetal Neonatal Ed **2010**; 96(1): F9-F14.
144. Lamagni TL, Keshishian C, Efstratiou A, et al. Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991-2010. Clinical Infectious Diseases **2013**; 57(5): 682-8.
145. Weisner AM, Johnson AP, Lamagni TL, et al. Characterization of group B streptococci recovered from infants with invasive disease in England and Wales. Clin Infect Dis **2004**; 38(9): 1203-8.
146. Meehan M, Cafferkey M, Corcoran S, et al. Real-time polymerase chain reaction and culture in the diagnosis of invasive group B streptococcal disease in infants: a retrospective study. European Journal of Clinical Microbiology and Infectious Diseases **2015**; 34 (12): 2413-20.
147. Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. BMJ (Clinical research ed) **2002**; 325(7359): 308.
148. Okike IO, Johnson AP, Henderson KL, et al. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clinical Infectious Diseases **2014**; 59(10): e150-7.
149. Heath PT, Balfour G, Weisner AM, et al. Group B streptococcal disease in UK and Irish infants younger than 90 days. Lancet **2004**; 363(9405): 292-4.
150. Early-onset and late-onset neonatal group B streptococcal disease--United States, 1996-2004. MMWR Morb Mortal Wkly Rep **2005**; 54(47): 1205-8.

151. Castrodale L, Gessner B, Hammitt L, Chimonas MA, Hennessy T. Invasive early-onset neonatal group B streptococcal cases--Alaska, 2000-2004. Matern Child Health J **2007**; 11(1): 91-5.
152. Chen KT, Puopolo KM, Eichenwald EC, Onderdonk AB, Lieberman E. No increase in rates of early-onset neonatal sepsis by antibiotic-resistant group B Streptococcus in the era of intrapartum antibiotic prophylaxis. Am J Obstet Gynecol **2005**; 192(4): 1167-71.
153. Cordero L, Rau R, Taylor D, Ayers LW. Enteric gram-negative bacilli bloodstream infections: 17 years' experience in a neonatal intensive care unit. Am J Infect Control **2004**; 32(4): 189-95.
154. Hyde TB, Hilger TM, Reingold A, Farley MM, O'Brien KL, Schuchat A. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. Pediatrics **2002**; 110(4): 690-5.
155. Mayor-Lynn K, Gonzalez-Quintero VH, O'Sullivan MJ, Hartstein AI, Roger S, Tamayo M. Comparison of early-onset neonatal sepsis caused by Escherichia coli and group B Streptococcus. Am J Obstet Gynecol **2005**; 192(5): 1437-9.
156. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. JAMA **2008**; 299(17): 2056-65.
157. Puopolo KM, Madoff LC, Eichenwald EC. Early-onset group B streptococcal disease in the era of maternal screening. Pediatrics **2005**; 115(5): 1240-6.
158. Perinatal group B streptococcal disease after universal screening recommendations--United States, 2003-2005. MMWR Morb Mortal Wkly Rep **2007**; 56(28): 701-5.
159. Stoll BJ, Hansen N, Fanaroff AA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med **2002**; 347(4): 240-7.
160. Jordan HT, Farley MM, Craig A, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. Pediatr Infect Dis J **2008**; 27(12): 1057-64.
161. Trends in perinatal group B streptococcal disease - United States, 2000-2006. MMWR Morb Mortal Wkly Rep **2009**; 58(5): 109-12.
162. Ecker KL, Donohue PK, Kim KS, Shepard JA, Aucott SW. The impact of group B Streptococcus prophylaxis on early onset neonatal infections. Journal of Neonatal-Perinatal Medicine **2013**; 6(1): 37-44.
163. Greenhow TL, Hung YY, Herz AM, Greenhow TL, Hung Y-Y, Herz AM. Changing epidemiology of bacteremia in infants aged 1 week to 3 months. Pediatrics **2012**; 129(3): e590-6.
164. Greenhow TL, Hung YY, Herz AM, et al. The changing epidemiology of serious bacterial infections in young infants. Pediatric Infectious Disease Journal **2014**; 33(6): 595-9.
165. Mukhopadhyay S, Eichenwald EC, Puopolo KM. Neonatal early-onset sepsis evaluations among well-appearing infants: projected impact of changes in CDC GBS guidelines. Journal of Perinatology **2013**; 33(3): 198-205.
166. Mukhopadhyay S, Dukhovny D, Mao W, et al. 2010 perinatal GBS prevention guideline and resource utilization. Pediatrics **2014**; 133(2): 196-203.
167. Tudela CM, Stewart RD, Roberts SW, et al. Intrapartum evidence of early-onset group B streptococcus. Obstetrics & Gynecology **2012**; 119(3): 626-9.
168. Weston EJ, Pondo T, Lewis MM, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008. Pediatric Infectious Disease Journal **2011**; 30(11): 937-41.
169. Stafford IA, Stewart RD, Sheffield JS, et al. Efficacy of maternal and neonatal chemoprophylaxis for early-onset group B streptococcal disease. Obstetrics and gynecology **2012**; 120 (1): 123-9.
170. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early Onset Neonatal Sepsis: The Burden of Group B Streptococcal and E. coli Disease Continues. Pediatrics **2011**; 127(5): 817-26.
171. Parente V, Clark RH, Ku L, et al. Risk factors for group B streptococcal disease in neonates of mothers with negative antenatal testing. Journal of perinatology : official journal of the California Perinatal Association **2017**; 37(2): 157-61.
172. Wortham JM, Hansen NI, Schrag SJ, et al. Chorioamnionitis and culture-confirmed, early-onset neonatal infections. Pediatrics **2016**; 137 (1) (no pagination)(e20152323).

ARTICLE 3

Maternal carriage of Group B streptococcus and Escherichia coli in a district hospital in Mozambique

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KEYWORDS

Group B streptococcus, *Escherichia coli*, Recto-vaginal colonization, maternal colonization, maternal risk factors.

Abbreviated title

Maternal colonization by *GBS* and *E. coli in southern Mozambique*

Running head title

Maternal colonization by *GBS* and *E. coli*

FINANCIAL DISCLOSURE

Quique Bassat had during the duration of the study a fellowship from the program Miguel Servet of the ISCIII (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269). Lola Madrid had a fellowship from the program Río Hortega of the ISCIII (CM13/00260) and Sara M. Soto has a fellowship from the program I3, of the ISCIII while the study was conducted. SM, AV, ES, SM, AC, RV, AN, NM, RA, KM, CM, EM and CM have nothing to declare.

ABSTRACT

Background: In low-income countries, data on prevalence and effects of *Group B streptococcus (GBS)* and *Escherichia coli* (*E. coli*) colonization among pregnant women are scarce, but necessary to formulate prevention strategies. We assessed prevalence of *GBS* and *E. coli* colonization and factors associated among pregnant women, its effect in newborns and acceptability regarding the utilized sampling methods in a semirural Mozambican hospital.

Methods: Pregnant women were recruited from June 2014 to January 2015, during routine antenatal clinics at gestational age ≥34 weeks (n=200); or upon delivery (n=120). Maternal risk factors were collected. Vaginal and vagino-rectal samples for *GBS* and *E. coli* determination were obtained and characterized in terms of antimicrobial resistance and serotype. Anti-GBS antibodies were also determined. Neonatal follow-up was performed in the first three months after birth. Semi-structured interviews were performed to investigate acceptability of sample collection methods.

Results: 21.3% of women recruited were *GBS* carriers, while 16.3% were positive for *E. coli*. Prevalence of HIV was 36.6%. No association was found between being colonized by *GBS* and *E. coli* and maternal risk factors. *GBS* isolates were fully susceptible to penicillin and ampicillin. Serotypes V (32.4%), Ia (14.7%) and III (10.3%) were the most commonly found and 69.2% of the women tested had immunoglobulin G antibodies against *GBS*. *E. coli* isolates showed resistance to ampicillin in 28.9% and co-trimoxazol in 61.3% of the cases.

Conclusion: Prevalence of *GBS* and/or *E. coli* colonization among pregnant women is high in this semirural community and comparable to those reported in similar settings. Four serotypes accounted for nearly 70% of all isolates of *GBS*. Population based data on infant *GBS* infections would enable the design of prevention strategies for *GBS* disease in Mozambique.

BACKGROUND

In 2016, 5.6 million children under the age of five died with nearly half of those deaths occurring in the first 28 days of life, the so-called neonatal period[1]. Neonatal deaths are disproportionately distributed across the globe, with 95% of them taking place in developing regions and infections remain a major contributor to this preventable mortality[2, 3].

Vertical transmission of bacteria that are normal commensal flora or

pathogens of the maternal genitourinary and gastrointestinal tracts, such as *Group B streptococcus* (GBS) or *Escherichia coli* (*E. coli*) are leading determinants of neonatal morbidity and mortality, causing invasive bacterial infections that can manifest as sepsis, pneumonia and meningitis[4, 5]. *GBS* and *E. coli* are particularly associated with early-onset neonatal disease (EOD, 0-6 days after birth[6]), but can also cause late-onset disease (LOD, 7-89 days[7]), preterm birth and very-low-birth-weight[8, 9], all of which are responsible for substantial morbidity and mortality in sub-Saharan Africa (SSA)[2, 10, 11].The estimated incidence of *GBS* neonatal disease in SSA countries suggests a burden at least comparable to that found in high-income countries (HIC) before the implementation of the preventive strategies[12].

Maternal *GBS* carriage during the period closely related to the delivery has consistently been demonstrated to determine the risk of vertical transmission, and thus of ensuing neonatal disease. Prevalence of maternal colonization varies from 6.5 to 36%[13] in Europe and has been reported higher than 20% in Sub-Saharan countries, although precise regional maternal carriage data for this continent are scarce[12, 14, 15].Maternal risk factors associated with higher prevalence of *GBS* colonization are controversial. Both younger[16] and older maternal ages[17] have been reported as maternal characteristics associated with higher risk of *GBS* colonization, as well as higher education[17], higher income[18], and high sexual activity[17]. The relation between HIV infection and risk for *GBS* maternal colonization has yet to be fully elucidated. Studies conducted in the United States[19] or in Zimbabwe[15] did not find an increased risk among HIV infected individuals, whereas researchers from South Africa[20] found a lower colonization prevalence among HIV-infected mothers. Vertical transmission of *GBS* may significantly increase (up to a 64% higher) among HIV-exposed infants compared with non-HIV exposed ones[12].

The primary intervention to reduce *GBS*-associated EOD involves the administration of intrapartum antibiotic prophylaxis (IAP) to women identified to either 1) be *GBS* carriers through microbiological screening (35-37 weeks' gestation)[21] of samples obtained from their genito-urinary or gastrointestinal lower tract; or 2) fulfil any of the different risk factors associated with neonatal disease[22-24]. In HIC, the widespread implementation of the IAP strategy has significantly reduced *GBS* EOD among those babies born to women in whom it was correctly applied. The IAP strategy has however not demonstrated any impact on *GBS*-associated LOD, or in the

prevention of *E. coli* neonatal disease of any kind[12, 13]. In low and lower-middle income countries (LIC and LMIC), the fragility of the health systems and the generalized lack of microbiology facilities, in the absence of a reliable rapid point of care test for *GBS*, hinders the applicability of the IAP strategy, therefore jeopardising the prevention of life-threatening *GBS* neonatal infections[12].

Despite SSA having the highest incidence of neonatal sepsis worldwide[12], epidemiological data on *GBS* and *E. coli* maternal colonization in this continent are scarce. In Mozambique, as a paradigmatic example, a Pubmed search only provides five results from studies reporting *GBS* data[12, 25-28], and only two of those related to maternal colonization, describing a prevalence of colonization as low as 1.8%[25] or even lower (1%)[27], difficult to contextualize among much higher prevalence data from neighboring sub-Saharan African countries[12]. Additionally, and to our knowledge, no articles reporting *E. coli* colonization prevalence in pregnant women in Mozambique have been published and only one multicenter study conducted in South Africa, Kenya and Rwanda have determined simultaneously the vaginal *GBS* and *E. coli* carriage rates in SSA[29, 30]. Such data, however, appear necessary for a better and more evidence-based design of preventive strategies, based on the resources and infrastructures available.

This study aimed to determine the prevalence of pregnant women colonized by *GBS* and *E. coli* attending a semi-rural Mozambican hospital, analyze risk factors associated to higher risk of carriage by these pathogens and characterize the isolates in terms of antimicrobial resistance and serotype distribution. As secondary objectives, we determined the neonatal outcomes and assessed the feasibility and acceptability of collecting vaginal and vagino-rectal samples among pregnant women, with the idea of generating locally-relevant data useful to guide national preventive strategies and policies to reduce transmission and the toll of such potentially life-threatening infections in the newborn.

METHODS

Study site

The study was conducted in Manhiça, a semi-rural site in Southern Mozambique. The Manhiça Health Research Center (CISM) runs a Demographic Surveillance System (DSS) in the area and a morbidity surveillance system (MSS) at the Manhiça District Hospital (MDH), across

the street. A detailed description of MDH, CISM and the study area can be found elsewhere[31]. MDH is the referral hospital for the Manhiça district, covering a population of circa 183,000 inhabitants. The MDH includes adult and paediatric wards, together with a maternity, where between 3500-4000 deliveries take place annually. Institutional delivery rates are around 85-90% in the study area. MDH also includes an outpatient department and an antenatal care (ANC) clinic where pregnant women are routinely followed. As part of the National policy, all pregnant women are invited to attend antenatal consultations during their pregnancy, where HIV testing and other screening of infections and conditions are routinely offered, in addition to intermittent preventive treatment during pregnancy (IPTp) for malaria prevention, a disease highly endemic in the area. Manhiça district has one of the highest prevalence rates of HIV in the world, with HIV prevalence during pregnancy having been estimated at around 29% during antenatal consultations[32]. No strategy to prevent neonatal sepsis is currently implemented in Mozambique. The hospital has recently introduced WHO-recommended Option B+ for the prevention of mother-to-child HIV transmission, which is offered to mothers free of charge.

Study design and population

This observational prospective study was conducted at the ANC and delivery wards of MDH, between June 15 2014 and January 15 2015, running continuously during working hours (8:00–16:00) and working days. We recruited pregnant women at two different time-points during their pregnancy. One group during routine antenatal care with a minimum estimated gestational age ≥34 weeks, as measured by fundal height >=32cm, 2 cm above the midpoint between umbilicus and xiphoyd process. A second group of women was recruited upon delivery (regardless of gestational age) if they were not recruited at ANC clinics, in order to understand real life risk for vertical transmission rate of *GBS* or *E.coli* to their offspring with no interference of antibiotic treatments. Participants were eligible for inclusion if they lived in the study area, were in good physical and mental health, able and willing to participate in the study and to provide informed consent. All women fulfilling inclusion criteria were eligible to participate in the study, and in order to obtain a more representative sample of the study population, the first two women seen every day were approached for recruitment.

Definitions

GBS colonization was considered in the event of a positive vaginal or vagino-rectal culture for *GBS*. *E. coli* colonization was considered when the positive vaginal culture grew *E. coli*. *E. coli* urinary tract infection was diagnosed when *E. coli* grew (>105 colony-forming units/mL) in the urine samples of pregnant women. Abortion was defined as pregnancy termination prior to 20 weeks' gestation or a foetus born weighting less than 500 g[33]. A preterm baby was defined as that with a gestational age at birth <37 weeks and stillbirth as intrauterine deaths occurring after 28 weeks of gestational age. Low-birth weight was defined as weight at birth <2,500 grams.

Study procedures

Sampling procedures

Microbiological swab samples were obtained from each participant (ANC or upon admission in labour at the delivery wards, but always prior to delivery) without the use of antiseptic solution or a speculum. A sample from the lower third of the vagina and a fresh urine sample were taken for *E. coli* determination. For *GBS* determination, samples included a lower vaginal swab (vaginal sample), and a single swab for the vagino-rectal sample, consisting on a sample of the vagina first and then the rectum obtained performing a brief rotation of the swab through the outer sphincter. Both kinds of swabs were collected in all women in order to compare the prevalence of *GBS* colonization detected by the two samples. Swabs were immediately placed in Amies transport medium and sent to the laboratory within 24 hours. The vaginal and vagino-rectal samples for *GBS* determination were inoculated directly onto Granada medium (Group B Streptococcus Differential Agar, Becton Dickinson, Erembodegem, Belgium) incubated anaerobically at 37ºC for 24 hours. Vaginal samples for E. coli determination were spread onto MacConkey agar and urine samples were inoculated onto agar Cysteine lactose electrolyte deficient (CLED) and MacConkey agar and incubated at 37ºC overnight without C02. *E. coli* isolates were identified based on colony appearance, Gram stain, latex agglutination with the Pastorex Strepto kit (Bio-rad Laboratories®, Marnes-la-Coquette, France) and standard biochemical tests for E. coli determination. Both, *GBS* and *E. coli* isolates were confirmed by MALDI-TOF. Resistance profiles were determined via Kirby-Bauer disk diffusion method following the Clinical & Laboratory Standards Institute (CSLI) guidelines.

Determination of the *GBS* capsular type or serotype implied the utilization of a multiplex-PCR using a set of primers described previously[34]. DNA of each isolate was obtained using the High Pure PCR Template Preparation kit (Roche, Spain). Briefly, this procedure consisted in performing three PCR reactions using specific primers for 10 different serotypes. Reaction 1 detects [Ia, Ib, II, III and IV], reaction 2 [V, VI, VII, VIII and IX] and reaction 3 is the amplification control. PCR conditions involved an initial step of 95ºC for 3 min, followed by 30 cycles of 95º C for 1 min, 57º C for 1 min and 72º C for 2 min, and a final step of 72º C for 10 min The PCR products were visualized by electrophoresis using 1% agarose gels. Antibody (AB) determination was identified in blood samples of mothers recruited at delivery. They were performed by ELISA using whole bacteria as antigens. This procedure is a modification based on the protocol proposed by Baker et al[33], using an optical density to 450 nm with a correction at 620 nm. The cut-off value for positivity was chosen to be ≥ 1 OD units, in order to be more strict than the one proposed by Baker (>0.125).
Maternal HIV infection status was determined and recorded if not previously known. Other screening tests routinely performed at ANC, such as syphilis or hemoglobin determination were also performed and recorded.

Communication of results to mothers and case management
Clinical assessment and management of patients was done following international guidelines for countries with no clear screen-and-treat national rules, both at the ANC and at the maternity. For those women identified as carriers of *GBS* in vagino-rectal swabs collected at the ANC, a field worker delivered to the mother at home a study card detailing the microbiological findings, together with indications of what to do during delivery, so that intrapartum antibiotics could be administered to the mother, following the CDC guidelines[35]. Urinary tract infections secondary to *E. coli* were also reported and treated according to national guidelines for pregnant women. All recruited women were encouraged to deliver at hospital and clinical staff was trained to identify them. Any child born to a recruited mother and found to be sick at delivery was assessed by a study clinician, and routine screening for bacterial surveillance (including a blood culture and a lumbar puncture to obtain cerebrospinal fluid (CSF) performed and clinical management organized according to MDH guidelines.
Although the aim of this study was not to assess the efficacy of IAP (already known), due to ethical considerations, IAP was started in GBS infected women upon labour initiation, according to CDC guidelines[35]. In

cases where IAP could not be adequately performed, we followed Spanish recommendations and prophylactic antibiotic treatment (50,000 IU of intramuscular penicillin as a single dose for a newborn weighting >2000 grams, or 25.000 IU if weight <2000 grams) was administered within the first hour after birth to the newborns of mothers with confirmed GBS colonization[36]. Such children were observed at hospital for a minimum of 24h. For women recruited at delivery, culture results were not available until at least 24-48h after recruitment. In such cases (and also in cases of women with pending *GBS* result recruited prior to delivery) we kept the newborns under observation for a minimum of 24 hours after delivery, and provided clear recommendations to mothers regarding the need for a follow up visit should the newborn become sick in the first weeks of life. Babies born to study participant mothers were followed-up during the first three months after birth.

Assessment of the acceptability of vaginal and vagino-rectal sampling

The study included a simple socio-behavioural component to evaluate the acceptability of collecting samples (vaginal and vagino-rectal swabs) during pregnancy. "Non-participant observations" were conducted, whereby a member of the study team observed the procedures being conducted (excluding genital examinations), and complemented by semi-structured interviews to a small sample of pregnant women not participating in the study but contemporaneously attending the ANC. Finally, semi-structured interviews were conducted among some participants who had accepted to provide vaginal and rectal samples. Questions, themes and probes arising from the non-participant observation, other than stated in the interview guide, were included in the semi-structured interviews.

Statistical analysis

All data were prospectively collected using standardized questionnaires, which were double entered in specific study databases, created using Openclinica© software. Discrepancies were solved after comparison with the original source documents by a senior data clerk, and in close collaboration with the study clinicians. Statistical analyses were performed using StataCorp. 2015. *Stata Statistical Software: Release 14* (College Station, TX: StataCorp LP). Study variables were counted and summarized in frequency tables. Qualitative variables were compared using a Chi-squared test or Fisher's exact test. Continuous variables were described as mean (standard deviation, SD) or median values (interquartile range, IQR) and were compared using

the test for normal distributions or the Mann Whitney test for skewed data. Logistic regression univariate and multivariate analyses were performed to identify risk factors for GBS or E. coli colonization, separately. Variables that were found to be significantly associated with GBS or E. coli in the univariate analysis together with those related at a significance level of p<0.10 were entered into a multivariate model. Age and gestational age at recruitment were also included in the multivariate analysis based on previous studies[9, 15-17]. A separate univariate and multivariate analysis of risk factor associated to *GBS* or *E. coli* colonization among HIV pregnant women was also performed.

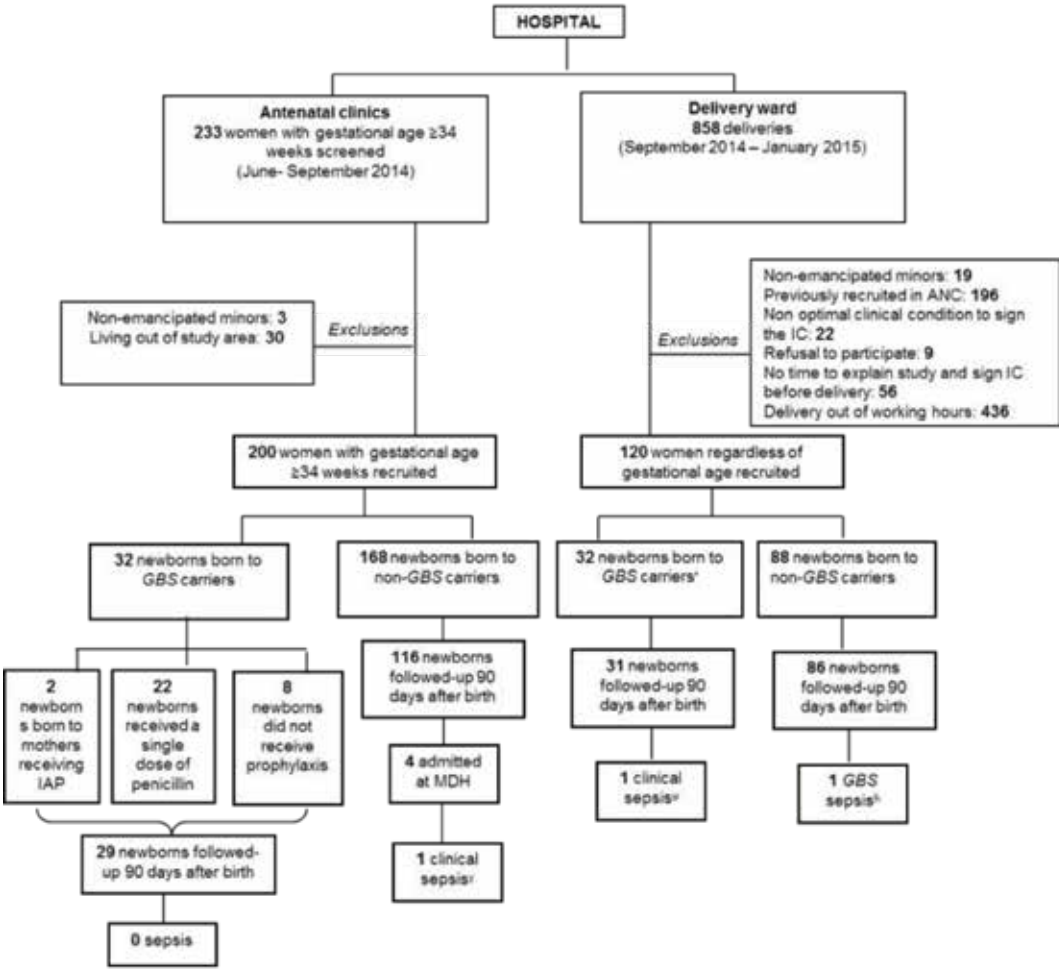
Ethical considerations

This protocol and all supporting documentation (Informed consent documents, Study questionnaires) were approved by the local bioethics committee of CISM (Comité Institucional de Bioética para Saúde do CISM (CIBS-CISM), and by the National Bioethics Committee of Maputo (CNBS) in Mozambique; and by the Ethics Committee of the Hospital Clínic in Barcelona, Spain. Written information and consent forms in the local language were provided to the women. After the interview, participants were asked to express their willingness to participate in the study by signing (or thumb-printing in case they were illiterate) the consent form. Participation in this study was voluntary, and study-related procedures did not interfere with the pregnant women's or children's standard clinical care.

RESULTS

Between June 15 2014 and January 15 2015, 320 pregnant women were recruited at MDH (Study profile in figure 1). Table 1 summarizes the socio-demographic and clinical characteristics of participants. Median age of recruited women was 24 years (Interquartile range, IQR 20-31), with no significant differences according to recruitment place. No major differences could be found in relation with recruitment site, with the exception of a higher frequency of higher education among women recruited upon delivery compared with those recruited at ANC (7.0% vs. 26.7%, p<0.001). More than one third of women (117/320, 36.6%) were HIV positive.

Figure 1. Study profile.



ANC: antenatal clinics; IC: informed consent; IAP: intrapartum antibiotic prophylaxis. MDH: Manhiça district hospital. [x]Microbiologically not confirmed. [i]None received any kind of prophylaxis; [y]neonate died before taking samples.[h] *GBS* sepsis without meningitis developed in the first 24h of life.

Table 1. Socio-demographic and clinical characteristics of all pregnant women recruited at antenatal clinics or directly upon delivery.

	Overall n=320 n (%)	Antenatal clinics recruitment n=200 n (%)	Delivery recruitment n=120 n (%)	p value[δ]
Socio-demographic characteristics				
Age in years				**0.040**
< 21	112 (35.0)	61 (30.5)	51 (42.5)	
22 to 29	110 (34.4)	69 (34.5)	41 (34.2)	
≥30	98 (30.6)	70 (35.0)	28 (23.3)	
Seconday or tertiary education	46 (14.4)	14 (7.0)	32 (26.7)	**<0.001**
Employment	17(5.3)	11 (5.5)	6 (5.0)	0.85
Obstetric History				
Age of first pregnancy (mean±SD)	18.6 (±2.6)	18.6 (±2.9)	18.7 (±2.7)	0.67
Gravidity (mean±SD)	2.8 (±1.8)	2.9 (±1.7)	2.7 (±1.9)	0.43
Previous abortion	24 (7.5)	12 (6.0)	12 (10.0)	0.19
History of current pregnancy				
Gestational age in weeks at recruitment (mean±SD)	37.1 (±2.0)	36.0 (±1.2)	38.9 (±1.6)	**<0.001**
At least 3 antenatal visits during the pregnancy	165 (51.6)	95 (47.5)	70 (58.3)	**0.007**
Gestational hypertension	23 (7.2)	16 (8.0)	7 (5.8)	0.51
Vaginal itching	21 (6.6)	21 (10.5)	0 (0)	**<0.001**
Vaginal discharge	125 (39.1)	123 (61.5)	2 (1.7)	**<0.001**
Urinary symptoms	3 (0.9)	3 (1.5)	0 (0.0)	0.18
Antibiotic usage[ι]	13 (4.0)	10 (5.0)	3 (2.5)	0.10
Investigations during pregnancy				
Syphilis positive	2 (0.6)	1 (0.5)	1 (0.5)	0.70
HIV positive	117 (36.6)	79 (39.5)	38 (31.7)	0.16
HIV positive on HAART[ϕ]	111 (94.9)	77 (97.5)	34 (89.5)	**0.024**
Anemia (<11g/dL)[h]	206 (64.4)	152 (76.0)	54 (45.0)	**0.047**
Neonatal Outcome				
Gestational age at birth				
Term newborn	290 (90.6)	177 (88.5)	113 (94.2)	0.09
Pre term newborn	30 (7.8)	23 (9.5)	7 (5.0)	
Stillbirth	5 (1.6)	4 (2.0)	1 (0.8)	0.26
Low birth weight (<2500g)	31 (9.7)	9 (4.5)	22 (18.3)	**<0.001**
Death after birth[m]	7 (2.7)	5 (3.6)	2 (1.7)	**<0.001**

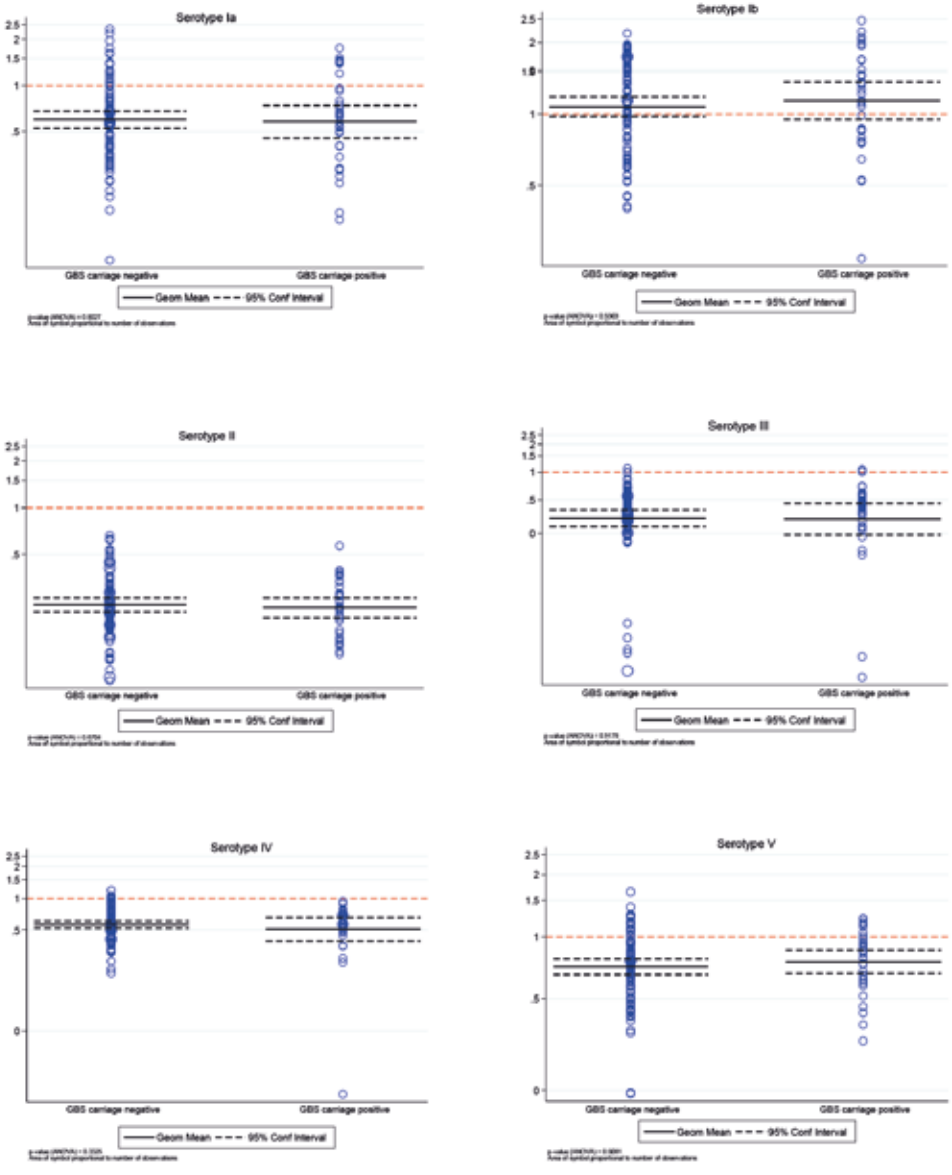
NA: not applicable; [ϕ]HAART: highly active antiretroviral therapy; [δ]P-value was derived from Chi² test for categorical variables and t-test for quantitative variables.[ι]Antibiotic usage two weeks before sample collection. Data available for 259 women. [m]Based on data for 262 newborns followed-up 90 days after birth.

Prevalence of GBS and E. coli colonization among pregnant women
More than a fifth (68/320; 21.3%) of all recruited women were colonized by *GBS*, detected in both samples in 33 women, in 15 in the vaginal one only, and in 20 in the vagino-rectal one only. A non-statistically significant higher proportion of *GBS* were isolated from the vagino-rectal sample (16.6%) as compared to the vagina (15.0%, p=0.81). Prevalence of *GBS* colonization was borderline significantly higher among women recruited upon delivery compared to those recruited at ANC (32/120 (26.7%) vs. 36/200 (18.0%), p=0.07). Fifty-two women had E. coli vaginal colonization (16.3%), being significantly more common among women recruited at delivery (22.5% vs. 12.5%, p=0.019) and 10/320 (3.1%) had a positive E. coli urine culture. Among HIV positive pregnant women recruited, *GBS* colonization was found in 26/117 (22.2%). *E. coli* vaginal colonization was determined in 18/117 (15.4%) HIV-positive women.

Anti-group B streptococcus antibodies
Antibodies against *GBS* were detected in 83/120 (69.1%) women recruited at delivery. Of them, 23/32 (71.9%) were among GBS colonized mothers and 60/88 (68.2%) among non-colonized women (figure 2). Among HIV positive participants, AB anti-GBS were detected in 25/38 (65.8%) of those tested. Forty women had AB against more than one GBS serotype, being the most frequent AB against serotype Ia (24/120, 20%), against serotype Ib (76/120, 63.3%) and against serotype V (27/120, 22.5%). Correlating presence of antibodies to the homotypic GBS serotype, 2/4 (50%) women colonized by serotype Ia had AB against it, 6/6 (100%) for serotype Ib, 0/3 (0%) for serotype III and 7/14 (50%) of those carriers of Ib serotype had AB against their homotypic serotype.

Risk factors associated to GBS and E. coli carriage
Table 2 presents the univariate analyses of associations of the different socio-demographics, clinical and laboratory variables with vaginal *GBS* and *E. coli* carriage. In the final multivariate *GBS* model (Table 3), no risk factors were significantly associated with *GBS* carriage. Similarly, no risk factors appeared to be independently associated with maternal vaginal *E. coli* carriage (table 4). The univariate and multivariate analyses performed to identify risk factors of GBS or E. coli colonization but restricted to HIV-infected women showed no differences compared to those including all women (data not shown).

Figure 2. Bubble plot demonstrating antibodies against GBS serotype Ia, Ib, II, III, IV and V in blood samples from women recruited at delivery (n=120).



OD: optical density to 450 nm with a correction at 620 nm. Cut-off value for positivity was ≥ 1 OD units. GBS: *Group B streptococcus.* Area of bubbles is proportional to the number of observations overlapping on the same coordinates.

Table 2. Univariate analysis of socio-demographic and clinical variables among women colonized by *GBS or E. coli*

	GBS colonized n= 68, n (%)	GBS uncolonized n=252, n (%)	Crude OR (95%CI)	p value[δ]	*E. coli* colonized n= 52, n (%)	*E. coli* uncolonized n=268, n (%)	Crude OR (95%CI)	p value[δ]
Socio-demographic characteristics								
Age in years				0.70				0.70
< 21	21 (30.9)	91 (36.1)	1.00		18 (34.6)	94 (35.1)	1.00	
22 to 29	24 (35.3)	86 (34.1)	1.21 (0.6 - 2.3)		18 (34.6)	92 (34.3)	1.02 (0.5- 2.1)	
≥30	23 (33.8)	75 (29.8)	1.33 (0.7 - 2.6)		16 (30.8)	82 (30.6)	1.02 (0.5 - 2.1)	
Seconday or tertiary education	10 (14.7)	36 (14.3)	1.03 (0.5-2.2)	0.93	12 (23.1)	34 (12.7)	2.06 (1.0-4.3)	**0.05**
Employment	5 (7.3)	12 (4.8)	1.59 (0.5-4.7)	0.40	2 (3.9)	15 (5.6)	0.67 (0.1-3.1)	0.61
History of current pregnancy								
Place of recruitment								
Antenatal clinic	36 (52.9)	164 (65.1)	1.00	0.07	25 (48.1)	175 (65.3)	1.00	**0.02**
At delivery	32 (47.1)	88 (34.9)	1.66 (0.9 - 2.9)		27 (51.9)	93 (34.7)	2.03 (1.1-2.7)	
Gestational age at recruitment (weeks) (mean±SD)[γ]	37.3 (±2.2)	37.0 (±1.9)	1.08 (0.9-1.2)	0.38	37.3 (±0.3)	37.0 (±0.1)	1.07 (0.9-1.2)	0.36
At least 3 antenatal visits during the pregnancy	37 (54.4)	128 (50.8)	1.39 (0.8-2.5)	0.29	29 (64.4)	136 (57.6)	1.33 (0.7-2.6)	0.40
Gestational hypertension	4 (5.9)	19 (7.5)	0.75 (0.2-2.3)	0.26	4 (8.0)	19 (7.2)	1.13 (0.4-3.5)	0.84
Vaginal itching	2 (2.9)	19 (7.5)	0.37 (0.1 – 1.6)	0.17	4 (7.7)	17 (6.3)	1.23 (0.4 – 3.8)	0.13
Vaginal discharge	22 (32.4)	103 (40.9)	0.69 (0.4-1.2)	0.20	14 (26.9)	111 (41.4)	0.52 (0.3-1.0)	**0.05**
Urinary symptoms	0 (0.0)	3 (1.2)	0 (0.0)	0.37	1 (1.9)	2 (0.8)	2.61 (0.2-29.5)	0.42
Antibiotic usage[ι]	3 (4.4)	10 (4.0)	1.07 (0.3 -4.0)	0.92	2 (3.8)	11 (4.1)	0.78 (0.2-2.9)	0.69
Investigations during pregnancy								
Syphilis positive	0 (0.0)	2 (0.8)	0 (0.0)	0.46	0 (0.0)	2 (0.8)	0 (0.0)	0.53
HIV positive	26 (38.2)	91 (36.1)	1.09 (0.6-1.9)	0.75	18 (34.6)	99 (36.9)	0.90 (0.5-1.7)	0.75
Anemia[λ]	38 (55.9)	168 (66.7)	0.65 (0.3-1.3)	0.21	25 (64.1)	181 (80.4)	0.43 (0.2-0.9)	**0.02**
Antibodies anti-GBS[θ]	23 (71.9)	60 (68.2)	1.19 (0.5 - 2.9)	0.70	19 (70.4)	64 (68.8)	1.08 (0.4 - 2.8)	0.89
GBS colonization	NA	NA	NA		13 (25)	55 (20.5)	1.29 (0.6-2.6)	0.47
***E. coli* colonization**	13 (19.1)	39 (15.5)	1.29 (0.6-2.6)	0.52	NA	NA	NA	
Outcome								
Gestational age at birth								
Term newborn	62 (91.2)	228 (90.5)	1.00	0.86	46 (88.5)	244 (91.0)	1.00	0.56
Pre term newborn	6 (8.8)	24 (9.5)	0.92 (0.4-2.3)		6 (11.5)	24 (9.0)	1.33 (0.5-3.4)	
Stillbirth	1 (1.4)	4 (1.6)	0.86 (0.1-7.9)	0.89	0 (0.0)	5 (2.4)	0 (0.0)	0.28
Low birth weight (<2500g)	4 (5.9)	27 (10.7)	0.52 (0.2-1.5)	0.24	7 (13.5)	24 (9.0)	1.58 (0.6-3.9)	0.31
Infant hospitalized in the first 90 days after birth[ω]	2 (3.4)	6 (3.0)	1.15 (0.2-5.9)	0.86	3 (6.1)	5 (2.4)	2.7 (0.6-11.9)	0.17
Death after birth[ω]	1 (1.4)	6 (2.4)	0.57 (0.1-4.8)	0.60	0 (0.0)	7 (3.3)	0 (0.0)	0.19

[γ]Gestational age is presented as mean and SD. [δ]P-value was derived from Chi2 test for categorical variables and t-test for quantitative variables.[λ]Data available for 260 women. [ι]Antibiotic usage two weeks before sample collection. Data available for 259 women. [ω]Data available for 264 women. [θ]Antibodies results available for 120 women recruited at delivery; [ω]data for 262 newborns 90 days after birth.

Table 3. Multivariate analysis of socio-demographic and clinical variables of women colonized by *GBS.*

Risk factors for *GBS* colonization	*GBS* positive n (%), N=68	Adjusted OR	95% CI		p-value[θ]
			Lower	Upper	
Age in years					
< 21	21 (30.9)	1.00			
22 to 29	24 (35.3)	1.26	0.65	2.56	0.69
≥30	23 (33.8)	1.46	0.74	2.89	
Gestational age at recruitment (mean ±SD)	37.3 (±2.2)	0.98	0.81	1.19	0.82
Place of recruitment					
Antenatal clinics	200 (62.5)	1.00			
Delivery ward	120 (37.5)	1.85	0.84	4.08	0.125

[θ]P-value was derived from likelihood ratio test.

Table 4. Multivariate analysis of socio-demographic and clinical variables of women colonized by *E. coli.*

Risk factors for *E.coli* colonization	*E. coli*, n (%), N=52	Adjusted OR	95% CI		p-value[θ]
			Lower	Upper	
Age in years					
< 21	18 (34.6)	1.00			
22 to 29	18 (34.6)	0.99	0.47	2.06	0.97
≥30	16 (30.8)	1.14	0.53	2.47	
Gestational age at recruitment (mean ±SD)	37.3 (±0.3)	0.88	0.71	1.10	0.27
Seconday or tertiary education					
Negative	40 (76.9)	1.00			0.26
Positive	12 (23.1)	1.58	0.71	3.49	
Place of recruitment					
Antenatal clinic	25 (48.1)	1.00			0.15
At delivery	27 (51.9)	2.12	0.77	5.84	
Vaginal discharge					
No	38 (73.1)	1.00			
Yes	14 (26.9)	0.83	0.36	1.95	0.67
Anemia					
No	14 (26.9)	1.00			
Yes	25 (48.1)	0.49	0.23	1.05	0.18
Unknown	13 (25.0)	0.77	0.31	1.91	

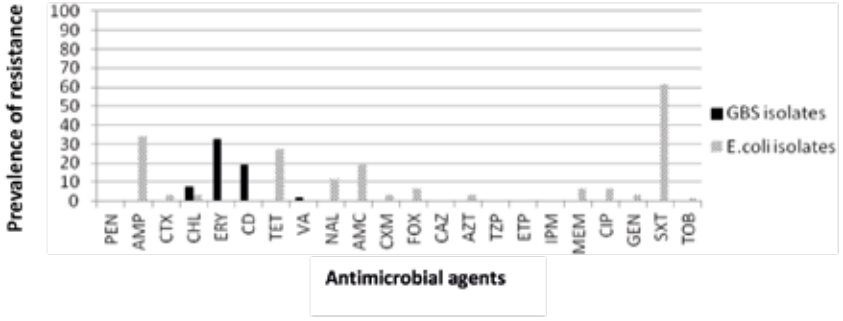
[θ]P-value was derived from likelihood ratio test.

Antimicrobial susceptibility and serotyping

One hundred and one specimens were found to be positive for *GBS* (48 vaginal and 53 vagino-rectal). All *GBS* isolates were fully sensitive to penicillin, ampicillin and ceftriaxone. Thirty-four (32.7%) isolates were resistant to erythromycin and 20 (19.2%) isolates to clindamycin. Seven isolates showed erythromycin-induced resistance to clindamycin. All the *E. coli* isolates were screened for susceptibility to 18 antimicrobial agents. Susceptibility to all antimicrobial agents tested was seen in 14 isolates (22.6%). *E. coli* was resistant to ampicillin in 21 (38.9%) cases, ceftriaxone in 2 (3.2%) cases, amoxicillin/clavulanate acid in 12 cases (19.4%), ciprofloxacin in 4 cases (6.5%) and co-trimoxazole in 38 cases (61.3%). Figure 3 summarizes the distribution of antimicrobial resistance (classifying isolates showing intermediate levels of susceptibility as resistant). Details of the resistance profiles of *GBS* and *E. coli* isolates are shown in Supplementary material table S1.

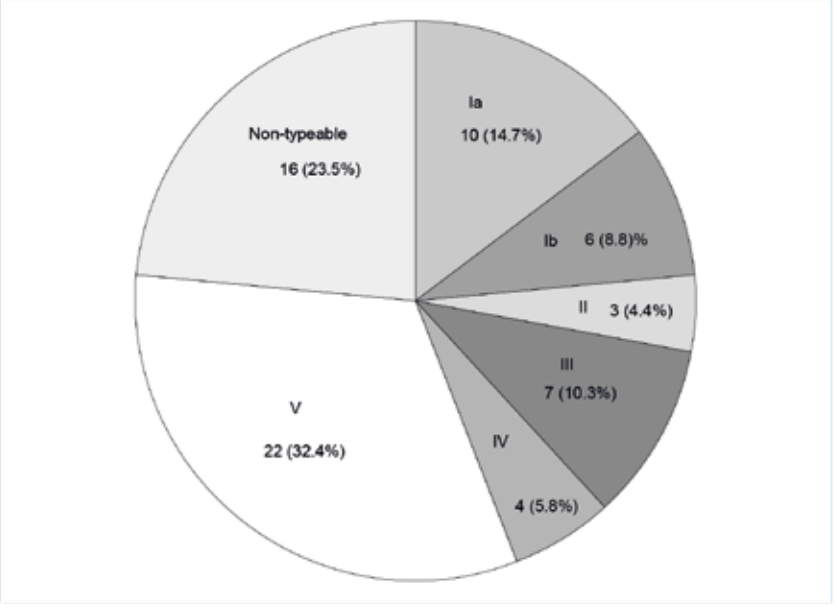
The serotype distribution of the *GBS* isolates is presented in Fig 4 and Fig S1 in the Supplementary material. The most prevalent serotypes were V (32.4%), Ia (14.7%), III and Ib (10.3% and 8.8%, respectively). Sixteen isolates (23.5%) were non-typeable. Twenty-six women had the same serotype detected both in the vaginal and vagino-rectal swabs, while in seven cases infections were serotype-discordant.

Figure 3. Distribution of antimicrobial resistance among GBS and *E. coli* isolates



PEN, penicillin; AMP, ampicillin; CTX, ceftriaxone; ERY: erythromycin; CD, clindamycin; TET, tetracycline; VA, vancomycin; NAL, nalidixic acid; AMC, amoxicillin/clavulanic acid; CXM, cefuroxime,; FOX, cefoxitine; CAZ, ceftazidime; AZT, aztreonam; TZP, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TOB, tobramycin

Figure 4. Serotype distribution of vaginal and recto-vaginal GBS isolates of 64 study participants (taking the serotype of recto-vaginal as reference in case of discordance (n (%)).



Neonatal outcomes

Three hundred and twenty neonatal outcomes from 316 pregnant women wererecorded (98.8%). The delivery outcomes of four women in the ANC group were not registered at MDH. Neonatal outcomes included four pair of twins, 290 term babies, 25 preterm and 5 cases of stillbirths. Figure 1 illustrates neonatal outcomes and follow-up in detail. Characteristics of neonates born of mothers participating in the study may be found in table 1 and 2. Thirty-two neonates born of 36 (88.9%) GBS carriers recruited at ANC were born at MDH, and 4 outside of the health system. Due to lack of qualified clinical staff, work saturation and advanced stage of labor, IAP strategy as recommended by CDC[35] was feasible only in two known *GBS* carriers at time of delivery, we administered a single dose of penicillin to 22 neonates in the first hour after birth. Two hundred and sixty-two infants (81.9%) were followed-up until 90 days of age and 8/262 (3.1%) were admitted in the hospital during this period. Seven infants died among those followed-up until 3 months after birth (2.7%), being five of them HIV-exposed (one clinical sepsis, one perinatal asphyxia and 3 unknown causes). A significantly higher risk for death among those neonates born of

mothers recruited at ANC compared to those recruited at delivery (3.6% vs. 1.7%, p<0.001) was found.

Acceptability of vaginal and vagino-rectal sampling

Fifteen study participant women and five non-study pregnant women were recruited for the social component. Acceptability of collecting vaginal and vagino-rectal samples was 100%. Facilitators for acceptance included: a) Willingness to know whether they had a reproductive tract infection; b) Being interested in understanding the objectives of collecting vaginal and vagino-rectal samples; and c) Willingness to be treated and accompanied to the hospital in case of reproductive tract infection and avoiding transmitting them to their offsprings. Only a few women felt uncomfortable with sample collection, referring to feeling of burning and/or pain. Although all participants of the social component accepted sample collection, possible barriers for acceptance of future vagino-rectal sample collection were explored and these included: a) fear in relation to the first time being submitted to this procedure; b) worries regarding being seen at the hospital (stigma); c) lack of privacy at the ANC at time of sample collection.

DISCUSSION

To our knowledge, this is the first study presenting data on *GBS* maternal colonization, antibodies against *GBS* and characterization of isolates in a rural area of Mozambique and the first time concomitantly examining E. coli colonization in pregnant women in the country. Maternal rate of *GBS* colonization found in this study, 21.3%, was as high as previous work in other countries in Sub-Saharan Africa reported[12]. However, two previous studies performed in the capital of Mozambique, Maputo, reported an extremely low prevalence of GBS colonization among pregnant women of 1% in 1995[27] and 1.8% in 2008[25]. Smaller sample sizes, different study population, and very likely laboratory and microbiology procedures utilized for *GBS* detection, may all contribute to explain the significant increase in terms of overall prevalence found in our study. Our findings are in close agreement with a systematic review on *GBS* disease in sub-Saharan Africa[12], which included 18 studies reporting data on maternal *GBS* colonization, finding an average *GBS* carriage of 21.8% (95% CI: 18.3 - 25.5) among pregnant women across the region. These results are also similar to general prevalence data from other regions, including the United States[9] and Europe[13], or from other neighboring countries in Sub-Saharan Africa such as South Africa (with similar prevalence of HIV[20, 37, 38], Zimbabwe[15] or

Malawi[39], supporting the credibility of these data. The yield of vagino-rectal sampling was better for *GBS* colonization than using only vaginal samples as previously reported[40, 41] and recommended[35].

No risk factors independently associated with higher prevalence of *GBS* colonization were found in this study. We adjusted the multivariate analysis by gestational age as previous studies examining the influence of advancing gestation on *GBS* colonization have observed that colonization rates appear to change overtime during pregnancy[15, 42, 43]. However, no associations between gestational age and colonization risk by *GBS* were found. Colonization prevalence was similar among age groups, in contrast to what has been described by some studies[16, 17, 44] but in concordance to a recent multicenter study performed in African settings[37], reinforcing the idea that colonization rates are quite stable across a wide variety of African settings. We did not find higher education to be a risk factor for *GBS* colonization as other studies have reported[18], a finding possibly influenced by the homogeneity of lower education backgrounds in our setting. Importantly, this study further contributes to expose the fact that current understanding on maternal risk factors for colonization is incomplete. No increased risk of *GBS* colonization among HIV-infected women was found in this study, a finding supported by other studies in USA and Zimbabwe[15, 19]. However, a South African study reported that maternal GBS carriage was lower in HIV-positive women and among those with lower CD4 counts in Malawi[20, 39]. This fact could be related to the fact that *GBS* carriage is inversely associated with the use of prophylactic co-trimoxazole among HIV-infected women. Information about co-trimoxazole use in this study was not recorded but due to high prevalence of HIV in our cohort it is likely that a high proportion of participants were routinely taking co-trimoxazole. Although HIV appears not to be a risk factor for maternal colonization during pregnancy, a recent South African study found that incidence of GBS neonatal disease may be up to 64% higher among HIV-exposed infants compared with non-HIV exposed ones[12]. As of today, no data are available regarding incidence of neonatal GBS invasive disease and HIV co-infection in Mozambique. However, studies conducted in South Africa[20, 45], with a similar HIV prevalence to the one reported in southern Mozambique[32], found an incidence of *GBS* invasive disease among infants higher than that reported in other resource-constrained settings[44, 46]. Hence, it would appear reasonable to expect a high incidence of *GBS* invasive disease in this particularly HIV-struck study area. However, a low incidence of *GBS* invasive

cases in neonates born to GBS infected women was found in this study. Reasons for this low incidence could be the high prevalence of antibodies against *GBS* found in the studied cohort (69.2%). Maternal antibody levels have been associated with protection against invasive *GBS* disease in high[47] and lower-middle income settings[48] and it has been documented that GBS placental transfer appears not to be affected by HIV infection[49]. It is difficult to correlate our *GBS* AB results with what is known regarding *GBS* maternal colonization and infant disease. The highest proportion of women with anti-*GBS* AB was against serotype Ib, Ia and V, consistent with predominant serotypes among carriers in our cohort. Although we did not examine antibody correlation between mothers and newborns, the higher prevalence of antibodies in our cohort could also potentially explain this low incidence of *GBS* invasive disease among our neonate cohort. In addition, prevalence of carriers of serotype III in this population, the known serotype causing more infant invasive disease[50], was lower than reported in other African studies[12], which would be also consistent with a lower incidence among infants. Another reason could be the attempt to implement IAP strategy to those colonized *GBS* mothers delivering at MDH. None of the neonates who received a single dose of penicillin after birth developed symptoms of sepsis. Understanding that this strategy is not generally recommended on account of the risks of enhancing antimicrobial resistance, and in spite of the small sample, it could be argued that for settings were access to health is problematic, but where GBS maternal carriage can be confirmed, such a strategy could prove effective in decreasing neonatal early morbidity by blocking the infection's transmissibility at a moment where the baby is still under the surveillance of the health system. The only *GBS* case in our study was a newborn developing symptoms in the first 24 hours, born to a mother recruited at delivery with negative *GBS* screening. This mother was HIV positive and likely was taking co-trimoxazole as prophylaxis of opportunistic infections, suggesting an intrauterine infection with a subsequent negativization.

On the other hand, the prevalence of *E. coli* found in this study was lower than reported from other authors in different African settings[20, 37] but comparable with the prevalence reported by Karou in Togo[51]. No risk factors were found to be independently associated with a higher risk of *E. coli* vaginal carriage among pregnant women. Some studies have reported specific risks factors for E. coli colonization, including sexual practices such as anal intercourse during pregnancy[52] or being a sexual worker[37]. Such factors were however not explored in our study.

Importantly, *GBS* continues to be susceptible to penicillin, ampicillin, and ceftriaxone in this setting. Previous studies in Ethiopia[53, 54] and South Africa([18, 51]) also reported full susceptibility of GBS strains to penicillin. Rarer cases of decreased susceptibility to penicillin have been reported in Japan and the United States([55]). A study in Zimbabwe found almost 100 % of isolates sensitive to penicillin, with 2% showing intermediate susceptibility to penicillin. Resistance to erythromycin resistance among invasive GBS isolates in Europe ranges from 3.8% to 21.2%([13]) and from 7% to 25% in the USA and Canada([24]). High levels of resistance to erythromycin (~33%) were found in this study which could be related to its use in sexual transmission infections and other common diseases and mass drug administration (MDA) of azithromycin for trachoma control in sub-Saharan Africa, since development of macrolide-resistant pathogens after more than one round of mass treatment has already reported([56, 57]). Erythromycin resistance is frequently associated with clindamycin resistance([24]). The emergence of non-susceptible GBS strains has important public health implications. *GBS* is still susceptible to penicillin and ampicillin which are the antibiotics of choice. Erythromycin and clindamycin are the drugs of choice for penicillin-hypersensitive patients and resistance to these antibiotics is emerging.

As other studies have reported[12], serotypes Ia, Ib, II, III and V were predominant. However, the most frequent serotype (V) found in this study differs from those found in the majority of studies conducted in other countries, revealing the need to identify prevalent serotypes in each region, as a prerequisite of establishing the potential coverage, impact and implementation requirements of future anti GBS vaccination strategies.

Characterization of *E. coli* isolates from this study has been described by Saez et al([58]). *E. coli* isolates showed significant resistance to co-trimoxazole, as a previous study on diarrhoeagenic *E. coli*([59]) conducted in Manhiça already described. Reasons for such high co-trimoxazole resistance levels may include its extensive use as treatment of community-acquired infections, or as prophylaxis of HIV-related opportunistic infections([60]).

This study has several limitations. Only women attending the MDH (and no other maternities) were included, and recruitment was not conducted after working hours, these being potential sources of selection bias and limiting the generalization of our results to the entire district. The first two women fulfilling inclusion criteria every day were invited to participate in

the study, leading to only 200 women being recruited at ANC, and 120 additional ones upon delivery, an estimated 10% of all deliveries per year attended at MDH. Pregnant women are less likely to attend ANC at the end of their pregnancies, and some women who attend antenatal care in other maternities do actually choose MDH to deliver. Altogether this justifies our sampling strategy, but it is important to highlight that this convenience sample may not be truly representative of the entire pregnancy cohort in the area. We did not collect information about population not sampled and we were unable to compare it with our population in order to assess such potential selection bias. However, the maternity at MDH is the biggest one in the study area and women seen there come from different places of the district and a sample of women attended at delivery was also recruited, minimizing bias. Other studies have reported association of other sexually transmitted infections such as gonorrhea or bacterial vaginosis[37] or socio-economic status[44] with GBS colonization in pregnant women, but we did not measure these variables. However, an attempt was made to explore the majority of potential risk factors described by other authors. Finally, and albeit this not being an objective of the study, it was impossible to assess the risk of GBS and E. coli transmission in this cohort, due the lack of denominator.

CONCLUSION

This study shows GBS and E. coli carriage among near term pregnant women is high in southern Mozambique. HIV infection was not a risk factor for GBS or E. coli colonization. Presence of anti-GBS antibodies, administration of single dose of penicillin to neonates born to colonized mothers or use of prophylactic co-trimoxazole among HIV-infected pregnant women could be reasons explaining the low incidence of GBS invasive disease among our cohort of newborns.

Screening mothers near term and providing appropriate antimicrobial prophylaxis could prevent potential adverse neonatal outcomes. Unfortunately, the fragility of the health system in LIC hinders the applicability of such approaches, and calls for innovative ideas to tackle these vertically transmitted infections. Serotype V was the most prevalent in our community and four serotypes cause the majority of cases of GBS colonization. The development and implementation of a conjugate vaccine incorporating the most commonly found serotypes globally, could enhance the transfer of maternal antibodies to the baby and protect their health in those critical first moments for survival.

SUPPLEMENTARY MATERIAL

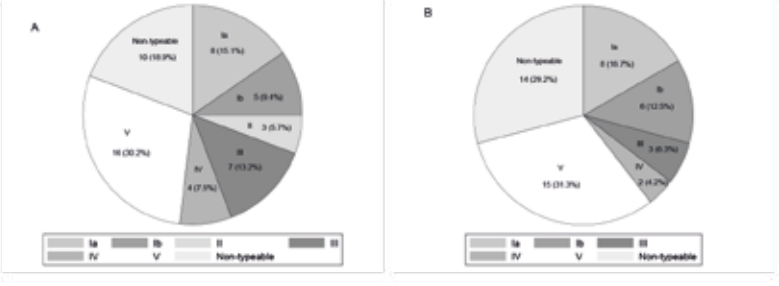
Table S1. Resistance profile of group B streptococcal and Escherichia coli isolates.

Antimicrobial agents	*GBS isolates*, N=104		*E.coli isolates*, N=62	
	Recto-vaginal, n(%), N=54	Vaginal, n(%), N=50	Vaginal, n(%), N=52	Urine, n(%), N=10
Penicillin				
Full resistant	0 (0)	0 (0)	NA	NA
Intermediate resistant	0 (0)	0 (0)	NA	NA
Ampicillin				
Full resistant	0 (0)	0 (0)	17 (32.7)	4 (40.0)
Intermediate resistant	0 (0)	0 (0)	0 (0)	0 (0)
Ceftriaxone				
Full resistant	0 (0)	0 (0)	0 (0)	0 (0)
Intermediate resistant	0 (0)	0 (0)	1 (1.9)	1 (10.0)
Chloramphenicol				
Full resistant	1 (1.9)	0 (0)	2 (3.9)	0 (0)
Intermediate resistant	3 (5.6)	4(8.0)	0 (0)	0 (0)
Erythromycin				
Full resistant	14 (25.9)	10(20.0)	NA	NA
Intermediate resistant	4 (7.4)	6 (12.0)	NA	NA
Clindamycin				
Full resistant	10 (18.5)	7 (14.0)	NA	NA
Intermediate resistant	1 (1.9)	2 (4.0)	NA	NA
Tetracycline				
Full resistant	NA	NA	15 (28.9)	2 (20.0)
Intermediate resistant	NA	NA	0.50	2.16
Vancomycine				
Full resistant	0 (0)	0 (0)	NA	NA
Intermediate resistant	2 (3.7)	0 (0)	NA	NA
Nalidixic acid				
Full resistant	NA	NA	3 (5.8)	3 (30.0)
Intermediate resistant	NA	NA	1 (1.9)	0 (0.0)
Amoxicillin/clavulanic acid				
Full resistant	NA	NA	11 (21.2)	1 (10.0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Cefuroxime				
Full resistant	NA	NA	1 (1.9)	1 (10.0)
Intermediate resistant	NA	NA	0.50	2.16
Cefoxitine				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	3 (5.8)	1 (10.0)
Ceftazidime				
Full resistant	NA	NA	0 (0)	0 (0)

Intermediate resistant	NA	NA	0 (0)	0 (0)
Aztreonam				
Full resistant	NA	NA	1 (1.9)	1 (10.0)
Intermediate resistant	NA	NA	0.50	2.16
Piperacillin-Tazobactam				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Ertapenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Imipenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Meropenem				
Full resistant	NA	NA	0 (0)	0 (0)
Intermediate resistant	NA	NA	4 (7.7)	0 (0)
Ciprofloxacine				
Full resistant	NA	NA	1(1.9)	2 (20.0)
Intermediate resistant	NA	NA	1(1.9)	0 (0)
Gentamicine				
Full resistant	NA	NA	2 (3.9)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)
Trimethoprim/ sulfamethoxazole				
Full resistant	NA	NA	34 (65.4)	4 (40.0)
Intermediate resistant	NA	NA	0.50	2.16
Trobramicin				
Full resistant	NA	NA	1(1.9)	0 (0)
Intermediate resistant	NA	NA	0 (0)	0 (0)

NA: not applicable

Fig S1. Serotype distribution of vaginal and vagino-rectal GBS isolates



A)Serotype distribution of vagino-rectal isolates (53samples). B) Serotype distribution of vaginal isolates (48 samples).

REFERENCES

1. United Nations. Levels & Trends Child Mortality. Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation. Report 2017. Available at: http://www.childmortality.org/index.php?r=site/index ; (accessed Nov 11, 2017). . In: Fund UNCs, ed. New York2017.
2. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*. 2016;388:3027-3035.
3. UNICEF. Levels & Trends in child mortality [Internet]. 2015 [cited 2017 Apr 26]. Available from: https://data.unicef.org/wp-content/uploads/2015/12/IGME-report-2015-child-mortality-final_236.pdf 2015.
4. Neto MT. Group B streptococcal disease in Portuguese infants younger than 90 days. *Arch Dis Child Fetal Neonatal Ed*. 2008;93:F90-93.
5. Stoll BJ, Hansen N, Fanaroff AA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *N Engl J Med*. 2002;347:240-247.
6. Van Dyke MK, Phares CR, Lynfield R, et al. Evaluation of universal antenatal screening for group B streptococcus. *N Engl J Med*. 2009;360:2626-2636.
7. Guilbert J, Levy C, Cohen R, Delacourt C, Renolleau S, Flamant C. Late and ultra late onset Streptococcus B meningitis: clinical and bacteriological data over 6 years in France. *Acta Paediatr*. 2009;99:47-51.
8. Acosta CD, Kurinczuk JJ, Lucas DN, et al. Severe maternal sepsis in the UK, 2011-2012: a national case-control study. *PLoS Med*. 2014;11:e1001672.
9. Krohn MA, Thwin SS, Rabe LK, Brown Z, Hillier SL. Vaginal colonization by Escherichia coli as a risk factor for very low birth weight delivery and other perinatal complications. *J Infect Dis*. 1997;175:606-610.
10. Beck S, Wojdyla D, Say L, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. *Bull World Health Organ*. 2010;88:31-38.
11. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. *Lancet*. 2006;367:1066-1074.
12. Sinha A, Russell LB, Tomczyk S, et al. Disease Burden of Group B Streptococcus Among Infants in Sub-Saharan Africa: A Systematic Literature Review and Meta-analysis. *Pediatr Infect Dis J*. 2016;35:933-942.
13. Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal group B streptococcal colonisation in European countries. *Acta Obstet Gynecol Scand*. 2008;87:260-271.
14. Kwatra G, Adrian PV, Shiri T, Buchmann EJ, Cutland CL, Madhi SA. Serotype-specific acquisition and loss of group B streptococcus recto-vaginal colonization in late pregnancy. *PLoS One*. 2014;9:e98778.
15. Mavenyengwa RT, Afset JE, Schei B, et al. Group B Streptococcus colonization during pregnancy and maternal-fetal transmission in Zimbabwe. *Acta Obstet Gynecol Scand*. 2010;89:250-255.
16. Anthony BF, Okada DM, Hobel CJ. Epidemiology of group B Streptococcus: longitudinal observations during pregnancy. *J Infect Dis*. 1978;137:524-530.
17. Regan JA, Klebanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group. *Obstet Gynecol*. 1991;77:604-610.
18. Stapleton RD, Kahn JM, Evans LE, Critchlow CW, Gardella CM. Risk factors for group B streptococcal genitourinary tract colonization in pregnant women. *Obstet Gynecol*. 2005;106:1246-1252.
19. Shah M, Aziz N, Leva N, Cohan D. Group B Streptococcus colonization by HIV status in pregnant women: prevalence and risk factors. *J Womens Health (Larchmt)*. 2011;20:1737-1741.
20. Cutland CL, Schrag SJ, Zell ER, et al. Maternal HIV infection and vertical transmission of pathogenic bacteria. *Pediatrics*. 2012;130:e581-590.

21. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med*. 2000;342:15-20.
22. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. *MMWR Recomm Rep*. 1996;45:1-24.
23. Melin P, Schmitz M, De Mol P, Foidart JM, Rigo J. [Group B streptococcus, primary cause of life-threatening infections in infants. Epidemiology and prevention strategy]. *Rev Med Liege*. 1999;54:460-467.
24. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Recomm Rep*. 2002;51:1-22.
25. de Steenwinkel FD, Tak HV, Muller AE, Nouwen JL, Oostvogel PM, Mocumbi SM. Low carriage rate of group B streptococcus in pregnant women in Maputo, Mozambique. *Trop Med Int Health*. 2008;13:427-429.
26. Sigauque B, Roca A, Mandomando I, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J*. 2009;28:108-113.
27. Osman NB, Folgosa E, Bergstrom S. An incident case-referent study of threatening preterm birth and genital infection. *J Trop Pediatr*. 1995;41:267-272.
28. Nhantumbo AA, Cantarelli VV, Caireao J, et al. Frequency of Pathogenic Paediatric Bacterial Meningitis in Mozambique: The Critical Role of Multiplex Real-Time Polymerase Chain Reaction to Estimate the Burden of Disease. *PLoS One*. 2015;10:e0138249.
29. Capan M, Mombo-Ngoma G, Akerey-Diop D, et al. Epidemiology and management of group B streptococcal colonization during pregnancy in Africa. *Wien Klin Wochenschr*. 2012;124 Suppl 3:14-16.
30. Stoll BJ, Schuchat A. Maternal carriage of group B streptococci in developing countries. *Pediatr Infect Dis J*. 1998;17:499-503.
31. Sacoor C, Nhacolo A, Nhalungo D, et al. Profile: Manhica Health Research Centre (Manhica HDSS). *Int J Epidemiol*. 2013;42:1309-1318.
32. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med*. 2012;13:581-588.
33. Schorge John O SJI, Halvorson Lisa M, Hoffman Barbara L, Bradshaw Karen D, Cunningham F Gary. . *6. First-Trimester Abortion*: McGraw-Hill Medical; 2008.
34. Poyart C, Tazi A, Reglier-Poupet H, et al. Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. *J Clin Microbiol*. 2007;45:1985-1988.
35. Verani Jennifer R ML, Schrag Stephanie J. . Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports*. 2010;59:1-36.
36. Alos Cortes JI, Andreu Domingo A, Arribas Mir L, et al. [Prevention of Neonatal Group B Sreptococcal Infection. Spanish Recommendations. Update 2012. SEIMC/SEGO/SEN/SEQ/SEMFYC Consensus Document]. *Enferm Infecc Microbiol Clin*. 2013;31:159-172.
37. Cools P, Jespers V, Hardy L, et al. A Multi-Country Cross-Sectional Study of Vaginal Carriage of Group B Streptococci (GBS) and Escherichia coli in Resource-Poor Settings: Prevalences and Risk Factors. *PLoS One*. 2016;11:e0148052.
38. Cutland CL, Madhi SA, Zell ER, et al. Chlorhexidine maternal-vaginal and neonate body wipes in sepsis and vertical transmission of pathogenic bacteria in South Africa: a randomised, controlled trial. *Lancet*. 2009;374:1909-1916.
39. Gray KJ, Kafulafula G, Matemba M, Kamdolozi M, Membe G, French N. Group B Streptococcus and HIV infection in pregnant women, Malawi, 2008-2010. *Emerg Infect Dis*. 2011;17:1932-1935.
40. Gupta C, Briski LE. Comparison of two culture media and three sampling techniques for sensitive and rapid screening of vaginal colonization by group B streptococcus in pregnant women. *J Clin Microbiol*. 2004;42:3975-3977.
41. Wang X, Ma LK, Song YN, Liu JT, Xu YC, Yi J. [Rapid Group B streptococcus screening methods in late pregnancy and the maternal-neonatal outcomes]. *Zhonghua Yi Xue Za Zhi*. 2016;96:1188-1191.

42. Baker CJ, Barrett FF, Yow MD. The influence of advancing gestation on group B streptococcal colonization in pregnant women. *Am J Obstet Gynecol*. 1975;122:820-823.
43. Hansen SM, Uldbjerg N, Kilian M, Sorensen UB. Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants. *J Clin Microbiol*. 2004;42:83-89.
44. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with Streptococcus agalactiae and associated stillbirth and neonatal disease in coastal Kenya. *Nature microbiology*. 2016;1:16067.
45. Dangor Z, Lala SG, Cutland CL, et al. Burden of invasive group B Streptococcus disease and early neurological sequelae in South African infants. *PLoS ONE*. 2015;10 (4) (no pagination).
46. Gray KJ, Bennett SL, French N, Phiri AJ, Graham SM. Invasive group B streptococcal infection in infants, Malawi. *Emerg Infect Dis*. 2007;13:223-229.
47. Dangor Z, Kwatra G, Izu A, Lala SG, Madhi SA. Review on the association of Group B Streptococcus capsular antibody and protection against invasive disease in infants. *Expert Rev Vaccines*. 2015;14:135-149.
48. Dangor Z, Kwatra G, Izu A, et al. Correlates of protection of serotype-specific capsular antibody and invasive Group B Streptococcus disease in South African infants. *Vaccine*. 2015;33:6793-6799.
49. Le Doare K, Taylor S, Allen L, et al. Placental transfer of anti-group B Streptococcus immunoglobulin G antibody subclasses from HIV-infected and uninfected women to their uninfected infants. *AIDS*. 2016;30:471-475.
50. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet*. 2012;379:547-556.
51. Karou SD, Balaka A, Bamoke M, et al. Epidemiology and antibiotic resistance of bacterial meningitis in Dapaong, northern Togo. *Asian Pac J Trop Med*. 2012;5:848-852.
52. Tameliene R, Barcaite E, Stoniene D, et al. Escherichia coli colonization in neonates: prevalence, perinatal transmission, antimicrobial susceptibility, and risk factors. *Medicina (Kaunas)*. 2012;48:71-76.
53. Alemseged G, Niguse S, Hailekiros H, Abdulkadir M, Saravanan M, Asmelash T. Isolation and anti-microbial susceptibility pattern of group B Streptococcus among pregnant women attending antenatal clinics in Ayder Referral Hospital and Mekelle Health Center, Mekelle, Northern Ethiopia. *BMC Res Notes*. 2015;8:518.
54. Mengist A, Kannan H, Abdissa A. Prevalence and antimicrobial susceptibility pattern of anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia. *BMC Res Notes*. 2016;9:351.
55. Dahesh S, Hensler ME, Van Sorge NM, et al. Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to beta-lactam antibiotics. *Antimicrob Agents Chemother*. 2008;52:2915-2918.
56. Bojang E, Jafali J, Perreten V, et al. Short-term increase in prevalence of nasopharyngeal carriage of macrolide-resistant Staphylococcus aureus following mass drug administration with azithromycin for trachoma control. *BMC Microbiol*. 2017;17:75.
57. Skalet AH, Cevallos V, Ayele B, et al. Antibiotic selection pressure and macrolide resistance in nasopharyngeal Streptococcus pneumoniae: a cluster-randomized clinical trial. *PLoS Med*. 2010;7:e1000377.
58. Saez-Lopez E, Cossa A, Benmessaoud R, et al. Characterization of Vaginal Escherichia coli Isolated from Pregnant Women in Two Different African Sites. *PLoS One*. 2016;11:e0158695.
59. Mandomando IM, Macete EV, Ruiz J, et al. Etiology of diarrhea in children younger than 5 years of age admitted in a rural hospital of southern Mozambique. *Am J Trop Med Hyg*. 2007;76:522-527.
60. Chintu C, Bhat GJ, Walker AS, et al. Co-trimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial. *Lancet*. 2004;364:1865-1871.

ARTICLE 4

Congenital and perinatally-acquired infections in resource-constrained settings

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REVIEW

Congenital and perinatally-acquired infections in resource-constrained settings

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ABSTRACT

Introduction: Congenital and perinatal infections are a leading cause of neonatal and infant morbidity and mortality. Maternal screening, vaccines or treatment where available, constitute effective prevention strategies to reduce the burden of these diseases. Data on the burden of congenital and perinatal infections are very limited for low and middle-income regions.

Areas covered: This review aims to summarize the burden of congenital and perinatal infections and the main challenges for their control in resource-limited settings. Articles were identified through the main electronic databases and cover the period 1971–2016.

Expert commentary: Estimates from low and middle-income countries indicate that the burden of congenital infections may be higher in these regions than in industrialized countries. As preventive and curative strategies are available to tackle some of these infections, efforts at the international and national levels must be made to implement those and thus reduce their burden in resource-limited countries.

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1. Background

Fetal, perinatal, and childhood morbidity and mortality are significantly influenced by infections acquired *in utero* or in the immediate postnatal period. These infections may be mild or subclinical for the mother, but if they are vertically transmitted can result in devastating consequences for the newborn [1,2]. Transmission of the pathogens may occur prenatally (through transplacental passage of organisms), perinatally (direct contact with blood and maternal secretions or by ascending route from the birth channel), and postnatally (from exposure to infected breast milk while breast-feeding) [2]. Infections acquired *in utero* are categorized under 'congenital' infections, whereas those acquired around the time of delivery and the immediate postpartum period are called 'perinatal' infections [1].

Congenital and perinatal infections are well-described causes of perinatal morbidity and stillbirths. The acronym 'TORCH' was introduced by Nahmias in 1971 to underline a group of pathogens that cause congenital and perinatal infections: ***T**oxoplasma gondii* (*T. gondii*), **R**ubella virus, **C**ytomegalovirus (CMV), and **H**erpes simplex virus (HSV) [3]. The diagnostic problems encountered in the evaluation of a suspected perinatal infection and the complexities of the evaluation process for the original four TORCH agents made it necessary to expand the original TORCH complex and include new agents [4]. Currently, TORCH stands for the following: ***T**. gondii*; **O**ther: syphilis, hepatitis B virus (HBV), hepatitis C virus (HCV), varicella zoster virus (VZV), human immunodeficiency virus (HIV), parvovirus B19 (B19V), enteroviruses, lymphocytic choriomeningitic virus, tuberculosis, listeriosis, and other less recognized pathogens such as *Chlamydia trachomatis* and *Ureaplasma urealyticum*; **R**ubella virus; **C**MV; and **H**SV [2,5,6]. A further expansion of this acronym, CHEAPTORCHES, was proposed by Ford-Jones and Kellner in 1995 but it is not widely used [7]. It includes **C** – **C**hickenpox and shingles; **H** – **H**epatitis B, C, D) E; **E** – **E**nterovirus; **A** – **A**IDS (HIV infection); **P** – B19V; **T** – **T**oxoplasmosis; **O** – **O**ther (Group B Streptococcus, Listeria, Candida, Lyme disease); **R** – **R**ubella; **C** – **C**MV; **H** – **H**erpes simplex; **E** – **E**verything else sexually transmitted (gonorrhea, chlamydia infection, *U. urealyticum*, human papillomavirus); **S** – **S**yphilis.

Although there are no specific estimates regarding the global burden of congenital and perinatal infections and their impact on disability and/or neonatal cause-of-death, they may contribute to death due to prematurity and low birth weight (35.7% of neonatal cause-of-death [8]), neonatal infections, and congenital abnormalities (15.6% and 10.5%, respectively) [8]. A study conducted in the United Kingdom over a 21-year period demonstrated that viruses contributed to 4.2% of stillbirths and 13.2 of infant deaths, confirming parvovirus and CMV, vertically transmitted, as the commonest viruses associated to stillbirth and neonatal deaths [9].

Producing global estimates of the attributable stillbirth, neonatal births or disability due to congenital infections in low-income countries appears challenging, primarily because of the scarcity of prevalence or risk data. In those settings, it would seem reasonable to assume a higher burden of congenital infections, partly may be assumed because of the higher underlying prevalence of HIV (frequently associated to CMV [10], HSV [11], and hepatitis B [12] vertical transmission), but also in relation to the challenges in the implementation of effective vaccines, such as for instance the anti-rubella vaccine.

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The presence of infections that may not be endemic in more industrialized parts of the world may also play a role. For instance, in sub-Saharan Africa, malaria is estimated to cause ~20% of stillbirths (global estimate 8.2%) and HIV could account for about 0.7% in this region. Similarly, maternal syphilis may be responsible for 7.7% of stillbirths worldwide and 11.2% in sub-Saharan Africa [13]. So, a first step to prevent these fetal deaths should be to improve prevention and treatment of malaria, syphilis, and HIV.

Some experts consider the acronym TORCH outdated [5], largely due to the growing number of infections listed under the 'other' category. However, the use of this acronym reminds clinicians that several infectious agents can produce similar and potentially devastating effects on the fetus and the nervous system in development.

The manifestations of congenital infections depend on several independent factors such as the effect of the pathogen on organogenesis, the timing of infection with respect to gestational age, the presence or absence of maternal immunity, the immaturity of the fetal immune response, and the mode of acquisition and load of the infection [14]. While each of the congenital and perinatal infections can cause distinct clinical manifestations and *sequelae*, some of these infections share characteristics (Table 1).

For many of these pathogens, prenatal screening is available and treating the infected pregnant women appears as an effective measure to prevent vertical transmission to their offspring. Early recognition of congenital disease in newborns is key and treatment and/or prevention strategies are internationally recognized and available, although not necessarily implemented in low- and middle-income countries (LMIC) (Table 2).

This review aims to summarize the burden of congenital and perinatal infections and the main challenges for their control in resource-limited settings. It includes congenital infections that are present at the time of delivery as well as perinatally acquired ones transmitted during or immediately after delivery. Although HIV can also be vertically transmitted, we will only describe it briefly, focused in the context of its impact on the control of other pathogen's transmission, since, due to its burden and impact, congenital HIV deserves a separated review. Assuming a higher burden of congenital infections in LMIC, we aim to highlight key aspects of their epidemiology, diagnosis, management, and prevention in resource-limited countries.

We have included data on incidence for those infections when available, but this review has not been designed to estimate absolute risk, since data have not been abstracted in a systematic manner.

2. Search methodology

Articles were identified through electronic searches of PubMed, Health InterNetwork Access to Research Initiative, and The Cochrane Library without any language or date restrictions, covering a period of 45 years (from 1971 to 2016). PubMed was searched through the use of a broad sensitive filter using the following combination of search terms: 'congenital,' 'infection,' 'developing' and 'countries,' 'low-income' and 'countries,' and 'lower-middle income' and 'countries' yielding 353 results, while the same search found out 20,789 results when the terms 'developing' and 'countries,' 'lower-middle-income' and 'countries,' and 'low-income' and 'countries' were dropped. Limits were applied to exclude studies on animals. The references of the retrieved papers were further hand-searched for additional studies. Unpublished and/or gray literature was not reviewed. Diseases of interest for this article were restricted to congenital infections belonging to the TORCH complex. Searching specific congenital infections, 'congenital' and 'toxoplasmosis' were used as search terms for congenital toxoplasmosis (CT), yielding 3466 articles; 'congenital' and 'rubella' 3217 results; 'congenital,' 'cytomegalovirus,' and 'infection' provided 2917 results; and 'congenital' 'herpes simplex' and 'infection,' 637. Search of others congenital infections as syphilis was performed using search terms 'congenital' and 'syphilis,' yielding 3346 results; varicella congenital infection using 'congenital,' 'varicella' and 'syndrome,' yielding 194 articles; 'congenital' and 'hepatitis b' 455 results; 'congenital' parvovirus B19" yielding 211; and other infections vertically transmitted (Hepatitis C, Zika virus [ZIKV], Dengue, and Chagas) yielded 1100 articles. Our outcomes of interest were related to the epidemiology and challenges in diagnosis and treatment of congenital infections, focusing on lower middle and low-income countries rather than more industrialized ones. We used the concept 'resource-constrained environment' defined as the setting in which production activities cannot exceed the volume of available resources. These constraints may be of a physical or technical nature [53]. A total of 170 articles were finally included in this review (Figure 1).

Table 1. Commonalities among vertically transmitted infections.

Transmission	Cause of infection	Maternal disease	Fetal and/or infant manifestations	Diagnosis
Placental via Direct contact during delivery	Virus Bacteria Parasite	Paucisymptomatic Asymtomatic Symptomatic in immunocompromised women	Depending on the timing of the infection: **<20 weeks of GA:** more severe illness presentation and may cause multiple malformations **Later times of pregnancy:** prematurity, IUGR, or CNSD **Perinatally:** sepsis, pneumonitis, jaundice, hepatosplenomegaly, thrombocytopenia, etc. [2–6]	Serology Molecular biology techniques Cell or pathogen culture

GA: Gestational age; IUGR: intrauterine growth restriction; CNSD: central nervous system disorders.

Table 2. Diagnosis, treatment, and prevention of some vertically transmitted infections.

Disease	Diagnosis	Treatment	Prevention
Congenital toxoplasmosis	**Mother** *Toxoplasma gondii*-specific IgG and IgM followed by the low IgG avidity test **Infant** Definitive diagnosis of congenital toxoplasmosis is made by organism isolation from the placenta, serum, and CSF of infants. Presence of *T. gondii*-specific IgM, or persistence of IgG beyond 12 months of age, is suggestive of congenital infection [5,15]	Pyrimethamine and sulfadiazine for 1 year ± folinic acid [5,15]	**Mother** Improved hygiene and avoidance of raw meat consumption during pregnancy [16,17] *Treatment*: *1. First trimester*: Spyramycin *2. Second/Third trimester*: Pyrimethamine and sulphadiazine
Congenital rubella syndrome	Diagnosed based on rubella-specific IgM, which is usually positive at birth and up to 3 months of age for congenital infection. This diagnosis is confirmed by stable or increasing serum concentrations of rubella-specific IgG over the first 7–11 months of life, although false-positive IgM positivity can occur. Low-avidity anti-rubella IgG suggests recent infection. The virus can also be isolated in culture or by PCR, common practice in the Western world [5,18–21]	Specific treatment not available Supportive care	**Mother** Women of childbearing age and pregnant women should have evidence of immunity to rubella. If they are found to be nonimmune, they should be vaccinated with 1 dose [2,21] **Infant** *Monovalent vaccine*: 1 dose regimen offers high response (≥95%) and long-term protection [22] *Combination vaccines*: 2-dose regimen combination with vaccines against measles (MR); measles and mumps (MMR); or measles, mumps, and varicella (MMRV)
Congenital CMV infection	Culture-based methods and PCR from saliva, urine, blood, and CSFs specimens should be obtained within the first 2 weeks of life to confirm intrauterine infection [23] A retrospective diagnosis of congenital CMV infection by detecting the presence of CMV-DNA in the dried blood spot samples collected and stored from the neonatal screening period [1] Serology (IgM anti-CMV and antigenemia) is also diagnostic but must be confirmed by culture or PCR [24]	*First choice*: GCV and val-GCV [25] *Other options*: foscarnet and cidofovir [26]	**Mother** Improved hand washing during pregnancy [27]. *Screening*: Antenatal screening of limited impact [27,28] *Treatment*: Maternal treatment with oral val-GCV yet to be evaluated [29] CMV hyperimmunoglobulin to pregnant women with fetal infection after primary CMV infection [30]
Neonatal herpes	**Mother** Culture and PCR from genital lesions Serology to distinguish primary infection from reactivation [31] **Infant** Viral culture (conjunctivae, nasopharynx, mouth, and anus) is the definitive method to identify newborns with HSV. PCR can also confirm the diagnosis [31] Little value of serological tests [32]	Parenteral acyclovir [31]	**Mother** Prevention of maternal HSV acquisition during pregnancy *Treatment*: Maternal treatment with oral acyclovir [32] Cesarean delivery Prevention of postnatal acquisition [31]
Congenital syphilis	According with the WHO guidelines [33], case definitions of CS are as follows: *Probable*: (1) an infant whose mother had untreated or inadequately treated syphilis during pregnancy (regardless of signs in the infant) or (2) an infant or child with a reactive treponemal test and any one of the following: evidence of syphilis infection in the newborn on physical examination, long-bone X-rays compatible with CS, a reactive VDRL-CSF, an elevated CSF cell count or protein (without other cause), a reactive FTA-ABS 19S-IgM antibody test, a reactive IgM ELISA, or a reactive IgM treponemal Western blot *Confirmed*: *Treponema pallidum* in darkfield microscopy, fluorescent antibody, or other specific stains in specimens from lesions, placenta, umbilical cord, or autopsy material In recent years, point-of-care rapid diagnostic tests for syphilis have appeared and could revolution the early recognition of this congenital infection	**Cases confirmed or probable of CS**: Crystalline penicillin G or procaine penicillin **Cases not probable or not confirmed**: a single dose of benzathine penicillin [34]	**Mother** *Screening*: Antenatal syphilis screening is highly cost-effective [35]. Diagnostic tests are effective and require minimal logistic support *Treatment*: Parenteral penicillin G [36]

(*Continued*)

Table 2. (Continued).

Disease	Diagnosis	Treatment	Prevention
Congenital varicella syndrome	Diagnosing congenital varicella starts with recognizing the disease in the pregnant mother. VZV can be identified by PCR or by immunofluorescence techniques in skin scrapings or from vesicle fluid. Acute and convalescent IgM titers can diagnose in hindsight but will not identify acute disease [5]	Parenteral acyclovir [37]	**Mother** *Screening* Immunization is contraindicated during pregnancy [38] *Treatment*: Acyclovir [38] **Infant** Postexposure prophylaxis with immunoglobulin and/or antivirals [37]
Hepatitis B infection	**Mother** The presence of HBsAg signifies that the mother has an acute or chronic. Presence of HBeAg implies higher risk of transmission [39] **Infant** The diagnosis of HBV infection is based on the detection of viral particles (HbsAg, HbeAg, HBV-DNA) and antibodies (anti-Hbc IgM, IgG, anti-HBs). In children exposed to HBV, an HBeAg positive or a high viral load can confirm the diagnosis [40]	Lamivudine is approved for treating CHB in children 2 years of age and older [41] There is no treatment for acute HBV infection	**Mother** Maternal antiviral therapy [42] **Infant** Hepatitis B vaccine (3 dose-regime) [43] and hepatitis B Ig prophylaxis [43]
Hepatitis C infection	**Mother** Routine screening of pregnant women is not advocated. However, targeted screening in high-risk women is recommended [44] **Infant** *Anti-HCV IgG*: To test the children after 18 months, since persistence of maternal antibodies can be as long as 18 months *HCV-RNA PCR*: May be performed at 1–2 months of life and should be repeated after 12 months of age, because up to 30% of infants may clear their infection [44,45]	Interferon-based therapy combined with ribavirin approved for children over 3 years. Not indicated in infants [45]	Therapy in pregnant women is not indicated to prevent perinatal transmission [44]
Congenital B19V	Fetal hydrops and aplastic anemia are highly indicative of intrauterine fetal infection. Anti-B19V IgG and IgM should be investigated in the mother, although maternal IgM may be negative at the onset of hydrops fetalis [46]. Fetal cord blood and amniotic fluid samples are suitable for definitive diagnosis, performed by PCR [47]	Supportive care Treatment of B19V-induced fetal anemia with intrauterine erythrocyte transfusion [48]	Interventions to control and prevent this disease are limited
HIV infection	**Mother** HIV serological assays should be included in the routine panel of prenatal screening tests for all pregnant women. Repeat screening in the third trimester is recommended in regions with elevated rates of HIV infection among pregnant women [49] **Infant** *HIV serological assays*: <18 months of age: used as a screening assay to determine HIV exposure >18 months of age: used as a diagnostic assay *HIV DNA or HIV RNA*: <18 months of age: used as a diagnostic assay. It should be done to all HIV-exposed infants at 4–6 weeks of age [50]	HAART should be initiated among all children living with HIV, regardless of WHO clinical stage or at any CD4 cell count [51]	**Mother** Option B+: all HIV-1-infected pregnant and breast-feeding women begin lifelong antiretroviral therapy regardless of their HIV stage [51] **Infant** Daily NVP or AZT from birth through age 4–6 weeks regardless of infant's feeding method [52]

HBV: Hepatitis B virus; HCV: hepatitis C virus; VZV: varicella zoster virus; HIV: human immunodeficiency virus; Ig: immunoglobuline; CSF: cerebrospinal fluid; GCV: ganciclovir; val-GCV: valganciclovir; B19V: parvovirus B19; HAART: highly active antiretroviral therapy; HBeAg: hepatitis B e antigen; CS: congenital syphilis; VDRL: venereal disease research laboratory; FTA-ABS: fluorescent treponemal antibody absorption; AZT: zidovudine; NVP: nevirapine.

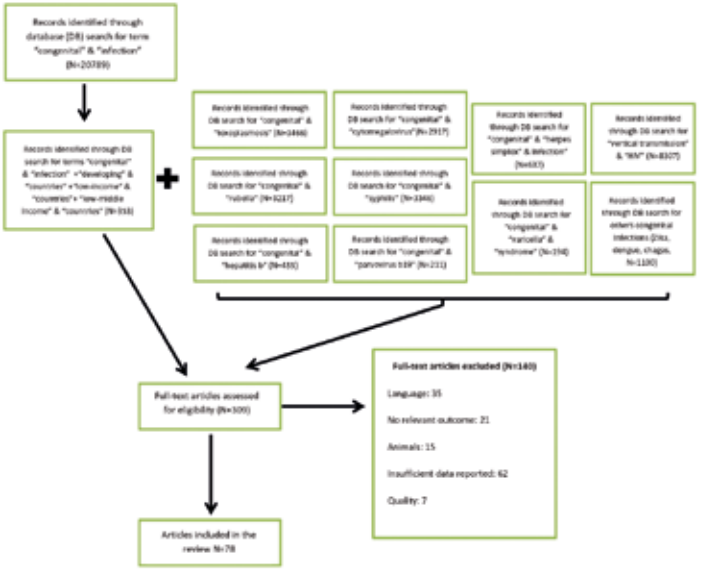

Figure 1. Flow chart diagram for articles selection process.

3. Burden of congenital and perinatal infections in lower middle and low-income countries

3.1. Congenital toxoplasmosis

Toxoplasmosis infection is usually asymptomatic in humans; however, fetal infection can result in severe disease causing CT. The global estimated incidence of CT is 190,100 annual cases (95% CI: 179,300–206,300), approximately 1.5 cases of CT per 1000 live births [54]. Seroprevalence for toxoplasma among pregnant women varies among countries. The highest prevalence is noted in regions with tropical climates, where the toxoplasma oocysts can survive in soil, as well as countries with dietary customs of raw or unprocessed meat [5]. Circa 50–80% of women of childbearing age in Brazil have antibodies to *T. gondii* [55], 44% in France [56], or 9.1% in the USA [57].

Regarding the annual incidence, the global burden of CT was estimated to be 9.6 disability-adjusted life years (DALYs) (95% CI: 5.8–15) per 1000 live births [54]. However, there are important differences among regions, peaking at 19 DALYs (95% CI: 13–22) per 1000 live births, in South America, 17 DALYs (95% CI: 8.5–26) in Eastern Mediterranean region, and 15 DALYs (95% CI: 8.3–24) in some low-income African countries compared with only 2.8 DALYs (95% CI: 1.3–4.3) per 1000 live births in some European countries [54]. Although evidence suggests seroprevalence is decreasing in high-income countries [54], regions, where a rapid process of industrialization is occurring and where meat consumption is growing, could witness an increase of the risk of exposure to *T. gondii*.

South America is suffering the highest burden of CT. Both incidence and frequency of *sequelae* secondary to CT are higher in this region [58]. Three genotypes of *T. gondii* have been isolated. Genotype 2 is predominant in Europe while non-type-2 genotypes, which are common in America, appear to associate more frequent and more severe sequelae [54].

3.2. Congenital rubella syndrome

Rubella remains an important pathogen worldwide, with roughly 100,000 cases of congenital rubella syndrome (CRS) estimated to occur every year [18]. The highest risk of CRS is found in countries with high rates of susceptibility to rubella among women of childbearing age [59,60]. The incidence of CRS in developing countries was reported as 0.6–2.2 per 1000 live births which is not far from the rates reported in Western countries in the pre-vaccine era [60]. Since the introduction of the vaccine, congenital rubella cases reported have decreased drastically. Nevertheless, estimates suggest that the burden of CRS in regions that have not yet introduced rubella-containing vaccine could be very high, although CRS are substantially underreported [61]. The number of member states reporting CRS cases increased from 75 in 2000 to 129 in 2012 [61]. In 2012, substantially more cases were reported in Europe (30,536 cases) and Western Pacific region (44,275 cases) than in other regions (19,219 cases) [61].

3.3. Congenital CMV infection

Congenital CMV infection is the most prevalent congenital infection worldwide, although it remains a neglected public health problem. Although the global prevalence of congenital CMV infection has been reported to vary from approximately 0.2% to 2% (mean 0.65%) [62,63], most of these studies have

been conducted in high-income regions of Europe, the USA, or Japan. Data from low-income countries vary substantially, with some reported prevalence as high as 6–14% [10,64,65]. Higher overall rates are found in countries with higher maternal CMV seroprevalence [62,63,66]. Those rates lead in seropositive hosts to an increased chance of reactivation or reinfection. Furthermore, seronegative hosts within the general population are at greater risk for primary infection.

Most congenital CMV cases in developing countries result from women with preexisting antibodies, a transmission mechanism more poorly understood as a cause of congenital CMV disease. Emerging data from populations with very high seroprevalence, usually from LMIC countries, suggest that prevalence and incidence of congenital CMV infection is higher than in developed countries. Consequently, congenital CMV infection could be an important cause of hearing loss (on the associated consequences of this infection) in resource-limited settings [67,68]. Studies that accurately estimate disability, mortality burden, and how it may be affected by other prevalent conditions such as HIV infection, malnutrition, or malaria in resource-constrained settings are scarce. Children born to mothers coinfected with HIV type 1 present higher prevalence of congenital CMV infection than those born to HIV-negative mothers, increasing from 2.3% to 10.3% [69–73]. Studies in industrialized countries support an increased risk for congenital CMV infection in neonates born to HIV/CMV coinfected mothers [69,70] and CMV may act as a cofactor for HIV disease progression in HIV/CMV coinfected newborns [74]. In addition, among HIV-infected children there is an impairment of the immunological response to CMV infection with a delay in the clearance of CMV viremia and high CMV peak loads. Apparently, the relationship between HIV and CMV is bidirectional [75]. In sub-Saharan Africa, the burden of HIV-1 in women of reproductive age is alarming, reaching 40% in some regions [76] and mother-to-child transmission (MTCT) risks may range from 25.8% (no antenatal antiretroviral drugs) to 10.9% (Options B/B+) [77]. However, the prevalence of CMV infection has decreased over time among neonates exposed but not infected with HIV-1. This prevalence has reached levels similar to those observed in the general population, following the introduction and increasing use of highly active antiretroviral therapy (HAART) for prevention of MTCT of HIV [70,78].

Unfortunately, maternal and birth CMV prevalence and long-term follow-up data for congenitally infected children for many parts of the world are scarce, likely underestimating the global impact of congenital CMV infection.

3.4. Neonatal herpes

Neonatal herpes is usually the result of HSV-2 infection, which is the primary type of HSV associated with genital infection, although recent studies indicate that HSV-1 may also play a major role in causation [79]. HSV-2 is a major global health concern, with a number of negative health impacts. The overall prevalence of HSV-2 remains high worldwide although varies by country [80]. Following primary infection, both types of herpes establish lifelong latent infections, which periodically reactivate and may be associated with recurrent episodes of disease. Approximately 20% of pregnant women are infected genitally with HSV-2, and most of them are unaware of this [81]. It is estimated that one neonate in 3200 live births has HSV-2 infection [82], and without treatment, 80% of infants with disseminated disease die and those who do survive are often severely brain damaged [31,83]. In addition to being the main cause of genital ulcer disease, HSV-2 is a major cofactor fuelling the HIV epidemic, mainly in sub-Saharan Africa, and may account for 40–60% of new HIV infections in high HSV-2 prevalence populations [84]. Seroepidemiological studies of HSV-2 show that prevalence of HSV-2 is at least twofold higher in sub-Saharan Africa (40% of Tanzanian women infected with HSV-2 [85]) than in the USA or Europe [86,87]. As a result, MTCT of both pathogens can be significantly increased [88,89], being a preventable cause of neonatal morbidity and mortality.

3.5. Congenital syphilis

Congenital syphilis (CS) occurs after infection of the placenta in pregnant women who have a primary or secondary syphilis infection. In 2008, the WHO estimated that, worldwide, approximately 1.4 million pregnant women had 'probable active syphilis' or syphilis infections sufficiently active to result in MTCT [90]. Several models have been proposed to estimate a prevalence of 75% (range: 50–80%) [91] of adverse pregnancy outcomes in untreated maternal syphilis. Such maternal infections would cause globally an estimated 521,000–1,575,000 new CS cases, and approximately 521,000 adverse perinatal outcomes, characterized as 212,000 stillbirths, 92,000 neonatal deaths, 65,000 preterm or low birth weight infants, and 152,000 syphilis-infected newborns [36,91–93]. The proportion of pregnant women globally receiving adequate testing and treatment for syphilis is currently unknown. WHO is monitoring syphilis testing and treatment coverage through the HIV Universal Access reporting system, but quality data are not yet available from all countries [90]. Sixty-three of 149 LMIC reported on coverage of syphilis testing during antenatal care (ANC) in 2011, with a median of 68% of women in the reporting countries being tested for syphilis at their first ANC visit [94]. Seroprevalence during pregnancy is generally low in Europe (0.02%) and the United States (4.5%) but may increase up to 18% in sub-Saharan Africa [94].

Overall, estimates suggest that untreated maternal syphilis may result in approximately 304,091 fetal or perinatal deaths and 216,814 syphilis-infected infants at risk for early death [90]. Regarding adverse outcome by region, most of adverse outcomes (87%) would be in Africa and Asia. CS also remains an important cause of severe psychomotor disability and death in infants, especially in resource poor settings. Case fatality rates of 15% in Africa and 6.4% in the United States have been reported [94].

3.6. Congenital varicella syndrome

Maternal chickenpox in the first 20 weeks of pregnancy is associated with an incidence of congenital varicella syndrome (CVS) of 0.91%, although this incidence could increase among pregnant women from tropical countries where the seroprevalence in adulthood is lower [95]. Few congenital infections have been reported, possibly because

most women of childbearing age are immune in developed countries and cases from developing countries remain severely underreported. No data have been found related to prevalence of CVS in developing countries. It is known that in temperate climes, this percentage is lower [96]. Several studies performed in the United Kingdom among South Asian-migrant women and British women showed lower seroprevalence of VZV in migrant women born in South Asia [96,97]. These women have a higher risk of VZV infection during pregnancy if they migrate to settings with higher prevalence of VZV and this infection could produce CVS or perinatal chickenpox [97].

3.7. Hepatitis B infection

HBV is the most serious type of viral hepatitis causing a potentially life-threatening liver infection and eventually leading to chronic liver disease and liver cancer [98]. Perinatal HBV infection occurs in ~1% of infants and is strongly associated with hepatitis B e antigen (HBeAg) positivity among childbearing women [40]. Babies born to HBeAg-positive mothers have the highest risk of developing chronic HBV infection (CHB) and becoming chronic HBV carriers [99]. Overall prevalence of HBeAg in childbearing women reaches to 20–50% in some regions. HBV coinfection among HIV-positive pregnant women is a recognized public health issue, increasing mortality and morbidity in this population [100].

There are 387 million chronic carriers of HBV worldwide [101]. A recent systematic review has shown that the prevalence of CHB worldwide is 3.61%, being the highest endemicity in countries of the African region (8.83%) and Western Pacific region (5.26%) [102], whereas less than 1% of the population in Western Europe and North America is chronically infected [98,101,102]. This prevalence has clearly declined in many countries after the implementation of routine infant immunization programs and the achievement of high coverage [103]. However, despite improvements, CHB remains highly prevalent in some countries in Africa (South Sudan, 22.38%), or in the Pacific (Kiribati 22.70% and Papua New Guinea 14.59%) [102]. Perinatal transmission occurs regionally in different magnitudes. The risk of perinatal transmission is lower in Africa than in Asia, a disparity that could be due to a lower prevalence of HBeAg and other differences in the pathogenic characteristics of circulating HBV genotypes [12]. A study performed in Libya showed HBsAg positivity in 1.5% and maternal to child transmission in 60.9% of the cases [104] while in Ghana, with higher prevalence (16%), the transmission to neonates occurred only in 8.4% [105]. However, in HIV endemic settings, HBV prevalence and MTCT may be increased. Perinatal HBV infection is associated with a 90% risk of CHB as compared with a risk of less than 5% among adults with intact immunity. CHB accounts for an estimated 21% of HBV-related deaths, ranging from 13% in the Eastern Mediterranean region to 26% in the Western Pacific region [106].

3.8. Hepatitis C infection

It has been estimated that the global prevalence of hepatitis C infection varies between 2% and 3% and that around 120–200 million people live with chronic hepatitis C infection worldwide [107]. The prevalence of that infection varies considerably by country and region, and the true burden of disease is not well established in many countries. The highest prevalence of HCV appears to be in sub-Saharan Africa (5.3%), followed by the Eastern Mediterranean (4.6%), Western Pacific (3.9%), and Southeastern Asia (2.15%) regions. Europe and the United States have the lowest prevalence of HCV (1.03% and 1.6%, respectively) [108]. HIV coinfection accelerates the progression of HCV and represents a major public health challenge. Estimates of HCV prevalence among HIV-infected persons range from 5% to 33% [107]. A global systematic review of hepatitis C seroprevalence and HIV coinfection estimated a seroprevalence of 4% among pregnant women [109]. Presence of maternal HCV viremia is a critical factor in MTCT of HCV, and maternal HIV coinfection is an important risk factor [110]. A recent meta-analysis estimated the pooled risk of HCV vertical transmission from HCV antibody and HCV RNA-positive women who were HIV negative at 5.8% as opposed to a 10.8% risk of HCV vertical transmission in children born to HIV-positive women. However, the risk to children born to HCV antibody-positive, RNA-negative mothers was negligible [110]. The natural history and histopathology of HCV-related liver disease in children are still conflicting and variable but generally not as severe as in adults [111].

3.9. Congenital B19V infection

B19V infection is common in childhood, with serologic evidence of previous infection seen in about approximately 35–53% of pregnant women, offering, in these cases, no risk to the fetus. The incidence of B19V infection in pregnancy has been estimated at 3.3–3.8% [112]. No available data of congenital B19V infection in developing countries have been found as part of this review.

3.10. HIV infection

In 2014, 36.9 million people were living with HIV. Although globally, the incidence of HIV is decreasing, the total number of people living with HIV continues to increase, in large part because more people globally are accessing antiretroviral therapy and as a result are living longer, healthier lives. New HIV infections have fallen by 35% since 2000 (by 58% among children) and AIDS-related deaths have fallen by 42% since the peak in 2004 [113].

The region more affected is sub-Saharan Africa, where 1.4 million people were newly infected out of a total of 2 million new HIV infections in 2014 globally, mainly HIV-1 infections. However, the incidence in this region has decreased by 41% since 2000. In Europe and the USA, the number of new infections has remained fairly stable since 2000 and in regions as Eastern Europe and central Asia and Middle East and North Africa, new infections rose by 30% between 2000 and 2014 [113].

Globally, 3.2 million children under 15 were living with HIV in 2013, comprising 9.1% of all people living with HIV. Most of the HIV-infected children acquired the infection from their

mothers. In 2013, an estimated 1.5 million women living with HIV gave birth, a figure virtually unchanged from those in 2009 [114].

Of the 3.2 million children living with HIV, 91% live in sub-Saharan Africa, 6% live in Asia and the Pacific, and the remaining 3% are situated in the rest of the world [114]. Globally, HIV causes 63.8 per 1000 deaths in children aged 1–59 months [115].

3.11. Other congenital infections, emerging or neglected

3.11.1. Congenital Chagas disease

Congenital Chagas disease (CD) is a neglected disease. The global epidemiologic profile of CD is the result of domestic vector-borne transmission mainly in Latin America and large-scale rural-to-urban migration over the past 50 years [116].

The estimated global prevalence of *Trypanosoma cruzi* infection declined from 18 million in 1991, when the first regional control initiative began, to 5.7 million in 2010 [117,118]. CD has been estimated to cause more than 10,000 deaths annually and 30–40% of people either have or will develop cardiomyopathy, digestive mega syndromes, or both [118,119].

At least, two million childbearing women are estimated to be chronically infected with *T. cruzi* in Latin America with the incidence of congenital infection being at least 15,000 cases/year [120]. Vertical transmission may be repeated in each pregnancy (family clustering of congenital cases) and can occur from one generation to another (vertical transmission).

Materno–fetal transmission rates range from 0% to 5.2% [121]. Higher prevalence of MTCT of CDs is found in Brazil, Paraguay, Bolivia, and Argentina [121]. Outside endemic areas, CD often is unrecognized because pregnant women may be asymptomatic. As a consequence of migration of infected women, mainly from Bolivia, congenital transmission has also been recorded from non-endemic countries. A systematic review found that in several studies conducted in Spain, 4.3% of children born to infected mothers were infected [121]. Associated symptoms in the infant include prematurity/low birth weight, respiratory distress syndrome, hepato- and splenomegaly, and neurologic signs [121].

3.11.2. Neonatal dengue

Dengue in pregnancy and its adverse outcomes are another example of a severely neglected public health issue. Dengue causes 100 millions of infections annually, 250,000 cases of dengue hemorrhagic fever, and 25,000 deaths [122]. The life-long immunity developed after infection with one of the four virus types is type-specific, and progression to more serious disease is frequently, but not exclusively, associated with secondary infection by heterologous types [123]. Dengue transmission is ubiquitous throughout the tropics, with the highest risk zones in the Americas and Asia [124]. Studies reporting prevalence data of dengue infection in pregnant women and adverse pregnancy outcomes are scarce. Data from highly endemic areas have reported a prevalence of dengue infection in pregnancy of 2.5% with a vertical transmission rate of 1.6% [125].

illness in newborns or infants, and adverse maternal o
[125,126].

3.11.3. Zika virus congenital infection

The emerging ZIKV epidemic that began in Brazil in 2
now spread rapidly to more than 30 countries in the A
and the Caribbean, infecting more than 2 million inh
[127,128]. The first major outbreak outside of
occurred in 2007 in the Yap Islands of Micronesia [1
another large outbreak in 2013 occurring in French P
[130].The WHO predicts that millions of cases of ZIKV
to occur in the Americas during the following month
projections, in conjunction with a possible ass
between ZIKV infection during pregnancy and micro
cases in newborns [131,132], prompted WHO to dec
epidemic a public health emergency of international

The explosive nature of recent outbreaks and co
links to Guillain–Barre syndrome and microcephaly ar
pletely understood. Incidence data from Brazil indic
reports of suspected microcephaly in Brazil best correl
ZIKV incidence around week 17 of pregnancy, altho
correlation does not demonstrate causation [131].
2010 and 2014, the whole of Brazil reported an av
163 children with microcephaly, whereas in 2015, th
3530 cases reported from 20 states and the federal
with a particular clustering in Pernambuco state, Nort
Brazil [133]. The relative importance of sexual transm
ZIKV and asymptomatic ZIKV infections to the overall
of transmission [134] is also unknown and further stu
urgently needed to confirm this association and estal
consequences of ZIKV congenital infection [132].

Other pathogens which may be vertically tra
remain neglected because they occur, almost exclus
limited-resource countries. Characteristics of some
can be found in Table 3.

4. Challenges in the control of congenital an perinatal infections in lower middle and low-countries

4.1. Prevention of congenital and perinatal infec lower middle and low-income countries

Vaccines are the most effective and cost-saving tools
ease prevention. The public health potential of vac
tackle and reduce vaccine-preventable infections
known. For instance, rubella virus is a candidate for w
eradication because human beings are the only kno
and a safe and highly effective vaccine is available. Go
of this is that endemic transmission in the Americas h
interrupted since 2009 [140]. The last WHO recomme
advise to introduce rubella vaccination into the routi
hood immunization schedule and in combination
vaccination of older age groups who are susceptible t
[59]. However, rubella control or elimination goals a
be established in the African, Eastern Mediterrane
Southeast Asia regions. While vaccination coverage

Table 3. Neglected congenital infections.

Organism	Category	Description	Region
Plasmodium (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae*)	Protozoa	**Neonatal malaria** *P. falciparum* and to a lesser extent also *P. vivax* are associated with malaria infection during pregnancy. Placental infection ranges between 4.6% and 5.6% in HIV-negative women with IPTp of malaria [135] and 7.4% in HIV-infected women with no preventive treatment [136]. Prevalences of congenital malaria range from 8% to 33%, but increasing trends of congenital malaria may be the result of increasing drug resistance, increasing virulence of the parasite, HIV, or increased reporting or detection of cases [137]. Associated symptoms in the infant include prematurity/low birth weight, IUGR, still birth, anemia and sepsis-like syndromes [138]	Sub-Saharan Africa More data needed in Asia and the Americas
Mycobacterium tuberculosis	Mycobacteria	**Congenital tuberculosis** The incidence of tuberculosis in pregnant women could be as high as in the general population, but congenital tuberculosis is a rarity. Transmission can be via hematogenous spread through the umbilical vein, or ingestion or aspiration of infected amniotic fluid. Congenital tuberculosis may simulate bacterial/viral sepsis or other congenital infections such as syphilis and human cytomegalovirus infection [139]	Worldwide

IPTp: Intermittent preventive treatment.

vaccine, 41 are African countries [141] (Table 3). Vaccination is also the most effective measure to reduce the global incidence of hepatitis B. Compared to other health-care interventions, vaccination is, in terms of cost-effectiveness, an economically advantageous option [43]. In 1991, the WHO recommended that all countries introduce a policy of universal hepatitis B vaccination to prevent and control HBV infection. By the end of 2014, 184 countries had included the hepatitis B vaccine in their national immunization programs. Considering that, in highly endemic areas, hepatitis B is most commonly spread from mother to child at birth and the development of chronic infection is very common in these cases, rapid delivery of the first dose of this vaccine soon after birth is essential [142]. A birth dose has been introduced in 96 countries, reaching up to 80% coverage in the Western Pacific but only 10% in African countries [143] (Table 4). Various candidate CMV vaccine trials have also been conducted in the last decade but it is unclear, in light of emerging findings on the epidemiology of congenital CMV, whether a CMV vaccine would provide substantial reductions in morbidity [144,145].

Strategies for prevention of non-vaccine preventable congenital infectious diseases such as CMV, HSV, or toxoplasmosis are not uniform across different countries or even within a country. Measures which involve prenatal education of pregnant or childbearing women, sexual behavior measures to avoid HSV-2 or syphilis in pregnancy, hand washing, filtering water, and veterinary public health interventions such as labeling to indicate toxoplasma-free meat and improved farm hygiene to reduce animal infection may be difficult to manage in resource-constrained countries. In these settings, hygiene is

Table 4. Available vaccines against pathogens potentially causing congenital infections.

Organism	Type of vaccine	Coverage by WHO region	Countries that have introduced vaccine by region		
Rubella	Combination vaccine → MR	140 countries by the end of 2014 (of 194 countries members of WHO) Global coverage 46% in 2014 90% America and Europe <10% Africa [143]	**WHO region** [141]	**Yes**	**No**
			Africa	6	41
			America	35	0
			East Mediterranean	15	6
			Europe	53	0
			South East Asia	6	5
			Western Pacific	*24*	*3*
			Total	**139**	**55**
Hepatitis B	1. Monovalent vaccine → birth dose 2. Pentavalent vaccine → Hepatitis B + DTPa + Hib	184 countries by the end of 2014 (of 194 countries members of WHO) Global coverage of 3 doses 82% in 2014 Birth dose was introduced in 96 countries by 2013 Global coverage 38% Western Pacific 80% Africa 10% [143]	Data not available		
Varicella	1. Monovalent vaccine 2. Combination vaccine → MMR + varicella	A limited number of industrialized countries have introduced varicella vaccination into their childhood immunization programs [146] No data of routine childhood varicella vaccination found in low- and middle-income countries	Data not available		

MR: Measles and rubella; MMR: measles + rubella + mumps.

limited, national health systems and governments are weak and clinical staff, besides scarce, is not always sufficiently qualified or motivated to reinforce behavioral measures.

Prevention of MTCT strategies in those mothers infected during pregnancy remains a challenge in low-income countries. Inadequate disease surveillance in ANC clinics of pregnant women for hepatitis B, toxoplasmosis, HSV-2, or rubella infection poses an important barrier to the control of congenital infections in these settings, where clinical staff is unaware of the real underlying burden of congenital infections and screening tests are not always available. Other potential congenital infections such as CMV or B19V remain neglected because universal screening at the ANC has not yet been established even in high-income countries [27].

Other potential MTCT diseases such as syphilis or HIV, whose screening and treatment policies are better established, have shown moderate-to-high successes in increasing the number of women diagnosed, after successful introduction of point-of-care screening methods [147,148]. It is known that antenatal screening for syphilis is cost beneficial and cost-effective and penicillin (the drug of choice) is effective, cheap, and readily available, as opposed to treating CS, which is expensive [91]. Screening, detecting, and treating pregnant women can also contribute to prevent clinical consequences in these women and their partners. However, given that screening and treatment for preventing MTCT of syphilis is not 100% effective, primary prevention of syphilis in pregnant women is also an important strategy that needs to be addressed to truly eliminate CS.

Unfortunately, CS still occurs due to a serious of reasons, including the fact that antenatal visits are too late to avert an adverse outcome, clinicians may not have offered testing, testing may not have been affordable, women may not have followed up or received their test results, treatment may not have been available, or treated women may have been reinfected by untreated sexual partners. All of these reasons mostly occur in the poorest and most resource-constrained settings [149].

Regarding to vertical transmission of HIV, in 2013, 54% of pregnant women in LMIC did not receive an HIV test, a key step to accessing HIV prevention, treatment, and care [150]. Additionally, in 2014, 73% of all pregnant women living with HIV globally received medicines to prevent transmission to their babies. In the absence of any interventions during these stages, rates of HIV transmission from mother-to-child can range between 15% and 45% [150]. In 2013, the 'Option B +' program was introduced, whereas all HIV-1-infected pregnant and breast-feeding women should begin lifelong antiretroviral therapy regardless of their HIV stage, and it has now become the standard of care, with initiation of treatment recommended as soon as HIV-1 is diagnosed [51]. With improved strategies such as this one for the prevention of MTCT, the number of newly infected infants has decreased by 58% worldwide, from an estimated 520,000 in 2000 to 220,000 in 2014; 41% of this decline occurring between 2010 and 2014 [151]. The implementation of the use of the newer point-of-care rapid syphilis tests could be a highly advantageous approach, as they may allow for same-day treatment and address logistical barriers to testing encountered with standard tests [147]. It is important to highlight, however, that adequate screening may not necessarily increase the proportion of women adequately treated if the required drugs are not readily available. Regional syphilis screening rates in Haiti, Kenya, Mozambique, and the United Republic of Tanzania were increased with introduction of on-site testing but evaluation showed that there were still obstacles to access treatment [91].

Although screening of Chagas is not fully implemented in high-endemic areas, some successful programs of maternal diagnosis and follow-up were conducted in Brazil [152]. In some countries of the Western world, similar programs have been put in place to screen for congenitally transmitted CD among pregnant women coming from highly endemic areas [153].

Finally, in order to control the unabated ZIKV epidemic, the Pan-American Health Organization and the WHO are now recommending aggressive vector control measures to reduce mosquito populations and avoiding bites, which occur mainly during the day [154].

Current recommendations to prevent malaria, another important cause of MTCT infection, in African pregnant women rely on the use of insecticide-treated nets and good compliance with intermittent preventive treatment guidelines [155].

4.2. Challenges in the diagnosis and treatment of congenital and perinatal infections in lower middle and low-income countries

An ideal approach for identification of infected pregnant women would be to screen women during the first trimester with a panel of highly sensitive serological tests for the most common pathogens potentially transmissible to the fetus, and again early in the third trimester. Another optimal approach would be to retest women who are at high risk or from high-prevalence areas closer to delivery as primary infection may occur after initial screening [1–6]. Most of ANC programs in low-income countries do not contemplate the screening for toxoplasma, rubella, HSV, or hepatitis B in pregnant women, which are routinely tested at the ANC of high-income countries [156]. In fact, in rural settings of very poor countries, access to ANC may be severely restricted; reasons ranging from fear of medical care to nonexistence of ANC. In addition, neonatal screening of those congenital infections named previously does not exist in these countries [156]. Congenital infections are neglected because there is no awareness of their burden. Diagnosis of children infected with such congenital infections is not straightforward, and differential diagnosis with other common infections of the neonatal period appears challenging [2].

Focusing on specific diseases, early detection of hearing loss resulting for instance from CMV congenital infections, can limit long-term disabilities; furthermore, PCR-based newborn screening to identify those infected, and thus at risk of *sequelae*, deserves consideration. Abnormal cranial computed tomography (CTX) findings are associated with congenital CMV infection and long-term *sequelae* [157]. However, CTX scans cannot be performed routinely in

limited-resource settings, and infants with congenital CMV infection presenting with central nervous system disorders may be more likely to remain undiagnosed. However, it would be premature to consider newborn CMV screening in resource-poor settings because the disease burden from congenital CMV and the cost/benefit ratio of long-term follow-up have not been well defined. In addition, the cost and the competing health priorities for these settings make it difficult to envision such a screening program. On the other hand, recommended treatment for congenital CMV is seldom available in low-income countries. Regarding rubella and measles, certain initiatives, such as the WHO Global Measles Rubella Laboratory Network created by The Measles and Rubella Initiative, support the elimination of measles and rubella through the introduction of high-quality laboratory testing of suspected measles and rubella cases [158]. However, conditions for specimen collection, processing, and testing can be suboptimal in low-income countries and cause laboratory results to be less accurate.

Although national and international guidelines are more uniform in order to prevent other MTCT diseases such as HIV, the access to diagnosis of HIV in infants is available to a very limited number of children in need, with only 15% of exposed infants in LMIC receiving a virological test [159]. According to the updated WHO guidelines, HAART should be initiated among all children living with HIV, regardless of WHO clinical stage or at any CD4 cell count [51]. Of the estimated 600,000 children who require HAART in sub-Saharan Africa, less than 5% are receiving therapy. Without treatment, about one-third of children living with HIV die by their first birthday, and half die by their second [114].

In order to approach other neglected congenital infections such as Chagas, a consensus has been established on the control strategy [160]. Since congenital infection with *T. cruzi* is mostly asymptomatic but may progress to severe chronic CD later in life and an effective treatment is available within the first year of life (benznidazol or nifurtimox), its early diagnosis is of upmost importance [121]. Conventional tests, such as ELISA, indirect immunofluorescence, and indirect hemagglutination, are available for diagnosis but its performance depends on good-quality testing kits and good laboratory practice [121].

4.3. The impact of HIV in control of congenital and perinatal infections

Perinatal HIV transmission rates have declined in industrialized countries after the introduction of HAART and elective caesarean sections [161]. However, sub-Saharan Africa remains the region of the world most heavily affected by HIV-1; more than 90% of all children who acquired HIV-1 in 2011 are residing in this region [162].

As described above, studies have suggested that perinatal HIV transmission is more frequent among newborns with congenital CMV infection [12] and among HSV-2-seropositive women [11]. In addition, MTCT of other viruses highly endemic in sub-Saharan Africa, such as HBV, may be increased in children born of HIV-infected women [163].

Although WHO recommends that countries should ensure the provision of the Option B+ to all HIV-infected pregnant women, many countries in sub-Saharan Africa are still only able to offer single-dose nevirapine to prevent HIV perinatal transmission [164]. Measures to strengthen national and international policies should be established, especially in areas where treatment of all HIV-infected pregnant women is not the standard of care. The benefits of treatment may be multiple: reduction of MTCT of HIV and decreasing the incidence of congenital infections such as CMV or HSV-2. Strategies such as the birth dose of hepatitis B vaccine [142] or acyclovir treatment to prevent neonatal herpes [165] should be considered in highly endemic countries for HIV, HBV, and HSV.

5. Global strategies to reduce congenital and perinatal infections

5.1. Ongoing strategies to reduce congenital and perinatal infections

Global strategies to reduce congenital and perinatal infections are urgently needed. International institutions, such as the WHO, the Global Alliance for Vaccine and Immunization, the Measles and Rubella Initiative, and other organizations are working together to control and prevent some of these infections (Table 5).

6. Five-year view: strategies and goals to tackle a major public health issue: congenital and perinatal infections in lower middle and low-income countries

The way forward in the following years should focus on the necessary efforts to reduce congenital infections in high-risk populations. At the international level, prevention of MTCT of infections should be made a health system priority, and links with international groups resulting in focused and coordinated international effort should be encouraged, mainly in highly endemic countries.

6.1. Global strategies

6.1.1. Access to ANC with emphasis on early access

Access to ANC in resource-constrained countries is highly variable but may be jeopardized in many settings because of fears of medical care to nonexistence of ANC programs. The first step to reduce congenital infections in these settings is ensuring a universal access to ANC while reinforcing the control of diseases already included and enlarging the screening to other prevalent diseases such as syphilis or HBV. In addition, strengthening health national systems and education of population would also be a main priority.

6.1.2. Surveillance and screening ANC

Monitoring of diseases using effective surveillance tools should be conducted both at the ANC and in postnatal encounters with the newborn, such as for instance, during the first vaccination scheduled as part of the expanded program on immunization. Congenital infections can be prevented by an early detection of infection in pregnant

Table 5. Strategies to reduce congenital infections currently ongoing.

Disease	Strategies to fight against the disease	Goal	Institutions involved	Region
Rubella [158]	1. Achieve and maintain high levels of population immunity by providing high vaccination coverage, including mass immunization of adolescent and young adults (male and females) as carried out in the Americas [166] 2. Monitor disease using effective surveillance and evaluate programmatic efforts to ensure progress → Measles and Rubella Laboratory Network 3. Develop and maintain outbreak preparedness, respond rapidly to outbreaks, and manage cases 4. Communicate and engage to build public confidence and demand for immunization 5. Perform research and development needed to support cost-effective operations and improve vaccination and diagnostic tools	To achieve rubella elimination in at least 5 (of 6) WHO regions by the end of 2020	**WHO** **MRI** **GAVI**	Worldwide
CS [167]	1. Ensure advocacy-sustained political commitment to achieving goal of elimination CS 2. Increases access to, and quality of, maternal and newborn health services ensuring that all pregnant women are adequately screened and treated and decrease the frequency of missed opportunities for screening women outside maternal and newborn care 3. Use of diagnostic test that are effective, affordable, and require minimal logistical support, with effective management of all infected women and their partners and the treatment of infants born to seropositive mothers 4. Establish surveillance, monitoring, and evaluation systems (improving surveillance systems, developing indicators, and strengthening monitoring and evaluation systems)	To achieve CS elimination	**WHO**	Worldwide
Hepatitis B [142]	Introducing hepatitis B vaccine (3 doses) including birth dose into national immunization services	Controlling HBV worldwide to decrease the incidence of HVB-related chronic liver disease and hepatocellular carcinoma, more likely become in person infected during infancy	**WHO** **GAVI**	Worldwide
Congenital toxoplasmosis [168,169]	Maternal Screening Program Neonatal Screening Program	To reduce the rate of vertical transmission and/or fetal development impairment To reduce sequelae in infected newborn	**National health systems**	Some countries in Europe (Austria, France, Slovenia, Germany, Italy, Spain, Belgium) and some states of Brazil Poland, Denmark, some states of the United States, and some states of Brazil

WHO: World Health Organization; GAVI: Global Alliance for Vaccination and Immunization; MRI: The Measles and Rubella Initiative; CS: congenital syphilis.

women. Programs promoting safe sex or control of STIs, hygiene, nutritional and educational measures will prevent maternal infection, but if women become infected, only proactive screening programs can detect and prevent the deleterious effects of maternal infections on the fetus. A minimal requirement would be that all women should undergo one screening test in their early pregnancy and if this does not happen, they should be tested at delivery. Hence, these programs must be implemented during ANC [170].

6.1.3. Control of STIs

Since the incidence of some congenital infections such as CS, neonatal herpes, HIV, or hepatitis B is directly related to the prevalence of STIs in the population, strategies aiming to reduce the burden of congenital infections should be complemented by adequate programs to prevent, control, and treat STIs.

6.1.4. Surveillance in neonates

Improving the early detection and diagnosis of congenital infections should be a priority if an impact on their associated morbidity and mortality is to be achieved. Additionally, better estimates of the global burden of congenital infections in developing countries are urgently required, so as to highlight the impact that such infections play in the global health scenario. Better training of clinical staff in the diagnosis and management of children with suspected congenital infection, together with enhanced awareness from the general

population regarding the burden and risks of congenital infections in their environment, should lead to the adoption of better management and preventive strategies.

6.1.5. Diagnosis

There is a need to ensure a wide availability of diagnostic tests for diseases such as HIV, syphilis, or HBV, for which there are rapid and well-functioning diagnostic tests [147,148]. Reference laboratories for each region (sentinel laboratory) should be established to offer diagnostic possibilities for diseases, which do not yet have readily accessible or cheap diagnostic schemes.

6.1.6. Treatment

The disassociation between testing and treatment administration observed in HIV and syphilis screening programs can be solved using on-site testing in ANC and allowing early receipt of reports leading to earlier treatment of mothers attending clinics late in their pregnancy. Treatment also should be available at the peripheral health post level.

6.1.7. Vaccines

For those vaccine-preventable congenital infections, it appears critical to achieve and maintain high levels of population immunity by providing high vaccination coverage. Since vaccines are supposed to be the most effective measure to prevent infections, endeavors to ensure rubella and birth dose of hepatitis B vaccines introduction in child immunization national programs should be encouraged.

7. Expert commentary

Although enormous efforts are being made at the international and national levels to reduce the burden of some congenital infections, these infections remain far from a public health priority. The recent ZIKV outbreak and its possible association with microcephaly in children born of women infected during pregnancy has generated worldwide alarm and has highlighted the lack of global awareness regarding the impact of infections transmitted from mother to child. It therefore appears essential to adequately describe the global and local burden of each perinatally transmitted infection, through the improvement of maternal and neonatal morbidity surveillance systems at the ANC and child services. Ensuring early diagnosis, treatment, and vaccination (when available) as the key preventive strategies is also of paramount importance. In order to achieve an effective surveillance, clinical staff must be trained to suspect cases both in pregnant women and their children, so that reporting of cases becomes routine. More effective diagnostic tests, for which more research is urgently needed, could contribute to achieve this goal. Reference laboratories need to be established, where specimens of suspected cases can be sent, ensuring reliable results and rapid feedback. The measles and rubella laboratory network is a successful example of these reference laboratories [158]. For prompt receipt of results, tested women must return to clinic, or there must be a notification system in place; but unfortunately, neither of these generally occurs.

As vaccines stand as the best method to reduce rubella and hepatitis B infections, campaigns to introduce them in pediatric immunization programs must be a high priority in places which have not yet done so. Currently, a single manufacturer of measles–rubella vaccine (MRV) exists [158]. Many countries have introduced only measles-containing vaccines (MCV) and rely on donors to pay for theses MCV vaccines. Costs to ensure MRV introduction should be ensured by national and international institutions. Research should also focus in the development of new vaccines against other common and devastating congenital infections, such as for instance, CMV and toxoplasmosis. Finally, national governments and international institutions should engage in a collaborative manner to successfully achieve all of these goals.

Key issues

- The TORCH complex typically comprises Toxoplasmosis, Rubella, CMV, HSV and other pathogens including Treponema pallidum (which causes syphilis), and other viruses (HIV, HBV, VZV, parvovirus B19).
- Mother to child transmitted infections remain neglected worldwide and especially in low-income countries where cases of children infected are underreported.
- No specific estimates regarding the global burden of congenital infections and their impact on disability and/or neonatal cause-of-death exist.
- Great burden of congenital disease is assumed in low-income countries where co-infection with HIV or other maternal conditions as malnutrition, malaria or poor hygiene may favour MTCT.
- Vertical transmission of some viruses such as CMV, rubella or HSV may be devastating for the infant but most of these cases are not diagnosed.
- Screening of some of infections such as HIV, syphilis or HBV can be easily done at the ANC level and ensuring early diagnosis and treatment (when available) is the key preventive strategy.
- Effective surveillance can be improved, strengthening local health systems and training clinical staff.Global endeavors are needed to introduce and ensure anti-rubella and anti-HBV vaccines at EPI worldwide and research should be focus to develop new vaccines to avoid other congenital infections.
- Global strategies should be established in order to reduce the burden of congenital infections worldwide and especially in low-income countries.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Shet A. Congenital and perinatal infections: throwing new light with an old TORCH. Indian J Pediatr. 2011;78(1):88–95.
2. Neu N, Duchon J, Zachariah P. TORCH infections. Clin Perinatol. 2015;42(1):77–103, viii.
• **This article is of considerable interest because it constitutes one of the most updated reviews on TORCH infections.**
3. Nahmias A, Walls KW, Stewart J, et al. The ToRCH complex-perinatal infections associated with toxoplasma and rubella, cytomegol-and herpes simplex viruses. Ped Research. 1971;5:405–406.
4. Kinney JS, Kumar ML. Should we expand the TORCH complex? A description of clinical and diagnostic aspects of selected old and new agents. Clin Perinatol. 1988;15(4):727–744.
5. Del Pizzo J. Focus on diagnosis: congenital infections (TORCH). Pediatr Rev. 2011;32(12):537–542.
6. Adams Waldorf KM, McAdams RM. Influence of infection during pregnancy on fetal development. Reproduction. 2013;146(5):R151–R162.
7. Ford-Jones EL, Kellner JD. 'Cheap torches': an acronym for congenital and perinatal infections. Pediatr Infect Dis J. 1995;14(7):638–640.
8. Oza S, Lawn JE, Hogan DR, et al. Neonatal cause-of-death estimates for the early and late neonatal periods for 194 countries: 2000–2013. Bull World Health Organ. 2015;93(1):19–28.
•• **This article provides global estimates of causes of neonatal mortality.**
9. Williams EJ, Embleton ND, Clark JE, et al. Viral infections: contributions to late fetal death, stillbirth, and infant death. J Pediatr. 2013;163(2):424–428.
10. Gumbo H, Chasekwa B, Church JA, et al. Congenital and postnatal CMV and EBV acquisition in HIV-infected Zimbabwean infants. PLoS One. 2014;9(12):e114870.
11. Bollen LJ, Whitehead SJ, Mock PA, et al. Maternal herpes simplex virus type 2 coinfection increases the risk of perinatal HIV transmission: possibility to further decrease transmission? AIDS. 2008;22(10):1169–1176.
12. Hoffmann CJ, Thio CL. Clinical implications of HIV and hepatitis B co-infection in Asia and Africa. Lancet Infect Dis. 2007;7(6):402–409.
13. Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet. 2016;387:587–603.
14. Menson E, Lyall H. Clinical presentation of congenital viral infections. Current Paediatrics. 2005;15(2):163–170.
15. Kravetz J. Congenital toxoplasmosis. BMJ Clin Evid. 2013;2013:1–6.
16. Cortina-Borja M, Tan HK, Wallon M. Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. PLoS Med. 2010;7(10):e1000351.
17. Wallon M, Peyron F, Cornu C, et al. Congenital toxoplasma infection: monthly prenatal screening decreases transmission rate and improves clinical outcome at age 3 years. Clin Infect Dis. 2013;56(9):1223–1231.
18. Lambert N, Strebel P, Orenstein W, et al. Rubella. Lancet. 2015;385(9984):2297–2307.
19. (CDC) CfDCaP. Vaccine and immunizations: congenital rubella syndrome. MMWR Morb Mortal Wkly Rep. 2009;2014:983–986.
20. Best JM. Rubella. Semin Fetal Neonatal Med. 2007;12(3):182–192.
21. Banatvala JE, Brown DW. Rubella. Lancet. 2004;363(9415):1127–1137.
22. Kremer JR, Schneider F, Muller CP. Waning antibodies in measles and rubella vaccinees–a longitudinal study. Vaccine. 2006;24(14):2594–2601.
23. Boppana SB, Pass RF, Britt WJ, et al. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr Infect Dis J. 1992;11(2):93–99.
24. Baquero-Artigao F. [Consensus document from the Spanish Society of Paediatric Infectious Diseases (SEIP) on the diagnosis and treatment of congenital cytomegalovirus infection]. An Pediatr (Barc). 2009;71(6):535–547.
25. Kimberlin DW, Jester PM, Sanchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. N Engl J Med. 2015;372(10):933–943.
26. Mareri A, Lasorella S, Iapadre G, et al. Antiviral therapy for congenital cytomegalovirus infection: pharmacokinetics, efficacy and side effects. J Matern Fetal Neonatal Med. 2016;29(10):1657–1664.
27. Wang C, Zhang X, Bialek S, et al. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis. 2011;52(2):e11–e13.
28. Mussi-Pinhata MM, Yamamoto AY, do Carmo Rego MA, et al. Perinatal or early-postnatal cytomegalovirus infection in preterm infants under 34 weeks gestation born to CMV-seropositive mothers within a high-seroprevalence population. J Pediatr. 2004;145(5):685–688.
29. Adler SP, Finney JW, Manganello AM, et al. Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. Pediatr Infect Dis J. 1996;15(3):240–246.
30. Nigro G, Adler SP. Hyperimmunoglobulin for prevention of congenital cytomegalovirus disease. Clin Infect Dis. 2013;57 Suppl 4:S193–S195.
31. Brown Z. Preventing herpes simplex virus transmission to the neonate. Herpes. 2004;11 Suppl 3:175A–186A.
32. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. N Engl J Med. 2009;361(14):1376–1385.
33. Organization WH. Guidelines for sexually transmitted infections surveillance. Geneve: WHO; 1999.
34. Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep. 2015;64(RR-03):1–137.
35. Kuznik A, Muhumuza C, Komakech H, et al. Antenatal syphilis screening using point-of-care testing in low- and middle-income countries in Asia and latin america: a cost-effectiveness analysis. PLoS One. 2015;10(5):e0127379.
36. Watson-Jones D, Gumodoka B, Weiss H, et al. Syphilis in pregnancy in Tanzania. II. The effectiveness of antenatal syphilis screening and single-dose benzathine penicillin treatment for the prevention of adverse pregnancy outcomes. J Infect Dis. 2002;186(7):948–957.
37. Lamont RF, Sobel JD, Carrington D, et al. Varicella zoster virus (chickenpox) infection in pregnancy. BJOG. 2011;118(10):1155–1162.
38. Marin M, Willis ED, Marko A, et al. Closure of varicella-zoster virus-containing vaccines pregnancy registry - United States, 2013. MMWR Morb Mortal Wkly Rep. 2013;63(33):732–733.
39. Gentile I, Borgia G. Vertical transmission of hepatitis B virus: challenges and solutions. Int J Womens Health. 2014;6:605–611.
40. Schillie S, Walker T, Veselsky S, et al. Outcomes of infants born to women infected with hepatitis B. Pediatrics. 2015;135(5):e1141–e1147.
41. Sokal EM, Roberts EA, Mieli-Vergani G, et al. A dose ranging study of the pharmacokinetics, safety, and preliminary efficacy of lamivudine in children and adolescents with chronic hepatitis B. Antimicrob Agents Chemother. 2000;44(3):590–597.
42. Zhang H, Pan CQ, Pang Q, et al. Telbivudine or lamivudine use in late pregnancy safely reduces perinatal transmission of hepatitis B virus in real-life practice. Hepatology. 2014;60(2):468–476.
43. Kang G, Ma F, Chen H, et al. Efficacy of antigen dosage on the hepatitis B vaccine response in infants born to hepatitis B-uninfected and hepatitis B-infected mothers. Vaccine. 2015;33:4093–4099.
44. Jhaveri R, Swamy GK. Hepatitis C virus in pregnancy and early childhood: current understanding and knowledge deficits. J Pediatr Infect Dis Soc. 2014;3 Suppl 1:S13–S18.
45. Mack CL, Gonzalez-Peralta RP, Gupta N, et al. NASPGHAN practice guidelines: diagnosis and management of hepatitis C infection in infants, children, and adolescents. J Pediatr Gastroenterol Nutr. 2012;54(6):838–855.

46. Young NS, Brown KE. Parvovirus B19. N Engl J Med. 2004;350(6):586–597.
47. de Haan TR, van den Akker ES, Porcelijn L, et al. Thrombocytopenia in hydropic fetuses with parvovirus B19 infection: incidence, treatment and correlation with fetal B19 viral load. BJOG. 2008;115(1):76–81.
48. Fairley CK, Smoleniec JS, Caul OE, et al. Observational study of effect of intrauterine transfusions on outcome of fetal hydrops after parvovirus B19 infection. Lancet. 1995;346(8986):1335–1337.
49. Branson BM, Handsfield HH, Lampe MA, et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. MMWR Recomm Rep. 2006;55(RR-14):1–17; quiz CE1–14.
50. WHO. WHO recommendations on the diagnosis of HIV infection in infants and children. In: Strengthening health services to fight HIV/AIDS. Geneve: programme, HA; 2010.
51. WHO. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Geneva: WHO Guidelines Approved by the Guidelines Review Committee; 2015.
52. UNICEF. Options b and b+ key considerations for countries to implement an equity focused approach. UNICEF; 2012.
53. Kornai J. Resource-constrained versus demand-constrained systems. Econometrica. 1979;47(4):801–819.
54. Torgerson PR, Mastroiacovo P The global burden of congenital toxoplasmosis: a systematic review. Bull World Health Organ. 2013;91(7):501–508.
• **This article is considered of interest because it provides estimates on the global burden of three congenital infections for which scarce data are available, namely congenital toxoplasmosis, congenital rubella syndrome, and syphilis.**
55. Dubey JP, Lago EG, Gennari SM, et al. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology. 2012;139(11):1375–1424.
56. Thiebaut R, Leproust S, Chene G, et al. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. Lancet. 2007;369(9556):115–122.
57. Jones JL, Kruszon-Moran D, Rivera HN, et al. Toxoplasma gondii seroprevalence in the United States 2009-2010 and comparison with the past two decades. Am J Trop Med Hyg. 2014;90(6):1135–1139.
58. Gilbert RE, Freeman K, Lago EG, et al. Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl Trop Dis. 2008;2(8):e277.
59. WHO. Rubella vaccines: WHO position paper. Wkly Epidemiol Rec. 2011;86(29):301–316.
60. Cutts FT, Robertson SE, Diaz-Ortega JL, et al. Control of rubella and congenital rubella syndrome (CRS) in developing countries, Part 1: burden of disease from CRS. Bull World Health Organ. 1997;75(1):55–68.
• **This article is considered of interest because it provides estimates on the global burden of three congenital infections for which scarce data are available, namely congenital toxoplasmosis, congenital rubella syndrome, and syphilis.**
61. (CDC) CfDCaP. Rubella and congenital rubella syndrome control and elimination - global progress, 2000-2012. MMWR Morb Mortal Wkly Rep. 2013;62(48):983–986.
62. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol. 2007;17(5):355–363.
63. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17(4):253–276.
64. Zhang XW, Li F, Yu XW, et al. Physical and intellectual development in children with asymptomatic congenital cytomegalovirus infection: a longitudinal cohort study in Qinba mountain area, China. J Clin Virol. 2007;40(3):180–185.
65. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol. 1991;44(5):366–369.
66. Waters A, Jennings K, Fitzpatrick E, et al. Incidence of congenital cytomegalovirus infection in Ireland: implications for screening and diagnosis. J Clin Virol. 2014;59(3):156–160.
67. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatr Infect Dis J. 2011;30(12):1043–1046.
68. Mussi-Pinhata MM, Yamamoto AY, Moura Brito RM, et al. Birth prevalence and natural history of congenital cytomegalovirus infection in a highly seroimmune population. Clin Infect Dis. 2009;49(4):522–528.
69. Duryea EL, Sanchez PJ, Sheffield JS, et al. Maternal human immunodeficiency virus infection and congenital transmission of cytomegalovirus. Pediatr Infect Dis J. 2010;29(10):915–918.
70. Guilbert G, Warszawski J, Le Chenadec J et al. Decreased risk of congenital cytomegalovirus infection in children born to HIV-1-infected mothers in the era of highly active antiretroviral therapy. Clin Infect Dis. 2009;48(11):1516–1525.
•• **This article describes how MTCT of CMV can occur even in women CMV infected before pregnancy.**
71. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med. 1999;341(2):77–84.
72. Mussi-Pinhata MM, Yamamoto AY, Figueiredo LT, et al. Congenital and perinatal cytomegalovirus infection in infants born to mothers infected with human immunodeficiency virus. J Pediatr. 1998;132(2):285–290.
73. Manicklal S, van Niekerk AM, Kroon SM, et al. Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. Clin Infect Dis. 2014;58(10):1467–1472.
74. Nigro G, Krzysztofiak A, Gattinara GC, et al. Rapid progression of HIV disease in children with cytomegalovirus DNAemia. AIDS. 1996;10(10):1127–1133.
75. Schleiss MR, Schleiss MR. HIV and cytomegalovirus co-infection in congenitally infected children: copathogens fanning each other's flames? AIDS. 2009;23(16):2215–2217.
76. Gonzalez R, Augusto OJ, Munguambe K et al. HIV Incidence and Spatial Clustering in a Rural Area of Southern Mozambique. PLoS One. 2015;10(7):e0132053.
• **This article describes the results of a multicenter clinical trial assessing the efficacy of intermittent preventive treatment for malaria in HIV women.**
77. Ciaranello AL, Perez F, Maruva M, et al. WHO 2010 guidelines for prevention of mother-to-child HIV transmission in Zimbabwe: modeling clinical outcomes in infants and mothers. PLoS One. 2011;6(6):e20224.
78. Mwaanza N, Chilukutu L, Tembo J, et al. High rates of congenital cytomegalovirus infection linked with maternal HIV infection among neonatal admissions at a large referral center in sub-Saharan Africa. Clin Infect Dis. 2014;58(5):728–735.
79. Kropp RY, Wong T, Cormier L, et al. Neonatal herpes simplex virus infections in Canada: results of a 3-year national prospective study. Pediatrics. 2006;117(6):1955–1962.
80. Looker KJ, Garnett GP, Schmid GP. An estimate of the global prevalence and incidence of herpes simplex virus type 2 infection. Bull World Health Organ. 2008;86(10):805–812, A.
81. Yahya-Malima KI, Evjen-Olsen B, Matee MI, et al. HIV-1, HSV-2 and syphilis among pregnant women in a rural area of Tanzania: prevalence and risk factors. BMC Infect Dis. 2008;8:75.
82. Brown ZA, Wald A, Morrow RA, et al. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. JAMA. 2003;289(2):203–209.
83. Pinninti SG, Kimberlin DW. Preventing herpes simplex virus in the newborn. Clin Perinatol. 2014;41(4):945–955.
84. del Mar Pujades Rodriguez M, Obasi A, Mosha F, et al. Herpes simplex virus type 2 infection increases HIV incidence: a prospective study in rural Tanzania. AIDS. 2002;16(3):451–462.

85. Obasi A, Mosha F, Quigley M, et al. Antibody to herpes simplex virus type 2 as a marker of sexual risk behavior in rural Tanzania. J Infect Dis. 1999;179(1):16–24.
86. (CDC) CfDCaP. Seroprevalence of herpes simplex virus type 2 among persons aged 14-49 years–United States, 2005–2008. MMWR Morb Mortal Wkly Rep. 2010;59(15):456–459.
87. Pebody RG, Andrews N, Brown D, et al. The seroepidemiology of herpes simplex virus type 1 and 2 in Europe. Sex Transm Infect. 2004;80(3):185–191.
88. Wald A, Link K. Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis. J Infect Dis. 2002;185(1):45–52.
89. Strick LB, Wald A, Celum C. Management of herpes simplex virus type 2 infection in HIV type 1-infected persons. Clin Infect Dis. 2006;43(3):347–356.
90. Newman L, Kamb M, Hawkes S et al. Global estimates of syphilis in pregnancy and associated adverse outcomes: analysis of multinational antenatal surveillance data. PLoS Med. 2013;10(2):e1001396.
    - **This article is considered of interest because it provides estimates on the global burden of three congenital infections for which scarce data are available, namely congenital toxoplasmosis, congenital rubella syndrome, and syphilis.**
91. WHO. The global elimination of congenital syphilis: rationale and strategy. Geneva: World Health Organization; 2007.
92. Schulz KF, Cates W Jr., O'Mara PR. Pregnancy loss, infant death, and suffering: legacy of syphilis and gonorrhoea in Africa. Genitourin Med. 1987;63(5):320–325.
93. Ham DC, Lin C, Newman L, et al. Improving global estimates of syphilis in pregnancy by diagnostic test type: a systematic review and meta-analysis. Int J Gynaecol Obstet. 2015;130 Suppl 1:S10–S14.
94. Saloojee H, Velaphi S, Goga Y, et al. The prevention and management of congenital syphilis: an overview and recommendations. Bull World Health Organ. 2004;82(6):424–430.
95. Tan MP, Koren G. Chickenpox in pregnancy: revisited. Reprod Toxicol. 2006;21(4):410–420.
96. Talukder YS, Kafatos G, Pinot de Moira A, et al. The seroepidemiology of varicella zoster virus among pregnant Bangladeshi and white British women in the London Borough of Tower Hamlets, UK. Epidemiol Infect. 2007;135(8):1344–1353.
97. Pembrey L, Raynor P, Griffiths P, et al. Seroprevalence of cytomegalovirus, Epstein Barr virus and varicella zoster virus among pregnant women in Bradford: a cohort study. PLoS One. 2013;8(11):e81881.
98. WHO. Hepatitis B. Surveillance, DoCD, Response. Geneve; 2015. Available from: http://www.who.int/mediacentre/factsheets/fs204/en/
99. Chang MH. Hepatitis B virus infection. Semin Fetal Neonatal Med. 2007;12(3):160–167.
100. Okeke TC, Obi SN, Okezie OA, et al. Coinfection with hepatitis B and C viruses among HIV positive pregnant women in Enugu south east, Nigeria. Niger J Med. 2012;21(1):57–60.
101. Kourtis AP, Bulterys M, Hu DJ, et al. HIV–HBV coinfection — a global challenge. N Engl J Med. 2012;366(19):1749–1752.
102. Schweitzer A, Horn J, Mikolajczyk RT, et al. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet. 2015;386(10003):1546–1555.
103. WHO. Progress towards meeting the 2012 hepatitis B control milestone: WHO Western Pacific Region, 2011. Wkly Epidemiol Rec. 2011;86(19):180–188.
104. El-Magrahe H, Furarah AR, El-Figih K, et al. Maternal and neonatal seroprevalence of Hepatitis B surface antigen (HBsAg) in Tripoli, Libya. J Infect Dev Ctries. 2010;4(3):168–170.
105. Candotti D, Danso K, Allain JP. Maternofetal transmission of hepatitis B virus genotype E in Ghana, west Africa. J Gen Virol. 2007;88(Pt 10):2686–2695.
106. Franco E, Bagnato B, Marino MG, et al. Hepatitis B: Epidemiology and prevention in developing countries. World J Hepatol. 2012;4(3):74–80.
107. Basnayake SK, Easterbrook PJ. Wide variation in estimates of global prevalence and burden and death estimates from chronic HBV, HCV and coinfection with HIV in literature. J Viral Hepat. 2016;23:545–559.
108. Uhanova J, Tate RB, Tataryn DJ, et al. A population-based study of the epidemiology of hepatitis C in a North American population. J Hepatol. 2012;57(4):736–742.
109. Platt L, Easterbrook P, Gower E, et al. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. Lancet Infect Dis. 2016;16:797–808.
110. Benova L, Mohamoud YA, Calvert C, et al. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. Clin Infect Dis. 2014;59(6):765–773.
111. El-Shabrawi MH, Kamal NM. Burden of pediatric hepatitis C. World J Gastroenterol. 2013;19(44):7880–7888.
112. Gratacos E, Torres PJ, Vidal J, et al. The incidence of human parvovirus B19 infection during pregnancy and its impact on perinatal outcome. J Infect Dis. 1995;171(5):1360–1363.
113. UNAIDS. AIDS by the numbers. Geneva: UNAIDS; 2015.
114. UNAIDS. Children and pregnant women living with HIV. Geneva: UNAIDS; 2014.
115. Collaborators GMaCoD. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117–171.
116. Bern C. Antitrypanosomal therapy for chronic Chagas' disease. N Engl J Med. 2011;364(26):2527–2534.
117. WHO. Chagas disease in Latin America: an epidemiological update based on 2010 estimates. Wkly Epidemiol Rec. 2015;90(6):33–43.
118. Rassi A Jr., Rassi A, Marin-Neto JA. Chagas disease. Lancet. 2010;375(9723):1388–1402.
119. Stanaway JD, Roth G. The burden of Chagas disease: estimates and challenges. Glob Heart. 2015;10(3):139–144.
120. OMS/OPS. Estimación cuantitativa de la enfermedad de Chagas en las Américas. Montevideo: Salud, OMdlSaOPdl; 2006.
121. Carlier Y, Sosa-Estani S, Luquetti AO, et al. Congenital Chagas disease: an update. Mem Inst Oswaldo Cruz. 2015;110(3):363–368.
122. Wilder-Smith A, Schwartz E. Dengue in travelers. N Engl J Med. 2005;353(9):924–932.
123. WHO. Dengue: guidelines for diagnosis, treatment, prevention and control: new edition. Geneva: WHO; 2009.
124. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–507.
125. Tan PC, Rajasingam G, Devi S, et al. Dengue infection in pregnancy: prevalence, vertical transmission, and pregnancy outcome. Obstet Gynecol. 2008;111(5):1111–1117.
126. Pouliot SH, Xiong X, Harville E, et al. Maternal dengue and pregnancy outcomes: a systematic review. Obstet Gynecol Surv. 2010;65(2):107–118.
127. Anderson KB, Thomas SJ, Endy TP. The emergence of Zika virus: a narrative review. Ann Intern Med. 2016. [Epub ahead of print].
128. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. Emerg Infect Dis. 2015;21(10):1885–1886.
129. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008;14(8):1232–1239.
130. Oehler E, Watrin L, Larre P, et al. Zika virus infection complicated by Guillain-Barre syndrome–case report, French Polynesia, December 2013. Euro Surveill. 2014;19(9):20720.
131. Faria NR, Azevedo Rdo S, Kraemer MU, et al. Zika virus in the Americas: early epidemiological and genetic findings. Science. 2016;352(6283):345–349.
132. Schuler-Faccini L, Ribeiro EM, Feitosa IM, et al. Possible association between Zika virus infection and microcephaly - Brazil, 2015. MMWR Morb Mortal Wkly Rep. 2016;65(3):59–62.
133. PAHO/WHO. epidemiological update: neurological syndrome, congenital anomalies and Zika virus infection. Washington (DC): Organisation, PAHOWH; 2016.
134. Sikka V, Chattu VK, Popli RK, et al. The emergence of Zika virus as a global health security threat: a review and a consensus statement

ARTICLE 5

Congenital cytomegalovirus, parvovirus and enterovirus infection in Mozambican newborns at birth: a cross-sectional survey

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Congenital cytomegalovirus, parvovirus and enterovirus infection in Mozambican newborns at birth: a cross-sectional survey

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ABSTRACT

Background: Congenital cytomegalovirus (cCMV) infection is the most prevalent congenital infections acquired worldwide, with higher incidence in developing countries and among HIV-exposed children. Less is known regarding vertical transmission of parvovirus B19 (B19V) and enteroviruses (EV). We aimed to assess the prevalence of CMV, B19V and EV vertical transmission and compare results of screening of congenital CMV obtained from two different specimens in a semirural Mozambican maternity.

Methods: A cross sectional study was conducted among pregnant mothers attending Manhiça District Hospital upon delivery. Information on maternal risk factors was ascertained. Dried umbilical cord (DUC) samples were collected in filter paper for CMV, B19V and EV detection by real-time polymerase chain reaction (RT-PCR), and nasopharyngeal aspirates (NPA) to test for CMV by RT-PCR. Maternal blood samples and placental biopsy samples were also obtained to investigate CMV maternal serology, HIV status and immunopathology.

Results: From September 2014 to January 2015, 118 mothers/newborn pairs were recruited. Prevalence of maternal HIV infection was 31.4% (37/118). CMV RT-PCR was positive in 3/115 (2.6%) of DUC samples and in 3/96 (6.3%) of NPA valid samples obtained from neonates. The concordance of the RT-PCR assay through DUC with their correspondent through NPA was moderate (Kappa = 0.42 and $p<0.001$. No differences on cCMV prevalence were found among HIV-exposed and unexposed. All (100%) mothers were seropositive for CMV IgG. RT-PCR of EV and B19V in DUC were both negative in all screened cases. No histological specific findings were found in placental tissues. No risk factors associated to vertical transmission of these viral infections were found.

Conclusions: This study indicates the significant occurrence of vertical transmission of CMV in southern Mozambique. Larger studies are needed to evaluate the true burden, clinical relevance and consequences of congenital infections with such pathogens in resource-constrained settings.

BACKGROUND

Despite the impressive reduction in child mortality in the last decades, neonatal mortality has declined more slowly and now accounts for nearly half (45%) of all under-5 child deaths[1]. Congenital and perinatal infections are well-known causes of neonatal morbidity and mortality and stillbirths in high-income countries (HIC)[2-4]. However, estimates of the global burden of congenital infections and attributable stillbirths, neonatal disease, disability or deaths due to mother-to-child transmission (MTCT) of these infections in low-income countries (LIC) are limited on account of the generalized scarcity of data[5]. Despite the positive impact in terms of health outcomes shown by the introduction of screening and treatment policies for several pregnancy-relevant infections such as human immunodeficiency virus (HIV), syphilis or malaria[6, 7]; pathogens that may also be vertically transmitted beyond these infections are rarely the focus of clinical practice and research[8, 9].

Several viruses, including among others cytomegalovirus (CMV), parvovirus B19V (B19V) and enterovirus (EV), may cause mild and self-limiting clinical manifestations among infected pregnant women, but more severe or even life-threatening disease in their offsprings. The Zika virus epidemic of 2016 in Latin America has contributed to highlight the emerging threat that maternal viral infections may carry for the health of the foetus and newborn[10].

Despite being the most prevalent congenital infection worldwide, congenital CMV infection (cCMV) it remains largely neglected in the developed and developing world[11]. Although the global prevalence of cCMV has been reported to vary from approximately 0.2% to 2% (mean 0.65%), most of these studies have been conducted in high-income regions of Europe, USA or Japan were prevalence of cCMV ranges between 0.6-0.7%[12-14]. Data from LIC varies substantially, with some estimates peaking at 6–14%[15-17]. Higher overall rates of cCMV are found in countries with higher maternal CMV seroprevalence[12, 13, 18] and among infants exposed to HIV during pregnancy. Indeed, maternal HIV infection is thought to significantly increase (from 2.3% to 10.3%) the prevalence of cCMV in those HIV-exposed infants compared to those born to HIV-negative mothers [19-23]. Furthermore, CMV infection seems to play a role as a cofactor for HIV disease progression in HIV/CMV co-infected newborns[24]. Different methods have been evaluated for cCMV screening based on saliva, urine and blood specimens, being saliva and urine the generally considered most appropriated samples[25].

Virus isolation from saliva or urine in rapid culture has been traditionally considered the standard method for identification of infants with cCMV but such methods appear unfeasible to perform for large screening efforts [26]. In contrast, real-time polymerase chain reaction (RT-PCR), also considered as gold standard[26,28], allows large numbers of specimens to be screened at a relatively low cost. However, RT-PCR based methods applied to dried-blood-spot (DBS), tested in countries where DBS are routinely collected for newborn metabolic screening, have shown a low sensitivity as a screening methodology[26]. Dried umbilical cord (DUC) sample-based PCR assays have demonstrated utility for diagnosis of cCMV, as part of retrospective investigations of the underlying aetiology of hearing impairment[29, 30].

Considering CMV is excreted through the nasopharynx, other samples such as nasopharyngeal aspirates (NPA), easily obtained and simple to store, could be used for CMV screening, while additionally allowing the investigation of other respiratory pathogens of public health importance[31, 32]. Until now and to our knowledge, NPAs have not been evaluated as a screening methodology for cCMV diagnosis, although the Child Health and Mortality Prevention Surveillance Network (CHAMPS) aiming to know cause of death through innovative techniques such as the minimally invasive tissue sampling (MITS), proposes NPA as a standard specimen for diagnosis of cCMV[33, 34].

Knowledge gaps regarding the burden of other congenital infections associated to fatal foetal outcomes, such as B19V and EV, remain significant. B19V can cause a variety of foetal complications including spontaneous abortion, non-immune hydrops foetalis or intrauterine foetal death[35]. The epidemiology of B19V infection in pregnancy has been well studied in industrialized countries whereby prevalence has been estimated to vary from 1 to 5% in pregnant women with transmission rates to the foetus ranging between 17–33%[36]. However, the burden of this infection during pregnancy in LIC has been rarely documented and studies investigating in these settings active B19V infection in newborns have not been conducted. Enteroviruses, which include coxsackieviruses and echoviruses, cause about one billion infections every year worldwide but their consequences during pregnancy have been seldom described[37]. Transplacental transmission of EV has been associated to stillbirths, non-immune hydrops foetalis and also severe neonatal infections, although the epidemiology and characterization of neonatal outcomes is not well documented[37]. Although data in LIC remain insufficient, higher burden of congenital infections may be assumed

in regions like Southern Mozambique where HIV is highly prevalent and effective vaccines against pathogens such as rubella are partially or even not implemented[15, 38-41].

This is a pilot study exploring congenital acquisition of CMV, B19V and EV determined at birth. We additionally aimed to compare the results of two simple screening methodologies using RT-PCR for cCMV investigation, using DUC and NPA specimens obtained from the newborns.

METHODS

Study site

The study was conducted in Manhiça, a semi-rural site in Southern Mozambique. The Manhiça Health Research Centre (CISM) runs a Demographic Surveillance System (DSS) in the area linked to a Hospital Morbidity Surveillance System (HMSS) ongoing at the Manhiça District Hospital (MDH) including all admitted children. A detailed description of MDH, CISM and the study area can be found elsewhere[42]. MDH is the referral hospital for the Manhiça district, covering a population of circa 183,000 inhabitants. The MDH includes adult and paediatric wards, together with a maternity, where an average of 3500-4000 deliveries takes place annually. Around 85% of all deliveries are institutionalized (A. Nhacolo, personal communication). It also includes an outpatient department and an antenatal care (ANC) clinic where pregnant women are routinely followed. As part of the National policy, all pregnant women are invited to attend ANC clinic during their pregnancy, where HIV testing and syphilis screening are routinely offered. Malaria transmission of moderate intensity is perennial with some seasonality and, intermittent preventive treatment during pregnancy (IPTp) for malaria prevention is recommended[42]. HIV prevalence in Manhiça district is amongst the highest in the world, with rates estimated at around 29% at the ANC clinic[40]. In 2013, MDH introduced WHO-recommended Option B+ for the prevention of mother-to-child HIV transmission[43], which is offered to mothers free of charge. No proactive strategies to screen for risk factors of neonatal sepsis or to prevent it are currently implemented in Mozambique.

Study design and population

This observational pilot study was conducted at the delivery wards of MDH, between September 15th 2014 and January 15th 2015, running continuously during working hours (8:00–16:00) and working days. We

recruited pregnant women upon delivery (regardless of gestational age (GA) and their offspring. Participants were eligible for inclusion if they were >18 years old and able and willing to participate in the study and to provide informed consent after an explanation of the study. As this was a pilot study, in order to obtain a more representative sample of the study population, the three first women seen every day were approached for recruitment.

Definitions

Gestational age was defined using fundal height, measured from the top of the mother's uterus to the top of the mother's pubic symphysis and assessed by a nurse specialist in maternal child health. A preterm baby was defined as that with a gestational age at birth of <37 weeks and a stillbirth case as an intrauterine death occurring after 28 weeks of GA. Low-birth weight was defined as weight at birth <2.500 grams[44]. Microcephaly was defined following the WHO growth standards[45]. Congenital CMV, B19V or EV infections were defined as detection of viral DNA/RNA by RT-PCR in dried cord umbilical samples obtained from neonates at birth[33]. Although cCMV diagnosis through NPA has not been validated, we also explored prevalence of CMV through positive RT-PCR in NPA specimens. Positive HIV status was defined according to national guidelines, which required for mothers two positive rapid testing (an initial discriminatory diagnostic test (Determine®) and a confirmatory test (Unigold®); and for children ≤ 18 months of age a positive rapid test in addition to a confirmatory positive PCR test.

Study procedures

A placental biopsy and two drops of umbilical cord blood collected in filter paper (Whatman 903®, Florham Park, NJ) were obtained immediately after delivery in order to determine DNA/RNA of CMV, B19V and EV and assess placental histopathology. In addition, a NPA was collected from neonates within the first two hours from birth, using a bulb aspiration kit conveniently pre-filled with sterile saline (M-PRO NPAK NASOPHARYNGEAL ASPIRATION KIT®) . At least 1ml of NPA specimen mixed with sterile saline was collected and stored with the objective of screening for CMV DNA. A blood sample was collected from mothers for assessing anti-CMV antibodies. Maternal HIV status was determined and recorded if not previously registered in antenatal source documents. Other screening test results routinely performed at the ANC clinic, such as syphilis screening (using Rapid Plasma Reagin) or haemoglobin determination were also recorded.

DNA Extraction and Real Time -PCR for viral amplification

CMV and B19V DNA and EV RNA were extracted from 1 drop of DUC (around 50 ul) and CMV from 400 ul of NPA specimen by using the NucliSENS®easyMag® (bioMérieux, Marcy l'Etoile, France) instrument according to the manufacturer's procedure and eluted in 25 µl of elution buffer for DUC and 50 ul for NPA. Samples were tested for the presence of CMV DNA ,B19V DNA and EV RNA with three different real-time PCR, CMV Q-PCR alert Kit®, Elitech Group Molecular Diagnostics, RealStar® Parvovirus B19V kit®, Altona Diagnostics and and a published in-house real-time RT-PCR assay for EV detection [46]. Positive results with cycle threshold values above 40 were classified as negative. Appropriate positive and negative controls were included in all the experiments. Samples were considered as valid when a clear positive or negative result was obtained.

Seroprevalence of CMV among pregnant women

Serum collected from the mothers was tested for the presence of anti-CMV immunoglobuline G (IgG) antibodies (AB) using the electrochemiluminescence immunoassay "ECLIA" intended for use on Elecsys and cobas immunoassay analyzers, according to the manufacturer's instructions. Positive, negative, and cut-off controls were included in all runs, and positive samples were retested to confirm initial positive result.

Placental biopsy

A placental biopsy was obtained with a scalpel blade and immediately placed into 10% buffered formalin for transport to the laboratory. Each placental sample was embedded in paraffin wax, sectioned in 5µm tissue pieces and stained with standard haematoxylin–eosin for histopathologic examination. Different histopathological parameters were assessed including a macroscopic description (weight, oedema, haemorrhage, infarct and calcifications) and a microscopic description (inflammation and presence of microorganisms). Additional sections were saved for histochemical staining (PAS, Grocott and Gram) performed in an automated stainer (BenchMark Special Stains- Ventana Roche), following commercial recommendations. An immunohistochemical assay was performed on formaline-fixed paraffin-embedded tissue sections according to standard procedures, using the Ventana-Roche automated immunostainer system (BenchMark XT-Ventana Roche). Antigen retrieval was performed by heat-induced epitope retrieval and B19V (Master Diagnostica, Granada, SP) and CMV (Cell Marque, Rocklin, CA, USA) antibodies were applied according to manufacturing instructions.

Infant follow-up

Clinical examination of all newborns, including the evaluation of head circumference and Apgar score at minute 1, 5 and 10, was performed at birth. Dubowitz score for postnatal GA determination was done in live neonates at least 12h after birth[47]. Maternal and child HIV status were registered. All participants were followed using the HMSS in order to check any hospital admissions to MDH during the first six months after birth.

Statistical analysis

All data were prospectively collected using standardized questionnaires, which were double entered in specific study databases, created using Openclinica© software. Discrepancies were solved after comparison with the original source documents by a senior data clerk, and in close collaboration with the study clinicians. Statistical analyses were performed using Stata 14.1 (Stata Corp., College Station, TX). Study variables were counted and summarized in frequency tables. Univariate and multivariate analyses were performed to identify risk factors for CMV, B19V and EV neonatal infection, separately. Firth logistic regression was used in order to address issues of separability, small sample sizes and bias of the parameter estimates. Variables that were found to be significantly associated with CMV, B19V and EV acquisition in the univariate analysis together with those related at a significance level of $p<0.10$ were entered into a multivariate model. Agreement between the results of DUC RT-PCR assays and those of NPA RT-PCR was assessed through Kappa statistic.

Ethical considerations

This protocol and all supporting documentation (Informed consent documents, Study questionnaires) were approved by the local bioethics committee of CISM (Comité Institucional de Bioética para Saúde) and by the National Bioethics Committee of Maputo in Mozambique, and by the Ethics Committee of the Hospital Clínic in Barcelona, Spain. Participants were asked to express their willingness to participate in the study by signing (or thumb-printing in case they were illiterate) a consent form. Participation in this study was voluntary, and study-related procedures did not interfere with the pregnant women's or children's standard clinical care.

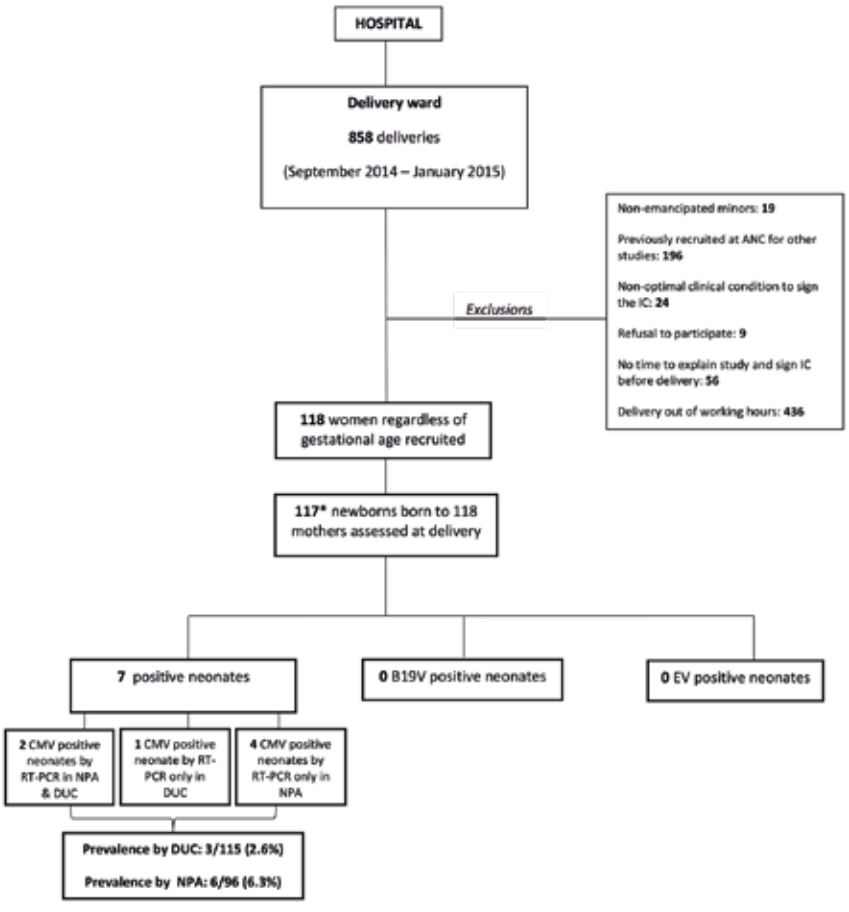
RESULTS

Maternal characteristics

During the study period, 118 pregnant women were recruited upon delivery

at MDH (Fig 1). Table 1 summarizes the socio-demographic and clinical characteristics of participant mothers. Median age of recruited women was 22 years (Interquartile range, IQR 19-29), with >75% being younger than 30 years of age. Nearly a third of women (31.4%, 37/118) were confirmed HIV positive and only one of 118 had a positive syphilis test. Malaria test results were registered as negative in 5/118 women and no information about this disease was obtained for the rest of mothers. CMV IgG AB were detected in all (100%) women. Distal villous hypoplasia was observed in 27/117 (23.0%) of placental tissue samples evaluated. Neither microorganisms nor other significant findings were detected after evaluating tissue sections with standard tissue staining or in the immunohistochemical studies (Fig 2).

Fig 1. Study profile.



ANC: antenatal clinics; IC: informed consent; *One stillbirth whose mother refused to take samples. RT-PCR: real-time polymerase chain reaction, DUC: dried umbilical cord, NPA: nasopharyngeal aspirate.

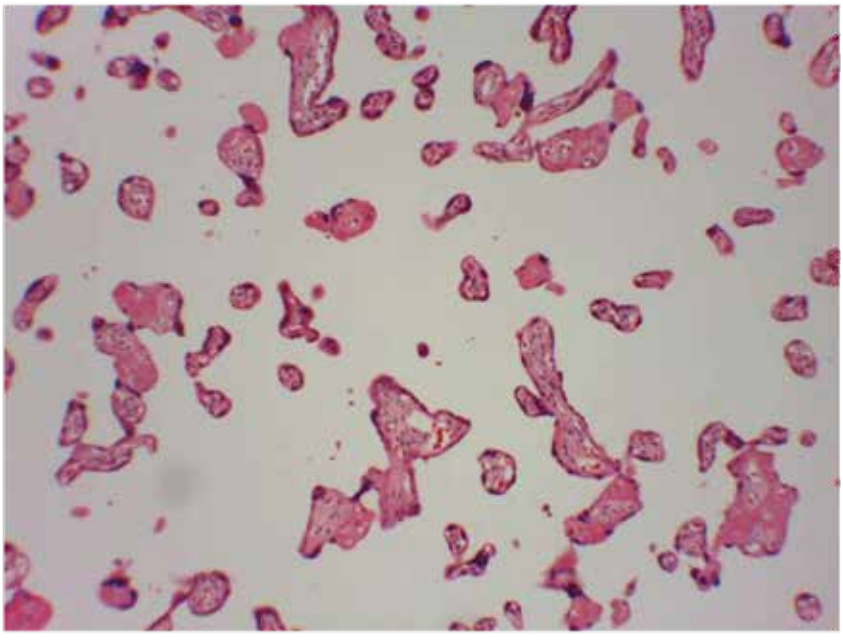
Table 1. Socio-demographic and clinical characteristics of mothers participating in the study and univariate analysis of maternal risk factors associated to congenital CMV infection measured by different specimens.

	Total mothers recruited n=118	Mothers of neonates CMV positive by DUC, n=3*	Crude OR (95%CI)[&]	p-value[&]	Mothers of neonates CMV positive by NPA, n=6^	Crude OR (95%CI)[&]	p-value[&]
Socio-demographic characteristics	n (%)	n (%)			n (%)		
Age in years				0.61			0.61
< 21	51 (43.2)	2 (66.7)	1.00		4 (66.7)	1.00	
22 to 29	40 (33.9)	0 (0)	0.23 (0.01 - 4.92)		1 (16.7)	0.23 (0.01 - 4.92)	
≥30	27 (22.9)	1 (33.3)	1.05 (0.13 - 8.42)		1 (16.7)	1.05 (0.13 - 8.42)	
Seconday or tertiary education	30 (25.4)	0 (0.0)	0.40 (0.02 – 8.06)	0.55	3 (50.0)	3.23 (0.68 - 15.36)	0.14
Employment	6 (5.1)	0 (0.0)	2.34 (0.11 – 50.28)	0.59	0 (0.0)	1.92 (0.89 - 41.36)	0.68
Obstetric History	n (%)	n (%)			n (%)		
Age of first pregnancy (median±IQR))	18.0 (17-20)	18.0 (17-18)	0.91 (02.7 – 3.11)	0.89	18.0 (18-18)	1.03 (0.76 - 1.41)	0.84
Gravidity (mean±SD)	2.7 (±0.2)	3.7 (±2.2)	1.27 (0.81 – 2.01)	0.30	1.5 (±0.2)	0.63 (0.31 - 1.29)	0.21
Previous abortion n(%)	11 (9.3)	0 (0.0)	1.26 (0.61 – 25.97)	0.88	0 (0.0)	0.75 (0.89 - 14.43)	0.85
History of current pregnancy	n (%)	n (%)			n (%)		
At least 3 antenatal visits during the pregnancy	69 (58.5)	2 (66.7)	0.78 (0.98 - 6.15)	0.81	5 (83.3)	1.22 (0.19 – 8.01)	0.83
Gestational hypertension	7 (6.1)	0 (0.0)	2.73 (0.12 – 62.25)	0.53	1 (16.7)	3.38 (0.47 – 24.33)	0.23
Vaginal discharge	2 (1.7)	0 (0.0)	6.31 (0.25 - 157.6)	0.26	0 (0.0)	2.72 (0.12 - 62.86)	0.53
Investigations	n (%)	n (%)			n (%)		
Syphilis positive	1 (0.5)	0 (0.0)	9.95 (0.34 – 290.3)	0.32	0 (0.0)	5.12 (0.19 - 140.9)	0.33
HIV positive	37 (31.4)	1 (2.7)	1.26 (0.16 – 9.89)	0.83	3 (50.0)	2.19 (0.46 – 10.30)	0.32
HIV positive in HAART	33 (89.2)	1 (100.0)	1.25 (0.11- 4.43)	0.86	3 (100.0)	2.60 (0.48- 14.04)	0.25
Anemia (<11g/dL)	55 (72.4)	0 (0.0)	0.13 (0.01 - 3.26)	0.21	2 (66.7)	0.59 (0.07 - 4.88)	0.63
CMV IgG serum antibodies	118 (100)	3 (100)	0.03 (0.00 - 1.81)	0.09	6 (100)	0.07 (0.06 - 3.92)	0.20

IQR (interquartile range). SD: standard deviation. HAART: highly active antiretroviral therapy. CMV: cytomegalovirus. DUC: Dried umbilical cord. *Results based on 115 valid DUC samples. NPA: Nasopharingeal aspirate. ^Results based on 96 valid NPA samples. OR: odds ratio. CI: confidence intervals. [&]OR and P-value derived from Firth logistic regression.

No maternal risk factors associated to neonatal cCMV were found in the univariate analysis and thus, multivariate analysis was not performed (Table 1).

Fig 2. A placental tissue sample from a CMV infected infant. Distal villous hypoplasia: many terminal villi are extremely small, with reduced stroma and capillaries number.



Neonatal outcomes and infant follow-up

One hundred and eighteen delivery outcomes from the 118 pregnant women were recorded (100%) at MDH. Characteristics of neonates born of mothers participating in the study are shown in table 2. Neonatal outcomes included 110 live term babies, 7 preterm and 1 case of stillbirth born at 41 weeks of GA, whose mother refused permission to sample the foetus. Twenty-one (17.8%) newborns had low-birth-weight and three of them (2.5%) presented microcephaly at birth[48]. At least one specimen was collected from 117 neonates. A hundred and fifteen of the 117 DUC samples obtained were valid for viral determination. All of them were negative for B19V and EV. Three of the 115 (2.6%) valid DUC samples were positive for CMV. This virus was also detected in 6/96 (6.3%) valid NPA samples obtained. A total of 7/117 (6.0%) newborns tested had at least one positive sample for CMV. Prevalence of cCMV infection measured by DUC among HIV-exposed neonates was 2.7% (1/37) and 8.1% (3/37) when CMV was detected through NPA, although no significant difference was found compared to the prevalence among HIV-unexposed in both cases (Table 1). One of the NPA-CMV positive cases was born preterm and with low birth weight while all neonates DUC-CMV positive were healthy at

birth. CMV infection was associated to a higher risk of microcephaly at birth when CMV was determined by NPA (OR 9.65, 95% CI 1.07 - 87.12, p=0.04) in the univariate analysis (Table 2).

Table 2. Clinical characteristics of neonates born to mothers participating in the study.

	Neonates at birth n=118	Neonates CMV positive by DUC n=3[a]	Crude OR (95%CI)[d]	p-value[d]	Neonates CMV positive by NPA n=6[c]	Crude OR (95%CI)[d]	p-value[d]
Clinical characteristics of newborns	n (%)	n (%)			n (%)		
Gestational age at birth (live birth)				0.65			0.21
Term newborn	110 (94.1)	3 (100.0)	1.00		5 (83.3)	1.00	
Pre term newborn	7 (5.9)	0 (0)	2.01 (0.95 - 42.62)		1 (16.7)	3.54 (0.49 - 25.53)	
Stillbirth	1 (0.9)	0 (0)	–	–	0 (0)	–	–
Low birth weight (<2500gr)	21 (17.8)	0 (0)	0.61 (0.03 - 12.21)	0.75	1 (16.7)	1.23 (0.19 - 8.09)	0.22
Head circumference in cm (mean±SD)	35.6 (0.4)	39.7 (5.2)	1.13 (0.99 - 1.29)	0.08	36.2 (2.9)	2.69 (0.12 - 62.15)	0.54
Microcephaly	3 (2.5)	(0)	4.43 (0.19 - 103.20)	0.35	1 (16.7)	9.65 (1.07 - 87.12)	**0.04**
Perinatal asphyxia	2 (1.7)	0 (0)	10.62 (0.36 – 309.66)	0.17	0 (0)	2.69 (0.12 - 62.15)	0.54
Jaundice	0 (0)	0 (0)	–	–	0 (0)	–	–
Purpura	0 (0)	0 (0)	–	–	0 (0)	–	–
Dubowitz neurological score (mean±SD)[e]	30.8 (0.2)	31.0 (0.8)	0.88 (0.64 - 1.22)	0.46	31.7 (1.02)	1.14 (0.79 - 1.65)	0.47
Malformations at birth	2 (1.7)	0 (0)	6.25 (0.25 - 156.21)	0.26	0 (0)	2.69 (0.12 - 62.15)	0.54
Sick at birth	2 (1.7)	0 (0)	6.25 (0.25 - 156.21)	0.26	0 (0)	2.69 (0.12 - 62.15)	0.54
Outcome							
Admitted first 6 months of life	7 (5.9)	0 (0)	2.01 (0.95 - 42.62)	0.65	1 (16.7)	3.55 (0.49 - 25.52)	0.21
Death after birth[m]	2 (1.7)	0 (0)	6.25 (0.25 - 156.21)	0.26	0 (0)	2.69 (0.12 - 62.15)	0.54

CMV: cytomegalovirus. [a]Results based on 117 patients. Mother of a stillbirth refused to take sample of the baby. DUC: Dried umbilica cord. [b]Results based on 115 valid DUC samples. NPA: Nasopharingeal aspirate. [c]Results based on 96 valid NPA samples. OR: odds ratio. CI confidence intervals. [d]OR and P-value derived from Firth logistic. [e]Apgar at minute 1 <5. [f]Suboptimal neurological score following Dubowitz: <30.5. [m] Death (including stillbirth and any deaths in the first 6 months after birth).

Through HMSS, 7/117 infants born to mothers participating in the study were detected as admissions at MDH at least once during their first 6 months of life (Table 2). Two of them admitted within the first 24h after birth died due to clinical sepsis. Other causes of admission included perinatal asphyxia, bronchiolitis, diarrhoea and malaria. Among neonates were CMV was detected only the preterm baby with a positive NPA for CMV was admitted at birth. In all of these cases, children were discharged fully recovered. No additional follow-up investigations were conducted.

Comparison of dried umbilical cord and nasopharyngeal aspirate RT-PCR Assays for CMV determination

A total of 94 pairs of samples (DUC and NPA) from 94 neonates were available for testing CMV by RT-PCR assay and comparing results. Of these, 2/94 neonates (2.1%) were positive for CMV by any test. A newborn with a positive RT-PCR assay through DUC had a negative NPA result, and the NPA RT-PCR assay also identified four additional neonates as infected although their DUC RT-PCR were negative (Figure 1). The overall concordance of the RT-PCR assay through DUC with their correspondent through NPA was moderate (Kappa = 0.42 and $p<0.001$).

DISCUSSION

This study is a first attempt at proactively investigating vertical transmission of CMV, B19V and EV in Mozambique. The study was an opportunistic attempt to explore congenital transmission of these viruses, and was not specifically designed to evaluate the performance of the RT-PCR method of identification of cCMV in neonates as compared with the "gold-standard" for the detection of CMV (isolation of the virus in rapid cultures or PCR assay) since that has already been demonstrated[49-51]. The study shows a prevalence of cCMV infection assessed through dried umbilical cord samples of 2.6% and through NPA of 6.3%. Although specimens utilized in this study are not those currently recommended and even NPA has not been validated for its use in cCMV diagnosis, they highlight a high burden of vertical CMV transmission. Contrarily, B19V and EV congenital transmission in the newborns was not found in this cohort although only DUC samples were analysed.

The prevalence of cCMV in this study detected through DUC was likely underestimated, as it has been demonstrated that real-time dried-blood-spot PCR assay has a lower sensitivity compared with the standard saliva rapid culture[26]. The dried umbilical cord samples have previously been used for retrospective studies to diagnose cCMV[29, 30] and although this sample type has never been compared to other accepted specimens for cCMV diagnosis, it would however be reasonable to assume that a positive DUC sample is likely a true positive. On the other hand, NPA has not been ever assessed as a specimen valid for cCMV. The prevalence of 6.3% found in this study through NPA is likely overestimated due to until 30% of CMVseropositive women will secrete CMV in vaginal fluid and that could contaminate with CMV nasopharynx of neonates[52]. Then, considering cCMV

prevalence found through DUC a true prevalence, it was higher than the rate reported in newborns from industrialized countries (<1%)[14] and falls within the range reported in a systematic review for developing countries (0.6% - 6.1%)[53]. However, that review excluded studies reporting data from high at-risk populations for CMV transmission, such as HIV infected mothers, and restricted inclusion to studies having used the recommended gold standard specimens (saliva and urine) and techniques (cultures and PCR)[54]. Although it is likely that our cCMV prevalence was underestimated for the aforementioned reasons, other studies conducted in HIV endemic areas have reported similar congenital CMV prevalence to those shown here. A study conducted in Nigeria found a rate of 3.8% among neonates born to mothers with a low prevalence of HIV (4.8%)[55]. High prevalence of cCMV in HIV-exposed infants has been previously reported in two settings (South Africa and Zambia) with a maternal HIV prevalence similar to the one documented in our study area[23, 56-58]. The South African study was conducted among 748 HIV-exposed infants found a cCMV prevalence of 2.9%. No comparison with HIV-unexposed was performed[23]. Overall prevalence of cCMV among high-risk newborns admitted to a referral neonatal unit in Zambia was 3.8%, and 11.4% in those infants exposed to maternal HIV (Adjusted OR 6.66, 95% CI 2.13–20.9)[58]. HIV prevalence in our maternal cohort was very high (31.4%) and almost 90% of mothers were under highly active antiretroviral therapy (HAART), possibly explaining why the prevalence of cCMV was not higher among HIV-exposed newborns and why differences between HIV-exposed and unexposed-neonates (2.7% vs. 2.6%%, OR 1.26 95% CI 0.16–9.89, p=0.83) were not found[59]. Reasons to explain the difference between Zambian study results and our findings could be that the Zambian study was performed on admitted and therefore sick neonates and our study was conducted at time of birth. Another reason may be a better immune status of our HIV-infected mothers although information about HAART in this Zambian study was not available[58]. Immunosuppression in HIV-infected pregnant women likely leads to increased incidence of reinfection or reactivation, or prolonged CMV viral shedding, lengthening the opportunity for congenital transmission[21, 60]. Moreover, an association of CMV transmission with advanced maternal immunosuppression has been previously described[23].

No risk factors independently associated with cCMV were found. Primiparity, acute placental malaria, HIV-exposure and jaundice have all been reported as independent risk factors for cCMV infection by other authors[19, 28, 58, 61].

Caution is needed when interpreting our findings, since sample size was small and likely insufficient to detect significant differences among infected and uninfected neonates. It has been estimated that 90% of infected newborns do not have obvious clinical signs of CMV congenital infection and of them, only 15% will develop long-term neurological sequelae, especially, neurosensory hearing loss[14]. However, no further examinations and follow-up were performed beyond birth and burden of hearing loss was not explored. cCMV infection is an important cause of hearing loss and better strategies to detect children at risk of this complication in LIC should be developed.

Maternal CMV IgG seroprevalence in this study was 100%. Immune status of mothers in our cohort prior to pregnancy was unknown. In these cases, isolated detection of CMV IgG or detection of specific IgM AB are inadequate single measures to diagnose maternal primary infection[28]. Estimates suggest that around 75% of all cCMV cases in industrialized countries occur in babies born to women with non-primary maternal infection (those women who are CMV seropositive before pregnancy[28, 62-64] and the risk of intrauterine transmission has been estimated at 1% in CMV-seropositive mothers[14]. IgG CMV seroprevalence in developing countries is generally over 90% by adolescence and over 95% by early adulthood[53] and it has been demonstrated that the incidence of cCMV infection is parallel to maternal seroprevalence[28] suggesting that most of cases of vertical transmission of this virus result from non-primary maternal infection and may be due to reactivation of latent virus or reinfection with a new cytomegalovirus strain[28, 62-64]. This is the reason behind the current recommendations issued by The International Congenital Cytomegalovirus Recommendations Group of not conducting universal serological screening of pregnant women for primary CMV infection[28].

Maternal infections with B19V, CMV and EV have been associated with intrauterine foetal death[65]. This study did not focus in cases of abortion and only one stillbirth was registered during recruitment, which limits our capacity to associate them to the aforementioned pathogens. Our findings suggest a low prevalence of B19V and EV infections in Southern Mozambique. No B19V congenital infection in newborns was found in this study. To our knowledge, no prospective screening of congenital B19V and EV infections among neonates in developing countries has been conducted to date. A South African study exploring prevalence of

B19V infection among pregnant women found 20 asymptomatic neonates born to IgM positive mothers, although no samples from newborns were obtained[66]. Different African studies on maternal B19 V seroprevalence found IgG AB between 24.9 and 80% and IgM between 3 and 19%[66,70]. Unfortunately, maternal seroprevalence was not performed in this study and further research should be done in order to know the real burden of congenital B19V.

Similarly, all babies were negative for EV and maternal seroprevalence studies were not performed. Data on incidence and consequences of EV during pregnancy and clinical outcomes are globally scarce. Case reports and small case series have suggested that EV infection may cause foetal loss, and maternal infections during the 2nd and 3rd trimester may also lead to in utero foetal anomalies and death, but also to severe neonatal infections[37]. However, no prospective studies investigating EV maternal prevalence and risk of transmission have been conducted.

The few and unspecific anatomopathological findings documented in this study, consisting on an accelerated placental maturation, could have resulted from a variety of causes, including maternal preeclampsia or other states of maternal vascular underperfusion, or, more likely in our setting, due to malnutrition or infectious such as HIV or malaria. However, the lack of ovular membranes and umbilical cord in placental samples did not allow ruling out possible infections of these tissues.

Our study has several limitations. First, diagnosis of cCMV through DUC specimen is a methodology not recommended for neonatal CMV screening, particularly as urine and saliva have been demonstrated to be the most reliable specimens [26, 28]. The use of NPA for cCMV has not been validated and this specimen could be contaminated by maternal secretions contained CMV, overestimating cCMV prevalence. However, a similar issue occurs with saliva samples, since the risk of contamination of saliva samples with breast milk exists. We however chose to use RT-PCR methods, with known good performance, in those samples available from the study, both because the kind of samples and the molecular screening techniques could be a good approach to study several viruses simultaneously. Further studies to validate these particular specimens for cCMV diagnosis would help to know their specificity and positive predictive value, and shed a light on why an important number of samples provided invalid results. Second, further

follow-up and additional investigations beyond six months of life were not performed and hearing impairment, which is frequently progressive and usually develops later during infancy, was not measured, leading to a potential underestimation of CMV- associated morbidity. Third, the study lacked sufficient statistical power to detect independent risk factors associated to higher risk of congenital CMV, B19V and EV infections given the small sample size, and the few positive results. Additionally, it is known that both, B19V and EV show seasonal or even epidemic patterns[71, 72]. Considering that the epidemiology of these viruses in unknown in Mozambique, we may have failed, during the short study period, to capture natural transmission of the pathogens. Finally, B19V and EV were only assessed in dried umbilical cord samples at birth and maternal seroprevalence of these viruses or their presence in other fluids as amniotic fluid was not performed. Then, correlation between burden of maternal infection and vertical transmission of these viruses is not known and their prevalence in this cohort could be underestimated.

In conclusion, despite the small sample size and the use of non-standard specimens, this study demonstrates that the prevalence of vertical transmission of CMV may be high in southern Mozambique, although further research is needed to assess its clinical relevance in this area. Larger studies should demonstrate the real limitations and validate the real limitations and potential use of NPA specimen for cCMV diagnosis. Congenital B19V and EV infections seem to be less prevalent in this area. Further research to evaluate the consequences of vertical transmission of these viral infections in resource-constrained settings is needed.

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The study was designed by QB, CMA and LM. During the study period LM, SM and RV were involved in the diagnosis, recruitment and management of patients and collection of data. CMA, EJC, CE, CC, MI, BV, VF and CLD designed sampling procedures, processed samples and interpreted results. LM and QB led the analysis. LM, RV, CM and QB led the interpretation and writing up of this data set, and received input from all authors. All authors read and approved the initial and subsequent versions of the manuscript.

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REFERENCES

[1] L. Liu, S. Oza, D. Hogan, Y. Chu, J. Perin, J. Zhu, J.E. Lawn, S. Cousens, C. Mathers, R.E. Black, Lancet 388(10063) (2016) 3027-3035.

[2] K.M. Adams Waldorf, R.M. McAdams, Reproduction 146(5) (2013) R151-62.

[3] J. Del Pizzo, Pediatr Rev 32(12) (2011) 537-42.

[4] N. Neu, J. Duchon, P. Zachariah, Clin Perinatol 42(1) (2015) 77-103, viii. [5] J.E. Lawn, H. Blencowe, P. Waiswa, A. Amouzou, C. Mathers, D. Hogan, V. Flenady, J.F. Froen, Z.U. Qureshi, C. Calderwood, S. Shiekh, F.B. Jassir, D. You, E.M. McClure, M. Mathai, S. Cousens, Lancet (2016).

[6] E.M. Ansbro, M.M. Gill, J. Reynolds, K.D. Shelley, S. Strasser, T. Sripipatana, A.T. Ncube, G. Tembo Mumba, F. Terris-Prestholt, R.W. Peeling, D. Mabey, PLoS One 10(6) (2015) e0127728.

[7] N.M. Gous, L.E. Scott, J. Potgieter, L. Ntabeni, I. Sanne, W.S. Stevens, J Acquir Immune Defic Syndr (2015).

[8] E.T. Nkhoma, N.M. Bowman, L. Kalilani-Phiri, V. Mwapasa, S.J. Rogerson, S.R. Meshnick, Am J Trop Med Hyg 87(6) (2012) 1022-7.

[9] L. Newman, M. Kamb, S. Hawkes, G. Gomez, L. Say, A. Seuc, N. Broutet, PLoS Med 10(2) (2013) e1001396.

[10] V. Sikka, V.K. Chattu, R.K. Popli, S.C. Galwankar, D. Kelkar, S.G. Sawicki, S.P. Stawicki, T.J. Papadimos, J Glob Infect Dis 8(1) (2016) 3-15.

[11] M.M. Mussi-Pinhata, A.Y. Yamamoto, R.M. Moura Brito, M. de Lima Isaac, P.F. de Carvalho e Oliveira, S. Boppana, W.J. Britt, Clin Infect Dis 49(4) (2009) 522-8.

[12] S.C. Dollard, S.D. Grosse, D.S. Ross, Rev Med Virol 17(5) (2007) 355-63. [13] A. Kenneson, M.J. Cannon, Rev Med Virol 17(4) (2007) 253-76.

[14] S. Manicklal, V.C. Emery, T. Lazzarotto, S.B. Boppana, R.K. Gupta, Clin Microbiol Rev 26(1) (2013) 86-102.

[15] H. Gumbo, B. Chasekwa, J.A. Church, R. Ntozini, K. Mutasa, J.H. Humphrey, A.J. Prendergast, PLoS One 9(12) (2014) e114870.

[16] X.W. Zhang, F. Li, X.W. Yu, X.W. Shi, J. Shi, J.P. Zhang, J Clin Virol 40(3) (2007) 180-5.

[17] C. Bello, H. Whittle, J Clin Pathol 44(5) (1991) 366-9.

[18] A. Waters, K. Jennings, E. Fitzpatrick, S. Coughlan, E.J. Molloy, C.F. De Gascun, W.W. Hall,

S.J. Knowles, J Clin Virol 59(3) (2014) 156-60.
[19] E.L. Duryea, P.J. Sanchez, J.S. Sheffield, G.L. Jackson, G.D. Wendel, B.S. McElwee, L.F. Boney, M.M. Mallory, K.E. Owen, E.K. Stehel, Pediatr Infect Dis J 29(10) (2010) 915-8.
[20] G. Guibert, J. Warszawski, J. Le Chenadec, S. Blanche, Y. Benmebarek, L. Mandelbrot, R. Tubiana, C. Rouzioux, M. Leruez-Ville, C. French Perinatal, Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 48(11) (2009) 1516-25.
[21] A. Kovacs, M. Schluchter, K. Easley, G. Demmler, W. Shearer, P. La Russa, J. Pitt, E. Cooper, J. Goldfarb, D. Hodes, M. Kattan, K. McIntosh, N Engl J Med 341(2) (1999) 77-84.
[22] M.M. Mussi-Pinhata, A.Y. Yamamoto, L.T. Figueiredo, M.C. Cervi, G. Duarte, J Pediatr 132(2) (1998) 285-90.
[23] S. Manicklal, A.M. van Niekerk, S.M. Kroon, C. Hutto, Z. Novak, S.K. Pati, N. Chowdhury, N.Y. Hsiao, S.B. Boppana, Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 58(10) (2014) 1467-72.
[24] G. Nigro, A. Krzysztofiak, G.C. Gattinara, T. Mango, M. Mazzocco, M.A. Porcaro, S. Provvedi, J.C. Booth, AIDS 10(10) (1996) 1127-33.
[25] S.B. Boppana, S.A. Ross, M. Shimamura, A.L. Palmer, A. Ahmed, M.G. Michaels, P.J. Sanchez, D.I. Bernstein, R.W. Tolan, Jr., Z. Novak, N. Chowdhury, W.J. Britt, K.B. Fowler, D. National Institute on, C.S. Other Communication Disorders, N Engl J Med 364(22) (2011) 21118.
[26] S.B. Boppana, S.A. Ross, Z. Novak, M. Shimamura, R.W. Tolan, Jr., A.L. Palmer, A. Ahmed, M.G. Michaels, P.J. Sanchez, D.I. Bernstein, W.J. Britt, K.B. Fowler, D. National Institute on, C.M.V. Other Communication Disorders, S. Hearing Multicenter Screening, JAMA 303(14) (2010) 1375-82. [27] F. Baquero-Artigao, P. Grupo de estudio de la infeccion congenita por citomegalovirus de la Sociedad Espanola de Infectologia, An Pediatr (Barc) 71(6) (2009) 535-47.
[28] W.D. Rawlinson, S.B. Boppana, K.B. Fowler, D.W. Kimberlin, T. Lazzarotto, S. Alain, K. Daly, S. Doutre, L. Gibson, M.L. Giles, J. Greenlee, S.T. Hamilton, G.J. Harrison, L. Hui, C.A. Jones, P. Palasanthiran, M.R. Schleiss, A.W. Shand, W.J. van Zuylen, Lancet Infect Dis 17(6) (2017) e177e188.
[29] M. Tagawa, H. Tanaka, M. Moriuchi, H. Moriuchi, J Pediatr 155(5) (2009) 749-51.
[30] S. Koyano, A. Araki, Y. Hirano, K. Fujieda, T. Suzutani, K. Yagyu, K. Murono, N. Inoue, Pediatr Infect Dis J 23(5) (2004) 481-2.
[31] C. Wejse, N.H. Birkebaek, L.P. Nielsen, H.M. Andersen, Pediatr Infect Dis J 20(3) (2001) 256-9.
[32] T.J. Meerhoff, M.L. Houben, F.E. Coenjaerts, J.L. Kimpen, R.W. Hofland, F. Schellevis, L.J. Bont, Eur J Clin Microbiol Infect Dis 29(4) (2010) 365-71. [33] C.H.a.M.P.S. (CHAMPS), Determination of Cause of Death (DeCoDe) Diagnosis Standards: guidance for standardized interpretation of CHAMPS data. [cited 2017 Nov 17]. Available from: https://champshealth.org/wp-content/uploads/2017/10/CHAMPS-Diagnosis-Standardsfor-DeCoDe-1.pdf. , 2017.).
[34] T.H. Farag, J.P. Koplan, R.F. Breiman, S.A. Madhi, P.M. Heaton, T. Mundel, J. Ordi, Q. Bassat, C. Menendez, S.F. Dowell, Am J Trop Med Hyg 97(1) (2017) 3-5.

[35] O. Norbeck, N. Papadogiannakis, K. Petersson, T. Hirbod, K. Broliden, T. Tolfvenstam, Clin Infect Dis 35(9) (2002) 1032-8.
[36] Z. Ergaz, A. Ornoy, Reprod Toxicol 21(4) (2006) 421-35.
[37] J. Mereaux, O. Picone, C. Vauloup-Fellous, Z. Khediri, A. Benachi, L. Mandelbrot, J.M. Ayoubi, Gynecol Obstet Fertil Senol 45(4) (2017) 231-237. [38] L.J. Bollen, S.J. Whitehead, P.A. Mock, W. Leelawiwat, S. Asavapiriyanont, A. Chalermchockchareonkit, N. Vanprapar, T. Chotpitayasunondh, J.M. McNicholl, J.W. Tappero, N. Shaffer, R. Chuachoowong, AIDS 22(10) (2008) 1169-76.
[39] C.J. Hoffmann, C.L. Thio, Lancet Infect Dis 7(6) (2007) 402-9.
[40] R. Gonzalez, K. Munguambe, J. Aponte, C. Bavo, D. Nhalungo, E. Macete, P. Alonso, C. Menendez, D. Naniche, HIV Med 13(10) (2012) 581-8.
[41] C.L. Cutland, S.J. Schrag, E.R. Zell, L. Kuwanda, E. Buchmann, S.C. Velaphi, M.J. Groome, P.V. Adrian, S.A. Madhi, P.S.t.t. Po, C.L. Cutland, S.J. Schrag, E.R. Zell, L. Kuwanda, E. Buchmann, S.C. Velaphi, M.J. Groome, P.V. Adrian, S.A. Madhi, Pediatrics 130(3) (2012) e581-90.
[42] C. Sacoor, A. Nhacolo, D. Nhalungo, J.J. Aponte, Q. Bassat, O. Augusto, I. Mandomando, J. Sacarlal, N. Lauchande, B. Sigauque, P. Alonso, E. Macete, K. Munguambe, C. Guinovart, P. Aide, C. Menendez, S. Acacio, D. Quelhas, E. Sevene, T. Nhampossa, Int J Epidemiol 42(5) (2013) 1309-18. [43] WHO, Use of Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants., in: W.L.C.-i.-P. Data (Ed.) World Health Organization, Geneva, Switzerland, 2012.
[44] WHO, New born with low birth weight. [cited 2017 Nov 17]. Available from: http://www.who.int/whosis/whostat2006NewbornsLowBirthWeight.pdf, Geneva, Switzerland 2006.
[45] WHO, Partnership for Maternal, Newborn & Child Health, World Health Organization. Countdown to 2015: Building a Future for Women and Children, Mozambique Country Reports., World Health Organization, Geneve, 2012.
[46] L. Selva, A. Martinez-Planas, J.J. Garcia-Garcia, R. Casadevall, C. Luaces, C. Munoz-Almagro, Eur J Clin Microbiol Infect Dis 31(5) (2012) 715-9.
[47] L.M. Dubowitz, V. Dubowitz, C. Goldberg, J Pediatr 77(1) (1970) 1-10.
[48] L. Quinto, A.L. Garcia-Basteiro, A. Bardaji, R. Gonzalez, N. Padilla, F.E. Martinez-Espinosa, M. Arevalo-Herrera, E. Macete, C. Menendez, J Trop Pediatr (2017).
[49] G.J. Demmler, G.J. Buffone, C.M. Schimbor, R.A. May, J Infect Dis 158(6) (1988) 1177-84.
[50] C.H. Tsai, F.J. Tsai, Y.T. Shih, S.F. Wu, S.C. Liu, Y.H. Tseng, Acta Paediatr 85(10) (1996) 12413.
[51] W.P. Warren, K. Balcarek, R. Smith, R.F. Pass, J Clin Microbiol 30(4) (1992) 786-9.
[52] S. Stagno, R.F. Pass, M.E. Dworsky, C.A. Alford, Jr., Clin Obstet Gynecol 25(3) (1982) 56376.
[53] T.M. Lanzieri, S.C. Dollard, S.R. Bialek, S.D. Grosse, Int J Infect Dis 22 (2014) 44-8.
[54] L.M. McGovern, T.G. Boyce, P.R. Fischer, J Travel Med 14(2) (2007) 117-28.
[55] B.O. Olusanya, T.M. Slusher, S.B. Boppana, Pediatr Infect Dis J 34(3) (2015) 322-4.
[56] T. Phillips, K. Brittain, C.A. Mellins, A. Zerbe, R.H. Remien, E.J. Abrams, L. Myer, I.B. Wilson, AIDS Behav 21(2) (2017) 450-461.

[57] E.M. Stringer, N.T. Chintu, J.W. Levy, M. Sinkala, B.H. Chi, J. Muyanga, M. Bulterys, M. Bweupe, K. Megazzini, J.S. Stringer, Bull World Health Organ 86(9) (2008) 697-702.
[58] N. Mwaanza, L. Chilukutu, J. Tembo, M. Kabwe, K. Musonda, M. Kapasa, C. Chabala, S. Sinyangwe, P. Mwaba, A. Zumla, M. Bates, Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 58(5) (2014) 728-35.
[59] T. Frederick, J. Homans, L. Spencer, F. Kramer, A. Stek, E. Operskalski, A. Kovacs, Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55(6) (2012) 877-84.
[60] S.B. Mostad, J.K. Kreiss, A.J. Ryncarz, J. Overbaugh, K. Mandaliya, B. Chohan, J. NdinyaAchola, J.J. Bwayo, L. Corey, J Med Virol 59(4) (1999) 469-73.
[61] M.A. van der Sande, S. Kaye, D.J. Miles, P. Waight, D.J. Jeffries, O.O. Ojuola, M. Palmero, M. Pinder, J. Ismaili, K.L. Flanagan, A.A. Aveika, A. Zaman, S. Rowland-Jones, S.J. McConkey, H.C. Whittle, A. Marchant, PLoS One 2(6) (2007) e492.
[62] A. Ornoy, O. Diav-Citrin, Reprod Toxicol 21(4) (2006) 399-409.
[63] S. Stagno, R.F. Pass, M.E. Dworsky, R.E. Henderson, E.G. Moore, P.D. Walton, C.A. Alford, N Engl J Med 306(16) (1982) 945-9.
[64] C. Wang, X. Zhang, S. Bialek, M.J. Cannon, Clin Infect Dis 52(2) (2011) e11-3.
[65] K. Petersson, O. Norbeck, M. Westgren, K. Broliden, J Perinat Med 32(6) (2004) 516-21.
[66] B.D. Schoub, N.K. Blackburn, S. Johnson, J.M. McAnerney, S Afr Med J 83(7) (1993) 505-6.
[67] S.E. Emiasegen, L. Nimzing, M.P. Adoga, A.Y. Ohagenyi, R. Lekan, Mem Inst Oswaldo Cruz 106(2) (2011) 227-31.
[68] M.F. Mirambo M, Majigo M, Mushi MF, Moremi N, Seni J, Matovelo D, Mshana SE., BMC Pregnancy and Childbirth 17(176) (2017).
[69] T.F. Schwarz, L.G. Gurtler, G. Zoulek, F. Deinhardt, M. Roggendorf, Zentralbl Bakteriol 271(2) (1989) 231-6.
[70] F. Volker, P. Cooper, O. Bader, A. Uy, O. Zimmermann, R. Lugert, U. Gross, BMC Pregnancy Childbirth 17(1) (2017) 172.
[71] D. Shapiro, C.K. Bodinayake, A. Nagahawatte, V. Devasiri, R. Kurukulasooriya, J. Hsiang, B. Nicholson, A.D. De Silva, T. Ostbye, M.E. Reller, C.W. Woods, L.G. Tillekeratne, Am J Trop Med Hyg 97(1) (2017) 88-96.
[72] N.S. Young, K.E. Brown, N Engl J Med 350(6) (2004) 586-97.

ARTICLE 6

Hypoglycemia and risk factors for death in 13 years of pediatric admissions in Mozambique

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Hypoglycemia and Risk Factors for Death in 13 Years of Pediatric Admissions in Mozambique

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Abstract. Hypoglycemia is a life-threatening complication of several diseases in childhood. We describe the prevalence and incidence of hypoglycemia among admitted Mozambican children, establishing its associated risk factors. We retrospectively reviewed clinical data of 13 years collected through an ongoing systematic morbidity surveillance in Manhiça District Hospital in rural Mozambique. Logistic regression was used to identify risk factors for hypoglycemia and death. Minimum community-based incidence rates (MCBIRs) for hypoglycemia were calculated using data from the demographic surveillance system. Of 49,089 children < 15 years hospitalized in Manhiça District Hospital, 45,573 (92.8%) had a glycemia assessment on admission. A total of 1,478 children (3.2%) presented hypoglycemia (< 3 mmol/L), of which about two-thirds (972) were with levels < 2.5 mmol/L. Independent risk factors for hypoglycemia on admission and death among hypoglycemic children included prostration, unconsciousness, edema, malnutrition, and bacteremia. Hypoglycemic children were significantly more likely to die (odds ratio [OR] = 7.11; $P < 0.001$), with an associated case fatality rate (CFR) of 19.3% (245/1,267). Overall MCBIR of hypoglycemia was 1.57 episodes/1,000 child years at risk (CYAR), significantly decreasing throughout the study period. Newborns showed the highest incidences (9.47 episodes/1,000 CYAR, $P < 0.001$). Hypoglycemia remains a hazardous condition for African children. Symptoms and signs associated to hypoglycemia should trigger the verification of glycemia and the implementation of life-saving corrective measures.

INTRODUCTION

Critical illness seriously deranges metabolism in children and adults.[1–3] Alterations in blood glucose homeostasis are the most common metabolic abnormalities found in critically ill children,[4] and both hyper- and hypoglycemia are associated with poor outcomes.[1,3,4] Hyperglycemia in critically ill patients is an adaptive response to stress related to hypovolemia, surgery, sepsis, or trauma.[5,6] The prevalence in tropical settings has been estimated between 2.9% and 10.9%,[7,8] and its presence on admission is associated with mortality.[1,9,10] Insulin therapy is the treatment of choice, but is often unavailable in resource-constrained settings and can also cause iatrogenic hypoglycemia, potentially more harmful than sustained hyperglycemia.

On the other side of the spectrum, hypoglycemia is also a common and life-threatening complication of several diseases such as severe malaria, bacterial sepsis, severe malnutrition, and neonatal illness, among others.[11–15] Hypoglycemia has been extensively reported to have important influence on the outcome of very ill patients, both children and adults.[1,16,17] In Africa, its prevalence among pediatric admissions has been estimated to range between 1.8% and 7.3%.[7,18] Use of toxic herbal preparations and delays in seeking medical assistance may all cause or further aggravate hypoglycemia in these settings. Severe and prolonged hypoglycemia can result in mental retardation, neurological deficits, and recurrent seizures.[19,20] Management of hypoglycemia according to World Health organization (WHO) guidelines[11] includes rapid administration of exogenous glucose, preferably through an intravenous access. In the developing world, hypoglycemia remains an insufficiently recognized killer of children, as it is seldom diagnosed and whenever detected, often poorly managed mainly because of the lack of simple equipment or trained staff.

We analyzed data collected throughout 13 consecutive years of systematic morbidity surveillance among children admitted to a rural Mozambican hospital to determine prevalence, incidence, and risk factors associated with hypoglycemia on admission and mortality in those children.

MATERIAL AND METHODS

Study site and population. The study was conducted in Manhiça in southern Mozambique. The Manhiça Health Research Center (CISM) runs a demographic surveillance system (DSS) in the area and a morbidity surveillance system (MSS) at Manhiça District Hospital (MDH), which admits around 3,000 children annually. Malaria, pneumonia, diarrhea, malnutrition, and neonatal pathologies are among the main causes of admission and under-five mortality in Manhiça,[21] where human immunodeficiency virus (HIV) prevalence is among the highest in the world.[22] A detailed description of MDH, CISM, and the study area can be found elsewhere.[23] CISM provides personnel as well as valuable resources and laboratory diagnosis to MDH.

Study design. We present a retrospective analysis of data collected through the Manhiça MSS from children < 15 years, who were admitted to MDH during a 13-year-long study period (2001–2013).

Hospital surveillance system. A standardized admission questionnaire, which includes demographic, clinical, laboratory, and outcome data, was filled in for all hospitalized children < 15 years of age by a clinician. On arrival, a finger-prick blood sample was collected to measure packed cell volume (PCV) and blood glucose concentration, and thick and thin blood films were prepared to quantify *Plasmodium falciparum* parasitemia. Blood cultures were systematically performed for all children under the age of 2 years, or in older children with clinical severity, as part of the routine microbiological surveillance in MDH.

HIV status information was not routinely collected. On discharge or death, up to four final diagnoses—based on the

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International Classification of Diseases 10 —were recorded on the questionnaire after review of all available results.

Laboratory methods. Glycemia was determined using Accu-Chek® (Roche Inc., Manheim, Germany) at the bedside, with blood being usually collected by finger prick. Glycemia results were provided either in mmol/L or mg/dL units. To simplify the analysis, all mg/dL values were converted into mmol/L by multiplying them by 0.0555. PCV was measured using a microcentrifuge and a Hawksley hematocrit reader card (Hawksley and Sons Ltd., Lancing, United Kingdom). Thick and thin blood films for malaria diagnosis and blood cultures were processed as previously described.[24,25]

Clinical definitions. All case definitions were based on admission data from the standardized questionnaires. Children were categorized into three groups according to blood glucose levels: 1) hypoglycemia: blood glucose levels < 3.0 mmol/L (categorized as severe if < 2.5 mmol/L), 2) hyperglycemia: glycemia > 11 mmol/L, and 3) normoglycemia: values between 3 and 11 mmol/L.

A malaria case was defined as a child admitted with a clinical diagnosis of malaria with a *P. falciparum* asexual parasitemia > 0 parasites/μL. Prostration was defined as the inability to sit unsupported or breast-feed in children not yet capable of sitting. Impaired consciousness was defined as a child having a Blantyre coma score less than 5. Severe anemia was defined as a PCV < 15% on admission. Hypothermia was defined as axillary temperature < 35°C. Increased respiratory rate followed age-specific WHO definitions.[11] Respiratory distress included the presence of deep breathing or indrawing. Nutritional status was based on weight-for-age z scores (WAZ), calculated using the least mean square method and the WHO and Centers for Disease Control and Prevention growth charts.[26] Malnutrition was defined if WAZ was < −1 and severe malnutrition as WAZ of < −3.

Case management. Children with hypoglycemia were managed according to Mozambican national guidelines, based on the WHO guidelines,[11] which recommend a rapid intravenous correction with 5 mL/kg of 10% glucose or dextrose solution, repeated if necessary. Ten percent dextrose in normal saline or Ringer's lactate for maintenance infusion was used to prevent further episodes, and feeding encouraged as soon as possible. Facilities for intensive care are not available at MDH. All clinical assistance and treatment of admitted children is free of charge. Children requiring specialized care were transferred to Maputo Central Hospital.

Data management and statistical methods. All admission questionnaires were double entered using a program written in FoxPro version 5.0 (Microsoft Corp., Seattle, WA). Statistical analyses were done with Stata 13.1 (Stata Corp., College Station, TX).

Minimum community-based incidence rates (MCBIRs) for hypoglycemia were calculated referring cases to population denominators establishing time at risk (child years at risk [CYAR]) inferred from the DSS. Children did not contribute to the numerator or denominator for a period of 28 days after each episode of hypoglycemia or when they were outside the study area. The analysis of MCBIRs only takes into account children with a permanent identification number issued by the demography department allowing the linkage of their demographic data with the morbidity surveillance. Children not living within the study area were excluded for incidence calculations. Negative binomial regression models with random effects using likelihood ratio test were used to assess differences in incidence rates between calendar years and age groups.

Case fatality rates (CFRs) were calculated for different glycemia levels as the number of patients who died with a specific glycemia level divided by the total number of patients with known outcome admitted in the study period. These CFRs represent in-hospital mortality and do not include patients absconding or being transferred.

Qualitative variables were compared using a χ^2 test or Fisher's exact test. Means of normally distributed variables were compared using the Student *t* test or analysis of variance.

Multivariate logistic regression was used to investigate 1) adjusted associations for potential risk factors for hypoglycemia on admission and 2) adjusted associations for potential risk factors for hypoglycemia-related deaths. In the second analysis, given that the dependent variable was the final outcome (dead/alive), only children with a known outcome were included in the analysis (those absconding or being transferred were excluded). *P* values from analyses performed in large samples may be confounded because of their dependence on sample size and may reach the significance level even when the association is negligible. Thus, significant associations will be interpreted in accordance with the effect size (odds ratio [OR]) and will be classified as small (< 2), medium (2–3), or large (> 3).[27]

Ethics. This study retrospectively assessed data collected in the context of routine clinical practice. The morbidity surveillance in place at MDH has been approved by the Mozambican Ethics Committee. The analytical plan of this specific analysis was assessed and approved by Manhiça's Internal Scientific Committee.

RESULTS

During the 13-year study period (January 1, 2001 to December 31, 2013), 49,089 children < 15 years of age were admitted to MDH, including 17,115 infants and 2,774 newborns. Median age on admission was 18 months (interquartile range [IQR] = 8–35). Glycemia results were available for 45,573 (92.8%) children (Figure 1) and in-hospital outcome information was missing in 4,013 (8.2%) cases. Children without glycemia data were excluded from the analysis.

Prevalence of dysglycemia. On admission, 1,478/45,573 (3.2%) children had hypoglycemia, two-thirds of these episodes (972; 2.1%) being severe. Hyperglycemia was detected in 770/45,573 (1.7%) patients. By age group, hypoglycemia prevalence was the highest among newborns (8.8%), but present in all ages. Hyperglycemia was low and present in all age groups (Figure 2).

Clinical presentation and CFR of children with hypoglycemia admitted to the hospital. Table 1 compares some key characteristics in children with hypoglycemia and normoglycemia. A significantly higher proportion of the patients with hypoglycemia were newborns, but median age was not significantly different between the two glycemia groups. Children with hypoglycemia were significantly more prone to refer feeding difficulties, have hypothermia, or neurological impairment than their normoglycemic peers. They were also significantly more malnourished, with lower mean weight and WAZ scores, and, more frequently, severely anemic or bacteremic. Vomiting and diarrhea were not associated with having more

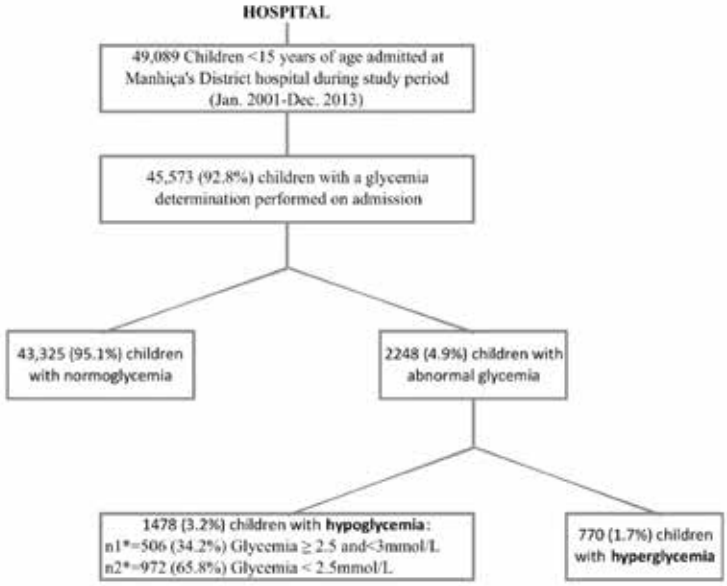

FIGURE 1. Study profile.

hypoglycemia on admission. The odds of dying in the hospital were higher among hypoglycemic children (OR = 7.11, 95% confidence interval [CI] = 6.11–8.27; associated CFR = 19.3% versus 3.3%, $P < 0.001$). Figure 3 summarizes CFRs by categorized glycemia. CFRs increased with decreasing glycemia, peaking at 33.3% in patients with values < 1 mmol/L. Importantly, CFRs also rose significantly for patients with hyperglycemia (associated CFR = 12.1%, $P < 0.001$, when compared with CFR of normoglycemia).

The multivariate analysis showed nine risk factors independently associated with the presence of hypoglycemia on admission (Table 2). History of seizures, unconsciousness, refusing to feed, malnutrition, edema, jaundice, prostration, *P. falciparum* infection, and having a positive blood culture

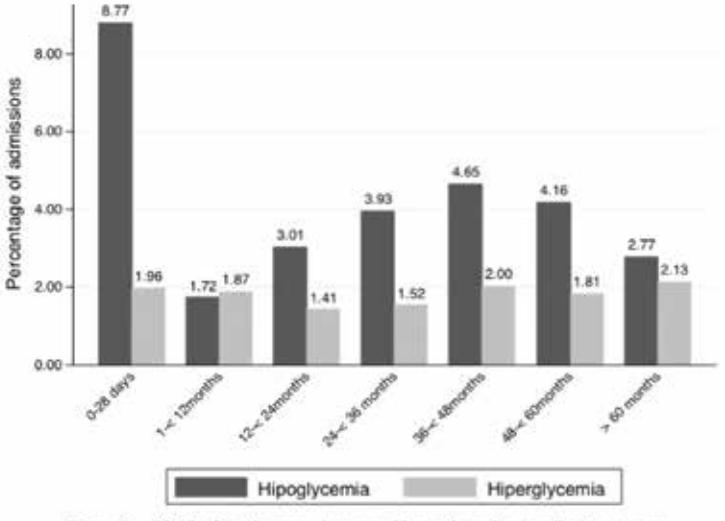

FIGURE 2. Distribution of normo-, hypo-, and hyperglycemia according to age group.

TABLE 1
Univariate analysis of clinical variables and diagnosis according to glycemia group

Variables	Normoglycemia (N = 43,325)	Hypoglycemia (N = 1,478)	OR and 95% CI	P value
Sociodemographic characteristics				
Age in months (median, IQR)	17 (8–34)	21 (8–37)	1.00	0.068*
Newborn, *n* (%)	2,304 (5.3)	226 (15.3)	3.21 (2.77–3.73)	**< 0.001**
Male, *n* (%)	23,249 (53.7)	841 (57.0)	1.14 (1.03–1.27)	**0.013**
Symptoms before admission				
Fever, *n* (%)	39,655 (91.5)	1,207 (81.7)	0.41 (0.36–0.47)	**< 0.001**
Cough, *n* (%)	28,429 (65.6)	825 (55.8)	0.66 (0.60–0.73)	**< 0.001**
Vomiting, *n* (%)	10,134 (23.4)	362 (24.5)	1.06 (0.94–1.20)	0.32
Diarrhea, *n* (%)	9,326 (21.5)	308 (20.8)	0.96 (0.84–1.09)	0.52
Difficulties to breast-feed/anorexia, *n* (%)	5,291 (13.5)	406 (30.1)	2.76 (2.45–3.12)	**< 0.001**
Seizures, *n* (%)	3,486 (8.1)	211 (14.3)	1.90 (1.64–2.21)	**< 0.001**
Anthropometrics				
Weight in kilogram (mean ± SD)	10.3 (5.9)	9.6 (5.9)	0.98 (0.97–0.99)	**< 0.001**
Malnutrition (WAZ < −1), *n* (%)	24,111 (55.7)	908 (61.4)	1.27 (1.14–1.41)	**< 0.001**
Severe malnutrition (WAZ < −3), *n* (%)	5,884 (14.6)	252 (19.4)	1.41 (1.22–1.62)	**< 0.001**
WAZ score (mean ± SD)	−1.41 (1.5)	−1.76 (1.4)	1.17 (1.13–1.22)	**< 0.001**
Symptoms and signs on admission				
Axillary temperature (°C) (mean ± SD)	37.9 (1.3)	37.6 (1.5)	0.86 (0.83–0.89)	**< 0.001**
Hypothermia (axillary temperature < 35°C), *n* (%)	99 (0.2)	26 (1.8)	7.82 (5.06–12.09)	**< 0.001**
Respiratory rate (mean ± SD)	45.5 (15.2)	47.0 (18.5)	1.01 (1.00–1.01)	**< 0.001**
Increased respiratory rate, *n* (%)	24,211 (55.9)	834 (56.5)	1.02 (0.92–1.14)	0.67
Respiratory distress, *n* (%)	9,373 (21.7)	427 (29.0)	1.47 (1.31–1.65)	**< 0.001**
Dehydration, *n* (%)	6,935 (16.0)	292 (19.8)	1.29 (1.14–1.47)	**< 0.001**
Pallor, *n* (%)	7,789 (18.0)	316 (21.4)	1.24 (1.09–1.41)	**< 0.001**
Jaundice, *n* (%)	613 (1.4)	55 (3.7)	2.69 (2.03–3.57)	**< 0.001**
Edema, *n* (%)	2,678 (6.2)	134 (9.1)	1.51 (1.26–1.81)	**< 0.001**
Stiff neck, *n* (%)	399 (0.9)	20 (1.4)	1.48 (0.94–2.33)	0.09
Prostration, *n* (%)	6,613 (15.3)	489 (33.1)	2.75 (2.45–3.07)	**< 0.001**
BCS on admission (mean ± SD)	4.9 (0.5)	4.5 (1.2)	4.10 (3.72–4.52)	**< 0.001**
Unconsciousness (BCS < 5), *n* (%)	2,270 (5.3)	289 (19.7)	4.42 (3.85–5.06)	**< 0.001**
Deep coma (BCS ≤ 2), *n* (%)	693 (1.6)	146 (9.9)	6.77 (5.61–8.17)	**< 0.001**
Investigation				
Plasmodium falciparum malaria, *n* (%)	22,186 (54.4)	716 (56.2)	1.07 (0.96–1.20)	0.21
HIV, *n* (%)	341 (22.1)	12 (27.9)	0.73 (0.37–1.45)	0.37
Severe anemia, *n* (%)	1,653 (4.2)	77 (6.5)	1.59 (1.26–2.02)	**< 0.001**
Positive blood culture, *n* (%)	2,803 (7.9)	177 (14.8)	2.02 (1.72–2.38)	**< 0.001**
Clinical severe pneumonia (WHO criteria), *n* (%)	9,934 (23.0)	370 (25.1)	1.12 (1.00–1.27)	0.053
Outcome				
Length of admission (mean ± SD)	4.8 (5.8)	5.0 (6.1)	1.00 (0.99–1.01)	0.21
Died, *n* (%)	1,307/40,063 (3.3)	245/1,267 (19.3)	7.11 (6.11–8.27)	**< 0.001**

BCS = Blantyre coma score; CI = confidence interval; HIV = human immunodeficiency virus; IQR = interquartile range; OR = odds ratio; SD = standard deviation; WHO = World Health Organization; WAZ = weight-for-age *z* score.
Significance: *P*-value of the statistical test comparing normoglycemic and hypoglycemic children on admission.
* Mann–Whitney test for the difference of two medians.

were all associated with hypoglycemia, while a history of cough was protective against it. All identified factors showed small effect size (OR = < 2 for all risk factors and OR = > 0.5 for history of cough), except unconsciousness, whose effect size was medium (OR = 2.13, 95% CI = 1.66, 2.72).

Risk factors for death in children admitted with hypoglycemia. Of the 1,267 children with hypoglycemia on admission and outcome results, 245 died, yielding an overall CFR of 19.3%. Independent risk factors for death in children admitted with hypoglycemia included anorexia and malnutrition with a small effect size (OR = < 2), prostration and edema with medium effect size (OR = 2–3), and unconsciousness, oral candidiasis-positive blood culture, and respiratory distress with large effect size (OR = > 3) (Table 3).

Minimum community-based incidence rates. Overall MCBIR throughout the study period was 1.57 episodes/1,000 CYAR. MCBIR trends for hypoglycemia in all pediatric age groups during the 13-year-long study period are shown in Figure 4. MCBIR peaked at 3.73/1,000 CYAR in 2001 (first year of the study) and significantly decreased subsequently, reaching the nadir in 2013 (0.50/1,000 CYAR; $P < 0.001$). MCBIR were significantly higher in newborns (9.47 episodes/1,000 CYAR) than in any other age group ($P < 0.001$; Table 4).

DISCUSSION

The MSS ongoing at MDH, in rural Mozambique, has allowed us to retrospectively review the prevalence of hypoglycemia in nearly 50,000 pediatric admissions, spanning across a 13-year-long period. This is perhaps the largest series examined in the developing world for the occurrence of this common (3.2% of children in this series) albeit insufficiently highlighted life-threatening complication. A study performed in an urban referral center in Mozambique two decades ago[28] showed a higher prevalence (7.1%), similar to the studies conducted in a rural Kenyan hospital[7] and in a Nigerian pediatric emergency ward.[29] Other more recent studies in a high-malaria-endemic area in Mali[30] or among febrile children in Tanzania[12] have shown hypoglycemia prevalence similar to this study. More important than its frequency, its associated mortality risk needs to be overemphasized. Indeed, in this series, a fifth of all hypoglycemia cases on admission ended

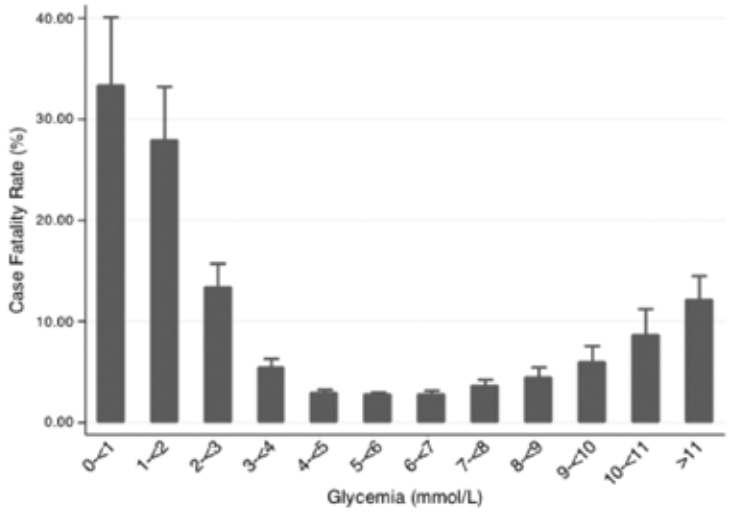

FIGURE 3. Case fatality rates (CFRs) according to glycemia level.

up in death. Although it remains to be seen how much did hypoglycemia contribute to each individual outcome, it is clear, and has robustly been shown, that hypoglycemia carries an excessive and unacceptable risk of death[7,12,24,28,29] even if detected only as a single episode.[31,32] The CFR associated with hypoglycemia was lower in this cohort (19.3%) than in other studies.[12,29] This possibly reflects variations in case management and access to health care, and also relates to the fact that no clear consensus has yet been reached regarding the glycemia cutoff level at which to define hypoglycemia. Indeed, different authors have proposed different hypoglycemia thresholds, regardless of the presence or absence of malaria in the area.[7,8,12,29] To allow comparisons, simplify guidelines, and homogenize management, the scientific community should align in defining a unique value. Irrespective of the threshold used, mortality dramatically increases with glycemia lower than 3 mmol/L, with a linear and steep inverse relationship between mortality and glycemia levels below this value. In our series, however, borderline values still carried a worse prognosis, with risk of death doubling in children with admission glycemia between 3 and 4 mmol/L when compared with those with higher levels.

Of all hypoglycemia episodes, 15.9% affected newborns, and 8.8% of all admitted newborns had hypoglycemia, highlighting

TABLE 2
Multivariate analysis of independent risk factors associated to hypoglycemia on admission

Risk factors	Hypoglycemia (N = 1,478), n (%)	Adjusted OR	95% CI		P value
			Lower	Upper	
Newborn	226 (15.3)	1.05	0.56	1.95	0.879
Male	841 (57.0)	1.12	0.96	1.30	0.143
Malnutrition (per unit WAZ decrease)	908 (61.4)*	1.12	1.07	1.18	**< 0.001**
History of fever	1,207 (81.7)	0.72	0.50	1.02	0.062
History of cough	825 (55.8)	0.82	0.69	0.97	**0.020**
Hypothermia on admission	26 (1.8)	3.21	0.79	12.94	0.101
History of seizures	211 (14.3)	1.47	1.18	1.84	**0.001**
Anorexia/refusing to feed	406 (30.1)	1.67	1.38	2.02	**< 0.001**
Unconsciousness (BCS < 5)	289 (19.7)	2.13	1.66	2.72	**< 0.001**
Edema	134 (9.1)	1.56	1.17	2.08	**0.002**
Oral candidiasis	66 (4.5)	1.14	0.74	1.74	0.550
Jaundice	55 (3.7)	1.95	1.25	3.03	**0.003**
Respiratory distress	427 (29.0)	1.22	0.97	1.54	0.092
Pallor	316 (21.4)	1.20	0.99	1.45	0.066
Dehydration	292 (19.8)	0.94	0.76	1.15	0.536
Neck stiffness	20 (1.4)	1.00	0.57	1.77	0.994
Positive blood culture	177 (14.8)	1.66	1.32	2.09	**< 0.001**
Plasmodium falciparum malaria	716 (56.2)	1.29	1.09	1.52	**0.003**
Prostration	489 (33.1)	1.64	1.36	1.98	**< 0.001**

BCS = Blantyre coma score; CI = confidence interval; OR = odds ratio; WAZ = weight-for-age z score.
Significance: P-value of the multivariate analysis of independent risk factors associated to hypoglycemia.
*Number and proportion of children with hypoglycemia who had severe malnutrition (i.e., WAZ < −3).

TABLE 3
Multivariate analysis of independent risk factors associated to mortality in children with hypoglycemia on admission

Risk factors for adverse outcome	Hypoglycemia deaths (N = 245), n/N (%)	Adjusted OR	95% CI		P value
			Lower	Upper	
Newborn	46/245 (18.8)	0.99	0.39	2.49	0.977
History of fever	182/245 (74.3)	0.52	0.23	1.15	0.106
History of seizures	53/245 (21.6)	1.08	0.59	1.98	0.798
Anorexia/refusing to feed	117/224 (52.2)	1.74	1.02	2.97	**0.042**
Hypothermia on admission	11/245 (4.5)	0.58	0.04	9.03	0.695
Unconsciousness (BCS < 5)	106/245 (43.3)	3.10	1.75	5.49	**< 0.001**
Prostration	160/245 (65.3)	2.34	1.30	4.23	**0.005**
Oral candidiasis	31/244 (12.7)	4.80	1.80	12.8	**0.001**
Edema	45/245 (18.4)	2.67	1.25	5.71	**0.011**
Pallor	72/245 (29.4)	1.40	0.81	2.42	0.225
Dehydration	67/245 (27.4)	1.06	0.59	1.89	0.846
Jaundice	17/245 (6.9)	2.43	0.84	7.06	0.102
Positive blood culture	75/211 (35.6)	3.24	1.85	5.66	**< 0.001**
Plasmodium falciparum malaria	77/206 (837.4)	0.62	0.37	1.04	0.069
Malnutrition (per unit WAZ decrease)	143/245 (58.4)*	1.21	1.03	1.42	**0.019**
Respiratory distress	139/245 (56.7)	2.03	1.25	3.30	**0.004**

BCS = Blantyre coma score; CI = confidence interval; OR = odds ratio; WAZ = weight-for-age Z score.
Significance: P-value of the multivariate analysis of independent risk factors associated to mortality in children with hypoglycemia.
*Number and proportion of children who died with hypoglycemia and severe malnutrition (i.e., WAZ < −3).

the importance of hypoglycemia in this age group. However, hypoglycemia also commonly affected—and killed—children of all ages, which justifies universal screening for hypoglycemia among all admitted children in the developing world.

Most studies describing the incidence of hypoglycemia and associated outcomes are based on the determination of glycemia at a fixed point, usually admission. Our results are also limited by this lack of follow-up. We probably missed children who were normoglycemic on admission, but developed hypoglycemia subsequently. So we may be underestimating the incidence of hypoglycemia and the OR of death associated with hypoglycemia occurring during hospitalization. Similarly, we were unable to determine how many recurrent hypoglycemia episodes occurred in our series. Few studies in developing countries have monitored glycemia throughout the whole hospitalization. This is now possible through the innovative use of continuous glucose monitoring (CGM). CGM can be performed through a subcutaneous sensor that measures the interstitial glucose level—closely related to the blood glucose level—every 5 minutes, uninterruptedly 24 hours a day, and for as long as a week. CGM is slowly being introduced in pediatric intensive care units of developed countries,[33,34] but has not reached yet in the developing world, possibly due to its high cost.

Our large sample size gave us sufficient power to assess the association of different clinical and laboratorial characteristics with the presence of hypoglycemia on admission or with hypoglycemia-related mortality. Although many of such

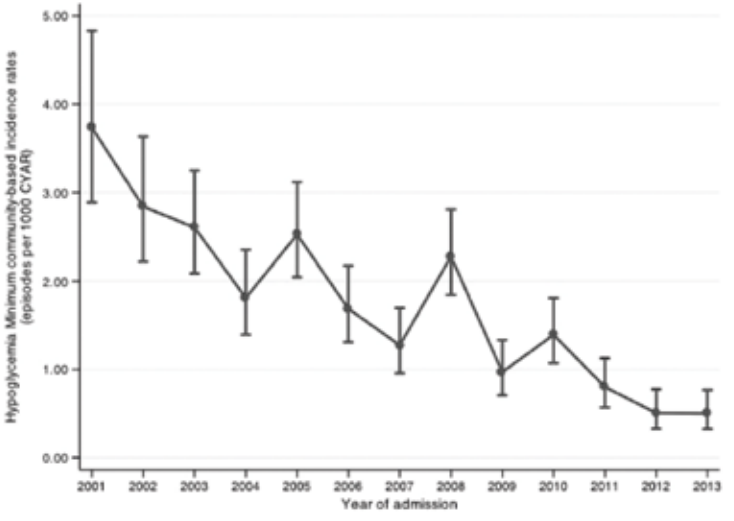

FIGURE 4. Minimum community-based incidence rates (MCBIRs) of hypoglycemia according to year (vertical bars indicate 95% confidence intervals [CI]).

TABLE 4
MCBIRs of hypoglycemia among children admitted to Manhiça District Hospital, according to age group

				Rate estimations		Model estimations		
Age groups	Subjects	Episodes	Time at risk (CYAR)	Incidence rate (episodes per 1,000 CYAR)	95% CI	IRR	95% CI	*P* value
Newborns	38,442	28	2,957.53	9.47	(6.54, 13.71)	1	–	< 0.0001
28 days to < 1year	42,682	117	33,651.90	3.48	(2.90, 4.17)	0.37	(0.24, 0.55)	
1 to < 2 years	41,429	170	34,865.01	4.88	(4.20, 5.67)	0.51	(0.34, 0.76)	
2 to < 3 years	39,774	139	33,825.08	4.11	(3.48, 4.85)	0.43	(0.29, 0.65)	
3 to < 4 years	38,418	113	33,157.82	3.41	(2.83, 4.10)	0.35	(0.23, 0.53)	
4 to < 5 years	37,461	55	32,422.22	1.70	(1.30, 2.21)	0.17	(0.11, 0.27)	
5 to < 15 years	63,103	82	276,806.07	0.30	(0.24, 0.37)	0.03	(0.02, 0.05)	
Total	90,472	704	447,685.62	1.57	(1.46, 1.69)	–	–	–

CYAR = child years at risk; CI = confidence interval; IRR = incidence rate ratio; MCBIRs = minimum community-based incidence rates.
P value from negative binomial regression model with random effects using likelihood ratio test.

factors have been previously described in the literature, three major groups of factors stand out as significantly associated in our series with the risk of having hypoglycemia: 1) not being able to feed[35] (as directly reported by the mother or as a consequence of an altered clinical condition decreasing the capacity to feed [altered consciousness or coma,[25,36–38] prostration, a history of seizures]); 2) malnutrition (including edema as a common sign typically associated with this condition); and 3) concomitant infections such as invasive bacterial disease or *P. falciparum* malaria. In addition, jaundice was also identified as an important risk factor for hypoglycemia, a finding also previously reported in Tanzania.[12]

Fasting is a recognized risk factor causing hypoglycemia in children.[35] During fasting, plasma glucose levels are maintained within narrow limits by a delicate balance between endogenous glucose production deriving from glycogenolysis and gluconeogenesis.[39] Studies performed in children with malaria have demonstrated a relationship between fasting, hypoglycemia, severity of disease, and mortality.[7,40] Conditions that decrease consciousness may also result in a prolonged fasting state, explaining the strong association found between consciousness level and the risk of hypoglycemia. However, as hypoglycemia per se is a cause of decreased consciousness, interpreting the direction of the association is not straightforward. Hypoglycemia should, however, always be investigated in the presence of a child unable to feed or unconscious.

The association between malnutrition and hypoglycemia has also been firmly established in the past.[11,13,41] In malnourished patients, glucose homeostasis[41] can be compromised in several ways, including a lack of exogenous nutritional intake, decreased absorption of disaccharides because of intestinal villous atrophy, increased oxidative stress, or glucose uptake compromised by intestinal bacteria. In our series, 62% of all hypoglycemic children were malnourished, with 19.4% being severely malnourished. Kenyan[7] and Tanzanian[12] studies had already shown a strong association between severe malnutrition (WAZ < −3) and hypoglycemia, but not with WAZ < −1.

The association of hypoglycemia with bacterial sepsis[14,18,25,26,38] or malaria[7,14,26] is also well documented. Our series also support these associations, as risk of hypoglycemia was increased by 68% among bacteremic patients and by 30% in malaria-infected patients. In bacterial disease, hypoglycemia has been attributed to a series of factors, including high circulating levels of cytokines such as tumor necrosis factor and interleukin-6, both powerful stimulators of insulin secretion, which can then cause among other things inhibition of the gluconeogenic pathways.[36,42] Decreased levels of glycemia secondary to the consumption of glucose by the *Plasmodium* parasite, hyperinsulinism caused by quinine, impaired gluconeogenesis, and lack of adequate supplementation/oral intake are possible explanations in malaria.[37,38,40,43]

Independent risk factors associated with hypoglycemia mortality are similar to those found associated with the risk of hypoglycemia. Again, factors related to feeding difficulties or the presence of a clinical condition hindering feeding (unconsciousness, prostration, respiratory distress), malnutrition, and concomitant severe infections (in this case only bacteremia) were all significantly and independently associated with a higher odds of death among patients with hypoglycemia. In these patients, the multivariate analysis also identified edema and oral candidiasis as important prognostic factors. The association between mortality and these factors has not been properly described in the literature, but it is likely that edema is associated with kwashiorkor (protein-deficient malnutrition) and oral candidiasis is a proxy of HIV coinfection, highly endemic in the Manhiça area[22] and highly prevalent among malnourished patients.[13]

Regarding hyperglycemia, we found a prevalence of 1.7%, and a significantly higher associated CFR (12.1%, $P < 0.001$) when compared with normoglycemia. Other studies in similar settings have shown similar[7] or higher[8,44] prevalence rates. Hyperglycemia is an insufficiently well-known risk factor for death[10] in the developing world, and efforts for its early detection and correction should parallel those devoted to hypoglycemia. Glucose variability has recently emerged as a new concept and is considered to have important influence on the outcome of critically ill patients[45,46] and possibly cause more harm than sustained hyperglycemia. More studies are required, using CGM, to specifically address this issue.

We report for the first time MCBIRs for hypoglycemia in sub-Saharan Africa. Overall MCBIR throughout the study period were 1.57 episodes/1,000 CYAR, peaking at 3.73 episodes/1,000 CYAR in 2001, underscoring the high burden of this particularly dangerous complication. Reasons for the decreasing trends observed throughout the study period still need to be clarified, although we could hypothesize a better and earlier access to medical care or lower incidence of malaria and/or malnutrition in the last years in the study area. Newborns showed the highest incidence rates of hypoglycemia, underscoring the need to carefully follow this complication in this particularly vulnerable age group.

Hypoglycemia is a silent and under-recognized killer of African children and needs to be properly exposed because the correction of hypoglycemia is simple and has rapid effects

on the health of the child. However, in resource-constrained settings where dextrose infusion is not readily available or is operationally challenging, other alternatives to intravenous administration should be investigated and promoted to correct hypoglycemia in children unable to feed. In this respect, sublingual sugar or preprepared dextrose gel appear as promising treatments for the prevention and correction of hypoglycemia in children with hypoglycemia.[30]

Our study had several limitations. There are concerns with the accuracy of commercial finger-prick blood glucose assays. Advances in glucose meter technology have resulted in significant improvement of accuracy and precision of meters. However, those meters are not available in developing countries due to higher cost. Another limitation is the use of the glucose meters instead of a laboratory serum or plasma glucose concentration to measure glycemia. It is known there are physical differences between the glucose concentration in serum or plasma and the glucose from capillary blood, but formal laboratory determination was not available in our settings. Unfortunately, study subject only had one determination of glycemia measured on admission, and we were unable to know if any recurrent hypoglycemia episodes occurred in our study. Because there is no universally applicable definition of hypoglycemia, we used the threshold established by national guidelines. Different glycemia cutoff levels could modify the results.

CONCLUSION

Hypoglycemia is a common complication of many conditions causing hospitalization in Mozambican children and is associated with unacceptable adverse outcomes. In settings similar to Manhiça, all admitted children should be screened for hypoglycemia and aggressively managed when found to be hypoglycemic. A single determination on admission is not enough, and glycemia should be recurrently screened during hospitalization. Better, cheaper, and more innovative diagnostic and therapeutic alternatives need to be urgently investigated to better address the consequences of hypoglycemia in developing countries.

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REFERENCES

1. Hirshberg E, Larsen G, Van Duker H, 2008. Alterations in glucose homeostasis in the pediatric intensive care unit: hyperglycemia and glucose variability are associated with increased mortality and morbidity. *Pediatr Crit Care Med 9:* 361–366.
2. Faustino EV, Hirshberg EL, Bogue CW, 2012. Hypoglycemia in critically ill children. *J Diabetes Sci Technol 6:* 48–57.
3. Ognibene KL, Vawdrey DK, Biagas KV, 2011. The association of age, illness severity, and glycemic status in a pediatric intensive care unit. *Pediatr Crit Care Med 12:* e386–e390.
4. Zijlmans WC, van Kempen AA, Serlie MJ, Kager PA, Sauerwein HP, 2013. Adaptation of glucose metabolism to fasting in young children with infectious diseases: a perspective. *J Pediatr Endocrinol Metab 27:* 5–13.
5. Kraft R, Herndon DN, Mlcak RP, Finnerty CC, Cox RA, Williams FN, Jeschke MG, 2014. Bacterial respiratory tract infections are promoted by systemic hyperglycemia after severe burn injury in pediatric patients. *Burns 40:* 428–435.
6. Hirata Y, Tomioka H, Sekiya R, Yamashita S, Kaneda T, Kida Y, Nishio C, Kaneko M, Fujii H, Nakamura T, 2013. Association of hyperglycemia on admission and during hospitalization with mortality in diabetic patients admitted for pneumonia. *Intern Med 52:* 2431–2438.
7. Osier FH, Berkley JA, Ross A, Sanderson F, Mohammed S, Newton CR, 2003. Abnormal blood glucose concentrations on admission to a rural Kenyan district hospital: prevalence and outcome. *Arch Dis Child 88:* 621–625.
8. Sambany E, Pussard E, Rajaonarivo C, Raobijaona H, Barennes H, 2013. Childhood dysglycemia: prevalence and outcome in a referral hospital. *PLoS One 8:* e65193.
9. Faustino EV, Apkon M, 2005. Persistent hyperglycemia in critically ill children. *J Pediatr 146:* 30–34.
10. Bhutia TD, Lodha R, Kabra SK, 2013. Abnormalities in glucose homeostasis in critically ill children. *Pediatr Crit Care Med 14:* e16–e25.
11. World Health Organization, 2013. *Pocket Book for Hospital Care of Children: Guidelines for the Management of Common Illness with Limited Resources*, 2nd edition. Geneva, Switzerland: World Health Organization.
12. Nadjm B, Mtove G, Amos B, Hildenwall H, Najjuka A, Mtei F, Todd J, Reyburn H, 2013. Blood glucose as a predictor of mortality in children admitted to the hospital with febrile illness in Tanzania. *Am J Trop Med Hyg 89:* 232–237.
13. Nhampossa T, Sigauque B, Machevo S, Macete E, Alonso P, Bassat Q, Menendez C, Fumado V, 2013. Severe malnutrition among children under the age of 5 years admitted to a rural district hospital in southern Mozambique. *Public Health Nutr 16:* 1565–1574.
14. Houin S, Rozance PJ, 2014. 50 years ago in the Journal of Pediatrics: the incidence of neonatal hypoglycemia in a nursery for premature infants. *J Pediatr 164:* 1485.
15. Harris DL, Weston PJ, Signal M, Chase JG, Harding JE, 2013. Dextrose gel for neonatal hypoglycaemia (the Sugar Babies Study): a randomised, double-blind, placebo-controlled trial. *Lancet 382:* 2077–2083.
16. Pisarchik AN, Pochepen ON, Pisarchyk LA, 2012. Increasing blood glucose variability is a precursor of sepsis and mortality in burned patients. *PLoS One 7:* e46582.
17. Badawi O, Waite MD, Fuhrman SA, Zuckerman IH, 2012. Association between intensive care unit-acquired dysglycemia and in-hospital mortality. *Crit Care Med 40:* 3180–3188.
18. Sigauque B, Roca A, Mandomando I, Morais L, Quinto L, Sacarlal J, Macete E, Nhamposa T, Machevo S, Aide P, Bassat Q, Bardaji A, Nhalungo D, Soriano-Gabarro M, Flannery B, Menendez C, Levine MM, Alonso PL, 2009. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J 28:* 108–113.
19. Ikeda T, Takahashi T, Sato A, Tanaka H, Igarashi S, Fujita N, Kuwabara T, Kanazawa M, Nishizawa M, Shimohata T, 2013. Predictors of outcome in hypoglycemic encephalopathy. *Diabetes Res Clin Pract 101:* 159–163.
20. Fong CY, Harvey AS, 2014. Variable outcome for epilepsy after neonatal hypoglycaemia. *Dev Med Child Neurol 56:* 1093–1099.

21. Sacarlal J, Nhacolo AQ, Sigauque B, Nhalungo DA, Abacassamo F, Sacoor CN, Aide P, Machevo S, Nhampossa T, Macete EV, Bassat Q, David C, Bardaji A, Letang E, Saute F, Aponte JJ, Thompson R, Alonso PL, 2009. A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique. *BMC Public Health 9:* 67.
22. Gonzalez R, Munguambe K, Aponte J, Bavo C, Nhalungo D, Macete E, Alonso P, Menendez C, Naniche D, 2012. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med 13:* 581–588.
23. Sacoor C, Nhacolo A, Nhalungo D, Aponte JJ, Bassat Q, Augusto O, Mandomando I, Sacarlal J, Lauchande N, Sigauque B, Alonso P, Macete E, Munguambe K, Guinovart C, Aide P, Menendez C, Acacio S, Quelhas D, Sevene E, Nhampossa T, 2013. Profile: Manhica Health Research Centre (Manhica HDSS). *Int J Epidemiol 42:* 1309–1318.
24. Bassat Q, Guinovart C, Sigauque B, Aide P, Sacarlal J, Nhampossa T, Bardaji A, Nhacolo A, Macete E, Mandomando I, Aponte JJ, Menendez C, Alonso PL, 2008. Malaria in rural Mozambique. Part II: children admitted to hospital. *Malar J 7:* 37.
25. Bassat Q, Guinovart C, Sigauque B, Mandomando I, Aide P, Sacarlal J, Nhampossa T, Bardaji A, Morais L, Machevo S, Letang E, Macete E, Aponte JJ, Roca A, Menendez C, Alonso PL, 2009. Severe malaria and concomitant bacteraemia in children admitted to a rural Mozambican hospital. *Trop Med Int Health 14:* 1011–1019.
26. Grummer-Strawn LM, Reinold C, Krebs NF, 2010. Use of World Health Organization and CDC growth charts for children aged 0–59 months in the United States. *MMWR Recomm Rep 59:* 1–15.
27. Sullivan GM, Feinn R, 2012. Using effect size—or why the *P* value is not enough. *J Grad Med Educ 4:* 279–282.
28. Solomon T, Felix JM, Samuel M, Dengo GA, Saldanha RA, Schapira A, Phillips RE, 1994. Hypoglycaemia in paediatric admissions in Mozambique. *Lancet 343:* 149–150.
29. Elusiyan JB, Adejuyigbe EA, Adeodu OO, 2006. Hypoglycaemia in a Nigerian paediatric emergency ward. *J Trop Pediatr 52:* 96–102.
30. Graz B, Dicko M, Willcox ML, Lambert B, Falquet J, Forster M, Giani S, Diakite C, Dembele EM, Diallo D, Barennes H, 2008. Sublingual sugar for hypoglycaemia in children with severe malaria: a pilot clinical study. *Malar J 7:* 242.
31. Krinsley JS, 2013. Glycemic control in the critically ill—3 domains and diabetic status means one size does not fit all! *Crit Care 17:* 131.
32. Rattarasarn C, 1997. Hypoglycemia in sepsis: risk factors and clinical characteristics. *J Med Assoc Thai 80:* 760–766.
33. Branco RG, Chavan A, Tasker RC, 2009. Pilot evaluation of continuous subcutaneous glucose monitoring in children with multiple organ dysfunction syndrome. *Pediatr Crit Care Med 11:* 415–419.
34. Bridges BC, Preissig CM, Maher KO, Rigby MR, 2010. Continuous glucose monitors prove highly accurate in critically ill children. *Crit Care 14:* R176.
35. Thien HV, Kager PA, Sauerwein HP, 2006. Hypoglycemia in falciparum malaria: is fasting an unrecognized and insufficiently emphasized risk factor? *Trends Parasitol 22:* 410–415.
36. Yelich MR, Filkins JP, 1980. Mechanism of hyperinsulinemia in endotoxicosis. *Am J Physiol 239:* E156–E161.
37. White NJ, Miller KD, Marsh K, Berry CD, Turner RC, Williamson DH, Brown J, 1987. Hypoglycaemia in African children with severe malaria. *Lancet 1:* 708–711.
38. White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, Krishna S, Williamson DH, Turner RC, 1983. Severe hypoglycemia and hyperinsulinemia in falciparum malaria. *N Engl J Med 309:* 61–66.
39. Robinson PJ, Rapoport SI, 1986. Glucose transport and metabolism in the brain. *Am J Physiol 250:* R127–R136.
40. Kawo NG, Msengi AE, Swai AB, Chuwa LM, Alberti KG, McLarty DG, Orskov H, 1990. Hypoglycaemia and cerebral malaria. *Lancet 336:* 1128–1129.
41. Bandsma RH, Mendel M, Spoelstra MN, Reijngoud DJ, Boer T, Stellaard F, Brabin B, Schellekens R, Senga E, Heikens GT, 2010. Mechanisms behind decreased endogenous glucose production in malnourished children. *Pediatr Res 68:* 423–428.
42. Romijn JA, Godfried MH, Wortel C, Sauerwein HP, 1990. Hypoglycemia, hormones and cytokines in fatal meningococcal septicemia. *J Endocrinol Invest 13:* 743–747.
43. Dekker E, Hellerstein MK, Romijn JA, Neese RA, Peshu N, Endert E, Marsh K, Sauerwein HP, 1997. Glucose homeostasis in children with falciparum malaria: precursor supply limits gluconeogenesis and glucose production. *J Clin Endocrinol Metab 82:* 2514–2521.
44. Willcox ML, Forster M, Dicko MI, Graz B, Mayon-White R, Barennes H, 2009. Blood glucose and prognosis in children with presumed severe malaria: is there a threshold for 'hypoglycaemia'? *Trop Med Int Health 15:* 232–240.
45. Rake AJ, Srinivasan V, Nadkarni V, Kaptan R, Newth CJ, 2010. Glucose variability and survival in critically ill children: allostasis or harm? *Pediatr Crit Care Med 11:* 707–712.
46. Wintergerst KA, Buckingham B, Gandrud L, Wong BJ, Kache S, Wilson DM, 2006. Association of hypoglycemia, hyperglycemia, and glucose variability with morbidity and death in the pediatric intensive care unit. *Pediatrics 118:* 173–179.

ARTICLE 7

Post-discharge mortality in children admitted to a rural Mozambican hospital: development of a prediction model to identify children at risk of dying

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Under review

Post-discharge mortality in children admitted to a rural Mozambican hospital: development of prediction models to identify children at risk of dying

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ABSTRACT

Background: Although the burden of post-discharge mortality (PDM) in low-income settings appears significant, no clear recommendations have been proposed in relation to follow-up care after hospitalization. We aimed to determine the burden of paediatric PDM and develop predictive models to identify children at risk of dying following discharge.

Methods: Deaths after hospital discharge among children aged <15 years in the last 16 years were reviewed in an area under demographic and morbidity surveillance in Southern Mozambique. We determined PDM over time (up to 90 days) and derived predictive models of PDM using easily collected variables upon admission.

Results: Overall PDM was 3.6%, with half of the deaths occurring in the first 30 days. One primary predictive model for all ages included young age, malnutrition, history of diarrhoea, clinical pneumonia symptoms, prostration, HIV and/or malnutrition associated symptoms, bacteraemia, positive HIV status, rainy season and transfer or absconding, with an area under the curve (AUC) ~80% during the whole follow-up. Alternative models for all ages including simplified clinical predictors had a similar performance. A model specific to infants <3 months identified as predictors: being a neonate, low WAZ score, difficulty breathing, hypothermia or fever, oral candidiasis and history of absconding or transfer to another hospital, with an AUC ~80% during the 90-days of follow-up.

Conclusions: Death following discharge is an important although poorly recognized contributor to child mortality. A simple predictive algorithm based on easy-to-collect variables could readily identify most infants and children at high risk of dying after discharge.

KEYWORDS

Post-discharge mortality, child mortality, low-income countries, predictive models, post-discharge follow-up.

KEY MESSAGES BOX

- Post-discharge mortality is as high as inpatient mortality in African rural context.
- Young age, malnutrition, history of diarrhoea, clinical pneumonia symptoms, prostration, HIV and/or malnutrition associated symptoms, bacteraemia, positive HIV status, rainy season and history of absconding or transfer to another hospital were predictors to identify children <15 years with higher risk of dying after a hospital admission.

• Being a neonate, low WAZ score, difficulty breathing, hypothermia or fever, oral candidiasis and history of absconding or transfer to another hospital were predictors to identify children <3 months with higher risk of dying after a hospital admission.

• Simple predictive algorithm based on easy-to-collect variables could be applied at hospital discharge and children at risk of dying could be identified through their use. This could allow designing a better post-discharge planning, health education to the families and follow-up care.

BACKGROUND

The last 25 years have witnessed a significant (56%) reduction in under-5 (U5) child mortality[1]. This decreasing trend has been observed in all regions, with great advances in Northern Africa, Eastern and Western Asia and Latin America. As positive as these trends may appear, many low-income countries (LIC), and particularly those in Sub-Saharan Africa, have failed to meet the two thirds reduction objective set by the fourth millennium development goal[2].

In the last decades, algorithms for diagnosis and treatment of sick children have been developed by policy makers in order to address the management of disease during the acute phase. Their wide implementation by health providers have contributed to improve child survival[3]. Such guidelines and recommendations, however, have historically failed to address the days immediately following hospitalization, a critical period for child survival4. The fragility of the health system and scarcity of the health force in LIC, the unaffordable high costs to provide care for acute illness and the generalised lack of awareness about the burden and implications of post-discharge mortality (PDM) by health care workers[4, 5] partly explain this neglect.
Contrary to what occurs in industrialized countries, where PDM is limited to certain small, high-risk groups[6, 7], children in resource-constrained countries appear to be at increased risk of mortality following hospitalisation for any illness[4, 5, 8-13]. Estimates of PDM in children living in LIC vary significantly among studies4, likely due to differences in the setting's characteristics, study design, target population or duration of follow-up[4]. Studies of all paediatric hospital admissions some decades ago estimated risk of PDM to range between 3.3% and 13%[10, 14, 15]. Studies on admissions due to malaria found a risk of PDM of around 2.0-2.6%[16, 17]. Estimates for other causes of admission including diarrhoea (2.9 to 7.0%)[18, 19], anaemia (2.7-11.6%)[20], pneumonia (1.0-15.0%)[15, 21] or invasive bacterial diseases (2.8%)[8] were

also unacceptably high. In Kenya, PDM was 3.3-4.5% in children aged under-15 years admitted with any disease and followed-up for one year[10] and in Uganda, almost 5% of children admitted due to acute infectious diseases died in the 6 months following discharge, with most of these deaths occurring in the first 30 days[5]. A systematic review on PDM in LIC found that history of previous hospitalizations, young age, HIV infection and hospitalizations related to malnutrition or pneumonia were the most important predictors of PDM4.

A rigorous follow-up of all hospital discharged children would be unfeasible and unaffordable for resource-constrained settings[5]. Thus, early identification of vulnerable children appears essential to design more targeted interventions to prevent PDM5. Some researchers have tried to develop simple risk-scoring algorithms5 or prediction models based on selected candidate predictor variables associated with PDM22. However, these tools have not been validated for global use. Improving the discharge process and post-discharge care will be a critical step to further continue reducing child mortality4.We therefore aimed to determine the burden of paediatric PDM in a semi-rural area from southern Mozambique, to identify predictors of mortality following discharge and to derive models that could efficiently stratify children according to PDM risk.

METHODS

Study site and population

This study was conducted in Manhiça, southern Mozambique, a semi-rural setting with a predominantly young population (45% <15 years of age). In 2015, the U5 mortality rate in Mozambique was 78.5/1000 live births[23], with available data for Manhiça district being very similar. The Manhiça Health Research Center (CISM) is one of the leading research centre in Africa with a well-developed clinical laboratory which supports the Manhiça District Hospital (MDH). CISM runs a demographic surveillance system (DSS) in the area, and a paediatric morbidity surveillance system (MSS) has been implemented for nearly two decades at MDH and five other peripheral health posts, accurately capturing standardized morbidity data for 3000 child admissions per year and > 75 000 annual outpatient visits. Main causes of paediatric admissions at MDH include malaria, pneumonia, diarrhoea, malnutrition, and neonatal conditions[24]. Human immunodeficiency virus (HIV) prevalence in the area is among the highest in the world[25] and, in some areas, up to 40% of pregnant women attending the antenatal care

clinics are HIV-infected[26]. Vertical transmission of HIV has been estimated at around 9%[27] and contributing 10% to the U5 mortality nationally[28]. A detailed description of CISM and the study area can be found elsewhere29.

Study design and definitions

A retrospective cohort study of children <15 years discharged from the MDH from 1st January 2000 to 31st December 2016 was conducted, using the DSS and MSS databases. We analysed burden of PDM over three different post-discharge time-periods: 1-30 days, 31-60 days and 61-90 days. PDM among infants less than three months old was also analysed separately to check whether identified predictors differed from those of the whole cohort. Only children living in the study area and having a permanent identification number were included. In-hospital deaths during the initial admission and deaths in the first 24 hours after discharge were excluded. Those readmissions of children already being followed-up did not count as new admissions to avoid re-starting the period at risk every time. In these cases, we used a single-discharge approach, considering the first admission as reference admission. Post-discharge death was defined as a death occurring more than 24 hours after and within 90 days of discharge from MDH; community death as a death ocurring outside a health facility within 90 days after discharge and facility death as a death ocurring at any health facility within 90 days following discharge from MDH. Chidren dying during a readmission within the follow-up period were considered as a hospital death within follow-up (supplementary figure S1). Other relevant definitions may be found in supplementary table S1.

Morbidity surveillance system

Morbidity surveillance data routinely collected for all children less than 15 years during the study period were analysed, including clinical data (medical history and physical examination), basic laboratory investigations (including malaria microscopy, haematocrit and glycaemia), ICD-10 based diagnoses, outcome and medication prescribed. For admitted children, blood culture results, systematically performed for all children under 2 years and, in older children, in the presence of severe symptoms or according to the admitting clinician's call, were available, and HIV status information, although not routinely collected, was available for those patients with suspected immunosuppression.

Demographic Surveillance System

CISM has been conducting demographic surveillance since the year 1996, covering an increasing proportion over time of the Manhiça District, and now covers 2380 km2 and a total population of ~183 000 inhabitants. During periods in which the entire district was not covered, analysis has excluded inpatients not part of the DSS. Through a unique identifier number, DSS and MSS databases can be linked. Data collected in the Manhiça DSS, updated through two annual rounds, includes household and individual variables, socio-economic status, vital data, migration, health history, cause and date of death, among others.

Data management and data analysis

Questionnaires were double-entered on site into databases using Foxpro and OpenClinica software. Both entries were compared and discrepancies resolved by referring to the original forms. A survival analysis was performed to model events within 90 days of discharge. The discharge date+1 day was used as date of entry to the study whilst post-discharge death, loss to follow-up or end of follow-up period (90 days after discharge) were used as the exit time. For each variable with a high proportion of missing values but suspected to be a strong confounder, a "missing" category was created. In order to achieve the study objectives, we split the data analysis in two parts: *a) Determining burden and identifying associations:* Descriptive statistics were calculated for all explanatory variables. Kaplan-Meier (KM) curves for all categorical predictors were produced to look for differences in survival with different values of the predictor. Associations between potential predictors and risk of death after discharge were explored in univariable Cox regression models. *b) Selecting and validating predictive models*: The dataset was randomly split into two subsets (training set containing 80% of data and the validation set with the remaining 20%) which were then compared to confirm that there were no important differences between the two subsets. A univariable analysis using the training set was performed as explained above. Those predictors showing evidence of an association (p-value ≤0.05) with the outcome in this univariable analysis were selected for potential inclusion in a multivariable Cox regression model (primary model). Those variables with the highest p-value were removed from the multivariable model one-by-one in a backward stepwise approach until all p-values were ≤0.05. Three additional models were also examined based on their suitability for different contexts: model 2), which uses the primary model as reference, but includes only variables with minimal costs; model 3) based exclusively on clinical variables collected on admission; and model 4)

predictors of PDM restricted to infants <3months. The area under the curve (AUC) was plotted for each model over time using the training set. Within-child clustering of repeated admissions during the study period was taken into account when assessing strengths of association. Analyses used Stata Statistical software (Release 15). Graphical representation of AUC curves was done in R (R Core Team; 2017) using the *survivalROC* package.

Ethical considerations

This study examined data collected in the context of routine clinical practice. DSS and MSS ongoing in the study area have been approved by the National Ethics Committee of Mozambique.

RESULTS

Overall characteristics of study population

From 1st January 2000 to 31st December 2016, 58 990 inpatient records were checked of which 29 574 (50%) were initially excluded (figure 1). 3097 observations of children living in study area readmitted within follow-up (supplementary figure S1), hospital deaths (2.5%, 662/26 319 of remaining children) and 25 deaths in the first 24 hours (<0.1%) were also excluded (figure 1). Thus, 25 632 inpatient records of 18 023 children <15 years old admitted to MDH were included in the analysis. Characteristics on admission of children included in the study are summarized in table 1. 2055 observations of 2049 infants <3months were also analysed separately (table 2). During the study period, 7609 children (42.2%) had more than one admission.

Incidence of post-discharge deaths and potential predictors of PDM

During the 90-day follow-up period, 935 (3.6%) deaths were documented after discharge among the 25 632 admissions, with 783/935 (83.7%) occurring at the community level and 488/935 (52.2%) within the first 30 days of discharge. The median time to death was 28 days (IQR 11-53). The risk of post-discharge deaths varied over time (figure 2A and supplementary figure S2) and by age (figure 2B). Overall, there was an increasing trend in risk until 2010, the year with the highest rate, followed by a progressive decline until 2016.

Forty-five variables were tested for their univariable association with PDM. The Kaplan-Meier survival curve according to age group revealed that infants <3months were more likely to die than older children (figure 3A). Similarly, poorer nutritional levels were clearly linked to PDM (figure 3B). Other variables associated with higher risk of PDM in the univariable analysis may be found in table 1.

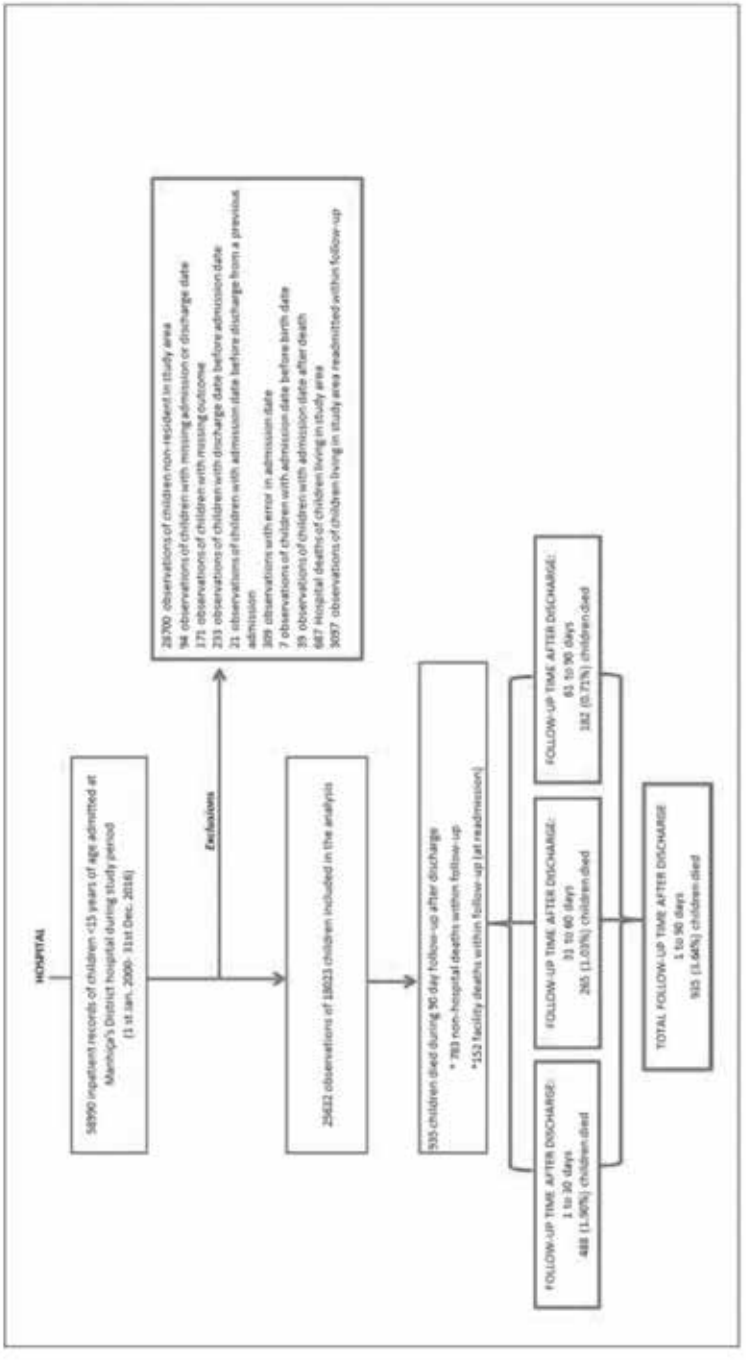


Figure 1. Study profile

Table 1. Socio-demographic, clinical characteristics and univariate analysis of predictors at admission associated to post-discharge mortality in southern Mozambique based on 25632 observations and 935 post-discharge deaths.

Characteristics at admission	Total observations included, N= 25632, n (%)	Children dying within 90 day after discharge[g] N= 935, n (%[h])	Univariate HR[a]	95 % CI*	p value[a]
Demographic characteristics					
Age					<0·001
< 3 months	2055 (8·0)	126 (6·1)	1·00		
4 to < 1year	5203 (20·3)	276 (5·3)	0·86	0·70 to 1·06	0·164
1 to 5 years	14558 (56·8)	450 (3·1)	0·50	0·41 to 0·60	<0·001
> 5 years	3816 (14·9)	83 (2·2)	0·35	0·26 to 0·46	<0·001
Sex					
Female	11571 (45·3)	425 (3·7)	1·01	0·89 to 1·15	0·838
Rainy season	15624 (61·0)	602 (3·9)	1·16	1·02 to 1·33	0·029
Anthropometric characteristics					
Weight for height-z score(mean ±SD[c])	-0·93 (0·01)	-1·99 (0·13)	0·63	0·57 to 0·69	<0·001
Nutritional status by WHZ[i] z-score					<0·001
>-1 SD[c]	5994 (23·4)	57 (0·9)	1·00		
>-2 to <-1 SD[c]	2749 (10·7)	35 (1·3)	1·34	0·88 to 2·04	0·172
>-3 to <-2 SD[c]	1486 (5·8)	34 (2·3)	2·42	1·58 to 3·71	<0·001
< -3 SD[c]	1030 (4·0)	57 (5·5)	5·94	4·12 to 8·57	<0·001
Unknown	14373 (56·1)	752 (5·2)	5·62	4·30 to 7·36	<0·001
History of current disease[e]					
History of fever	23424 (91·4)	797 (3·4)	0·54	0·45 to 0·64	<0·001
History of cough	16324 (63·7)	705 (4·3)	1·78	1·53 to 2·06	<0·001
History of diarrhoea	5015 (19·6)	336 (6·7)	2·36	2·06 to 2·69	<0·001
History of vomiting	6004 (23·4)	268 (4·5)	1·32	1·15 to 1·52	<0·001
History of difficulty breathing	5303 (20·8)	298 (5·6)	1·81	1·58 to 2·08	<0·001
Anorexia	1648 (6·5)	101 (6·1)	1·79	1·46 to 2·20	<0·001
Blood in urine	97 (0·4)	5(5·2)	1·43	0·59 to 3·44	0·429
History of seizures	2658 (10·4)	39 (1·5)	0·37	0·27 to 0·51	<0·001

Characteristics at admission	Total observations included, N= 25632, n (%)	Children dying within 90 day after discharge[§] N= 935, n (%[&])	Univariate HR[Ψ]	95 % CI*	p value[θ]
Symptoms and signs on admission					
Axillary temperature (ºC)					<0·001
Normothermia (35.5-37.4ºC)	9840 (37·0)	427 (4·3)	1·00		
Hypothermia (<35.5ºC)	513 (2·0)	30 (5·8)	1·31	0·90 to 1·89	0·158
Fever (≥37.5ºC)	15598 (61·0)	476 (3·1)	0·67	0·59 to 0·77	<0·001
Heart rate					0·647
Normal	16697 (65·5)	600 (3·6)	1·00		
Bradycardia	1902 (7·5)	75 (3·9)	1·10	0·87 to 1·40	0·436
Tachycardia	6876 (27·0)	259 (3·8)	1·05	0·90 to 1·21	0·518
Increased respiratory rate	11560 (45·3)	351 (3·0)	1·37	1·20 to 1·57	<0·001
Skin pinch goes back slowly	2007 (8·1)	186 (9·3)	3·03	2·58 to 3·56	<0·001
Dehydration	3907 (15·3)	248 (6·3)	2·04	1·77 to 2·37	<0·001
Pallor	4228 (16·5)	141 (3·3)	0·90	0·75 to 1·07	0·227
Jaundice	328 (1·1)	8 (2·4)	0·66	0·33 to 1·32	0·243
Oedema (any location)	1371 (5·4)	130 (9·5)	2·96	2·46 to 3·57	<0·001
Skin flaking off	464 (1·8)	46 (9·9)	2·90	2·15 to 3·90	<0·001
Depigmented or redish hair	1460(5·7)	216 (14·8)	5·30	4·55 to 6·18	<0·001
Oral candidiasis	493 (1·9)	108 (21·9)	7·44	6·08 to 9·09	<0·001
Swollen lymph nodes	827 (3·2)	113 (13·7)	4·33	3·56 to 5·25	<0·001
Concjuntivitis	416 (1·6)	24 (5·8)	1·62	1·08 to 2·43	0·020
Ear discharge	641 (2·5)	57 (8·9)	2·59	1·98 to 3·38	<0·001
Lower chest wall indrawing	5488 (21·4)	298 (5·4)	1·73	1·51 to 1·99	<0·001
Nasal flaring	4123 (16·1)	206 (5·0)	1·48	1·27 to 1·73	<0·001
Pathological breathing pattern	1018 (3·7)	54 (5·3)	1·50	1·14 to 1·97	0·004
Auscultatory crackles	5314 (20·8)	320 (6·0)	2·02	1·77 to 2·31	<0·001
Wheeze/roncus	3090 (12·1)	127 (4·1)	1·15	0·96 to 1·39	0·138
Heart gallop	882 (3·4)	32 (3·6)	0·99	0·70 to 1·41	0·972
Palpable liver	677 (2·6)	38 (5·6)	1·57	1·14 to 2·18	0·006
Palpable spleen	5378 (21·0)	145 (2·7)	0·69	0·58 to 0·82	<0·001
Neck stiffness	203 (0·8)	11 (5·4)	1·51	0·83 to 2·74	0·175
Abnormal fontanella (among applicable)	922 (8·7)	70 (7·6)	1·55	1·21 to 1·99	<0·001
Prostration	3261 (13·0)	146 (4·5)	1·29	1·08 to 1·54	0·005

Characteristics at admission	Total observations included, N= 25632, n (%)	Children dying within 90 day after discharge[δ] N= 935, n (%[h])	Univariate HR[Φ]	95 % CI*	p value[θ]
Symptoms and signs on admission					
BCS on admission					0.047
Normal (BCS=5)	24320 (95·0)	870 (3·6)	1·00		
Abnormal BSC (BCS=3-4)	873 (3·4)	39 (4·5)	1·26	0·91 to 1·73	0·164
Deep coma (BCS≤2)	396 (1·6)	22 (5·6)	1·57	1·03 to 2·41	0·037
Investigations					
Malaria diagnosis					<0·001
Negative	9431 (36·8)	581 (6·2)	1·00		
Positive	12232 (47·7)	202 (1·7)	0·26	0·22 to 0·31	<0.001
Test not done	3969 (15·5)	152 (3·8)	0·61	0·51 to 0·74	<0·001
Glycaemia					0.189
Normoglycaemia (2.5-11.0 mmol/l)	21384 (83·4)	798 (3·7)	1·00		
Hypoglycaemia (<2.5 mmol/l)	2413 (9·4)	83 (3·4)	0·92	0·73 to 1·15	0·471
Hyperglycaemia (>11.0 mmol/l)	1835 (7·2)	54 (2·9)	0·78	0·60 to 1·03	0·084
Blood culture					
Negative	24316 (94·9)	798 (3·3)	1·00		
Positive	1296 (5·1)	136 (10·5)	3·32	2·77 to 3·98	<0·001
Anaemia					0·902
No anaemia	8806 (34·4)	319 (3·6)	1·00		
Mild to moderate anaemia	13624 (53·1)	495 (3·6)	1·00	0·87 to 1·15	0·997
Severe anaemia	3202 (12·5)	121 (3·8)	1·04	0·84 to 1·28	0·709
HIV status					<0·001
Test not done	24128 (94·1)	867 (3·6)	1·00		
Negative	1246 (4·9)	25 (2·0)	0·55	0·37 to 0·82	0·004
Positive	258 (1·0)	43 (16·7)	4·97	3·59 to 6·88	<0·001
Outcome of the admission[η]					
					<0·001
Discharged alive	24145 (94·2)	666 (2·8)	1·00		
Absconded	805 (3·1)	161 (20·0)	8·18	6·87 to 9·74	<0·001
Transferred	682 (2·7)	108 (15·8)	6·30	5·12 to 7·75	<0·001

[ε]SD: standard deviation. [ι]WHZ: Weigh-for-height.See definitions in Table S1. [θ]It refers both communitary deaths and deaths in a readmission during follow-up period. [δ]Percentage represents risk among children with same chacarteristics. [Φ] HR: Hazard ratios. HR and confidence intervals were derived from a Cox regression model.*Confidence intervals. [θ] P-value was derived from Wald test. BCS: Blantyre coma score. [λ]History of current disease reported by the child carer. [η]Hospital deaths and deaths in the first 24h ommited.

Table 2: Socio-demographic, clinical characteristics and univariate analysis of predictors on admission associated to post-discharge mortality among 2055 observations of infants less than 3 months of age and 126 post-discharge deaths in southern Mozambique.

Characteristics at admission	Total observations included N= 2055, n (%)	Infants < 3months dying within 90 day after discharge[β] N= 126, n (%[h])	Univariate[x] HR[Φ]	95 % CI*	p value[θ]
Demographic characteristics					
Age					
< 28 days	945 (46·0)	80 (8·5)	1·00		
≥ 28 days to < 3months	1110 (54·0)	46 (5·3)	0·48	0·33 to 0·69	<0·001
Sex					
Female	934 (45·6)	61 (6·5)	1·12	0·79 to 1·60	0·502
Rainy season	1239 (60·3)	74 (6·0)	0·94	0·66 to 1·33	0·711
Anthropometric characteristics					
Weight for age-z score(mean ±SD[ε])	-0·22 (0·I	-1·55 (0·12)		0·46 to 0·58	<0·001
Nutrition status by WAZ[ξ] z-score					<0·001
>-1 SD[ε]	1458 (71·0)	38 (2·6)	1·00		
>-2 to <-1 SD[ε]	312 (15·2)	35 (11·2)	4·50	2·85 to 7·12	<0·001
>-3 to <-2 SD[ε]	155 (7·5)	29 (18·7)	7·90	4·87 to 12·83	<0·001
< -3 SD[ε]	61 (3·0)	17 (27·9)	12·14	6·90 to 21·35	<0·001
Unknown	69 (3·4)	7 (10·1)	4·05	1·81 to 9·07	<0·001
History of current disease[κ]					
Current breastfeeding	1489 (72·5)	91 (6·1)	0·43	0·25 to 0·75	0·003
History of fever	1660 (80·8)	101 (6·1)	0·96	0·62 to 1·49	0·853
History of cough	1326 (64·5)	95 (7·2)	1·71	1·14 to 2·56	0·010
History of diarrhoea	1706 (83·0)	28 (1·6)	1·44	0·94 to 2·19	0·094
History of vomiting	1713 (83·4)	35 (2·0)	1·98	1·34 to 2·93	0·001
History of difficulty breathing	748 (20·8)	64 (8·6)	1·81	1·28 to 2·58	0·001
Anorexia	168 (8·2)	19 (11·3)	2·07	1·27 to 3·39	0·004
History of seizures	42 (2·0)	3 (7·1)	1·16	0·37 to 3·61	0·795

Characteristics at admission	Total observations included N= 2055, n (%)	Infants < 3months dying within 90 day after discharge[β] N= 126, n (%[h])	Univariate[x] HR[ψ]	95 % CI*	p value[θ]
Symptoms and signs on admission					
Axillary temperature (ºC)					0·0361
Normothermia (35.5-37.4ºC)	1081 (52·7)	54 (5·0)	1·00		
Hypothermia (<35.5ºC)	51 (2·5)	6 (11·8)	2·46	1·05 to 5·73	0·037
Fever (≥37.5ºC)	919 (44·8)	65 (7·1)	1·43	1·00 to 2·06	0·051
Heart rate					0·647
Normal	1352 (65·8)	86 (6·4)	1·00		
Bradycardia	169 (8·2)	9 (5·3)	0·84	0·42 to 1·67	0·616
Tachycardia	534 (25·9)	31 (5·8)	0·91	0·60 to 1·36	0·634
Increased respiratory rate	1101 (53·8)	86 (7·8)	1·93	1·32 to 2·82	0·001
Skin pinch goes back slowly	113 (5·5)	13 (11.5)	2·03	1·14 to 3·60	0·015
Dehydration	244 (11·9)	25 (10·2)	1·90	1·22 to 2·95	0·004
Pallor	126 (6·1)	6 (4·8)	0·76	0·33 to 1·72	0·510
Jaundice	42 (2·0)	3 (7·1)	1·14	0·37 to 3·49	0·813
Oedema (any location)	28 (1·4)	2 (7·1)	1·14	0·29 to 4·48	0·845
Skin flaking off	61 (3·0)	4 (6·6)	1·06	0·40 to 2·84	0·906
Depigmented or redish hair	17 (0·8)	3 (17·6)	3·19	0·99 to 10·27	0·052
Oral candidiasis	79 (3·9)	22 (27·8)	6·18	3·87 to 9·85	<0·001
Swollen lymph nodes	23 (1·1)	6 (26·1)	4·73	2·16 to 10·36	<0·001
Conjunctivitis	69 (3·4)	6 (8·7)	1·46	0·64 to 3·31	0·366
Ear discharge	46 (2·2)	4 (8·7)	1·46	0·54 to 3·98	0·457
Lower chest wall indrawing	795 (38·8)	61 (7·7)	1·50	1·06 to 2·13	0·022
Nasal flaring	582 (28·3)	38 (6·5)	1·10	0·75 to 1·60	0·631
Pathological breathing pattern	138 (6·7)	11 (8·0)	1·34	0·72 to 2·49	0·350
Auscultatory crackles	572 (27·9)	49 (8·6)	1·68	1·17 to 2·40	0·005
Wheeze/roncus	361 (17·6)	22 (6·1)	0·99	0·63 to 1·57	0·977
Heart gallop	37 (1·8)	2 (5·4)	0·86	0·22 to 3·38	0·828
Palpable liver	35 (1·7)	3 (8·6)	1·41	0·45 to 4·35	0·554

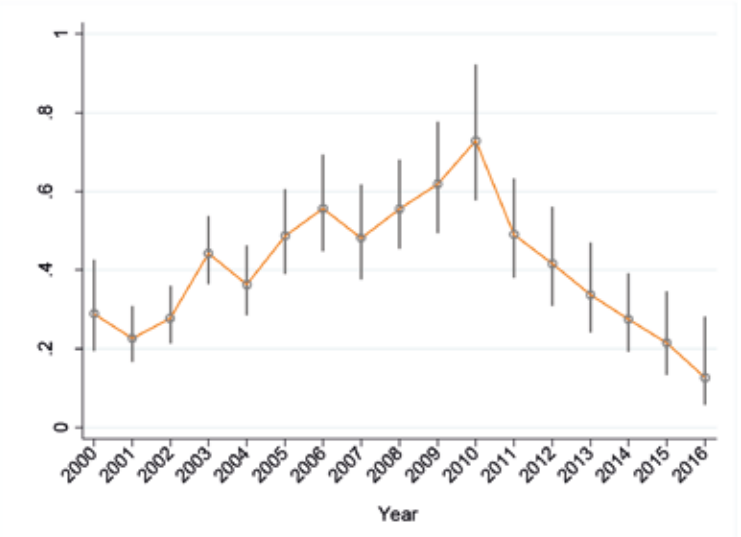
Characteristics at admission	Total observations included N= 2055, n (%)	Infants < 3months dying within 90 day after discharge[β] N= 126, n (%[h])	Univariate[k] HR[ϕ]	95 % CI*	p value[θ]
Symptoms and signs on admission					
Palpable spleen	158 (21·0)	9 (7·1)	0·92	0·47 to 1·80	0·799
Abnormal fontanella	112 (5·6)	7 (6·3)	1·02	0·47 to 2·21	0·958
Prostration	398 (20·5)	25 (6·3)	1·06	0·68 to 1·66	0·782
BCS on admission					0.923
Normal (BCS=5)	1851 (90·3)	115 (6·2)	1·00		
Abnormal BSC (BCS=3-4)	164 (8·0)	9 (5·5)	0·88	0·44 to 1·72	0·701
Deep coma (BCS≤2)	35 (1·7)	2 (5·7)	0·91	0·23 to 3·67	0·898
Investigations					
Malaria diagnosis					<0·072
Negative	1257 (61·2)	88 (7·0)	1·00		
Positive	414 (20·2)	16 (3·9)	0·54	0·32 to 0·93	0.025
Test not done	384 (18·7)	22 (5·7)	0·81	0·51 to 1·29	0·389
Glycaemia					0.988
Normoglycaemia (2.5-11.0 mmol/l)	1799 (87·5)	110 (6·1)	1·00		
Hypoglycaemia (<2.5 mmol/l)	163 (7·9)	10 (6·1)	1·00	0·53 to 1·93	0·984
Hyperglycaemia (>11.0 mmol/l)	93 (4·5)	6 (6·5)	1·07	0·47 to 2·45	0·877
Blood culture					
Negative	1881 (91·7)	110 (5·8)	1·00		
Positive	171 (8·3)	16 (9·4)	1·62	0·96 to 2·72	0.071
Anaemia					0·008
No anaemia	951 (46·3)	41 (4·3)	1·00		
Mild to moderate anaemia	845 (41·1)	64 (7·6)	1·78	1·20 to 2·63	0·004
Severe anaemia	259 (12·6)	21 (8·1)	1·90	1·12 to 3·21	0·017
HIV status					0·001
Test not done	1850 (90·0)	115 (6·2)	1·00		
Negative	185 (9·0)	6 (3·2)	0·52	0·23 to 1·18	0·115
Positive	20 (1·0)	6 (25·0)	4·50	1·94 to 11·04	0·001

Characteristics at admission	Total observations included N= 2055, n (%)	Infants < 3months dying within 90 day after discharge[β] N= 126, n (%[h])	Univariate[ᵃ] HR[Φ]	95 % CI*	p value[θ]
Outcome of the admission[n]					
					<0·001
Discharged alive	1906 (92·8)	91 (4·8)	1·00		
Absconded	85 (4·1)	19 (22·4)	5·23	3·19 to 8·59	<0·001
Transferred	64 (3·1)	16 (25·0)	6·31	3·61 to 11·02	<0·001

[i]SD: standard deviation. [l]WAZ: Weigh-for-age. [β]It refers both communitary deaths and deaths in a readmission during follow-up period. [h]Percentage represents risk among children with same chacarteristics. [a]Univariate model based on 2055 infants under 3 months and 126 deaths. [Φ]HR: Hazard ratios. Hazard ratios and confidence intervals were derived from a Cox regression model.*Confidence intervals. [θ] P-value was derived from Wald test. [n]Validated predictive model base of 80% of data (1636 infants). BCS: Blantyre coma score. [ᵃ]History of current disease reported by the child carer. [n]Hospital deaths ommited.

Figure 2: Post-discharge mortality trends over time during the 16 year long study period

A) yearly incidence for all ages



B) yearly incidence by age group.

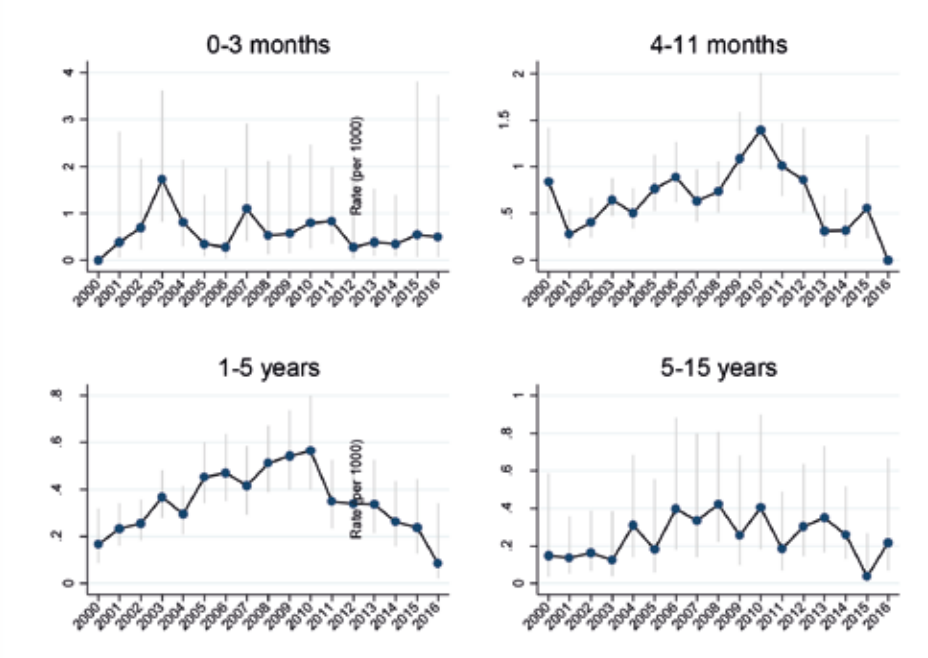
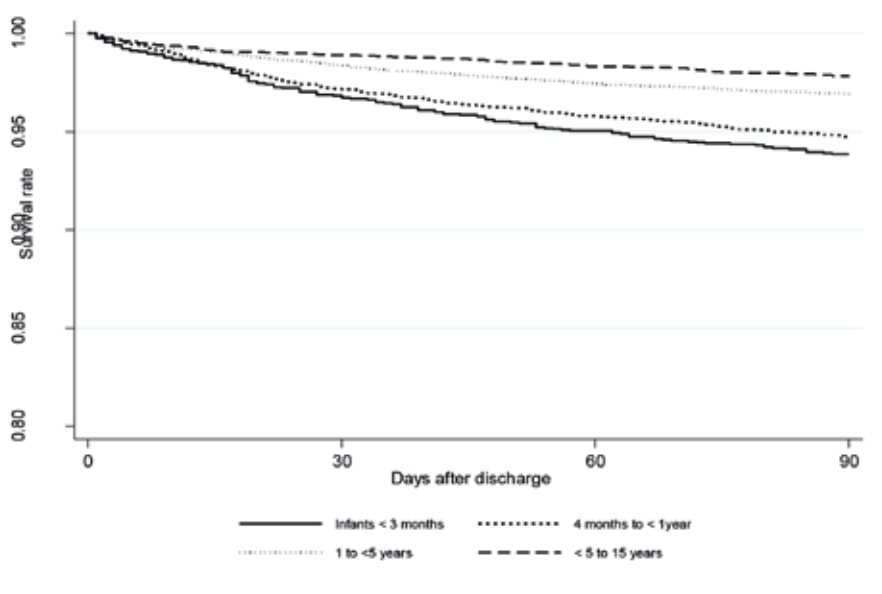
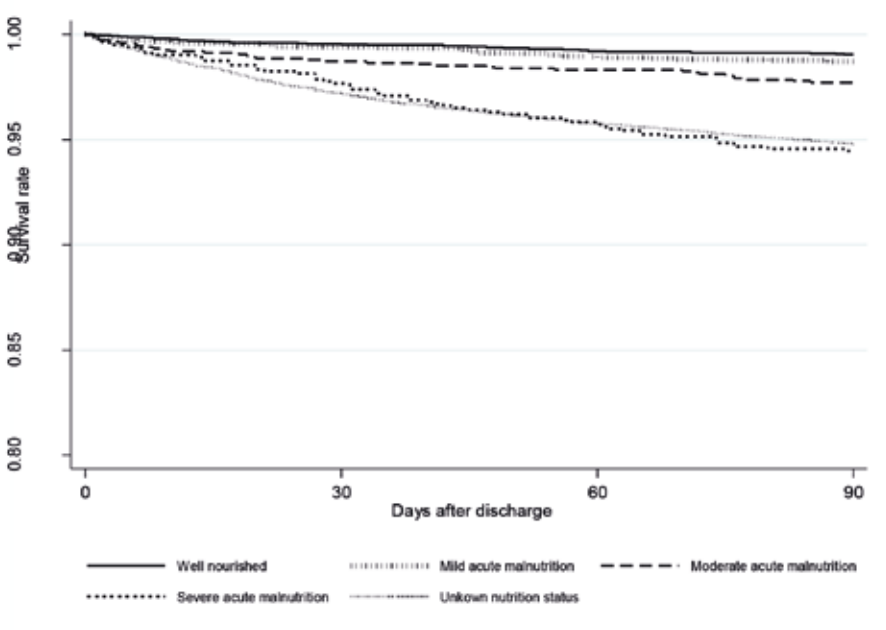


Figure 3: Kaplan-Meier failure estimates for 935 deaths during 90 days follow-up after discharge from Manhiça District Hospital.

A) by age groups;



B) by categories of nutritional status.



Predictive models and validation

The comparison of training and validation set may be found in supplementary table S2. Nineteen variables were associated with higher risk of PDM in the multivariable analysis (primary model, table 3). Infants less than 3 months had the highest rate of PDM and this decreased with increasing age (p<0.001). The rate of PDM varied between rainy and dry seasons (HR 1.22, 95% CI 1.03-1.43). Severe acute malnutrition (SAM) (HR 3.26, 95% CI (2.08-5.12) as well as other 13 clinical variables were also associated with PDM. Children with a positive blood culture (HR 1.68, 95% CI 1.33-2.12) and a positive HIV test (HR 1.77, 95% CI 1.07-2.91) also had a higher rate of death during the follow-up period. Absconding (HR 5.23, 95% CI 4.22-6.50) or referral to a higher level of health care (HR 4.48, 95% CI 3.31-6.05) were clearly associated with death. On the other hand, children with a malaria diagnosis had lower risk of PDM (HR 0.44, 95% CI 0.36-0.54). The AUC for this model was above 80% during the 90 days follow-up period (figure 4).

Two alternative models were estimated which selectively excluded certain variables from the primary model to ensure that predictions could be made in the absence of certain variables which may not be available in all centres, particularly in the absence of laboratories (table 3). Time-varying AUC of each additional model is compared in supplementary figure S3 A. Model 2, which excluded blood culture but maintained minimal cost tests, performed similarly to the primary model, with an AUC around 80% until 60 days after discharge. Model 3, which included only clinical variables, perforemd slightly less well, with AUC however maintained around 75% during the whole period. Model 4, limited to infants <3months, included variables such as breastfeeding and weight-for-age (WAZ) to assess nutritional status, on account of the excess of missing height data. Neonates appeared to have the highest risk of PDM among this age group (table 4). This model had an AUC reaching 85% in the first days after discharge, decreasing until 75% around day 15 and keeping stable over 80% through the remaining follow-up period (supplementary figure S3 B).

Table 3: Estimation of predictive models derived from primary model including predictors associated to post-discharge death among 20506 observations of children less than 15 years old and 750 deaths in the first 90 days following discharge.

	PRIMARY MODEL			MODEL 2			MODEL 3		
Characteristics at admission	**Adjusted HR[φ]**	**95 % CI***	**p value[θ]**	**Adjusted HR[φ]**	**95 % CI***	**p value[θ]**	**Adjusted HR[φ]**	**95 % CI***	**p value[θ]**
Demographic characteristics									
Age			<0·001			<0·001			0·002
< 3 months	1·00			1·00			1·00		
4 to < 1year	0·92	0·71 to 1·20		0·93	0·72 to 1·20	0·041	0·79	0·62 to 1·03	
1 to 5 years	0·69	0·53 to 0·91		0·71	0·54 to 0·92	<0·001	0·66	0·51 to 0·86	
> 5 years	0·54	0·38 to 0·76		0·54	0·38 to 0·76	<0·001	0·55	0·39 to 0·77	
Rainy Season	1.22	1.03 to 1.43	0·018	1.22	1.04 to 1.44	0·017	1.25	1·07 to 1·46	0·005
Anthropometric characteristics									
Nutrition status by WHZ[ɨ] z-score			<0·001			<0·001			<0·001
>-1 SD'	1·00			1·00			1·00		
>-2 to <-1 SD'	1·23	0·75 to 2·01		1·27	0·77 to 2·07		1·32	0·81 to 2·14	
>-3 to <-2 SD'	2·40	1·49 to 3·87		2·44	1·51 to 3·93		2·30	1·41 to 3·75	
< -3 SD'	3·26	2·08 to 5·12		3·28	2·08 to 5·16		4·16	2·71 to 6·40	
Unknown	2·99	2·12 to 4·21		3·09	2·19 to 4·35		3·72	2·64 to 5·23	
History of current disease[ᵃ]									
History of diarrhoea	1·72	1·45 to 2·03	<0·001	1·70	1·44 to 2·01	<0·001	1·58	1·32 to 1·89	<0·001
History of cough	1·32	1·07 to 1·62	0·009	1·31	1·07 to 1·61	0·010	1·25	1·02 to 1·53	0·030
History of difficulty breathing	—	—	—	—	—	—	1·36	1·09 to 1·70	0·007
Symptoms and signs on admission									
Increased respiratory rate	1·41	1·18 to 1·68	<0·001	1·42	1·19 to 1·69	<0·001	1·27	1·07 to 1·52	0·007
Skin pinch goes back slowly	—	—	—	—	—	—	1·51	1·20 to 1·90	<0·001
Nasal flaring	0·69	0·55 to 0·86	<0·001	0·69	0·56 to 0·87	0·001	0·79	0·65 to 0·97	0·022
Auscultatory crackles	1·37	1·12 to 1·67	0·002	1·41	1·16 to 1·71	<0·001	1·44	1·19 to 1·75	<0·001
Oral candidiasis	2·64	1·98 to 3·52	<0·001	2·72	2·03 to 3·64	<0·001	3·51	2·70 to 4·58	<0·001
Oedema (any location)	1·86	1·39 to 2·48	<0·001	1·83	1·67 to 2·44	<0·001	2·48	1·88 to 3·27	<0·001
Depigmented or redish hair	2·03	1·60 to 2·57	<0·001	2·08	1·64 to 2·64	<0·001	2·42	1·90 to 3·07	<0·001
Swollen lymph nodes	1·89	1·42 to 2·51	<0·001	1·87	1·41 to 2·49	<0·001	2·23	1·70 to 2·93	<0·001

	PRIMARY MODEL			MODEL 2			MODEL 3		
Characteristics at admission	**Adjusted HR[Φ]**	**95 % CI***	**p value[θ]**	**Adjusted HR[Φ]**	**95 % CI***	**p value[θ]**	**Adjusted HR[Φ]**	**95 % CI***	**p value[θ]**
Symptoms and signs on admission									
Ear discharge	1·76	1·20 to 2·58	0·004	1·74	1·16 to 2·59	0·007	1·76	1·24 to 2·49	0·001
Prostration	1·42	1·15 to 1·75	0·001	1·44	1·17 to 1·77	<0·001	1·41	1·15 to 1·73	0·001
Investigations									
Malaria diagnosis			<0·001			<0·001			
Negative	1·00			1·00					
Positive	0·44	0·36 to 0·54		0·43	0·35 to 0·52				
Test not done	0·86	0·46 to 0·73		0·84	0·68 to 1·04				
Positive blood culture									
Negative	1·00			EXCLUDED			EXCLUDED		
Positive	1·68	1·33 to 2·12	<0·001						
HIV status			<0·001			<0·001			
Test not done	1·00			1·00					
Negative	0·53	0·35to 0·80		0·53	0·35 to 0·80				
Positive	1·77	1·07 to 2·91		1·80	1·07 to 3·01				
Outcome of the admission[η]									
			<0·001			<0·001			
Alive	1.00			1.00			EXCLUDED		
Absconded	5·23	4·22 to 6·50		5·50	4·45 to 6·79				
Transferred	4·48	3·31 to 6·05		4·57	3·36 to 6·21				

[WHZ: weight for height. [SD: standard deviation. See definitions in Table S1. [β]It refers both communitary deaths and deaths in a readmission during follow-up period. [Φ]HR: Hazard ratios. HR and confidence intervals were derived from a Cox regression model. .*Confidence intervals. [θ] P-value was derived from Wald test. [Δ]History of current disease reported by the child carer. [η]Hospital deaths and deaths in the first 24h ommited. [δ]AUC: area under the curve.

Figure 4: The time-varying area under the curve of primary model comparing training and validation set

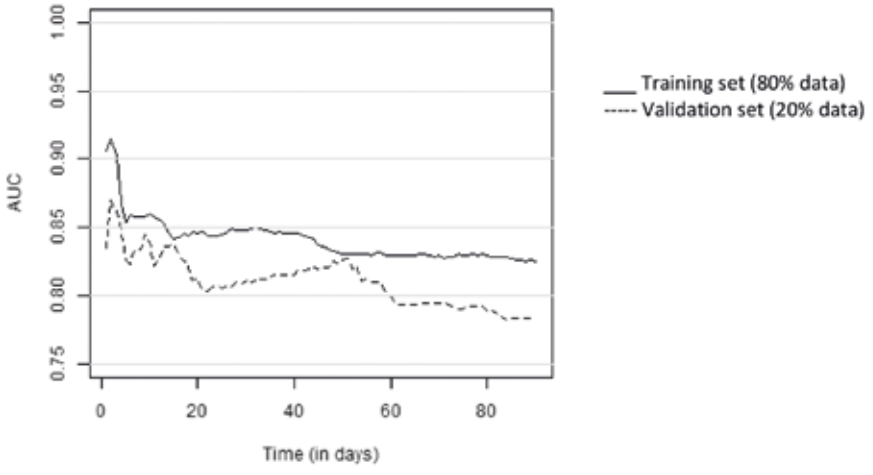


Table 4: Estimation of predictive model including predictors associated to post-discharge death among 1655 observations of infants less than 3 months old and 94 deaths in the first 90 days following discharge.

Characteristics at admission	Adjusted HR[Φ]	95 % CI*	p value[θ]
Demographic characteristics			
Age			
< 28 days	1·00		
≥ 28 days to < 3months	0·43	0·33 to 0·68	<0·001
Anthropometric characteristics			
Nutrition status by WAZ[ɨ] z-score			<0·001
>-1 SD[ɣ]	1·00		
>-2 to <-1 SD[ɣ]	4·53	2·51 to 8·19	
>-3 to <-2 SD[ɣ]	7·03	3·87 to 12·77	
< -3 SD[ɣ]	13·49	7·43 to 24·51	
Unknown	5·11	1·77 to 14·72	
History of current disease[ʌ]			
History of difficulty breathing	1·68	1·13 to 2·52	0·011
Symptoms and signs on admission			
Axillary temperature (ºC)			0·020
Normothermia (35.5-37.4ºC)	1·00		
Hypothermia (<35.5ºC)	2·30	1·04 to 5·11	
Fever (≥37.5ºC)	1·67	1·10 to 2·53	
Oral candidiasis	4·32	2·51 to 7·43	<0·001
Outcome of the admission[ɳ]			
			<0·001
Discharged alive	1·00		
Absconded	5·23	2·97 to 9·20	
Transferred	5·92	2·78 to 12·64	

[ɣ]SD: standard deviation. [ɨ]WAZ: Weigh-for-age. [β]It refers both communitary deaths and deaths in a readmission during follow-up period. [Φ]HR: Hazard ratios. HR and confidence intervals were derived from a Cox regression model.*Confidence intervals. [θ] P-value was derived from Wald test. BCS: Blantyre coma score. [ʌ]History of current disease reported by the child carer. [ɳ]Hospital deaths ommited.

DISCUSSION

To our knowledge, this is the largest study to date evaluating PDM in the first three months following hospital discharge from a rural district hospital in a LIC. This analysis, based on more than 20 000 hospital discharges and 935 post-discharge deaths, represents a systematic approach to ascertain predictors of PDM in a resource-constrained environment.

The cumulative three-month post discharge mortality found in this study (3.6%) is lower than that reported in the 1990s from other African settings, where incidence risk was estimated between 6.1% and 13%[14, 15], but similar to other more recent PDM studies8, 10, 11, 18. As an example, a study conducted in Kenya in children <15 years, reported PDM ranging from 3.3% to 4.5%[10]. Although cumulative mortality was similar in this study, follow-up time was one year (four times longer), making the first three month mortality likely much inferior. Differences among studies may derive from variations in the epidemiology and burden of diseases across populations and over time, age and follow-up periods. Importantly, inpatient mortality found in our cohort (2.5%) aligned more closely with that reported in other settings, likely due to the use of similar guidelines in similar type of settings[5, 14].

Risk of death appears to be greatest in the early weeks immediately following discharge[8, 11], with the first 30 days, where nearly half of all post-discharge deaths occur, being the most critical for survival[11, 15, 18], as our findings also highlight. Overall, trends of post-discharge mortality for all ages changed over time. PDM rates increased over the period from 2000-2010, subsequently declining. One could speculate that variations in the epidemiology of a single disease may have played a significant role in variations in PDM (Supplementary figure S4). For instance, malaria used to be highly endemic in the area at the beginning of the study period, with a subsequent declining trend observed from the year 2005 onwards, when highly effective new antimalarials were rolled out. Such trends appear inversely proportional to those of PDM curve, suggesting that children admitted with malaria, readily treatable and with a rapid recovery, are probably less likely to be associated with post discharge complications. This could partly explain why a diagnosis of clinical malaria has been shown to be an overall protective factor against PDM (HR 0.44, 95% CI 0.36-0.54) similarly to what Moisi et al described in Kenya[10]. Similarly, although HIV incidence is now much higher in the area than it was a decade ago, the

uptake of antiretroviral drugs may also explain a more controlled impact of this infection on PDM in recent years when more admissions due to HIV have been registered. Trends in the proportion of children admitted with other diseases with greater incidence of post-discharge deaths, such as SAM, showed an overall tendency parallel to the PDM curve over time. However, the declining trend of PDM seen at the end of the study period cannot be exclusively explained by malaria, HIV or SAM trends. The progressive introduction of other strongly protective interventions, such as vaccination against Hib (2009), pneumococcus (2013), and rotavirus (2015) have reduced hospital admissions and, improved hygiene practices in households and health centres, implementation of oral rehydration therapy for diarrhoeal diseases, antibiotics for pneumonia, or improved skill hospital attendance among others[30] may all have also contributed to these decreasing trends. PDM trends over time among infants <3 months differed slightly from other age groups, with a peak in 2003, and stable rates thereafter. Possible explanations include the fact that effective interventions specifically targeting this age group, and designed to reduce neonatal and infant mortality, have not been fully implemented in the study area. These include, among others, kangaroo care for preterm and LBW infants, induction of labor for prolonged pregnancy to avoid perinatal asphyxia, proper case management of neonatal sepsis[31], particularly those late-onset cases if cerebrospinal fluid sampling is not undertaken, thus hindering an adequate diagnosis of neonatal meningitis[32]. Other conditions which may have led to worse outcomes in this particularly vulnerable period relate to the relative high prevalence of low birth weight and premature deliveries, and the social burden of orphanhood secondary to the effects of the HIV epidemic, Although our dataset did not allow us to explicitly explore these conditions, previous data from the study area found a high prevalence of preterm births and an association between prematurity and higher risk of dying during the first year of life, especially during the neonatal period, results that could be applied to our population[33].

Our primary model, which included all available useful variables, performed very well, particularly to predict risk of dying in the first month following discharge. Applying a score based on this model to a population similar to ours, we could identify up to 80% of the children at risk of dying during the first 90 days, being this percentage even higher in the first 30 days. This model however includes blood culture results, an expensive determination that requires laboratory infrastructures seldom available in poor settings.

The model using only clinical variables (with no lab results) was the worst performing one, and model 2 which included information of discharge status (alive, referral or absconding) and HIV and malaria results, performed similarly well to the primary model, seems more applicable, and showed a good predictive capacity for PDM. Similarly to that described above for the other models, model 4, developed for infants <3 months old, is parsimonious, using only few variables, all of them easily and readily obtainable in most resource constrained contexts. Using a score based on this model at discharge in our population (or other with similar characteristics), 85% of infants <3months at risk of dying after discharge could be identified in the first days after discharge and almost 80% during the remaining follow-up period, a remarkable finding taking into account the high incidence risk of dying following hospital discharge in this age group.

Young age, SAM, history of diarrhoea, clinical symptoms of pneumonia, prostration, symptoms associated to HIV and /or malnutrition, bacteraemia, positive HIV status, rainy season and absconding or being transferred from the hospital were all variables associated to an excess mortality in the model including all post-discharge deaths. Conversely, nasal flaring and malaria diagnosis appear to be protective factors against PDM. The association between age and PDM has been previously reported[8, 10, 11, 15, 34], having older children less risk of death after hospitalization compared to infants. SAM is a recognized predictor of PDM[5, 9-11, 18] as our models confirm. Data from Manhiça district showed that nearly half of children attending seen as outpatients presented some level of malnutrition and 6% could be classified as severely malnourished[35]. The chronicity of malnutrition, the fact that it can predispose to an array of co-infections and complications, and its association with HIV infection in Manhiça[35], may all contribute to explain the prolonged risk for survival that it entails. Diarrhoea was related to reductions in post-hospital survival in our cohort similarly to other studies[9, 11, 18]. The GEMS multicentre study on the aetiology of diarrhoeal disease showed that diarrhoea burden in Manhiça is high and greatest among infants, with a strong association to mortality in the first 90 days after diagnosis[36]. HIV positivity, a clearly described predictor of PDM[5, 20], remained in our model a strong independent predictor, even after adjusting for malnutrition. Some studies have reported an association between sepsis and excess child mortality[8] but only one to date[10] found bacteraemia to predict post-discharge death as we found.

In our series, type of outcome at discharge, and particularly being transferred or absconding from hospital, were the greatest predictors of PDM. Transfer to a higher level health facility usually arises due to requirement for a more specialized evaluation or supportive care, typically on account of severity. As a result, their greatest PDM risk is an expected finding. Children absconding (3.1% of the study sample) against medical recommendations had an extremely high risk of post-discharge deaths, and represent one out of every five deaths in our series. Veirum et al in Guinea-Bissau[15] described absconding from the hospital as the dominating predictor of PDM, but a Kenyan study did not confirm this association[10]. Absconding is in Manhiça a cultural and financial phenomenon, typically occurring when families anticipate a bad outcome and prefer their children to die at home, additionally sparing costs associated with the transport of a corpse. Socio-behavioural studies trying to address absconding are essential as well as the perception by health staff of the serious consequences of this phenomenon. Rainy season was also associated to higher risk of PDM as more children are admitted during this season, likely due to greater number of admissions and severe disease occurring during this season, similarly to what was found in the Gambia[8], but differently from what was seen in Kenya[10]. Some of the disparities found among studies may reflect methodological differences such as study design, sample size or variables collected and epidemiological variations across populations and over time.

Infants under 3 months of age had the highest PDM risk, a result aligning with global trends of child mortality in the last two decades, whereby neonatal deaths have an increasing importance as the total proportion of U5 mortality[37]. Specific risk factors in this age group were also different, and included having a lower WAZ, a higher occurrence of hypo- or hyperthermia, oral candidiasis and respiratory distress. Surprisingly, bacteraemia was not found in our model to predict PDM. This is, to our knowledge, the first proposed predictive tool for PDM in this highly vulnerable age group.

The majority (83.7%) of deaths following hospital discharge occurred at the community, in the absence of any further contact with the health system. A study investigating cause of death in Manhiça using verbal autopsies documented that 53% of all paediatric deaths occurred at home[24]. These alarming figures reflect the generalized challenges in access to care, which become even more blatant following hospital discharge. A recent study trying to understand the context surrounding out-of-hospital deaths

and the barriers to health care access for children recently discharged from a hospital showed that resource limitations, health knowledge and perceptions of caregivers significantly influences timely access to care[38]. New studies in our setting could allow unveiling specific causes and guiding future interventions.

Algorithms based on predictive tools are commonly used in routine clinical practice in LMIC, but not without challenges. Diseases such as malaria, sepsis, pneumonia or severe malnutrition share many overlapping symptoms, hindering the development of highly specific differential algorithms to identify populations at risk of dying. Effective models for identifying children at risk of PDM should take into consideration the existing resources but consider illness as a continuum transcending the information that the admission snapshot can provide. Although the Integrated Management of Childhood Illness algorithm (IMCI) was not designed as a prediction tool *per se*, it is used in many countries as an algorithm-based approach for the screening and management of infectious diseases[3]. It has demonstrated an improvement of outcomes and reduction of infant mortality in settings where it has been implemented, although most data come from South Asia[39]. However, IMCI has little impact on nutritional status[39], which was a strong predictor of PDM in our study and fails to address the risk after discharge and therefore, does not provide any guidelines of follow-up care beyond admission. In any case, it is essential to follow the effective and entire implementation of the malnutrition guidelines. A similar algorithm or score based in predictive models and applied at discharge, could help to identify those children requiring a more rigorous follow-up after hospitalization. Once identified, these children at higher risk of PDM could benefit from strategies to prevent post-discharge death. A clinical trial conducted in Kenya explored the efficacy of daily co-trimoxazole prophylaxis in children admitted with complicated SAM without HIV and they found no reduction in mortality during the first year after admission[40]. Although this strategy has not worked for children with SAM, other promising strategies such as mass drug administration (MDA) of azithromycin to reduce child mortality could be adapted for children at risk of PDM decreasing risk of death in this population and reducing antibiotic resistance associated to MDA[41, 42]. Community-based interventions driven by community-health workers consisting in pre and post-natal home visits, supporting LBW infants and sepsis case management, facilitating referral in case of need have reduced neonatal and infant mortality in several countries[43]. Although

these interventions have not been explored in children after a hospital admission, their impact reducing PDM could be similar.

This study has several limitations. First of all, it is a retrospective study. Selection bias may arise due to the fact that almost half of children admitted to MDH between 2000 and 2016 were excluded, the majority of which (86%) on account of not being part of the study area under DSS. This might affect the representativeness of the study sample. Another limitation includes the fact that all clinical predictors were collected at the time of admission, and some these clinical variables may have changed over the course of admission. We decided to use a single-discharge approach within 90 days of follow-up to avoid double counting time at risk, excluding observations of children re-admitted during the follow-up period. This strategy may have resulted in a clearer picture of the true community PDM but also in an underestimation of the likely higher real-life true incidence, particularly in a setting such as Manhiça, where access to care is likely better as a result of the good linkages to the community established by CISM. Another possible factor potentially underestimating the real PDM rate relates to the exclusion of deaths occurring in the first 24 hours after discharge, as they were considered as hospital deaths. However, they accounted for <0.1%. The inclusion of children who were transferred or absconded from the hospital may be overestimating the incidence of PDM since they were not officially discharged, but this is an extremely frequent occurrence in African settings, and needs to be taken into consideration, particularly in the light of the strength and magnitude of the statistical association found. Importantly, we could not assess the role of low birth weight (LBW) in infants as a likely risk factor for PDM, since this information was not available. Finally, our predictive models lack an external validation, although they performed reasonably well.

In conclusion, this study highlights the importance and oversight of post discharge mortality as a significant portion of the childhood mortality cake. Simple models including predictors easily collected with minimal cost, such as those presented in this article, need to be prospectively validated in different circumstances and settings. Specific interventions targeting children identified to be at higher risk and guaranteeing their adequate follow-up at the hospital or even at the household level could possibly increase their survival risk. Implementation of such strategies could prevent avoidable deaths, especially among neonates and infants who suffer the highest burden of PDM.

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Authors´contributions

This paper was designed by LM, QB, LQ and SC. It is based on data obtained from a demographic surveillance system (DSS) and a morbidity surveillance system (MSS) going on in the study area since 1996. PA and CM designed DSS and MSS and they have led surveillance modifications over time. CS has been leading DSS in the last years. LM, CS, RV, AS, SA, TN, BS, EM and QB have participated in data collection and SM and IM in laboratory procedures and interpretation of results. Database management has been led by LQ. LQ, AC and LM have led the data analysis and LM, QB, LQ, AC, SC, CM and RV have interpreted data set results. Writing-up has been led by LM, QB and RV and have received input from all authors. All authors have read and approved the submitted version of the manuscript.

SUPPLEMENTARY INFORMATION

Figure S1. Flow chart on how hospital readmissions of children were selected.

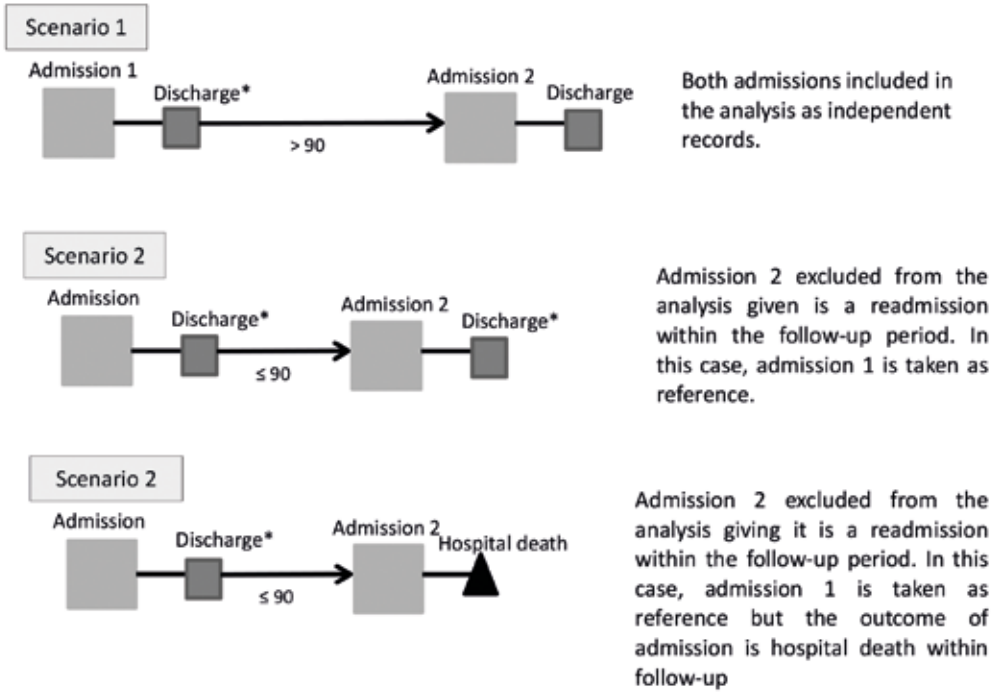


Figure S2: Number of admissions and post-discharge deaths in the first 90 days of follow-up by year.

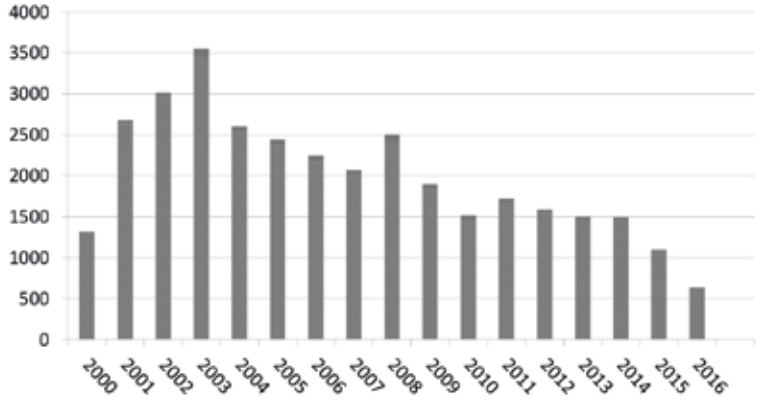
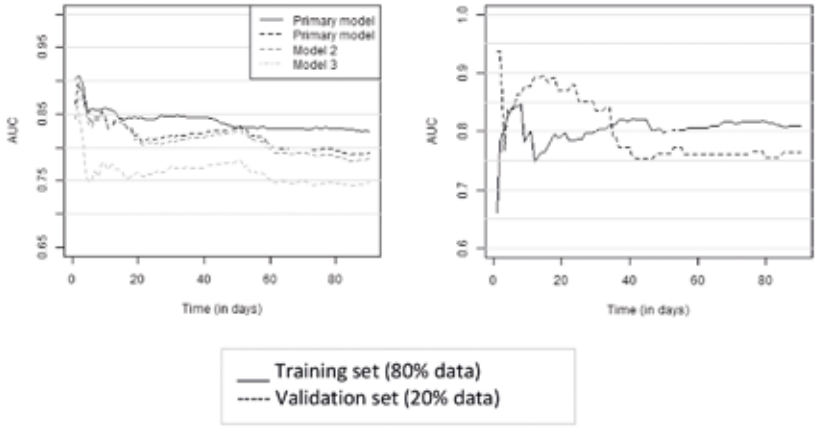


Figure S3: Time-varying area under the curve of predictive models estimated from primary model.



A) Comparison among primary model and estimated models 2 and 3. B) Comparison between training and validation set of estimated model 4 which includes exclusively infants <3 months.

Figure S4: Number of admissions due to specific diseases over time

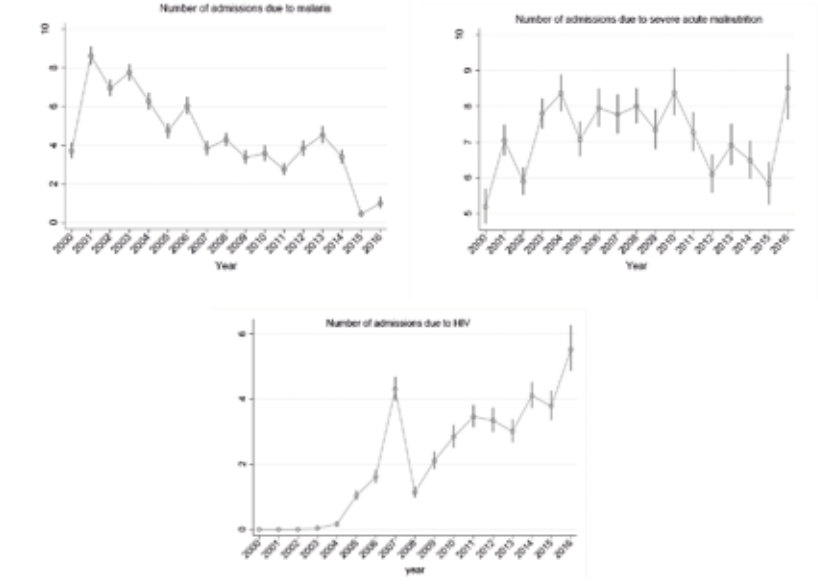


Table S1: Definitions

Rainy Season	November to April
Blantyre coma scale (BCS):	Based on pain response, cry or verbal response, eyes movement. Score 0 to 5 · Normal: 5 · Impaired consciousness: 3-4 · Coma: ≤ 2
Prostration	Inability to drink or breastfeed or sit if usually stable.
WHZ	Weight-for-height z-score calculated using the WHO growth chart · >-1 DS: well nourished · >-2 to <-1DS: mild acute malnutrition · >-3 to <-2DS: moderate acute malnutrition · <-3DS: severe acute malnutrition
WAZ	Weight-for-age z-score calculated using the WHO growth chart · <-3DS: underweight
Malaria	A malaria case was defined as a child admitted with a clinical diagnosis of malaria with a positive P. falciparum asexual parasitemia.
Increased Respiratory Rate **(IRR)**	• IRR in < 2 months old children = respiratory rate ≥ 60 • IRR in 2 - <12 months old children = respiratory rate ≥ 50 • IRR in 12 - <60 months old children = respiratory rate ≥ 40 • IRR in 60 - <120 months old children = respiratory rate ≥ 30 • IRR ≥ 120 months old children = respiratory rate ≥ 20
Heart rate normal ranges in beats per minute:	· 0 to 1 year: 110-160 · 1 -2 years: 100-150 · 2-5 years:95-140 · 5-12 years: 80-120 · >12 years: 60-100
Diarrhoea	Three or more abnormally loose stools in the previous 24 hours.
Dehydration	Pesence of two or more of the following signs: sunken eyes · unable to drink, drinks eagerly · skin pinch goes back slowly · irritability/restlessness
Clinical severe pneumonia	Cough or difficulty breathing + IRR + lower chest wall indrawing or crackles
Non-severe anaemia	· Children ≤ 28 days old with a packed cell volume (PCV) between 25- <42% · Children >28 days: PCV between 15- <33%
Severe anaemia	· Children ≤ 28 days old= PCV <25% · Children >28 days: PCV <15%
Hypoglycaemia	Blood glucose levels <3.0 mmol/L (categorized as severe if <2.5 mmol/L)
Hyperglycaemia	Blood glucose levels >11.0 mmol/L (categorized as severe if <2.5 mmol/L)
Hypothermia	Axillary temperature < 35°C

Table S2: Socio-demographic and clinical characteristics of children <15 years admitted at MDH comparing training (80% of data) and validation set (20% of data).

Characteristics at admission	Training set, N= 20506, n (%)	Validation set, N= 5126, n (%[h])	p value [θ]
Demographic characteristics			
Age			0·935
< 3 months	1655 (8·0)	400 (8·0)	
4 to < 1year	4162 (20·0)	1041 (20·0)	
1 to 5 years	11635 (57·0)	2923 (57·0)	
> 5 years	3054 (15·0)	762 (15·0)	
Sex			
Male	11234 (55·0)	2746 (54·0)	
Female	9206 (45·0)	2365 (46·0)	0·113
Rainy season	12464 (61·0)	3160 (62·0)	0·257
Anthropometric characteristics			
Nutrition status by WHZ[i] z-score			0·540
>-1 SD[e]	4822 (24·0)	1172 (23·0)	
>-2 to <-1 SD[e]	2210 (11·0)	539 (11·0)	
>-3 to <-2 SD[e]	1178 (6·0)	308 (6·0)	
< -3 SD[e]	808 (4·0)	222 (4·0)	
Unknown	11488 (56·0)	2885 (56·0)	
Nutrition status by WAZ[k] z-score			0·538
>-1 SD[e]	8326 (41·0)	2078 (41·0)	
>-2 to <-1 SD[e]	5379 (26·0)	1376 (27·0)	
>-3 to <-2 SD[e]	3375 (16·0)	815 (16·0)	
< -3 SD[e]	2314 (11·0)	599 (12·0)	
Unknown	1112 (5·0)	258 (5·0)	
History of current disease[a]			
Current breastfeeding	5976 (29·0)	1545 (30·0)	0·224
History of fever	18766 (92·0)	4658 (91·0)	0·147
History of cough	13061 (64·0)	3262 (64·0)	0·957
History of diarrhoea	4032 (20·0)	983 (19·0)	0·431
History of vomit	4827 (24·0)	1177 (23·0)	0·372
History of difficulty breathing	4258 (21·0)	1045 (20·0)	0·503

History of seizures	2105 (10·0)	553 (11·0)	0·278
Symptoms and signs on admission			
Axillary temperature (ºC)			0·366
Normothermia (35.5-37.4ºC)	7532 (37·0)	1948 (38·0)	
Hypothermia (<35.5ºC)	395 (2·0)	118 (2·0)	
Hyperthermia (≥37.5ºC)	12548 (61·0)	3050 (60·0)	
Heart rate (mean±SD)			0·414
Normal	13331 (65·0)	3366 (66·0)	
Bradycardia	1543 (8·0)	359 (7·0)	
Tachycardia	1375 (27·0)	5501 (27·0)	
Increased respiratory rate	9256 (45·0)	2304 (45·0)	0·791
Skin pinch goes back slowly	1591 (8·0)	416 (8·0)	0·397
Dehydration	3128 (15·0)	779 (15·0)	0·921
Pallor	3376 (16·0)	852 (17·0)	0·792
Jaundice	253 (1·0)	75 (1·0)	0·191
Oedema (any location)	1082 (5·0)	289 (6·0)	0·304
Skin flaking off	358 (2·0)	106 (2·0)	0·121
Depigmented or redish hair	1158 (6·0)	302 (6·0)	0·499
Oral candidiasis	379 (2·0)	114 (2·0)	0·080
Swollen lymph nodes	665 (3·0)	162 (3·0)	0·766
Conjuntivitis	333 (2·0)	83 (2·0)	0·981
Ear discharge	518 (3·0)	123 (2·0)	0·603
Lower chest wall indrawing	4406 (22·0)	1082 (21·0)	0·557
Nasal flaring	3297 (16·0)	826 (16·0)	0·957
Pathological breathing pattern	793 (4·0)	225 (4·0)	0·087
Auscultatory crackles	4253 (21·0)	1061 (21·0)	0·951
Wheeze/roncus	2480 (12·0)	610 (12·0)	0·709
Heart gallop	720 (4·0)	162 (3·0)	0·218
Palpable liver	523 (3·0)	154 (3·0)	0·007
Palpable spleen	4296 (21·0)	1082 (21·0)	0·804
Neck stiffness	167 (1·0)	36 (1·0)	0·417
Abnormal fontanella (among applicable)	760 (4·0)	162 (3·0)	0·158
Prostration	2606 (13·0)	655 (13·0)	0·987

BCS at admission			0·221
Normal (BCS=5)	19476 (95·0)	4844 (95·0)	
Abnormal BSC (BCS=3-4)	683 (3·0)	190 (4·0)	
Deep coma (BCS≤2)	308 (2·0)	88 (2·0)	
Investigations			
Malaria diagnosis			0·750
Negative	7529 (37·0)	1902 (37·0)	
Positive	9810 (48·0)	2422 (47·0)	
Test not done	3167 (15·0)	802 (16·0)	
Glycaemia			0.478
Normoglycaemia (2.5-11.0 mmol/l)	17124 (84·0)	4260 (83·0)	
Hypoglycaemia (<2.5 mmol/l)	1934 (9·0)	479 (9·0)	
Hyperglycaemia (>11.0 mmol/l)	1448 (7·0)	387 (8·0)	
Blood culture			0·473
Negative	19436 (95·0)	4880 (95·0)	
Positive	1054 (5·0)	242 (5·0)	
Test not done	16 (0·0)	4 (0·0)	
Anemia			0·420
Non anemia	7019 (34·0)	1787 (35·0)	
Non-severe anaemia	10900 (53·0)	2724 (53·0)	
Severe anaemia	2587 (13·0)	615 (12·0)	
HIV status			0·638
Test not done	19298 (94·0)	4830 (94·0)	
Negative	1006 (5·0)	240 (5·0)	
Positive	202 (1·0)	56 (1·0)	
Outcome of the admission[n]			
			0·217
Alive	19340 (94·0)	4805 (94·0)	
Absconded	637 (3·0)	168 (3·0)	
Transferred	529 (3·0)	153 (3·0)	

[s]SD: standard deviation. [t]WHZ: Weigh-for-height.See definitions in Table S1.[u]WAZ: Weight-for-age. [β]It refers both communitary deaths and deaths in a readmission during follow-up period. [n]Percentage represents risk among children with same chacarteristics. [ψ]Hazard ratios and confidence intervals were derived from a Cox regression model.*Confidence intervals. [θ] P-value was derived from Wald test. BCS: Blantyre coma score. [λ]History of current disease reported by the child carer. [η]Hospital deaths ommited.

REFERENCES

1. United Nations. *Levels & Trends Child Mortality. Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation*. Report 2017. Available at: http://www.childmortality.org/index.php?r=site/index ; (accessed Nov 11, 2017). New York; 2017.

2. United Nations. *Levels and trends in child mortality*. Report 2015. *New UN study released on 9 September 2015 reveals substantial progress in child mortality since 1990 but global MDG 4 Target missed by wide margin.* [cited 2017 Feb 27]. Available from: http://www.un.org/en/development/desa/population/publications/mortality/child-mortality-report-2015.shtml. 2015 [cited; Available from:

3. World Health Organization. *Integrated Management of Childhood Illness: distance learning course*. Geneva: World Health Organization; 2014.

4. Wiens MO, Pawluk S, Kissoon N, et al. Pediatric post-discharge mortality in resource poor countries: a systematic review. *PLoS One* 2013; 8: e66698.

5. Wiens MO, Kumbakumba E, Larson CP, et al. Postdischarge mortality in children with acute infectious diseases: derivation of postdischarge mortality prediction models. *BMJ Open* 2015; 5: e009449.

6. American Academy of Pediatrics Section on S, American Academy of Pediatrics Committee on F, Newborn, Lally KP, Engle W. Postdischarge follow-up of infants with congenital diaphragmatic hernia. *Pediatrics* 2008; 121: 627-32.

7. Chang RK, Rodriguez S, Lee M, Klitzner TS. Risk factors for deaths occurring within 30 days and 1 year after hospital discharge for cardiac surgery among pediatric patients. *Am Heart J* 2006; 152: 386-93.

8. Chhibber AV, Hill PC, Jafali J, et al. Child Mortality after Discharge from a Health Facility following Suspected Pneumonia, Meningitis or Septicaemia in Rural Gambia: A Cohort Study. *PLoS One* 2015; 10: e0137095.

9. Chisti MJ, Graham SM, Duke T, et al. Post-discharge mortality in children with severe malnutrition and pneumonia in Bangladesh. *PLoS One* 2014; 9: e107663.

10. Moisi JC, Gatakaa H, Berkley JA, et al. Excess child mortality after discharge from hospital in Kilifi, Kenya: a retrospective cohort analysis. *Bull World Health Organ* 2011; 89: 725-32, 32A.

11. Roy SK, Chowdhury AK, Rahaman MM. Excess mortality among children discharged from hospital after treatment for diarrhoea in rural Bangladesh. Br Med J (*Clin Res Ed*) 1983; 287: 1097-9.

12. West TE, Goetghebuer T, Milligan P, Mulholland EK, Weber MW. Long-term morbidity and mortality following hypoxaemic lower respiratory tract infection in Gambian children. *Bull World Health Organ* 1999; 77: 144-8.

13. Wiens MO, Kumbakumba E, Kissoon N, Ansermino JM, Ndamira A, Larson CP. Pediatric sepsis in the developing world: challenges in defining sepsis and issues in post-discharge mortality. *Clin Epidemiol* 2012; 4: 319-25.
14. Zucker JR, Lackritz EM, Ruebush TK, 2nd, et al. Childhood mortality during and after hospitalization in western Kenya: effect of malaria treatment regimens. *Am J Trop Med Hyg* 1996; 55: 655-60.
15. Veirum JE, Sodeman M, Biai S, Hedegard K, Aaby P. Increased mortality in the year following discharge from a paediatric ward in Bissau, Guinea-Bissau. *Acta Paediatr* 2007; 96: 1832-8.
16. Biai S, Rodrigues A, Gomes M, et al. Reduced in-hospital mortality after improved management of children under 5 years admitted to hospital with malaria: randomised trial. *BMJ* 2007; 335: 862.
17. Phiri K, Esan M, van Hensbroek MB, Khairallah C, Faragher B, ter Kuile FO. Intermittent preventive therapy for malaria with monthly artemether-lumefantrine for the post-discharge management of severe anaemia in children aged 4-59 months in southern Malawi: a multicentre, randomised, placebo-controlled trial. *Lancet Infect Dis* 2012; 12: 191-200.
18. Islam MA, Rahman MM, Mahalanabis D, Rahman AK. Death in a diarrhoeal cohort of infants and young children soon after discharge from hospital: risk factors and causes by verbal autopsy. *J Trop Pediatr* 1996; 42: 342-7.
19. Stanton B, Clemens J, Khair T, Shahid NS. Follow-up of children discharged from hospital after treatment for diarrhoea in urban Bangladesh. *Trop Geogr Med* 1986; 38: 113-8.
20. Phiri KS, Calis JC, Faragher B, et al. Long term outcome of severe anaemia in Malawian children. *PLoS One* 2008; 3: e2903.
21. Ashraf H, Alam NH, Chisti MJ, Salam MA, Ahmed T, Gyr N. Observational follow-up study following two cohorts of children with severe pneumonia after discharge from day care clinic/hospital in Dhaka, Bangladesh. *BMJ Open* 2012; 2.
22. Wiens MO, Kissoon N, Kumbakumba E, et al. Selecting candidate predictor variables for the modelling of post-discharge mortality from sepsis: a protocol development project. *Afr Health Sci* 2016; 16: 162-9.
23. World Health Organization. *Under-five mortality. [cited 2017 Jul 13]. Available from: http://www.who.int/gho/child_health/mortality/mortality_under_five_text/en/. 2015 [cited; Available from:*
24. Sacarlal J, Nhacolo AQ, Sigauque B, et al. A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique. *BMC Public Health* 2009; 9: 67.
25. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a

southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012; 13: 581-8.
26. Gonzalez R, Augusto OJ, Munguambe K, et al. HIV Incidence and Spatial Clustering in a Rural Area of Southern Mozambique. *PLoS One* 2015; 10: e0132053.
27. Moraleda C, de Deus N, Serna-Bolea C, et al. Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa. *J Acquir Immune Defic Syndr* 2014; 65: 182-9.
28. World Health Organization. *Partnership for Maternal, Newborn & Child Health, World Health Organization. Countdown to 2015: Building a Future for Women and Children, Mozambique Country Reports.* Geneve: World Health Organization; 2012.
29. Sacoor C, Nhacolo A, Nhalungo D, et al. Profile: Manhica Health Research Centre (Manhica HDSS). *Int J Epidemiol* 2013; 42: 1309-18.
30. World Health Organization. *Every Woman, Every Child, Every Adolescent: Achievement and Prospects. The Final Report of the independent Expert Review Group (iERG) on Information and Accountaiblity for Women's and Children's Health.* Geneva, Switzerland: Worl Health Organization; 2015.
31. Lassi ZS, Middleton PF, Crowther C, Bhutta ZA. Interventions to Improve Neonatal Health and Later Survival: An Overview of Systematic Reviews. *EBioMedicine* 2015; 2: 985-1000.
32. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; 65: S200-S19.
33. Garcia-Basteiro AL, Quinto L, Macete E, et al. Infant mortality and morbidity associated with preterm and small-for-gestational-age births in Southern Mozambique: A retrospective cohort study. *PLoS One* 2017; 12: e0172533.
34. Villamor E, Misegades L, Fataki MR, Mbise RL, Fawzi WW. Child mortality in relation to HIV infection, nutritional status, and socio-economic background. *Int J Epidemiol* 2005; 34: 61-8.
35. Nhampossa T, Sigauque B, Machevo S, et al. Severe malnutrition among children under the age of 5 years admitted to a rural district hospital in southern Mozambique. *Public Health Nutr* 2013; 16: 1565-74.
36. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013; 382: 209-22.

37. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; 388: 3027-35.
38. English L, Kumbakumba E, Larson CP, et al. Pediatric out-of-hospital deaths following hospital discharge: a mixed-methods study. *Afr Health* Sci 2016; 16: 883-91.
39. Gera T, Shah D, Garner P, Richardson M, Sachdev HS. Integrated management of childhood illness (IMCI) strategy for children under five. *Cochrane Database Syst Rev* 2016: CD010123.
40. Berkley JA, Ngari M, Thitiri J, et al. Daily co-trimoxazole prophylaxis to prevent mortality in children with complicated severe acute malnutrition: a multicentre, double-blind, randomised placebo-controlled trial. *Lancet Glob Health* 2016; 4: e464-73.
41. Coles CL, Mabula K, Seidman JC, et al. Mass distribution of azithromycin for trachoma control is associated with increased risk of azithromycin-resistant Streptococcus pneumoniae carriage in young children 6 months after treatment. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2013; 56: 1519-26.
42. Porco TC, Gebre T, Ayele B, et al. Effect of mass distribution of azithromycin for trachoma control on overall mortality in Ethiopian children: a randomized trial. *JAMA* 2009; 302: 962-8.
43. Lassi ZS, Kumar R, Bhutta ZA. Community-Based Care to Improve Maternal, Newborn, and Child Health. In: Black RE, Laxminarayan R, Temmerman M, Walker N, editors. *Reproductive, Maternal, Newborn, and Child Health: Disease Control Priorities, Third Edition (Volume 2).* Washington (DC); 2016.

05 | SUMMARY OF RESULTS & DISCUSSION

The studies included in this thesis provide important results and highlight knowledge gaps that, if correctly addressed, could contribute to improve our understanding of severe conditions causing death among African infants and children and for which interventions have not yet been implemented in the majority of low-income countries, including Mozambique, where the greatest part of the work of this thesis took place.

The first article comments on the overall global burden of neonatal mortality, and highlights the importance of reducing NMR in order to achieve the SDG's target for child survival by 2030. MDG4 was not fully achieved, possibly in relation to the fact that neonatal deaths decreased at a much slower rate than post-neonatal mortality in the last two decades[11], and that measures designed to prevent or specifically address these early neonatal deaths are less implemented and available than for deaths occurring among older children. In 2016, nearly half of the deaths among children U5 were babies in their first 28 days of life and, whereby infectious diseases appear to significantly drive neonatal mortality[61]. National and international efforts should focus on achieving universal coverage of those interventions found to be most effective for reducing neonatal deaths, especially in those countries with highest burden in order to succeed in advancing towards the ambitious SDG target for child mortality by 2030[161].

The systematic review and meta-analysis investigating the global incidence of infant invasive *GBS* disease and the associated serotypes showed a high incidence and CFR of *GBS* invasive disease among infants <90 days in every world region, yet likely considerably underestimated in settings with limited access to care and diagnostics. The overall estimated incidence of infant GBS disease, 0.49 per 1000 LB, is slightly lower than the previous global estimate of 0.53 per 1000 LB (95% CI 0.41-0.62)[68]. However, our review and meta-analyses represent an important update since it includes new data from low and middle-income contexts (18 new studies from 10 countries).

The reduction in overall incidence is likely driven by lower incidence of invasive infant *GBS* disease in the Americas, (0.43 per 1000 LB here vs. 0.67 per 1000 LB in the previous review), and Europe, (0.53 vs. 0.57/1000 LB)[68]. The highest incidence continued to be in Africa (1.12 per 1000 LB), and especially in Southern Africa (2.00; 95% CI 0.74-3.26). Conversely, the lowest incidences were found in Asia (0.31 per 1000 LB) and in Southeast Asia (0.21; 95% CI 0.09-0.32). This may reflect a true regional difference, which could be related to differences in lower overall prevalence of maternal colonization and/or lower prevalence of serotype III which is more commonly associated with *GBS* LOD disease and with the most virulent clone, clonal complex 17[87]. The meta-analysis on maternal colonization[87], part of the same wider project in which our meta-analysis is included, showed a lower prevalence of maternal colonization with serotype III in Asia, consistent with a true regional difference and possibly explaining the lower *GBS* LOD incidence among infants found in our review. Some of the differences may also be related to incomplete case-ascertainment, considering the challenges of following birth outcomes and newborns in settings where more home births occur, in comparison to Africa.

Overall, *GBS* EOD incidence was higher than *GBS* LOD although a higher proportion of associated meningitis was found among the latter. The highest incidence of *GBS* EOD was also in Africa and it was higher compared to that reported in the most recent worldwide review although *GBS* LOD incidence for the same region was similar in both reviews[68]. Reasons for this increase in *GBS* EOD incidence in Africa is likely multifactorial, possibly including as some of the explanations a increase in co-morbidities such as *HIV*[162] or improved data collection to detect early disease. This high incidence is important in terms of total burden, as CFR in Africa were also four times higher than in developed countries (18.9% and 4.7% respectively); thus the greatest burden of cases, and deaths, is in Africa.

Although our data from Africa are limited to a few studies, mostly in Southern and Eastern Africa, this review has included broader data from Africa compared to the previous one[108,162-167]. Among them, unpublished data for a 15-year long period from Manhiça, Mozambique (B. Sigaúque, personal communication), the study area where most of this thesis has been developed, were included. The incidence in this area was one of the highest in SSA, 1.20 per 1000 LB, only slightly lower than those reported in South Africa[162,164,165] and Malawi[168]. The early to late-onset disease ratio in

Mozambique was different to the entire African region (0.50 vs. 1.02). This difference could be partially explained because of the high prevalence and poorly controlled maternal HIV infection in the study area which predisposes to LOD[148,149] and reduced case ascertainment, especially the earliest-onset cases (<24h of birth), due to difficulties in access to care and rapid and high case fatality before reaching at hospital or taking samples. CFR of *GBS* invasive disease in Mozambique was lower than the average of the region (12% vs. 18.9%). However, *GBS* EOD CFR was higher (32% vs. 27%) likely due to the high mortality associated to cases with onset in the first day of life (53%). On the other hand, *GBS* LOD CFR in Mozambique was 3% compared to 12% in Africa. Case management and the medical staff at MDH, where the study was conducted, is not representative of the usual staff in other rural African hospitals, nor in quantity neither in training, likely explaining this difference.

In terms of serotypes causing invasive disease, serotype III accounted for over half of all disease-causing isolates followed by serotypes Ia, V and II. The five more prevalent serotypes causing *GBS* invasive disease among infants accounted for 97% of all cases. Disease-causing serotypes were similar in prevalence across different regions. Serotype distribution was similar to that reported in the previous review suggesting stability over time[68,94].

This paper is part of *The Worldwide Burden of Group B Streptococcus for Pregnant Women, Stillbirths, and Children* project which presents the first systematic estimates of the worldwide burden of GBS[84]. Our findings on the ratio of late-onset to early-onset invasive *GBS* disease, CFR and proportion of cases with meningitis were used to estimate the number of LOD *GBS* cases, deaths in EOD and LOD *GBS* cases and proportions of each serotype reported in each disease syndrome, respectively, through a four-step compartmental model approach (figure 16)[84,169].

Number of cases of invasive GBS diseases was higher in the compartmental model compared to cases found in the meta-analysis (figure 17). The compartmental model approach mitigates some of the very substantial problems with low case ascertainment for invasive infant disease, which can result in huge underestimation, especially in LIC. Our comparatively low estimates using infant incidence data are a result of cases being “missed” through lack of access to healthcare, inadequate clinical assessment and suspicion of infection, lack of diagnostic testing, and lack of appropriate laboratory detection methods such as high-quality blood cultures[84].

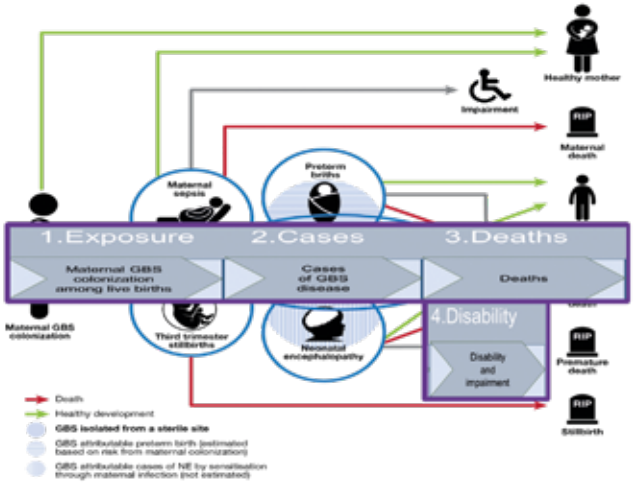


Figure 16. Disease schema for outcomes of perinatal group B Streptococcus. Abbreviations: GBS, group B Streptococcus; NE, neonatal encephalopathy. Adapted from Lawn et al Clinical Infectious Diseases 2017[169].

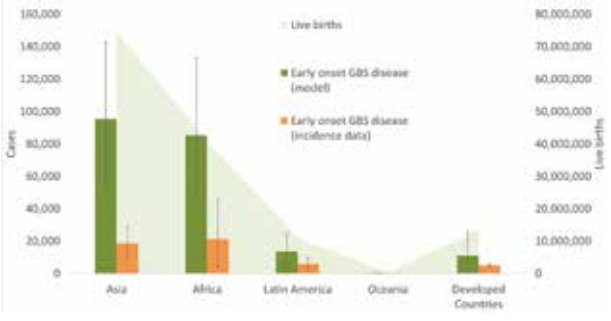


Figure 17. Comparing group B streptococcal (GBS) invasive diseases cases from model to incidence data. Adapted from Seale et al., Clinical Infectious Diseases 2017[84].

This series of papers also suggest that GBS is present in all regions of the world where it commonly infects pregnant women, with 21.7 million pregnant women colonized worldwide. In 2015 we estimated that, worldwide, there were at least 319 000 infants <3 months of age with this life-threatening infection resulting in 90 000 (UR 36 000-169 000) deaths plus at least 10 000 (UR 3000-27 000) children with disability related to *GBS* meningitis. In addition, it was estimated that 57 000 (UR 12 000-104 000) stillbirths were due to *GBS* disease.

A *GBS* maternal vaccination strategy using a *GBS* vaccine with 80% efficacy and 90% global coverage could prevent 231 000 (UR 14 000-507 000) infant and maternal *GBS* cases, 41 000 (UR 8000-75 000) stillbirths and 66 000 (UR 12 000-123 000) infant deaths annually (figure 18)[84].

GBS causes more deaths than those derived from mother-to child transmission of *HIV,* and more than the combined neonatal deaths from tetanus, pertussis, and RSV, for which maternal vaccines are already in use, or in advanced development[63].

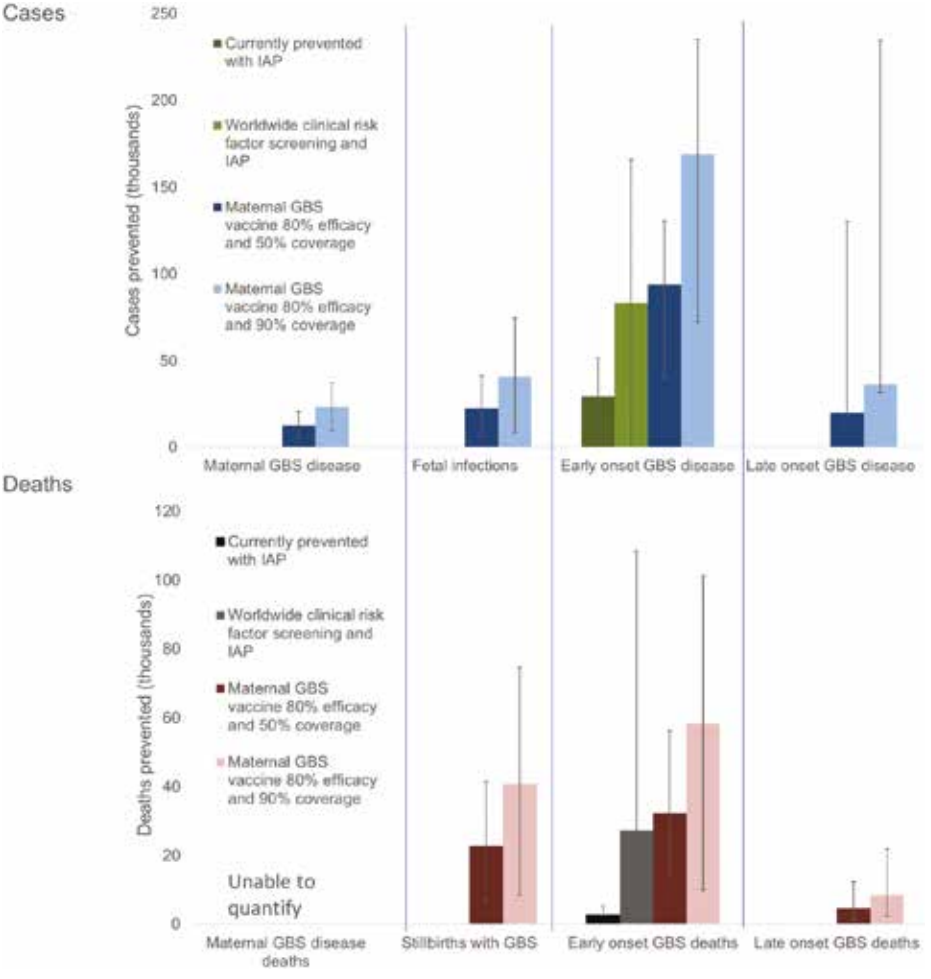


Figure 18. Scenarios of estimated cases of group B streptococcal (GBS) disease and deaths prevented with different intervention methods in a year. Adapted from Seale et al Clinical Infectious Diseases 2017[84].

The third article, aiming to characterize the burden of maternal carriage of *GBS* and *E. coli* among Mozambican pregnant women, showed a prevalence of maternal *GBS* colonization of 21.3%, as high as reported in other countries in SSA94 and probably more credible than the much lower estimates produced for this same country (ranging from 1 to 1.8%) in older studies (1995 and 2008, respectively)[45,170].

The yield of vagino-rectal sampling was better for detecting *GBS* colonization than using only vaginal samples as previously reported[171,172] and recommended[173]. Despite vaginal and vagino-rectal sample being uncommon practices in MDH, sample collection was well accepted by mothers recruited to the study, but also well perceived and found theoretically acceptable by mothers not included in the study but also interviewed in this respect. No risk factors independently associated with higher prevalence of GBS colonization were found in this study, both in the entire cohort and in the analysis restricted to HIV positive women.

We also found a low incidence of *GBS* invasive cases in neonates born to *GBS* infected women. Reasons for this low incidence could be the high prevalence of antibodies against *GBS* found in the studied cohort (69.2%), as antibodies against this infecton have been associated with protection against invasive *GBS* disease in HIC[174] and LMIC and LIC[175]. Another plausible explanation could be the attempt to implement preventive strategies to those colonized *GBS* mothers delivering at MDH by giving IAP or by administering a single dose of penicillin after birth to those neonates born to *GBS* colonized mothers as none of those babies developed symptoms of sepsis. Although this strategy is not generally recommended on account of the risks of enhancing antimicrobial resistance, it could be argued that for settings were access to health is problematic, but where *GBS* maternal carriage can be confirmed, such a strategy could prove effective in decreasing neonatal early morbidity and mortality.

On the other hand, the prevalence of *E. coli* found in this study was lower than that reported from other authors in different African settings[79,176] but comparable with the prevalence reported by Karou in Togo*177*. No risk factors were found to be independently associated with a higher risk of *E. coli* vaginal carriage among pregnant women.

GBS continues to be susceptible to penicillin, ampicillin, and ceftriaxone -the antibiotics of choice- in this setting, similarly to what previous studies in SSA have shown[177-180]. However, higher levels of resistance to erythromycin (~33%) were found in this study as many countries are also reported likely due to an increased use for common diseases[181-184]. Erythromycin resistance is frequently associated with clindamycin resistance[92] and these antibiotics are the drugs of choice for penicillin-hypersensitive patients. The emergence of non-susceptible *GBS* strains could have important public health implications, and therefore requires close monitoring.

Serotype V was the most prevalent serotype found in this study, a finding that differs from those in the majority of studies conducted in other countries[87]. This finding supports the need to identify prevalent serotypes in each region, as a pre-requisite of establishing the potential coverage, impact and implementation requirements of future anti *GBS* vaccination strategies.

The fourth article reviewed the epidemiology of congenital infections in LMIC and LIC covering a period of 45 years (from 1971 to 2016) and highlighted that the burden of congenital infections may be higher in these regions than in industrialized countries.

Although enormous efforts are being made at the international and national levels to reduce the burden of some congenital infections, their establishment as a public health priority remains distant. Recent outbreaks of congenital infections such as that involving the *Zika virus* and its possible association with microcephaly in newborns when women are infected during pregnancy, have generated worldwide alarm and have highlighted the generalized lack of attentiveness in the world about the impact of infections transmitted from mother to child. It is essential to adequately describe the global and local burden of each perinatally-transmitted infection, through the improvement of maternal and neonatal morbidity surveillance systems at the ANC clinics and child services. Ensuring early diagnosis, treatment and vaccination (when available) as the key preventive strategy are also of paramount importance.

Considering that vaccines are the best method to reduce *rubella* and *hepatitis B* infections, campaigns to introduce them in paediatric immunization programs must be a high priority in places where this has yet not been

properly done. Many countries have introduced only measles-containing vaccines (MCV) rather than the combined measles-rubella vaccine relying on donors to pay for them. Research should also focus in the development of new vaccines against other common and devastating congenital infections, such as for instance *CMV* and toxoplasmosis. Since preventive and curative strategies exist to tackle some of these infections, efforts at the international and national levels must be made to reduce their burden in resource-limited countries.

After reviewing the epidemiology of congenital infections in developing countries, the fifth article of this thesis assessed the prevalence of vertical transmission of some of these viruses in Manhiça, Mozambique. Among the 115 dried umbilical cord (DUC) samples from neonates obtained in this study, the prevalence of congenital CMV was 2.6% and 6.3% when assessed through nasopharyngeal aspirates (NPA). It has been demonstrated that real-time dried-blood-spot PCR assay has a lower sensitivity compared with the standard saliva rapid culture[185] and then, the prevalence of cCMV found in our cohort through this method is likely underestimated. On the other hand NPA has not been ever assessed as a specimen valid for cCMV diagnosis and it is know that until 30% of CMV-seropositive women secrete CMV in vaginal fluid and that may contaminate with CMV nasopharynx of neonates[186]. As consequence of this fact, the prevalence of cCMV found in this study through NPA is likely overestimated.

Taking in consideration both issues, it is reasonable to assume a cCMV prevalence in our cohort of at least 2.6%, higher than the rate reported in newborns from HIC (<1%)[187] and falling within the range reported in a systematic review for developing countries (0.6% - 6.1%)[188]. Although, the specimens utilized in this study are not the recommended sampling strategy for the screening of such infections, our results highlight a high burden of vertical CMV transmission. Urine and saliva have been demonstrated to be the most reliable specimens[185,189] although they are not exempt of problems. Saliva samples may be contaminated by breast milk and urine samples may be hard to collect. Larger studies should assess the limitations of NPA specimen and its potential use for cCMV diagnosis. The use of NPA and the molecular screening techniques could be a good approach to study several viruses simultaneously avoiding the risk of contamination of saliva samples and collection issues of urine samples.

Prevalence of cCMV among *HIV*-exposed children in our study was similar to that found in HIV-unexposed. However, no significant differences were found among these two groups (2.7% vs. 2.6%%, OR 1.26 95% CI 0.16–9.89, p=0.83). *HIV* prevalence in our maternal cohort was very high (31.4%) and almost 90% of mothers were under HAART, possibly explaining why the prevalence of cCMV was not even higher and why differences between HIV-exposed and unexposed-neonates were not found[190]. No risk factors independently associated with cCMV were found. However, caution is needed when interpreting our findings, since sample size was small and likely insufficient to detect significant differences among infected and uninfected neonates.

Congenital *B19V* and *EV* infections seem to be less prevalent in this area, although only dried umbilical cord samples were analysed for their screening. Further research to evaluate the real burden and consequences of vertical transmission of these viral infections in resource-constrained settings is needed.

The sixth article described the prevalence and incidence of hypoglycaemia among children admitted at MDH and reported a high prevalence of hypoglycaemia among a large cohort of nearly 50 000 children admitted to a rural Mozambican hospital across a 13-year long period. Such a life-threatening complication was even more common among newborns (overall prevalence 3.2% and neonatal prevalence 8.8%). This study was the first attempt to describe minimum-community based incidence rates (MCBIR) for hypoglycaemia in SSA. MCBIR were significantly higher in newborns (9.47 episodes/1000 child years at risk) than in any other age group ($P < 0.001$) underscoring the need to carefully follow this complication in this especially vulnerable age group. Particularly worrying was the excessive and unacceptable risk of death associated to hypoglycaemia, considering this complication is easily and readily treatable[46,191-194]. In our cohort the CFR was 19.3%, and likely underestimated as it was only detected through a single screening upon admission, and not throughout the rest of the hospitalization[195,196].

The seventh and last article included in this thesis was the largest study to date evaluating post-discharge mortality in the first three months following hospital discharge from a rural district hospital in a LIC. This analysis, based on more than 20 000 hospital discharges and 935 post-discharge deaths,

represents a systematic approach to ascertain predictors of PDM in a resource-constrained environment.

We found an overall post-discharge mortality of 3.6%, with half of the deaths clustering in the first 30 days post-discharge, similar to that reported in other studies on PDM[55,57,197,198] and higher than the in-hospital mortality observed in our cohort (2.5%). Overall, trends of PDM for all ages have decreased over time likely due to improved hygiene practices in households and better case management in health centres, among other reasons. However, PDM trends among infants <3 months have remained more or less stable over time. Possible explanations include the fact that effective interventions specifically targeting this age group, and designed to reduce neonatal and infant mortality, have not been fully implemented in the study area.

One primary predictive model for all ages included young age, malnutrition, history of diarrhoea, clinical pneumonia symptoms, prostration, *HIV* and/or malnutrition associated symptoms, bacteraemia, positive *HIV* status, rainy season and an outcome of transfer or absconding. Applying a score based on this model to a population similar to ours, we could identify up to 80% of the children at risk of dying during the first 90 days, being this percentage even higher in the first 30 days.

Two simplified alternative models, one including exclusively clinical predictors and another one including only variables with minimal costs, showed similar results. A specific infants <3 months model identified as risk factors being a neonate, having a low weight-for-age (WAZ) score, history of difficulty of breathing, hypothermia or fever, oral candidiasis and a history of absconding or transfer to another hospital. Using a score based on this model at discharge in our population, 85% of infants <3months at risk of dying after discharge could be identified in the first days after discharge and almost 80% during the remaining follow-up period, a remarkable finding taking into account the high incidence risk of dying following hospital discharge in this age group.

Predictive models presented in this study could be applied at hospital discharge and children at risk of dying could be identified through their use. This could allow designing a better post-discharge planning, health education to the families and follow-up care. However, the identification

of high risk does not imply that risk can be reduced. Future research should consider validation of these models in different contexts and prospectively assessing their accuracy to identify children at risk of dying after discharge in resource-constrained settings. Such an early identification could allow the design of preventive strategies to address the main causes of post-discharge mortality. Continued investment in child mortality data collection and understanding circumstances of child death following hospital discharge is needed in order to design innovative, effective and feasible strategies to reduce the risk of post-hospitalization deaths among children.

06 | CONCLUSIONS

1. In the absence of an accelerated progress in neonatal mortality, it will be challenging to significantly reduce overall child mortality by 2030, as established by SDGs. A significant increase of the coverage of several essential health interventions, with the aim of eventually ensuring universal coverage, has the potential to reduce neonatal deaths, benefit women and children after the first month, and reduce stillbirths.

2. *GBS* disease is an important cause of infant sepsis and meningitis despite the limitations in the data and uncertainties about the low incidence in Asia. Early-onset incidence is higher than late-onset one, although a higher proportion of meningitis is seen among late-onset cases. In Africa, where the incidence is highest, the mortality associated to *GBS* is also the highest, quadruplicating that of developed countries, suggesting this is the region where prevention strategies are most critical to introduce.

3. Serotype III accounted for over half of all disease-causing isolates and a vaccine covering the most common five global serotypes (Ia/Ib/II/III/V) could prevent up to 97% of *GBS* invasive cases in infants.

4. Prevalence of *GBS* and *E. coli* colonization among pregnant women in this semirural area of Mozambique is high and comparable to those reported in similar settings and in high-income countries. *HIV* infection was not identified as a specific or independent risk factor for *GBS* or *E. coli* colonization.

5. Serotype V was the most prevalent in Manhiça, Mozambique. This finding differs from results found in the majority of studies conducted in other countries, revealing the need to identify prevalent serotypes in each region, as a prerequisite of establishing the potential coverage, impact and implementation requirements of future anti *GBS* vaccination strategies.

6. Estimates from low and middle-income countries indicate that the burden of congenital infections may be higher in these regions than in industrialized countries. As preventive and curative strategies are available to tackle some of these infections, efforts at the international and national levels must be made to implement those and thus reduce their burden in resource-limited countries.

7. Vertical transmission of *CMV* in Southern Mozambique is higher than in HIC and at least as high as prevalence of congenital *CMV* infection in other similar settings in sub-Saharan Africa.

8. Congenital transmission of *parvovirus B19* and *enterovirus* seem to be less prevalent in Manhiça, Mozambique, although the small sample size is an important limitation of these results.

9. Hypoglycaemia is a common complication of many conditions causing hospitalization in Mozambican children, especially among newborns, and is associated with an excessive and unacceptable risk of death, being a treatable condition.

10. Death following discharge is an important although poorly recognized contributor to child mortality. Post-discharge mortality in the first 90 days after hospital discharge is higher than inpatient mortality, especially in the first 30 days after discharge from a rural hospital in Southern Mozambique.

11. Simple algorithms screening for determined combinations of the found risk factors could identify children at higher risk of dying after hospital discharge, thus facilitating their closer post-discharge monitoring.

07 | RECOMENDATIONS

1. In poor settings, implementation and scale-up of interventions focusing on helping neonates surviving through "the golden first 28 days of life" may facilitate in these settings a rapid decrease of neonatal mortality, and thus achieve the ambitious SDG of reducing neonatal deaths to a maximum of 12 per 1000 live births by 2030. If this is done at a large scale, up to 5 million neonatal lives could be saved throughout the period 2017-2030.

2. Existing preventive strategies using IAP are not feasible to implement in many resource-limited settings due to logistical issues, high number of home deliveries and late presentation to health facilities for delivery. Thus, alternative strategies are urgently needed.

3. Maternal vaccination appears as a very interesting alternative strategy, and the data we have generated suggest that a pentavalent conjugate vaccine (including serotypes Ia/Ib/II/III/V) would cover almost all disease-causing serotypes (97%) in young infants, worldwide.

4. *GBS* serotype distribution in pregnant women may differ among regions. Therefore, it is essential to identify prevalent serotypes in each region in order to know the potential coverage, impact and implementation requirements of future anti *GBS* vaccination strategies.

5. Larger studies are needed to evaluate the true burden, clinical relevance and consequences of congenital infections due to *CMV*, *parvovirus B19* and *enterovirus* in low-income countries. Cheap and highly sensitive tools for diagnosis of congenital infections, which may be feasible to use in resource-constrained settings are needed.

6. In settings similar to Manhiça, all admitted children should be screened for hypoglycaemia, and aggressively managed when found to be hypoglycaemic. A single determination on admission appears however insufficient, and glycaemia should be recurrently screened during hospitalization. Better, cheaper and more innovative diagnostic and therapeutic alternatives need to be urgently investigated to better address the consequences of hypoglycaemia in developing countries.

7. Child mortality following hospital discharge is unacceptably high and more attention needs to be put in this preventable cause of death. The use of algorithms to detect which children may require more attention following discharge is a good approach, and future research should consider validating predictive models in different contexts and prospectively assessing their accuracy to identify children at risk of dying. This could allow designing a better post-discharge planning, health education to the families and follow-up care.

8. Continued investment in child mortality data collection and understanding circumstances of child death following a hospital discharge is needed in order to design innovative, effective and feasible strategies to reduce the risk of post-hospitalization deaths among children.

08 | REFERENCES

1. Pozzi L FD. Infant and child mortality in the past. *Annales de démographie historique* 2015; **1**(129): 55-75.
2. H Le Bras. Naissance de la mortalité. L'origine politique de la statistique et de la démographie. Paris; 2000.
3. Garrett E GC, Shelton N, Woods R. Infant mortality: a continuing social problem. London: Ashgate; 2007.
4. Global Burden of Disease Child Mortality Collaborators. Global, regional, national, and selected subnational levels of stillbirths, neonatal, infant, and under-5 mortality, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**(10053): 1725-74.
5. UNICEF. UNICEF Data: Monitoring the Situation of Children and Women. Available at: https://data.unicef.org/topic/child-survival/neonatal-mortality/ (Accessed: 24th October 2017). 2017.
6. World Health Organization. Global Health Observatory Map Gallery. (cited 2017 Nov 03). Available from: http://gamapserver.who.int/mapLibrary/app/searchResults.aspx. 2015.
7. Salomon JA, Wang H, Freeman MK, et al. Healthy life expectancy for 187 countries, 1990-2010: a systematic analysis for the Global Burden Disease Study 2010. Lancet 2012; **380**(9859): 2144-62.
8. World Health Organization. Global Health Observatory data. Life Expectancy. [cited 2017 Nov 03]. Available from: http://www.who.int/gho/mortality_burden_disease/life_tables/situation_trends_text/en/ 2017.
9. United Nations. Conferences, meetings and events. Millennium submmit (6-8 September 2000). (cited 2017 nov 03). Available from: http://www.un.org/en/events/pastevents/millennium_summit.shtml. 2000.
10. Dieleman JL, Schneider MT, Haakenstad A, et al. Development assistance for health: past trends, associations, and the future of international financial flows for health. *Lancet* 2016; **387**(10037): 2536-44.
11. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet* 2016; **388**(10063): 3027-35.

12. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. *Lancet* 2014; **384**(9938): 189-205.
13. AbouZahr C, de Savigny D, Mikkelsen L, Setel PW, Lozano R, Lopez AD. Towards universal civil registration and vital statistics systems: the time is now. *Lancet* 2015; **386**(10001): 1407-18.
14. De Savigny D, Riley I, Chandramohan D, et al. Integrating community-based verbal autopsy into civil registration and vital statistics (CRVS): system-level considerations. *Glob Health Action* 2017; **10**(1): 1272882.
15. Murray CJ, Lopez AD, Barofsky JT, Bryson-Cahn C, Lozano R. Estimating population cause-specific mortality fractions from in-hospital mortality: validation of a new method. *PLoS Med* 2007; **4**(11): e326.
16. Byass P, Chandramohan D, Clark SJ, et al. Strengthening standardised interpretation of verbal autopsy data: the new InterVA-4 tool. *Glob Health Action* 2012; **5**: 1-8.
17. McCormick TH, Li ZR, Calvert C, Crampin AC, Kahn K, Clark SJ. Probabilistic Cause-of-death Assignment using Verbal Autopsies. *J Am Stat Assoc* 2016; **111**(515): 1036-49.
18. Liu L, Kalter HD, Chu Y, et al. Understanding Misclassification between Neonatal Deaths and Stillbirths: Empirical Evidence from Malawi. *PLoS One* 2016; **11**(12): e0168743.
19. Ordi J, Ismail MR, Carrilho C, et al. Clinico-pathological discrepancies in the diagnosis of causes of maternal death in sub-Saharan Africa: retrospective analysis. *PLoS Med* 2009; **6**(2): e1000036.
20. Taylor TE, Fu WJ, Carr RA, et al. Differentiating the pathologies of cerebral malaria by postmortem parasite counts. *Nat Med* 2004; **10**(2): 143-5.
21. Setel PW, Macfarlane SB, Szreter S, et al. A scandal of invisibility: making everyone count by counting everyone. *Lancet* 2007; **370**(9598): 1569-77.
22. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* 2015; **526**(7572): 207-11.
23. Lim SS, Fullman N, Stokes A, et al. Net benefits: a multicountry analysis of observational data examining associations between insecticide-treated mosquito nets and health outcomes. *PLoS Med* 2011; **8**(9): e1001091.
24. Ogbonna A, Uneke CJ. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. *Trans R Soc Trop Med Hyg* 2008; **102**(7): 621-7.
25. Fitzwater SP, Chandran A, Santosham M, Johnson HL. The worldwide impact of the seven-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis* J 2012; **31**(5): 501-8.
26. Sindhu KNC, Babji S, Ganesan SK. Impact of rotavirus vaccines in low and middle-income countries. *Curr Opin Infect Dis* 2017; **30**(5): 473-81.

27. Aaby P, Martins CL, Garly ML, et al. Measles vaccination in the presence or absence of maternal measles antibody: impact on child survival. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2014; **59**(4): 484-92.

28. Gumede-Moyo S, Filteau S, Munthali T, Todd J, Musonda P. Implementation effectiveness of revised (post-2010) World Health Organization guidelines on prevention of mother-to-child transmission of HIV using routinely collected data in sub-Saharan Africa: A systematic literature review. *Medicine* (*Baltimore*) 2017; **96**(40): e8055.

29. Liu L, Black RE, Cousens S, Mathers C, Lawn JE, Hogan DR. Causes of child death: comparison of MCEE and GBD 2013 estimates. *Lancet* 2015; **385**(9986): 2461-2.

30. Deloria Knoll M, Fu W, Shi Q, et al. Bayesian Estimation of Pneumonia Etiology: Epidemiologic Considerations and Applications to the Pneumonia Etiology Research for Child Health Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **64**(suppl_3): S213-S27.

31. Munos MK, Walker CL, Black RE. The effect of oral rehydration solution and recommended home fluids on diarrhoea mortality. *Int J Epidemiol* 2010; **39 Suppl 1**: i75-87.

32. World Health Organization. World Malaria Report 2016. (cited 2018 Jan 13). Available from: http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. Geneva: World Health Organization, 2016.

33. Pluess B, Tanser FC, Lengeler C, Sharp BL. Indoor residual spraying for preventing malaria. *Cochrane Database Syst Rev* 2010; (4): CD006657.

34. Garner P, Gulmezoglu AM. Drugs for preventing malaria in pregnant women. *Cochrane Database Syst Rev* 2006; (4): CD000169.

35. Drakeley C, Reyburn H. Out with the old, in with the new: the utility of rapid diagnostic tests for malaria diagnosis in Africa. *Trans R Soc Trop Med Hyg* 2009; 103(4): 333-7.

36. World Health Organization. Guidelines for the Treatment of Malaria 3rd ed. Geneva; 2015.

37. World Health Organization. Malnutrition. (cited 2018 Jan 13). Available from: http://www.who.int/mediacentre/factsheets/malnutrition/en/. 2017.

38. Seale AC. Every day and every gram counts at birth. *Lancet Infect Dis* 2016; 16(8): 876-7.

39. Pisarchik AN, Pochepen ON, Pisarchyk LA. Increasing blood glucose variability is a precursor of sepsis and mortality in burned patients. *PLoS One*; **7**(10): e46582.

40. Badawi O, Waite MD, Fuhrman SA, Zuckerman IH. Association between intensive care unit-acquired dysglycemia and in-hospital mortality. *Crit Care Med*; **40**(12): 3180-8.

41. Hirshberg E, Larsen G, Van Duker H. Alterations in glucose homeostasis in the pediatric intensive care unit: Hyperglycemia and glucose variability are associated with increased mortality and morbidity. *Pediatr Crit Care Med* 2008; **9**(4): 361-6.

42. Altman M, Vanpee M, Cnattingius S, Norman M. Neonatal morbidity in moderately preterm infants: a Swedish national population-based study. *J Pediatr* 2011; **158**(2): 239-44 e1.

43. Tam EW, Haeusslein LA, Bonifacio SL, et al. Hypoglycemia is associated with increased risk for brain injury and adverse neurodevelopmental outcome in neonates at risk for encephalopathy. *J Pediatr* 2012; **161**(1): 88-93.

44. Black RE, Allen LH, Bhutta ZA, et al. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008; **371**(9608): 243-60.

45. Sigauque B, Roca A, Mandomando I, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis* J 2009; **28**(2): 108-13.

46. Osier FH, Berkley JA, Ross A, Sanderson F, Mohammed S, Newton CR. Abnormal blood glucose concentrations on admission to a rural Kenyan district hospital: prevalence and outcome. *Arch Dis Child* 2003; **88**(7): 621-5.

47. Ikeda T, Takahashi T, Sato A, et al. Predictors of outcome in hypoglycemic encephalopathy. *Diabetes Res Clin Pract* 2013; **101**(2): 159-63.

48. Fong CY, Harvey AS. Variable outcome for epilepsy after neonatal hypoglycaemia. *Dev Med Child Neurol* 2014; **56**(11): 1093-9.

49. World Health Organization. Integrated Management of Childhood Illness: distance learning course. Geneva: World Health Organization, 2014.

50. Wiens MO, Kumbakumba E, Larson CP, et al. Postdischarge mortality in children with acute infectious diseases: derivation of postdischarge mortality prediction models. *BMJ Open* 2015; **5**(11): e009449.

51. Wiens MO, Pawluk S, Kissoon N, et al. Pediatric post-discharge mortality in resource poor countries: a systematic review. *PLoS One* 2013; **8**(6): e66698.

52. American Academy of Pediatrics Section on S, American Academy of Pediatrics Committee on F, Newborn, Lally KP, Engle W. Postdischarge follow-up of infants with congenital diaphragmatic hernia. *Pediatrics* 2008; **121**(3): 627-32.

53. Brown KL, Wray J, Knowles RL, et al. Infant deaths in the UK community following successful cardiac surgery: building the evidence base for optimal surveillance, a mixed-methods study. *NIHR Journals Library* 2016.

54. Chang RK, Rodriguez S, Lee M, Klitzner TS. Risk factors for deaths occurring within 30 days and 1 year after hospital discharge for cardiac surgery among pediatric patients. *Am Heart J* 2006; **152**(2): 386-93.

55. Chhibber AV, Hill PC, Jafali J, et al. Child Mortality after Discharge from a Health Facility following Suspected Pneumonia, Meningitis or Septicaemia in Rural Gambia:

A Cohort Study. *PLoS One* 2015; **10**(9): e0137095.

56. Chisti MJ, Graham SM, Duke T, et al. Post-discharge mortality in children with severe malnutrition and pneumonia in Bangladesh. *PLoS One* 2014; **9**(9): e107663.

57. Moisi JC, Gatakaa H, Berkley JA, et al. Excess child mortality after discharge from hospital in Kilifi, Kenya: a retrospective cohort analysis. *Bull World Health Organ* 2011; **89**(10): 725-32, 32A.

58. West TE, Goetghebuer T, Milligan P, Mulholland EK, Weber MW. Long-term morbidity and mortality following hypoxaemic lower respiratory tract infection in Gambian children. *Bull World Health Organ* 1999; **77**(2): 144-8.

59. Wiens MO, Kumbakumba E, Kissoon N, Ansermino JM, Ndamira A, Larson CP. Pediatric sepsis in the developing world: challenges in defining sepsis and issues in post-discharge mortality. *Clin Epidemiol* 2012; **4**: 319-25.

60. Akseer N, Lawn JE, Keenan W, et al. Ending preventable newborn deaths in a generation. *Int J Gynaecol Obstet* 2015; **131 Suppl 1**: S43-8.

61. United Nations. Levels & Trends Child Mortality. Estimates Developed by the UN Inter-agency Group for Child Mortality Estimation. Report 2017. Available at: http://www.childmortality.org/index.php?r=site/index ; (accessed Nov 11, 2017). New York, 2017.

62. Lee AC, Katz J, Blencowe H, et al. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *Lancet Glob Health* 2013; **1**(1): e26-36.

63. Miller JE, Hammond GC, Strunk T, et al. Association of gestational age and growth measures at birth with infection-related admissions to hospital throughout childhood: a population-based, data-linkage study from Western Australia. *Lancet Infect Dis* 2016; **16**(8): 952-61.

64. Blencowe H, Lee AC, Cousens S, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. *Pediatr Res* 2013; **74 Suppl 1**: 17-34.

65. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; **371**(9606): 75-84.

66. Garces AL, McClure EM, Perez W, et al. The Global Network Neonatal Cause of Death algorithm for low-resource settings. *Acta Paediatr* 2017; **106**(6): 904-11.

67. Menendez C, Castillo P, Martinez MJ, et al. Validity of a minimally invasive autopsy for cause of death determination in stillborn babies and neonates in Mozambique: An observational study. *PLoS Med* 2017; **14**(6): e1002318.

68. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet* 2012; **379**(9815): 547-56.

69. Krohn MA, Thwin SS, Rabe LK, Brown Z, Hillier SL. Vaginal colonization by Escherichia coli as a risk factor for very low birth weight delivery and other perinatal complications. *J Infect Dis* 1997; **175**(3): 606-10.

70. Subair O, Wagner P, Omojole F, Morgan H. Group B streptococcus disease in neonates: to screen or not to screen? *J Obstet Gynaecol* 2005; **25**(5): 462-4.

71. Stevens DL KE, editor. Streptococcal infections: clinical aspects, microbiology, and molecular pathogenesis. United States: Oxford University Press; 2000.

72. Williams PCM, Isaacs D, Berkley JA. Antimicrobial resistance among children in sub-Saharan Africa. *Lancet Infect Dis* 2017.

73. Guilbert J, Levy C, Cohen R, Delacourt C, Renolleau S, Flamant C. Late and ultra late onset Streptococcus B meningitis: clinical and bacteriological data over 6 years in France. *Acta Paediatr* 2009; **99**(1): 47-51.

74. Lin FY, Weisman LE, Troendle J, Adams K. Prematurity is the major risk factor for late-onset group B streptococcus disease. *J Infect Dis* 2003; **188**(2): 267-71.

75. Van Dyke MK, Phares CR, Lynfield R, et al. Evaluation of universal antenatal screening for group B streptococcus. *N Engl J Med* 2009; **360**(25): 2626-36.

76. Bisharat N, Jones N, Marchaim D, et al. Population structure of group B streptococcus from a low-incidence region for invasive neonatal disease. *Microbiology* 2005; **151**(Pt 6): 1875-81.

77. Schrag SJ, Farley MM, Petit S, et al. Epidemiology of Invasive Early-Onset Neonatal Sepsis, 2005 to 2014. *Pediatrics* 2016; **138**(6).

78. Berkley JA, Lowe BS, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* 2005; **352**(1): 39-47.

79. Cutland CL, Schrag SJ, Zell ER, et al. Maternal HIV infection and vertical transmission of pathogenic bacteria. *Pediatrics* 2012; 130(3): e581-90.

80. Dangor Z, Lala SG, Cutland CL, et al. Burden of invasive group B Streptococcus disease and early neurological sequelae in South African infants. *PLoS ONE* 2015; **10 (4) (no pagination)**(e0123014).

81. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. *Pediatrics* 2011; **127**(5): 817-26.

82. Sinha A, Russell LB, Tomczyk S, et al. Disease Burden of Group B Streptococcus among Infants in sub-Saharan Africa: A Systematic Literature Review and Meta-Analysis. *The Pediatric infectious disease journal* 2016.

83. Bianchi-Jassir F, Seale AC, Kohli-Lynch M, et al. Preterm Birth Associated With Group B Streptococcus Maternal Colonization Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S133-S42.

84. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children.

Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2017; **65**(suppl_2): S200-S19.ç

85. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. *Lancet* 2015; **385**(9966): 430-40.

86. Russell NJ, Seale AC, O'Sullivan C, et al. Risk of Early-Onset Neonatal Group B Streptococcal Disease With Maternal Colonization Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S152-S9.

87. Russell NJ, Seale AC, O'Driscoll M, et al. Maternal Colonization With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S100-S11.

88. Seale AC, Blencowe H, Bianchi-Jassir F, et al. Stillbirth With Group B Streptococcus Disease Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S125-S32.

89. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000; **342**(1): 15-20.

90. Prevention of perinatal group B streptococcal disease: a public health perspective. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 1996; **45**(RR-7): 1-24.

91. Melin P, Schmitz M, De Mol P, Foidart JM, Rigo J. [Group B streptococcus, primary cause of life-threatening infections in infants. Epidemiology and prevention strategy]. *Rev Med Liege* 1999; **54**(5): 460-7.

92. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Recomm Rep* 2002; **51**(RR-11): 1-22.

93. Schrag SJ, Verani JR. Intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease: experience in the United States and implications for a potential group B streptococcal vaccine. *Vaccine* 2013; **31 Suppl 4**: D20-6.

94. Sinha A, Russell LB, Tomczyk S, et al. Disease Burden of Group B Streptococcus Among Infants in Sub-Saharan Africa: A Systematic Literature Review and Meta-analysis. *Pediatr Infect Dis J* 2016; **35**(9): 933-42.

95. Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal group B streptococcal colonisation in European countries. *Acta Obstet Gynecol Scand* 2008; **87**(3): 260-71.

96. Kohli-Lynch M, Russell NJ, Seale AC, et al. Neurodevelopmental Impairment in

Children After Group B Streptococcal Disease Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S190-S9.

97. Melin P, Efstratiou A. Group B streptococcal epidemiology and vaccine needs in developed countries. *Vaccine* 2013; **31 Suppl 4**: D31-42.

98. Ekwempu CC, Lawande RV, Egler LJ. Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. *J Clin Pathol* 1981; **34**(1): 82-3

99. Karou SD, Djigma F, Sagna T, et al. Antimicrobial resistance of abnormal vaginal discharges microorganisms in Ouagadougou, Burkina Faso. *Asian Pac J Trop Biomed* 2012; **2**(4): 294-7.

100. Schellenberg JJ, Links MG, Hill JE, et al. Molecular definition of vaginal microbiota in East African commercial sex workers. *Appl Environ Microbiol* 2011; **77**(12): 4066-74.

101. Tameliene R, Barcaite E, Stoniene D, et al. Escherichia coli colonization in neonates: prevalence, perinatal transmission, antimicrobial susceptibility, and risk factors. *Medicina* (*Kaunas*) 2012; **48**(2): 71-6.

102. Bergstrom S. Infection-related morbidities in the mother, fetus and neonate. *J Nutr* 2003; **133**(5 Suppl 2): 1656S-60S.

103. Soto SM, Bosch J, Jimenez de Anta MT, Vila J. Comparative study of virulence traits of Escherichia coli clinical isolates causing early and late neonatal sepsis. *J Clin Microbiol* 2008; **46**(3): 1123-5.

104. Goldenberg RL, McClure EM, Saleem S, Reddy UM. Infection-related stillbirths. *Lancet* 2010; **375**(9724): 1482-90.

105. Mandomando I, Sigauque B, Morais L, et al. Antimicrobial drug resistance trends of bacteremia isolates in a rural hospital in southern Mozambique. *Am J Trop Med Hyg* 2010; **83**(1): 152-7.

106. Musicha P, Cornick JE, Bar-Zeev N, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998-2016): a surveillance study. *Lancet Infect Dis* 2017; **17**(10): 1042-52.

107. Sigauque B, Kobayashi M, Vubil D, et al. Invasive bacterial disease trends and characterization of group B streptococcal isolates among young infants in southern Mozambique, 2001-2015. *PLoS One* 2018; **13**(1): e0191193.

108. Kabwe M, Tembo J, Chilukutu L, et al. Etiology, Antibiotic Resistance and Risk Factors for Neonatal Sepsis in a Large Referral Center in Zambia. *Pediatr Infect Dis J* 2016; **35**(7): e191-8.

109. Wall SN, Lee AC, Carlo W, et al. Reducing intrapartum-related neonatal deaths in low- and middle-income countries-what works? *Semin Perinatol* 2010; **34**(6): 395-407.

110. World Health Organization. Congenital anomalies. (cited 2017 Nov 13). Available from: http://www.who.int/mediacentre/factsheets/fs370/en/. Geneva: World Health Organization, 2016.

111. Liu S, Joseph KS, Wen SW. Trends in fetal and infant deaths caused by congenital anomalies. *Semin Perinatol* 2002; **26**(4): 268-76.

112. Sikka V, Chattu VK, Popli RK, et al. The Emergence of Zika Virus as a Global Health Security Threat: A Review and a Consensus Statement of the INDUSEM Joint working Group (JWG). *J Glob Infect Dis* 2016; **8**(1): 3-15.

113. Adams Waldorf KM, McAdams RM. Influence of infection during pregnancy on fetal development. *Reproduction* 2013; **146**(5): R151-62.

114. Del Pizzo J. Focus on diagnosis: congenital infections (TORCH). Pediatr Rev 2011; 32(12): 537-42.

115. Neu N, Duchon J, Zachariah P. TORCH infections. *Clin Perinatol* 2015; **42**(1): 77-103, viii.

116. Doyle M, Atkins JT, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. *Pediatr Infect Dis J* 1996; **15**(12): 1102-6.

117. Barbi M, Binda S, Primache V, et al. Cytomegalovirus DNA detection in Guthrie cards: a powerful tool for diagnosing congenital infection. *J Clin Virol* 2000; **17**(3): 159-65.

118. Mussi-Pinhata MM, Yamamoto AY, do Carmo Rego MA, Pinto PC, da Motta MS, Calixto C. Perinatal or early-postnatal cytomegalovirus infection in preterm infants under 34 weeks gestation born to CMV-seropositive mothers within a high-seroprevalence population. *J Pediatr* 2004; **145**(5): 685-8.

119. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. *Rev Med Virol* 2007; **17**(5): 355-63.

120. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol* 2007; **17**(4): 253-76.

121. Waters A, Jennings K, Fitzpatrick E, et al. Incidence of congenital cytomegalovirus infection in Ireland: implications for screening and diagnosis. *J Clin Virol* 2014; **59**(3): 156-60.

122. Duryea EL, Sanchez PJ, Sheffield JS, et al. Maternal human immunodeficiency virus infection and congenital transmission of cytomegalovirus. *Pediatr Infect Dis J* 2010; **29**(10): 915-8.

123. Guibert G, Warszawski J, Le Chenadec J, et al. Decreased risk of congenital cytomegalovirus infection in children born to HIV-1-infected mothers in the era of highly active antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009; **48**(11): 1516-25.

124. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. *N Engl J Med* 1999; **341**(2): 77-84.

125. Manicklal S, van Niekerk AM, Kroon SM, et al. Birth prevalence of congenital cytomegalovirus among infants of HIV-infected women on prenatal antiretroviral prophylaxis in South Africa. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **58**(10): 1467-72.

126. Mussi-Pinhata MM, Yamamoto AY, Figueiredo LT, Cervi MC, Duarte G. Congenital and perinatal cytomegalovirus infection in infants born to mothers infected with human immunodeficiency virus. *J Pediatr* 1998; **132(**2): 285-90.

127. Mwaanza N, Chilukutu L, Tembo J, et al. High rates of congenital cytomegalovirus infection linked with maternal HIV infection among neonatal admissions at a large referral center in sub-Saharan Africa. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2014; **58**(5): 728-35.

128. Barbi M, Binda S, Caroppo S, Ambrosetti U, Corbetta C, Sergi P. A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. *Pediatr Infect Dis J* 2003; **22**(1): 39-42.

129. Boppana SB, Rivera LB, Fowler KB, Mach M, Britt WJ. Intrauterine transmission of cytomegalovirus to infants of women with preconceptional immunity. *N Engl J Med* 2001; **344**(18): 1366-71.

130. Boppana SB, Ross SA, Fowler KB. Congenital cytomegalovirus infection: clinical outcome. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2013; **57 Suppl 4**: S178-81.

131. Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF. Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. *Pediatrics* 1999; **104**(1 Pt 1): 55-60.

132. Maixenchs M, Anselmo R, Zielinski-Gutierrez E, et al. Willingness to Know the Cause of Death and Hypothetical Acceptability of the Minimally Invasive Autopsy in Six Diverse African and Asian Settings: A Mixed Methods Socio-Behavioural Study. *PLoS Med* 2016; **13**(11): e1002172.

133. Martinez MJ, Massora S, Mandomando I, et al. Infectious cause of death determination using minimally invasive autopsies in developing countries. *Diagn Microbiol Infect Dis* 2016; **84**(1): 80-6.

134. The Bill & Melinda Gates Foundation. The Bill & Melinda Gates Foundation to fund disease surveillance network in Africa and Asia to prevent childhood mortality and help prepare for the next epidemic. Seattle, USA. Available at: http://www.gatesfoundation.org/Media-Center/Press-Releases/2015/05/Child-Health-and-Mortality-Prevention-Surveillance-Network; (accessed Oct 23, 2017). 2015.

135. Bassat Q, Castillo P, Martinez MJ, et al. Validity of a minimally invasive autopsy tool for cause of death determination in pediatric deaths in Mozambique: An observational study. *PLoS Med* 2017; **14**(6): e1002317.

136. Lee AC, Kozuki N, Cousens S, et al. Estimates of burden and consequences of

infants born small for gestational age in low and middle income countries with INTERGROWTH-21st standard: analysis of CHERG datasets. *BMJ* 2017; **358**: j3677.

137. Global Burden of Disease Child Mortality Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016; **388**(10053): 1459-544.

138. Chou VB, Friberg IK, Christian M, Walker N, Perry HB. Expanding the population coverage of evidence-based interventions with community health workers to save the lives of mothers and children: an analysis of potential global impact using the Lives Saved Tool (LiST). *J Glob Health* 2017; **7**(2): 020401.

139. World Health Organization, UNICEF. Every newborn: An action plan to end preventable death. Available at: http://www.everynewborn.org/Documents/Full-action-plan-EN.pdf (Accessed: 10th November 2017). Geneva, 2014.

140. Lassi ZS, Middleton PF, Crowther C, Bhutta ZA. Interventions to Improve Neonatal Health and Later Survival: An Overview of Systematic Reviews. *EBioMedicine* 2015; **2**(8): 985-1000.

141. Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? *Lancet* 2014; **384**(9940): 347-70.

142. World Health Organization. Guideline: Managing Possible Serious Bacterial Infection in Young Infants When Referral Is Not Feasible. Geneva, 2015.

143. Soofi S, Cousens S, Turab A, et al. Effect of provision of home-based curative health services by public sector health-care providers on neonatal survival: a community-based cluster-randomised trial in rural Pakistan. *Lancet Glob Health* 2017; **5**(8): e796-e806.

144. World bank. World Development Indicators. Country profile: Mozambique: World Bank, 2017.

145. World Health Organization. Global Health Observatory indicator views. Under-five mortality rate (probability of dying by age 5 per 1000 live births) . [cited 2017 Dec 07]. Available from: http://apps.who.int/gho/data/node.imr.MDG_0000000007?lang=en. 2017.

146. World Health Organization. Global Health Observatory data. Newborn deaths. Data by country. [cited 2017 Dec 07]. Available from: http://apps.who.int/gho/data/view.main.GSWCAH05v. 2017.

147. World Health Organization. Mozambique: WHO statistical profile. Geneva: World Health Organization, 2013.

148. Gonzalez R, Munguambe K, Aponte J, et al. High HIV prevalence in a southern semi-rural area of Mozambique: a community-based survey. *HIV Med* 2012; **13**(10): 581-8.

149. Gonzalez R, Augusto OJ, Munguambe K, et al. HIV Incidence and Spatial Clustering in a Rural Area of Southern Mozambique. *PLoS One* 2015; **10**(7): e0132053.

150. Sacarlal J, Nhacolo AQ, Sigauque B, et al. A 10 year study of the cause of death in children under 15 years in Manhica, Mozambique. *BMC Public Health* 2009; **9**: 67.

151. INSIDA. National Survey on Prevalence, Behavioral Risks and Information about HIV and AIDS in Mozambique, 2009.

152. Lanaspa M, O'Callaghan-Gordo C, Machevo S, et al. High prevalence of Pneumocystis jirovecii pneumonia among Mozambican children <5 years of age admitted to hospital with clinical severe pneumonia. *Clin Microbiol Infect* 2015; **21**(11): 1018 e9- e15.

153. Sacoor C, Nhacolo A, Nhalungo D, et al. Profile: Manhica Health Research Centre (Manhica HDSS). *Int J Epidemiol* 2013; 42(5): 1309-18.

154. Moraleda C, de Deus N, Serna-Bolea C, et al. Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa. *J Acquir Immune Defic Syndr* 2014; 65(2): 182-9.

155. World health Organization. Use of Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants. Programmatic update. (cited 2018 Jan 13). Available from: http://www.who.int/hiv/pub/mtct/programmatic_update2012/en/. Geneva: World Health Organization, 2012.

156. Hall J, Adams NH, Bartlett L, et al. Maternal Disease With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S112-S24.

157. Le Doare K, O'Driscoll M, Turner K, et al. Intrapartum Antibiotic Chemoprophylaxis Policies for the Prevention of Group B Streptococcal Disease Worldwide: Systematic Review. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S143-S51.

158. Tann CJ, Martinello KA, Sadoo S, et al. Neonatal Encephalopathy With Group B Streptococcal Disease Worldwide: Systematic Review, Investigator Group Datasets, and Meta-analysis. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S173-S89.

159. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700.

160. Stevens GA, Alkema L, Black RE, et al. Guidelines for Accurate and Transparent Health Estimates Reporting: the GATHER statement. *PLoS Med* 2016; **13**(6): e1002056.

161. United Nations. Sustainable Development Goals. New York: United Nations, 2015, (accessed Oct 11, 2017). Available at: http://www.un.org/sustainabledevelopment/summit/. 2015.

162. Cutland CL, Schrag SJ, Thigpen MC, et al. Increased risk for group B Streptococcus sepsis in young infants exposed to HIV, Soweto, South Africa, 2004-2008(1). Emerging Infectious Diseases 2015; **21**(4): 638-45.

163. Akindolire AE, Tongo O, Dada-Adegbola H, Akinyinka O. Etiology of early onset septicemia among neonates at the University College Hospital, Ibadan, Nigeria. *Journal of infection in developing countries* 2016; **10**(12): 1338-44.

164. Dangor Z, Cutland CL, Izu A, et al. Temporal Changes in Invasive Group B Streptococcus Serotypes: Implications for Vaccine Development. *PLoS One* 2016; **11**(12): e0169101.

165. Frigati L, van der Merwe JL, Harvey J, Rabie H, Theron G, Cotton MF. A retrospective review of group B streptococcal infection in the Metro East area of the Western Cape province: 2010 to 2011. *Southern African Journal of Infectious Diseases* 2014; **29**(1): 33-6.

166. Le Doare K, Jarju S, Darboe S, et al. Risk factors for Group B Streptococcus colonisation and disease in Gambian women and their infants. *J Infect* 2016; **72**(3): 283-94.

167. Ojukwu JU, et.al. Neonatal septicemia in high risk babies in South-Eastern Nigeria. *JPerinatMed* 2005; **34**: 166-72.

168. Gray KJ, Bennett SL, French N, Phiri AJ, Graham SM. Invasive group B streptococcal infection in infants, Malawi. *Emerg Infect Dis* 2007; **13**(2): 223-9.

169. Lawn JE, Bianchi-Jassir F, Russell NJ, et al. Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children: Why, What, and How to Undertake Estimates? *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; **65**(suppl_2): S89-S99.

170. de Steenwinkel FD, Tak HV, Muller AE, Nouwen JL, Oostvogel PM, Mocumbi SM. Low carriage rate of group B streptococcus in pregnant women in Maputo, Mozambique. *Trop Med Int Health* 2008; **13**(3): 427-9.

171. Gupta C, Briski LE. Comparison of two culture media and three sampling techniques for sensitive and rapid screening of vaginal colonization by group B streptococcus in pregnant women. *J Clin Microbiol* 2004; **42**(9): 3975-7.

172. Wang X, Ma LK, Song YN, Liu JT, Xu YC, Yi J. [Rapid Group B streptococcus screening methods in late pregnancy and the maternal-neonatal outcomes]. *Zhonghua Yi Xue Za Zhi* 2016; **96**(15): 1188-91.

173. Verani Jennifer R ML, Schrag Stephanie J. . Prevention of perinatal group B streptococcal disease--revised guidelines from CDC, 2010. *MMWR Recommendations and reports: Morbidity and mortality weekly report Recommendations and reports* 2010; **59**(RR-10): 1-36.

174. Dangor Z, Kwatra G, Izu A, Lala SG, Madhi SA. Review on the association of Group B Streptococcus capsular antibody and protection against invasive disease in infants. *Expert Rev Vaccines* 2015; **14**(1): 135-49.

175. Dangor Z, Kwatra G, Izu A, et al. Correlates of protection of serotype-specific capsular antibody and invasive Group B Streptococcus disease in South African infants. *Vaccine* 2015; **33**(48): 6793-9.

176. Cools P, Jespers V, Hardy L, et al. A Multi-Country Cross-Sectional Study of Vaginal Carriage of Group B Streptococci (GBS) and Escherichia coli in Resource-Poor Settings: Prevalences and Risk Factors. *PLoS One* 2016; **11**(1): e0148052.

177. Karou SD, Balaka A, Bamoke M, et al. Epidemiology and antibiotic resistance of bacterial meningitis in Dapaong, northern Togo. *Asian Pac J Trop Med* 2012; **5**(11): 848-52.

178. Alemseged G, Niguse S, Hailekiros H, Abdulkadir M, Saravanan M, Asmelash T. Isolation and anti-microbial susceptibility pattern of group B Streptococcus among pregnant women attending antenatal clinics in Ayder Referral Hospital and Mekelle Health Center, Mekelle, Northern Ethiopia. *BMC Res Notes* 2015; **8**: 518.

179. Mengist A, Kannan H, Abdissa A. Prevalence and antimicrobial susceptibility pattern of anorectal and vaginal group B Streptococci isolates among pregnant women in Jimma, Ethiopia. *BMC Res Notes* 2016; **9**: 351.

180. Stapleton RD, Kahn JM, Evans LE, Critchlow CW, Gardella CM. Risk factors for group B streptococcal genitourinary tract colonization in pregnant women. *Obstet Gynecol* 2005; **106**(6): 1246-52.

181. Belard S, Toepfner N, Capan-Melser M, et al. Streptococcus agalactiae Serotype Distribution and Antimicrobial Susceptibility in Pregnant Women in Gabon, Central Africa. *Sci Rep* 2015; **5**: 17281.

182. Bolukaoto JY, Monyama CM, Chukwu MO, et al. Antibiotic resistance of Streptococcus agalactiae isolated from pregnant women in Garankuwa, South Africa. *BMC Res Notes* 2015; **8**: 364.

183. Lopez Y, Parra E, Cepas V, et al. Serotype, virulence profile, antimicrobial resistance and macrolide-resistance determinants in Streptococcus agalactiae isolates in pregnant women and neonates in Catalonia, Spain. *Enferm Infecc Microbiol Clin* 2017.

184. Pinheiro S, Radhouani H, Coelho C, et al. Prevalence and mechanisms of erythromycin resistance in Streptococcus agalactiae from healthy pregnant women. *Microb Drug Resist* 2009; **15**(2): 121-4.

185. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA* 2010; **303**(14): 1375-82.

186. Stagno S, Pass RF, Dworsky ME, Alford CA, Jr. Maternal cytomegalovirus infection and perinatal transmission. *Clin Obstet Gynecol* 1982; **25**(3): 563-76.

187. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The “silent” global burden of congenital cytomegalovirus. *Clin Microbiol Rev* 2013; 26(1): 86-102.

188. Lanzieri TM, Dollard SC, Bialek SR, Grosse SD. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. *Int J Infect Dis* 2014; **22**: 44-8.

189. Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. *Lancet Infect Dis* 2017; **17**(6): e177-e88.

190. Frederick T, Homans J, Spencer L, et al. The effect of prenatal highly active antiretroviral therapy on the transmission of congenital and perinatal/early postnatal cytomegalovirus among HIV-infected and HIV-exposed infants. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2012; **55**(6): 877-84.

191. Bassat Q, Guinovart C, Sigauque B, et al. Malaria in rural Mozambique. Part II: children admitted to hospital. *Malar J* 2008; **7**: 37.

192. Elusiyan JB, Adejuyigbe EA, Adeodu OO. Hypoglycaemia in a Nigerian paediatric emergency ward. *J Trop Pediatr* 2006; **52**(2): 96-102.

193. Nadjm B, Mtove G, Amos B, et al. Blood glucose as a predictor of mortality in children admitted to the hospital with febrile illness in Tanzania. *Am J Trop Med Hyg* 2013; **89**(2): 232-7.

194. Solomon T, Felix JM, Samuel M, et al. Hypoglycaemia in paediatric admissions in Mozambique. *Lancet* 1994; **343**(8890): 149-50.

195. Krinsley JS. Glycemic control in the critically ill - 3 domains and diabetic status means one size does not fit all! *Crit Care* 2013; **17**(2): 131.

196. Rattarasarn C. Hypoglycemia in sepsis: risk factors and clinical characteristics. *J Med Assoc Thai* 1997; **80**(12): 760-6.

197. Islam MA, Rahman MM, Mahalanabis D, Rahman AK. Death in a diarrhoeal cohort of infants and young children soon after discharge from hospital: risk factors and causes by verbal autopsy. *J Trop Pediatr* 1996; **42**(6): 342-7.

198. Roy SK, Chowdhury AK, Rahaman MM. Excess mortality among children discharged from hospital after treatment for diarrhoea in rural Bangladesh. *Br Med J* (Clin Res Ed) 1983; **287**(6399): 1097-9.

09 | ANNEXES

Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Estimates of the Burden of Group B Streptococcal Disease Worldwide for Pregnant Women, Stillbirths, and Children

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Background. We aimed to provide the first comprehensive estimates of the burden of group B *Streptococcus* (GBS), including invasive disease in pregnant and postpartum women, fetal infection/stillbirth, and infants. Intrapartum antibiotic prophylaxis is the current mainstay of prevention, reducing early-onset infant disease in high-income contexts. Maternal GBS vaccines are in development.

Methods. For 2015 live births, we used a compartmental model to estimate (1) exposure to maternal GBS colonization, (2) cases of infant invasive GBS disease, (3) deaths, and (4) disabilities. We applied incidence or prevalence data to estimate cases of maternal and fetal infection/stillbirth, and infants with invasive GBS disease presenting with neonatal encephalopathy. We applied risk ratios to estimate numbers of preterm births attributable to GBS. Uncertainty was also estimated.

Results. Worldwide in 2015, we estimated 205 000 (uncertainty range [UR], 101 000–327 000) infants with early-onset disease and 114 000 (UR, 44 000–326 000) with late-onset disease, of whom a minimum of 7000 (UR, 0–19 000) presented with neonatal encephalopathy. There were 90 000 (UR, 36 000–169 000) deaths in infants <3 months age, and, at least 10 000 (UR, 3000–27 000) children with disability each year. There were 33 000 (UR, 13 000–52 000) cases of invasive GBS disease in pregnant or postpartum women, and 57 000 (UR, 12 000–104 000) fetal infections/stillbirths. Up to 3.5 million preterm births may be attributable to GBS. Africa accounted for 54% of estimated cases and 65% of all fetal/infant deaths. A maternal vaccine with 80% efficacy and 90% coverage could prevent 107 000 (UR, 20 000–198 000) stillbirths and infant deaths.

Conclusions. Our conservative estimates suggest that GBS is a leading contributor to adverse maternal and newborn outcomes, with at least 409 000 (UR, 144 000–573 000) maternal/fetal/infant cases and 147 000 (UR, 47 000–273 000) stillbirths and infant deaths annually. An effective GBS vaccine could reduce disease in the mother, the fetus, and the infant.

Keywords. group B *Streptococcus*; infection; newborn; stillbirth; maternal.

The number of worldwide child deaths has declined, from an estimated 12.7 million in 1990 to 5.9 million in 2015 [1]. However, there has been less progress in reducing neonatal mortality and stillbirths, with 2.7 million neonatal deaths and 2.6 million stillbirths in 2015 [2, 3]. Maternal mortality remains unacceptably high, with an estimated 303 000 deaths in 2015. Most of this burden is in low-income settings, particularly in sub-Saharan Africa and South Asia [1, 2, 4].

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Infection is an important cause of maternal, fetal, and infant mortality in low- and middle-income contexts [1, 5–7]. However, in addition to the substantial burden of mortality, there is a mostly unquantified burden of infection-related short- and long-term morbidity [8]. Infections are also an important underlying contributor to preterm birth and neonatal encephalopathy, which, along with infections, are leading causes of neonatal mortality and subsequent adverse outcomes worldwide [8–11].

Understanding of specific infectious etiologies is, however, limited [12]. Quantifying the burden of individual etiologies is necessary to inform public health interventions. Group B *Streptococcus* (GBS) is an important perinatal pathogen [13, 14], yet to date no systematic estimates have been undertaken of its overall global burden [15].

GBS is a frequent colonizer of the maternal gastrointestinal and genital tracts. Overall, 18% (95% confidence interval [CI], 17%–19%) of women worldwide are estimated to be colonized, although there is regional variation in prevalence, ranging from a high prevalence in the Caribbean of 35% (95% CI, 35%–40%), to a much lower prevalence in Southern Asia and Eastern Asia (13% [95% CI, 10%–14%] and 11% [95% CI, 10%–12%], respectively) [16]. Ascending infection can cause maternal, fetal, and early-onset neonatal disease (days 0–6), leading to maternal death, stillbirth, and/or neonatal death [17–19]. In survivors of neonatal or young infant GBS disease, neurodevelopmental impairment may result [20]. In addition to causing invasive neonatal disease, maternal GBS colonization also increases the risk of preterm birth [21]. Neonatal encephalopathy (NE) may occur with invasive GBS disease, but maternal GBS colonization and ascending infection also increases the risk of NE [22].

Preventive measures aimed at reducing the risk of invasive early-onset GBS disease (EOGBS) in newborns have focused on intrapartum antibiotic prophylaxis (IAP), with intravenous antibiotics given to women in labor, based either on microbiological screening or clinical risk factors [23]. However, this depends on national policy and a health system with the capacity to implement either strategy with appropriate coverage. While reductions in EOGBS disease (days 0–6 after birth) in the United States have been observed [24], IAP does not prevent late-onset GBS disease (LOGBS; days 7–89) [25] and is unlikely to have an impact on stillbirth or preterm birth. GBS vaccines are in development [26] and, if given to women, could be effective in preventing these outcomes as well as infant and maternal invasive GBS disease [15]. Vaccine candidates include protein-based formulations and serotype-specific polysaccharide-protein conjugates [27] and thus an understanding of serotype distribution in maternal and infant disease worldwide is important.

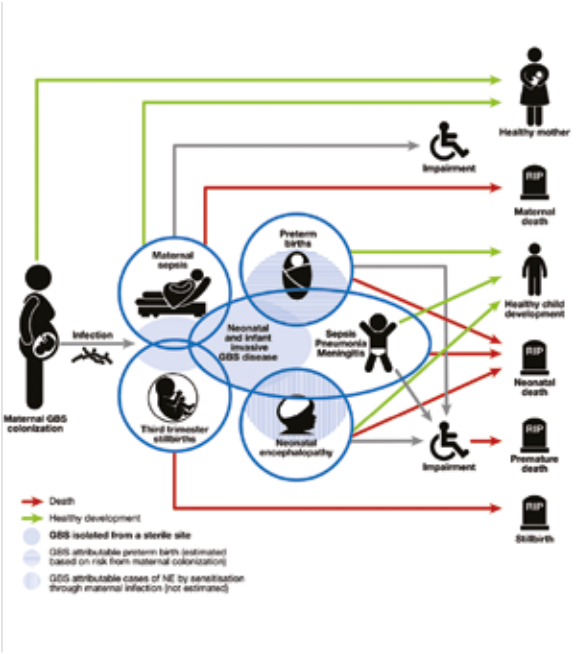
This is the last article in a supplement estimating the burden of invasive GBS disease in pregnant and postpartum women, stillbirths, and infants (Figure 1) [15]. The supplement includes systematic reviews and meta-analyses across the disease burden schema (Figure 2). These provide input parameters into the compartmental model described here, for infant GBS cases, deaths, and disability (Figure 3). We also estimate maternal GBS disease, stillbirths with GBS disease, the subset of cases of infant GBS disease who also have neonatal encephalopathy, and preterm birth attributable to GBS. These are reported according to international guidelines [28, 29].

OBJECTIVES

We aimed to:

1. Estimate national, regional, and worldwide numbers of infants in 2015 with invasive GBS disease (including those presenting with neonatal encephalopathy), and outcomes in terms of deaths and disability, using a compartmental model.

Figure 1. Overview of the articles in this supplement to estimate the worldwide burden of group B *Streptococcus*. Adapted from Lawn et al [15]. Abbreviations: GBS, group B *Streptococcus*; NE, neonatal encephalopathy.



Parameter	Case definitions used for estimates	Estimate
Group B *Streptococcus* maternal colonization	Isolation by culture of GBS from either the vagina (high or low), rectum or peri-anal region at any time during pregnancy	**21,734,000** (16,034,000-27,764,000) live births exposed
Maternal GBS disease	Laboratory isolation of GBS from sterile site in pregnant or postpartum woman (up to 42 days postpartum), with clinical signs of sepsis	**33,000** (13,000-52,000)
Stillbirth GBS invasive disease	Birth of a fetus weighting >1000g and/ or ≥28 weeks' gestation age with no signs of life and evidence of GBS invasive disease from a normally sterile site such as fetal blood, lung aspirate or cerebrospinal fluid	**57,000** (12,000-104,000)
Neonatal and infant GBS invasive disease	Laboratory isolation of Streptococcus agalactiae from a normally sterile site in an infant aged 0 to 89 days with signs of clinical disease, including meningitis, sepsis or bacteremic pneumonia	**319,000** (119,000-417,000)
Neonatal encephalopathy with invasive GBS disease	Laboratory isolation of GBS from a sterile site (blood, cerebrospinal fluid analysis or on a post mortem sample) in cases of neonatal encephalopathy, hypoxic-ischaemic encephalopathy and intrapartum-related death	**7,000** (300-19,000) as a subset of all invasive disease cases
Neurodevelopment impairment in children after GBS invasive disease	Cognitive and/or motor, vision or hearing impairment in survivors of invasive infant GBS disease isolated from a normally sterile site	**10,000** (3,000-27,000) cases after GBS meningitis. Unable to estimate for sepsis
Preterm birth associated with GBS maternal colonization	Delivery prior to completion of 37 weeks' gestation from mother with maternal GBS colonization isolated from vaginal, cervical and/or rectal swabs	**Up to 3.5 million**

Figure 2. Disease schema for outcomes of maternal group B *Streptococcus* colonization showing worldwide estimates for 2015. Adapted from Lawn et al [15]. Abbreviations: GBS, group B *Streptococcus*; NE, neonatal encephalopathy.

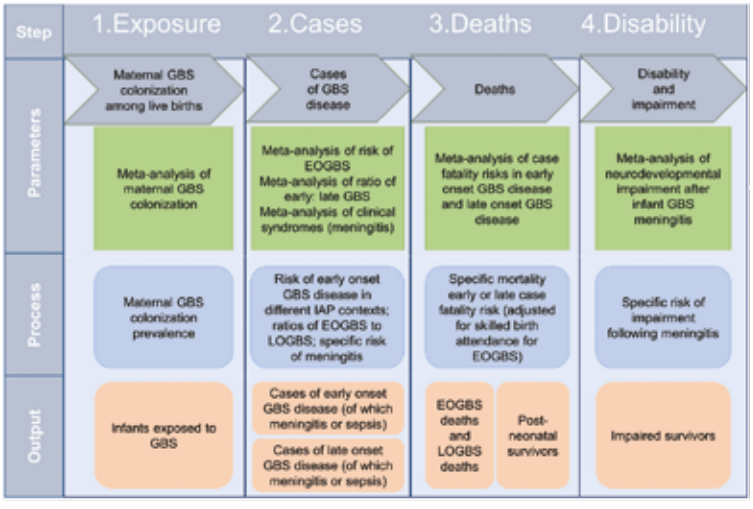


Figure 3. Compartmental model for estimating cases of infant group B streptococcal disease, deaths, and disability. Abbreviations: EOGBS, early-onset group B *Streptococcus*; GBS, group B *Streptococcus*; IAP, intrapartum antibiotic prophylaxis; LOGBS, late-onset group B *Streptococcus*.

2. Estimate national, regional, and worldwide numbers of cases in 2015, using pooled estimates of incidence, proportions or risk ratios, derived from meta-analyses for:
   a. maternal GBS disease,
   b. stillbirths with invasive GBS disease, and
   c. Preterm birth attributed to maternal GBS colonization.
3. Estimate the number of maternal and infant cases, infant deaths, and stillbirths currently prevented by IAP, and preventable cases and deaths with high worldwide IAP coverage and/or maternal GBS vaccination.
4. Describe GBS serotypes colonizing mothers and causing maternal and infant GBS disease, summarizing reported regional variation.

METHODS

We summarize our methods according to our 4 objectives as follows:

1. Estimate national, regional, and worldwide numbers of infants in 2015 with invasive GBS disease (including those presenting with neonatal encephalopathy) and outcomes in terms of deaths and disability, using a compartmental model.

Modeling Approach

We conceptualized the full burden of GBS disease (Figure 2) to include pregnant and postpartum women, fetal infections (based on stillbirths), and infants, as described in the first article in this supplement [15]. We took a compartmental model approach to modeling infant invasive GBS disease, deaths, and disability, with 4 steps as illustrated in Figure 3. For the first step in the model (maternal GBS colonization), the step where most data were available for national prevalence estimation, we also attempted a multivariable regression model to predict national maternal GBS colonization, as an alternative to using a subregional estimate when national-level data were limited (Appendix).

Data Inputs

We sought data inputs from the published literature through systematic reviews and unpublished sources through research databases and investigators worldwide, as summarized in the previous 10 articles (Figure 1). The specific methods used for each of these (database searches, inclusion and exclusion criteria, data characteristics, criteria used to assess bias and sensitivity analyses) are described in general [15] and reported elsewhere [16–23, 30]. We performed meta-analyses, to obtain estimates of maternal GBS colonization prevalence [16], the ratio of late-onset to early-onset invasive GBS disease [19], case fatality risks (CFRs) [19], proportion of cases with meningitis [19], proportion of infants with GBS meningitis who had moderate to severe neurodevelopmental impairment [20], incidence of maternal GBS disease in pregnant/postpartum women [17], prevalence of GBS disease in stillbirth [18], prevalence of GBS disease in neonatal encephalopathy [22], and the association between maternal GBS colonization and preterm birth [21]. We calculated pooled estimates using random-effects models [31] to allow for heterogeneity across studies by use of a statistical parameter representing the variation between studies.

Burden Estimation Applying the Compartmental Model

Step 1. Exposure to Maternal Group B Streptococcus Colonization

For the first step of the compartmental model, we determined maternal GBS colonization prevalence for countries, subregions (South America, Central America, Caribbean, Western Asia, Southern Asia, South-Eastern Asia, Eastern Asia, Oceania) and regions (Latin America, Asia, Africa, Oceania, developed) as described elsewhere [16], to apply to estimates of live births in 195 countries for 2015, using latest United Nations data [32]. The colonization data were adjusted for sampling site (rectal and/or vaginal) and laboratory culture methods [16]. Where data were considered sufficient (≥1000 mothers tested for rectovaginal colonization), we used an estimate for individual countries. Where data were limited (<1000 mothers tested for rectovaginal colonization), we used a subregional estimate, and where no subregional estimate was available, we used a regional estimate (Supplementary Table 1 for inputs by country).

Step 2. Cases of Invasive Early-Onset Disease and Late-Onset Disease in Different Intrapartum Antibiotic Prophylaxis Settings

For the second step of the compartmental model, we assessed IAP policies and their implementation in countries as described elsewhere in this supplement [23], and categorized 89 countries with data available into 1 of 4 categories, which were (1) microbiological screening for maternal GBS colonization with IAP and high implementation coverage (>50% of mothers screened and given IAP if appropriate); (2) clinical risk factor approach with IAP given to mothers with risk factors before delivery and high implementation coverage (>50% with risk factors receiving IAP); (3) microbiological screening for maternal GBS colonization with IAP and low implementation coverage (<50%); (4) clinical risk factor approach with IAP given to mothers with risk factors before delivery and low implementation coverage (<50%), or no IAP strategy in place. We assigned countries in the developed region with no data to category 1 as a conservative approach, and of those countries reporting these data, 21 of 31 developed countries were in group 1. We assigned countries, not in the developed region and with no data to group 4, as 51 of 59 countries not in the developed region reporting these data were in this group.

We then assessed the risk of EOGBS disease in studies reporting maternal GBS colonization data, and the use of IAP, as described elsewhere in this supplement [30]. We used the linear association between IAP use and risk of EOGBS disease described in [23] to estimate the risk of EOGBS disease in each of the 4 contexts, with specific risks for each group as follows: group 1 = 0.3% (95% CI, .0–.9%); group 2 = 0.6% (95% CI, .10%–1.2%); group 3 = 0.9% (95% CI, .4%–1.5%); group 4 = 1.1% (95% CI, .6%–1.5%). For each country, the number of cases of EOGBS was estimated by multiplying the estimated number of exposed babies by the appropriate risk for that country.

We used regional estimates of the ratio of early-onset to late-onset GBS cases [19] to then estimate the number of LOGBS cases. For Oceania, where data were lacking, we applied the estimate for Asia, as the most similar regional context. There were variations in estimates, with the highest ratio in Asia (5.99 [95% CI, 2.40–14.9]) suggesting more EOGBS than LOGBS, and lowest in Africa (1.02 [95% CI, 0.82–1.27]). We give parameters for each region in Table 1. These regional estimates could, however, be affected by low case ascertainment. This could reduce EOGBS disease cases, particularly those with home delivery, inadequate access to care and/or high rapid CFR, and/or late-onset cases, particularly if cerebrospinal fluid sampling is not undertaken, and cases of GBS meningitis are thus not detected. We therefore did a sensitivity analysis applying a worldwide ratio of early-onset to late-onset GBS disease from high-quality studies worldwide (1.11 [95% CI, 0.90–1.30] / 3.92) [19].

Step 3. Deaths in Early-Onset and Late-Onset Group B Streptococcus *Disease*

For the third step of the compartmental model, we applied region-specific CFRs to 3 different groups that differ considerably in terms of outcome: EOGBS cases delivered without a skilled birth attendant, EOGBS cases delivered with a skilled birth attendant, and LOGBS cases.

Case fatality risk for EOGBS: We applied percentages of skilled birth attendance for each country to EOGBS cases to determine EOGBS cases which would, and would not, have been attended by a skilled birth attendant. We applied a CFR of 0.9 (0.3–1.0) to estimated EOGBS cases born without a skilled birth attendant, based on expert opinion as to the likely high CFR in these "unseen" cases. To estimate deaths from EOGBS born with a skilled birth attendant (and for all developed countries), we estimated regional CFRs for EOGBS from facility-based data, as described elsewhere in this supplement [19]. We applied these regional CFRs to cases of EOGBS disease with skilled birth attendance. The highest CFR for EOGBS with skilled attendance was in Africa (0.27 [0.15–0.37]), then Latin America (0.17 [0.05–0.30]), Asia (0.14 [0.06–0.23]), and developed countries (0.05 [0.04–0.07]) (Table 1). For Oceania, where even regional data were lacking, we applied the risk in Asia, being the most geographically proximal.

Case fatality risk for LOGBS: We also estimated regional CFRs for LOGBS from facility-based data, as described elsewhere in this supplement [19]. Regional CFRs for LOGBS were lower than EOGBS overall, with the highest again in Africa (0.12 [0.05–0.19]) (Table 1). Due to insufficient data from Oceania, we applied the CFR for Asia.

Step 4. Disability or Impairment After Infant Group B Streptococcus *Meningitis*

We estimated moderate to severe neurodevelopmental impairment (NDI) after meningitis, only, because data were insufficient to estimate NDI after sepsis, as described elsewhere in this supplement [20]. To do this, we applied the percentage of infant cases of GBS disease which were meningitis, for early (12% [8%–15%]) and late-onset (42% [30%–55%]) GBS disease [18] to estimates of EOGBS and LOGBS survivors. We then applied an incidence risk of moderate to severe NDI at 18 months of age of 0.18 (0.13–0.22) [20]. These data were limited to developed countries; however, we applied this proportion worldwide, on the basis that this would be a minimum estimate as NDI was unlikely to be lower in settings with reduced levels of care.

Triangulation of Infant Invasive Group B *Streptococcus* Disease Cases From the Compartmental Model With Estimates Based on Incidence Data

We compared the results from the compartmental model for infant GBS disease cases with those estimated using incidence data on infant GBS disease [19]. To do this, we calculated subregional incidence, or regional incidence where subregional data were not available, of EOGBS and LOGBS disease. We applied these to estimates of live births for each country in 2015. Data inputs are given for each country in Supplementary Table 2.

Infants With Invasive Group B *Streptococcus* Disease Presenting With Neonatal Encephalopathy

To calculate the numbers of infants with invasive GBS disease and coexistent neonatal encephalopathy, we used previously published national incidences of neonatal encephalopathy and modeled uncertainties and adjusted these for births in 2015 [11]. Then using our new data, we calculated the proportion of invasive GBS disease among these cases of NE. In developed countries, among all NE cases included in cooling trials, 0.51% (95% CIs, 0.05%–0.97%) were also identified as having GBS disease [22]. Data inputs were limited for data from other regions (3/16 studies), so we used the worldwide estimate of 0.58% (95% CIs, 0.18%–0.98%) of NE cases with GBS disease to apply in Africa, Asia, Latin America, and Oceania. Since our case definition assumes that cases of NE with GBS count as a case of GBS invasive disease, we include these numbers within our estimates of GBS infant disease.

2. Estimate country, regional, and worldwide number of cases of GBS-associated maternal disease, stillbirths, and preterm birth, for births in 2015 using pooled estimates of incidence, proportions, or risk ratios, derived from meta-analyses.

Where a compartmental approach was not possible, we used incidence, prevalence, or risk ratios from pooled data applied to births in 2015 to make minimum estimates of worldwide, regional, and national estimates for cases attributable to GBS (Figure 2).

a. Maternal GBS disease

We calculated the pooled incidence of maternal GBS disease per 1000 maternities and applied this to a denominator of total

Table 1. Data Inputs to the Compartmental Model to Estimate Cases of Infant Group B Streptococcal Disease, Deaths, and Disability

		Asia					Africa					Oceania	Latin America and Caribbean			
		Southern Asia	Eastern Asia	Central Asia	West Asia	SE Asia	Northern Africa	Southern Africa	Eastern Africa	Western Africa	Mid. Africa	Oceania	Caribbean	Central America	South America	Developed
No. of countries		9	4	5	18	11	6	5	18	16	9	14	13	8	12	47
No. of live births in 2015		37M	18M	1.6M	5.8M	12.3M	6.1M	1.2M	13.9M	13.4M	6.0M	0.03M	0.7M	0.3M	7.0M	13.4M
Step 1																
Percentage of infants exposed to maternal GBS at birth [16]	Countries	4	3	0	5	4	2	1	4	3	2	1		1	11	21
	Datasets	44	41		32	14	8	7	22	18	3	1	5	6	35	83
	Pregnant women	15838	63289		15124	3591	1576	13218	14071	4860	2058	440	1137	3229	16141	144604
		12.5 (10.2–14.8)	11.1 (9.9–12.4)		14.7 (12.1–17.4)	14.4 (11.5–17.4)	22.9 (17.0–28.2)	28.9 (26.6–31.2)	19.4 (15.9–23.0)	17.5 (10.8–24.1)	23.9 (14.7–33.1)		34.7 (29.5–39.9)	17.1 (13.2–21.0)	18.4 (15.5–21.3)	19.2 (17.7–20.7)
Step2 IAP policy [23]	Countries	28					20						11			31
	IAP group where known	Group 2 = 1; group 3 = 1; group 4 = 26					Group 2 = 1; group 4 = 19						Group 1 = 4; group 3 = 1; group 4 = 6			Group 1 = 21; group 2 = 7; group 4 = 3
	Datasets	14 (from varying IAP policy contexts in 8 countries)														
	GBS cases	85														
	By IAP policy	Group 1 = 0.003 (0.0–0.009); group 2 = 0.006 (0.001–0.012); group 3 = 0.009 (0.004–0.015); group 4 = 0.011 (0.006–0.015)														
Ratio of EOGBS to LOGBS [30]	Countries	3					6						3			12
	Datasets	4					7						3			13
	GBS cases	123					1352						50			3217
		5.60 (2.40–14.9)					1.02 (0.82–1.07)						1.90 (0.98–3.69)			1.82 (1.29–2.57)
Proportion of meningitis cases in EOGBS [19]	Countries	30														
	Datasets	26														
	Meningitis cases	176														
		0.12 (0.08–0.15)														
Proportion of meningitis cases in [19]	Countries	17														
	Datasets	18														
	Meningitis cases	689														
		0.42 (0.30–0.55)														
Step 3		Case fatality risk in EOGBS without skilled birth attendance 0.9 (0.3–1.0) estimated														
Case fatality risk (proportion) in EOGBS in a facility [30]	Countries	8					5						7			18
	Datasets	12					6						9			19
	Deaths	36					131						15			123
		0.14 (0.06–0.23)					0.27 (0.15–0.37)						0.17 (0.05–0.30)			0.05 (0.04–0.07)
Case fatality risk (proportion) of LOGBS [30]	Countries	3					5						3			14
	Datasets	6					5						14			3
	Deaths	12					116						3			67
		0.05 (0.02–0.09)					0.12 (0.05–0.19)						0.00 (0–0.19)			0.04 (0.03–0.06)
Step 4 NDI risk (proportion) in infant meningitis (EOGBS and LOGBS) [20]	Countries															4
	Datasets															12
	Cases															80
																0.18 (0.13–0.22)

Data in parentheses represent the 95% confidence interval.
Abbreviations: EOGBS, early-onset group B *Streptococcus*; GBS, group B *Streptococcus*; IAP, intrapartum antibiotic prophylaxis; LOGBS, late-onset group B *Streptococcus*; NDI, neurodevelopmental impairment.

births worldwide to estimate cases. As described elsewhere [17], data were only available for developed countries, with a pooled estimate of 0.23 (95% CI, .09–.37) per 1000 maternities. We applied this to all regions, on the basis that maternal GBS disease was unlikely to be lower in settings with reduced levels of care.

b. Stillbirths with GBS disease

We calculated the pooled prevalence of GBS disease in stillbirths, equating also to the minimum number of fetal infections. Data were available from developed countries (1% [95% CIs, 0–2%]) and from Africa (4% [95% CIs, 2%–6%]) [18]. For regions with no data, we applied the prevalence of GBS in stillbirths from developed countries, on the basis that GBS-associated stillbirth was unlikely to be lower in settings with reduced levels of care. However, as this is a conservative approach, we did a sensitivity analysis applying the regional estimate from Africa (4% [95% CIs, 2%–6%]) [18] to regions with no data.

c) Preterm birth associated with maternal GBS colonization

We calculated pooled risk ratios or odds ratios for the association between maternal GBS colonization and preterm birth [21]. For cohort or cross-sectional studies, the risk ratio was 1.21 (95% CI, .99–1.48; $P = .061$), and for case-control studies, the odds ratio was 1.85 (95% CI, 1.24–2.77; $P = .003$). However, for preterm birth the results, in terms of the association between maternal colonization and preterm birth, are susceptible to confounding and bias. For preterm birth, we thus give a range for the number of cases, based on calculation of the population attributable fraction, which could be attributable to GBS given maternal GBS colonization [16] and incidence of preterm birth [33]. The ranges are based on the range in the 95% CIs of risk and odds ratios (1.0–2.8) for the association between maternal GBS colonization and preterm birth.

3. Estimate maternal and infant cases, stillbirths, and infant deaths, prevented by IAP at present, and preventable cases and deaths with high worldwide IAP coverage and/or maternal GBS vaccination.

We applied risks, without adjusting for IAP use, to estimates of live births for 2015 to calculate early-onset cases with no IAP use. We adjusted for skilled birth attendance as previously and applied regional facility CFRs to estimate deaths with no IAP use. We subtracted current cases and deaths in early infancy to calculate those currently prevented by IAP.

For IAP scale-up worldwide, we assumed that all births were being attended by a skilled birth attendant, able to provide careful clinical monitoring for risk factors at delivery and administer IAP, but we did not adjust CFRs for this. Given these assumptions, we applied risks of EOGBS disease with a clinical risk factor approach, with coverage >50% and IAP worldwide where microbiological screening and IAP was not already in place. We did not calculate cases prevented with IAP for pregnant or postpartum women or stillbirths, or late-onset cases as these are not the target of IAP and any effect is likely to be limited due to the timing of IAP administration.

For maternal GBS vaccination, we calculated cases prevented (with no IAP) by a maternal GBS vaccine with 80% efficacy and coverage at 50% and 90%, for births in 2015. No assumptions were made on skilled birth attendance and/or laboratory capacity.

4. Describe GBS serotypes colonizing mothers and causing maternal and infant GBS disease.

We calculated the prevalence of GBS serotypes (Ia/Ib/II–X) colonizing mothers and causing maternal and infant GBS disease from meta-analyses of proportions of each serotype reported in each disease syndrome [16, 17, 19]. We calculated the coverage of a pentavalent maternal GBS vaccine (Ia/Ib/II/III/V) based on these data.

Uncertainty Estimation

For the compartmental model, we included uncertainty at every step by taking 1000 random draws, assuming a normal distribution with a mean equal to the point estimate of the parameter, and standard deviation (SD) equal to the estimated standard error (SE) of the parameter. We present the 2.5th and 97.5th centiles of the resulting distributions as the uncertainty range (UR).

For the incidence or proportional approach, we estimated uncertainty around the point estimate with the same approach, taking 1000 random draws, assuming a normal distribution with a mean equal to the point estimate of the parameter, and SD equal to the estimated SE of the parameter. We present the 2.5th and 97.5th centiles of the resulting distributions as the UR.

Source Code

Code used for the estimation process is available online at https://doi.org/10.17037/data.51.

RESULTS

We summarize our results according to our 4 objectives as follows:

1. Estimate country, regional, and worldwide cases of invasive infant GBS disease, and outcomes in terms of deaths and disabilities for live births in 2015 using a compartmental model.

Step 1. Exposure to Maternal Group B *Streptococcus* Colonization

We estimated that, of 140 million live births in 2015, there were 21.3 million (UR, 16.4–27.0 million) infants exposed to maternal GBS colonization at delivery. There were 74.5 million live births in Asia with 8.9 million (UR, 6.7–10.7 million) infants exposed, 40.7 million live births in Africa with 8.0 million (UR, 5.3–10.3 million) infants exposed, 11.0 million live

births in Latin America with 2.1 million (UR, 1.7–2.5 million) infants exposed, 260 000 live births in Oceania with 33 000 (UR, 31–36 000) infants exposed and 13.4 million live births in developed countries with 2.8 million (UR, 2.3–3.2 million) infants exposed (subregional estimates in Supplementary Figure 3).

Step 2. Cases of Early-Onset and Late-Onset Disease in Different Intrapartum Antibiotic Prophylaxis Settings

We estimated that there were 319 000 cases (UR, 119 000–417 000) of infant invasive GBS disease worldwide. Most cases were EOGBS disease, with 205 000 (UR, 101 000–327 000) cases compared to 114 000 (UR, 44 000–326 000) LOGBS cases. With a high absolute number of births, and thus newborns exposed, Asia had the highest number of EOGBS disease cases, with 95 000 (UR, 53–143 000). Africa had fewer EOGBS cases 85 000 (UR, 44–133 000), but, because of the differences in early-onset to late-onset disease ratios, more LOGBS disease cases, with 84 000 (UR, 43–140 000) in Africa compared to 17 000 (UR, 0–146 000) in Asia. In contrast, developed countries had 11 000 (UR, 0–26 000) cases of EOGBS and 6000 (UR, 0–15 000) cases of LOGBS disease (Table 2; Figure 4; Supplementary Figure 4). Using a fixed worldwide ratio of early-onset to late-onset disease based only on high-quality studies (sensitivity analysis), we estimated a higher 184 000 (UR, 142–196 000) LOGBS infant cases. Asia accounted for this increase, with 84 000 (UR, 65–90 000) LOGBS disease cases (Supplementary Figure 5).

Step 3. Deaths in Early-Onset and Late-Onset Group B *Streptococcus* Disease

We estimated that there were 90 000 (UR, 36 000–169 000) deaths in infants due to invasive GBS disease worldwide. Africa accounted for 54 000 (UR, 22 000–98 000) of these, Asia 31 000 (UR, 13 000–60 000), Latin America 4000 (600–10 000), Oceania 200 (UR, 60–300), and developed countries 800 (UR, 0–2000).

In terms of deaths due to EOGBS, there were 51 000 deaths (UR, 23 000–89 000) in infants without access to healthcare worldwide. There were a further 27 000 (UR, 9000–50 000) deaths from EOGBS in facilities in developing countries. In contrast, there were 500 (UR, 0–1300) deaths in developed countries from EOGBS. In terms of LOGBS deaths, overall deaths were lower, with 12 000 (UR, 3–30 000) worldwide. Most of these 10 000 (UR, 3000–21 000) were in Africa. Regional and subregional estimates are given in Table 2 and Supplementary Figure 6 and illustrated in Figure 5. Countries with the highest number of cases are not always those with the highest number of deaths, as illustrated for Nigeria, Ethiopia, and Pakistan (Table 3).

Step 4. Disability: Calculation of Impairment After Infant Group B *Streptococcus* Meningitis

We estimated that a minimum of 10 000 (UR, 3000–27 000) infants worldwide had moderate to severe NDI after GBS meningitis. Of these, more than half were in Africa (6000 [UR, 3000–12 000]), with 3000 (UR, 0–11 000) in Asia, 700 (UR, 100–2300) in Latin America, 700 (UR, 0–1700) in developed countries, and <100 (UR, 0–100) in Oceania (Table 4; Supplementary Figure 7).

Triangulation of Infant Invasive Group B Streptococcus Disease Cases From the Compartmental Model With Estimates Based on Incidence Data

Applying pooled incidences of EOGBS and LOGBS to the 140 million live births for 2015, we estimated a much lower burden, particularly for EOGBS cases, than that estimated using the compartmental model. We estimated 51 000 (UR, 23 000–89 000) infants with EOGBS and 40 000 (UR, 12 000–75 000) infants with LOGBS worldwide (subregional estimates in Supplementary Figures 8 and 9). These are likely to be considerable underestimates as cases are systematically underascertained, particularly in low- and middle-income contexts, as described in Table 2 and Figure 6.

Infants With Invasive Group B *Streptococcus* Disease Presenting With Neonatal Encephalopathy

We estimated that there were a minimum of 7000 (300–19 000) infants with invasive GBS disease presenting with neonatal encephalopathy. There were an estimated 3400 (UR, 200–9000) cases in Asia, 3300 (UR, 100–8600) in Africa, 300 (UR, 0–1200) in Latin America, 100 (UR, 0–300) in developed countries, and 10 (UR, 0–40) in Oceania (subregional estimates are given in Supplementary Figure 10).

2. Estimate country, regional, and worldwide cases, for births in 2015 using pooled estimates of incidence, proportions, or risk ratios, derived from meta-analyses for maternal GBS disease, stillbirth with GBS disease, and preterm birth associated with maternal GBS colonization:

    a. Maternal GBS disease

We estimated that there were a minimum of 33 000 (UR, 13–52 000) cases of maternal invasive GBS disease worldwide. Estimates are given by subregion in Supplementary Figure 11 and region in Table 4.

    b. Stillbirth with GBS disease

We estimated that there were a minimum of 57 000 (UR, 12 000–104 000) cases of stillbirth with GBS disease worldwide, equating to a minimum of 57 000 (UR, 12 000–104 000) fetal infections. Of these, Africa accounted for 42 000 (UR, 10 000–71 000) and Asia 13 000 (UR, 1000–30 000) (Supplementary Figure 12 and Table 4). Applying the higher regional estimate for Africa to regions where there are no data (sensitivity analysis), the number of stillbirths with GBS disease was much higher, at 96 000 (UR, 26–168 000) worldwide, with Asia accounting for 50 000 (UR, 14 000–87 000) of these, almost all the increase (Supplementary Figure 13).

    c. Preterm birth attributable to GBS

We estimated that the range of cases of preterm birth attributable to GBS was 0–3.5 million. The cases of preterm birth

Table 2. Estimated Cases of Maternal, Fetal, and Infant Group B Streptococcal Disease in 2015

Region	Maternal GBS Disease	Fetal Infection[a]	EOGBS Disease	LOGBS Disease
Southern Asia	8700 (4000–14 000)	9700 (1200–21 300)	42 500 (23 000–65 400)	7600 (0–57 000)
Eastern Asia	4100 (1700–6700)	1300 (0–2300)	21 900 (12 700–32 900)	3900 (0–30 000)
Central Asia	400 (200–600)	200 (0–400)	2300 (1300–3200)	400 (0–3000)
Western Asia	1300 (600–2200)	800 (0–1700)	9200 (5100–13 800)	1600 (0–12 400)
South-Eastern Asia	2900 (1200–4600)	1500 (0–3300)	19 400 (10 800—28 700)	3500 (0–43 200)
Asia	35 900 (7 100–28 100)	13 400 (1200–29 600)	95 300 (52 800–142 900)	17 000 (0–145 600)
Oceania	60 (20–100)	40 (0–100)	400 (10 800–28 700)	100 (0–43 100)
Northern Africa	1400 (600–2300)	3900 (1000–6700)	15 400 (8600–22 400)	15 000 (8300–24 000)
Southern Africa	300 (100–500)	800 (200–1400)	4000 (2300–5500)	3900 (2300–6000)
Eastern Africa	3300 (1300–4600)	12 600 (3100–21 700)	26 400 (15 300–40 300)	25 900 14 700–42 700)
Western Africa	3200 (1300–5200)	18 300 (4500–30 800)	23 500 (10 200–39 000)	23 000 (10 300–41 000)
Middle Africa	1400 (3300–12 500)	6300 (1600–10 800)	15 900 (7900–25 600)	15 600 (7500–25 900)
Africa	9600 (6700–25 000)	42 000 (10 400–71 400)	85 200 (44 300–132 800)	20 700 (43 100–140 000)
Caribbean	200 (60–300)	100 (0–200)	2600 (1500–3700)	1300 (0–4300)
Central America	800 (300–1300)	200 (1700–13 400)	3100 (200–6430)	1700 (0–6100)
South America	1600 (700–2600)	600 (0–1200)	8000 (1700–14 400)	4200 4000–14 500)
Latin America	3700 (1000–4100)	900 (0–2000)	13 700 (3400–24 400)	5900 (4000–24 800)
Developed countries	3000 (1300–5000)	500 (0–800)	10 900 (0–25 800)	6000 (0–15 500)
Total	32 800 (13 400–52 100)	56 800 (11 600–103 900)	205 500 (44 200–326 000)	113 800 (0–11 100)

Data in parentheses represent the uncertainty range (UR).

Abbreviations: EOGBS, early-onset group B Streptococcus; GBS, group B Streptococcus; IAP, intrapartum antibiotic prophylaxis; LOGBS, late-onset group B Streptococcus; NDI, neurodevelopmental impairment.

[a]Stillbirths indicated a minimum estimate of cases of fetal infection.

attributable to GBS according to each risk ratio (in 0.2 increments [1.0–2.8]) are given in Supplementary Table 4.

3. Estimate maternal and infant cases, infant deaths, and stillbirths prevented by IAP at present, and preventable cases and deaths with high worldwide IAP coverage and/or maternal GBS vaccination.

Contingent in the limitations in our estimates, we estimated that 29 000 infants (UR, 0–51 000) with EOGBS and 3000 (UR, 0–108 000) infant deaths were prevented by intrapartum antibiotic prophylaxis worldwide in 2015. With worldwide application of a clinical risk factor–based approach (microbiological screening where already in place), and IAP (>50% coverage), we estimate that 83 000 (UR, 0–166 000) cases of EOGBS and 27 000 (UR, 0–110 000) deaths could be prevented worldwide (not adjusting CFRs for the changes in skilled birth attendance that IAP administration would require). With worldwide maternal vaccination (and no IAP assumed), a maternal GBS vaccine with 80% efficacy and 50% coverage would prevent 127 000 (UR, 63 000–282 000) infant and maternal GBS cases, 23 000 (UR, 6000–42 000) stillbirths, and 37 000 (UR, 15 000–68 000) infant deaths. A maternal vaccine with the same assumptions with 90% coverage would prevent 229 000 (UR, 114 000–507 000) infant and maternal GBS cases, 41 000

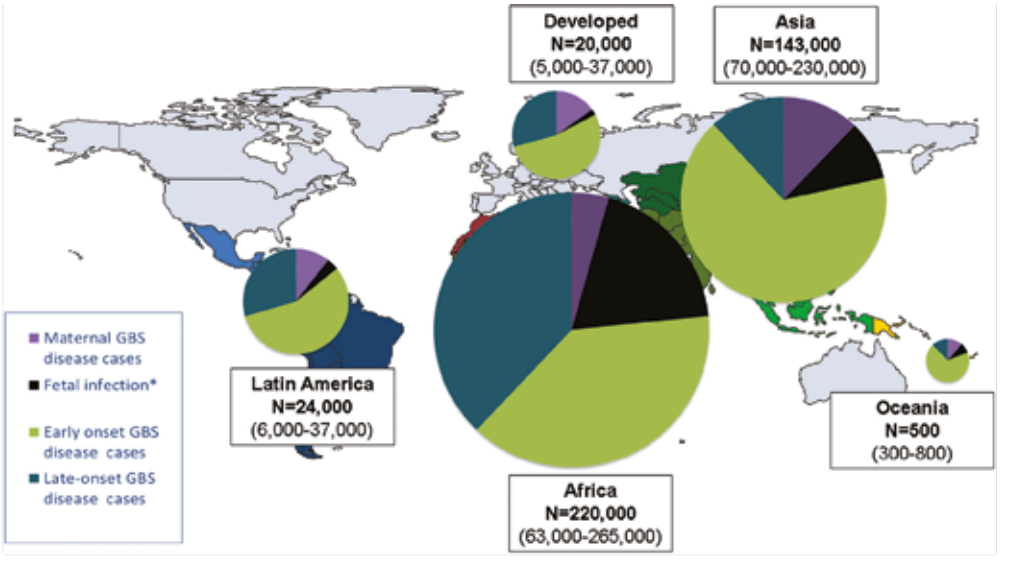


Figure 4. Cases estimated for group B streptococcal (GBS) disease in pregnant/postpartum women, fetuses, and infants in 2015, by United Nations Sustainable Development Goal region. *Stillbirths represent a minimum estimate of fetal infection cases. More details are shown in Supplementary Figures 4, 11, and 12.

(UR, 8000–75 000) stillbirths, and 67 000 (UR, 12 000–123 000) infant deaths (Figure 7).

4. Describe GBS serotypes colonizing mothers and causing maternal and infant GBS disease.

Serotype III is the most dominant serotype and colonizes 28% of mothers worldwide. It causes 48% of EOGBS, 74% LOGBS, and 29% of maternal GBS disease (Figure 8). A pentavalent vaccine (Ia/Ib/II/III/V) would cover 96% of worldwide colonizing

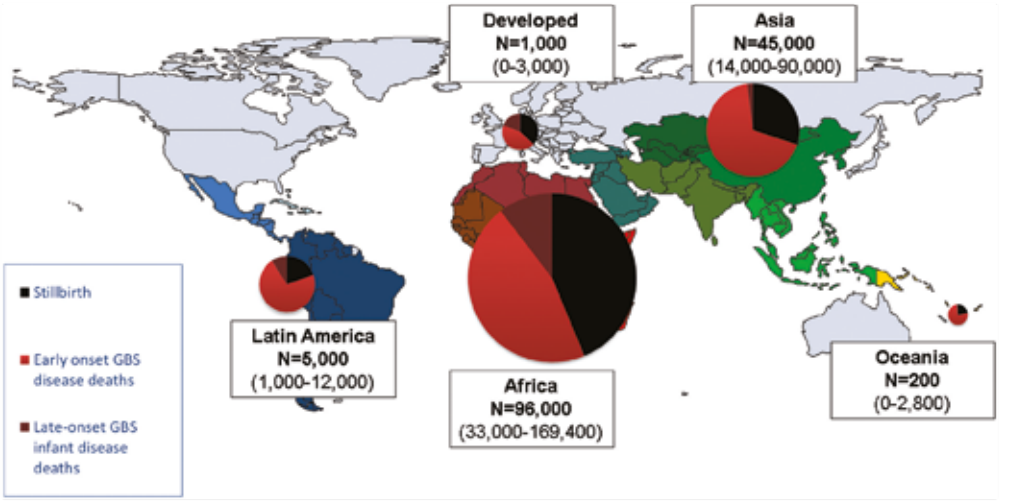


Figure 5. Deaths estimated from group B streptococcal (GBS) disease for infants and stillbirths in 2015, by United Nations Sustainable Development Goal region. Maternal deaths not estimated. More details are shown in Supplementary Figures 6 and 12.

Table 3. Countries With the Highest Estimated Numbers of Infant Group B Streptococcal Disease Cases and Deaths

Cases			Deaths		
Rank	Country	Infant Cases	Rank	Country	Infant Deaths
1	India	31 000 (0–75 000)	1	India	13 000 (5000–23 000)
2	China	25 000 (0–59 000)	2	Nigeria	8000 (2000–16 000)
3	Nigeria	22 000 (8000–39 000)	3	Ethiopia	4000 (2000–8000)
4	Democratic Republic of the Congo	16 000 (8000–39 000)	4	Democratic Republic of the Congo	4000 (2000–7000)
5	Egypt	14 000 (8000–21 000)	5	Pakistan	3000 (1000–6000)

Data in parentheses represent the uncertainty range.

Table 4. Stillbirth, Infant Deaths From Group B Streptococcal Disease and Resultant Disability Estimated in 2015

Region	Stillbirth	Early Infant Deaths	Late Infant Deaths	Disability
Southern Asia	9700 (1200–21 300)	19 600 (8500–34 400)	400 (0–2800)	1000 (0–4500)
Eastern Asia	1300 (0–2800)	3200 (1100–5800)	200 (0–1600)	700 (0–2400)
Central Asia	200 (0–400)	400 (100–600)	0 (0–200)	100 (0–300)
Western Asia	800 (0–1700)	2100 (900–10 900)	100 (0–700)	300 (0–1100)
South-Eastern Asia	1500 (0–3300)	5200 (2100–8900)	200 (0–1400)	600 (0–2300)
Asia	13 400 (1200–29 600)	30 400 (12 700–60 600)	900 (0–6600)	2600 (0–10 600)
Oceania	40 (0–90)	100 (60–200)	0 (0–30)	10 (0–40)
Northern Africa	4000 (1000–6700)	6600 (3100–10 900)	1800 (600–3600)	1200 (600–2100)
Southern Africa	800 (200–1400)	1200 (600–1900)	500 (100–900)	300 (200–500)
Eastern Africa	12 600 (3100–21 700)	15 600 (7500–26 800)	3100 (1000–6400)	2000 (1000–3600)
Western Africa	18 300 (4500–30 800)	13 400 (5000–24 500)	2800 (800–6000)	1800 (700–3300)
Middle Africa	6300 (1500–10 800)	7300 (3100–12 600)	1900 (600–3900)	1200 (500–2400)
Africa	42 000 (10 400–71 400)	44 000 (19 200–76 700)	10 000 (3100–20 800)	6400 (3000–11 900)
Caribbean	100 (0–200)	900 (300–1600)	100 (0–400)	100 (60–400)
Central America	200 (0–500)	800 (100–2000)	100 (0–600)	200 (0–600)
South America	600 (0–1200)	1600 (1400–3700)	300 (0–1400)	400 (60–1300)
Latin America	900 (0–2000)	3300 (1900–7200)	400 (0–2400)	700 (100–2300)
Developed countries	500 (0–800)	500 (0–1300)	200 (0–700)	700 (0–1700)
Total	56 800 (11 600–103 900)	78 400 (32 500–138 900)	11 500 (3100–30 500)	10 500 (3000–26 000)

Data are presented as estimate (uncertainty range).

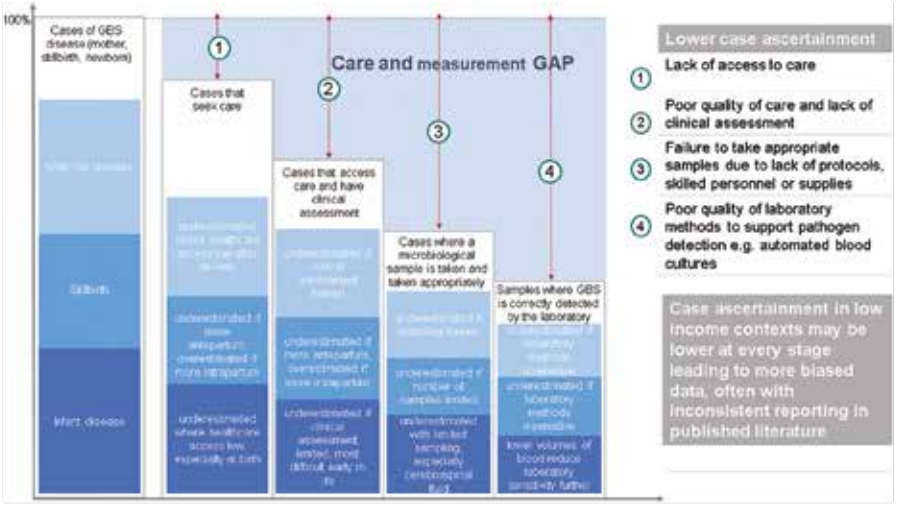


Figure 6. Care and measurement gap estimating cases from incidence and prevalence data. Adapted from Lawn et al [15]. Triangulation of estimates from compartmental model compared to incidence data for invasive infant disease is detailed in Supplementary Figures 8 and 9. Abbreviation: GBS, group B *Streptococcus*.

isolates, 86% of EOGBS disease, 93% of LOGBS disease, and 97% of maternal GBS disease. While there are a limited number of GBS capsular types (n = 10), the distribution by region varies, particularly for maternal GBS colonization; serotypes V, VI, VII, VIII, and IX are more commonly reported in South-Eastern Asia (23%) (Supplementary Table 5).

DISCUSSION

GBS is established as a leading cause of infant disease, particularly in the first week after birth, as evidenced by our estimation of 205 000 (UR, 101 000–327 000) neonates with EOGBS worldwide. Furthermore, there are a minimum 33 000 (UR, 13–52 000) maternal GBS cases, 57 000 (UR, 12 000–104 000) fetal infections/stillbirths, and 114 000 (UR, 44 000–326 000) infants with LOGBS. Up to 3.5 million preterm births could be attributable to maternal GBS infection/colonization worldwide (Figure 9).

Importantly, GBS is also a significant cause of death, with 57 000 (UR, 12 000–104 000) stillbirths and 90 000 (UR, 36 000–169 000) infant deaths estimated in 2015. IAP prevented an estimated 3000 (UR, 0–108 000) early neonatal deaths in 2015, mainly in high-income contexts. A maternal GBS vaccine, for which candidates are in development (Table 5), with 80% efficacy and 90% coverage could prevent 108 000 (UR, 20 000–198 000) fetal and infant deaths. GBS accounts for more than the total number of deaths from mother-to child transmission of human immunodeficiency virus, and more than the combined neonatal deaths from tetanus, pertussis, and respiratory syncytial virus (Table 6), for which maternal vaccines are already in use, or in advanced development.

The compartmental model approach mitigates some of the very substantial problems with low case ascertainment for invasive infant disease, which can result in huge underestimation, especially in low-income contexts. Our comparatively low estimates using infant incidence data are a result of cases being "missed" through lack of access to healthcare, inadequate clinical assessment and suspicion of infection, lack of diagnostic testing, and lack of appropriate laboratory detection methods such as high-quality blood cultures (Figure 6). Sensitivity of microbiological cultures is further reduced if there has been peripartum antibiotic exposure in the mother or infant. These biases are not included in the uncertainty around estimates from incidence or prevalence data, and thus the uncertainty bounds are likely too narrow. In contrast, the wide uncertainty bounds in the compartmental model, a result of including uncertainty at every step, better reflect the true uncertainty in estimation of these outcomes. In addition, in the compartmental model we addressed other sources of underestimation, by adjusting maternal GBS colonization for sampling site and laboratory methods [16] and only applying CFRs from facility data to births with a skilled birth attendant. Those born at home without access to a skilled birth attendant and who develop EOGBS will have very high, but unobserved, and thus unknown, CFR (Table 7). There are, however, limitations to the compartmental model approach, as described in general elsewhere [15], and in particular where parameters are derived from incidence data, such as the ratio of EOGBS to LOGBS disease. This ratio could be affected through differentially low case ascertainment in EOGBS compared to LOGBS disease or vice versa. EOGBS cases can be reduced with difficulties accessing care after birth and/or a high rapid CFR.

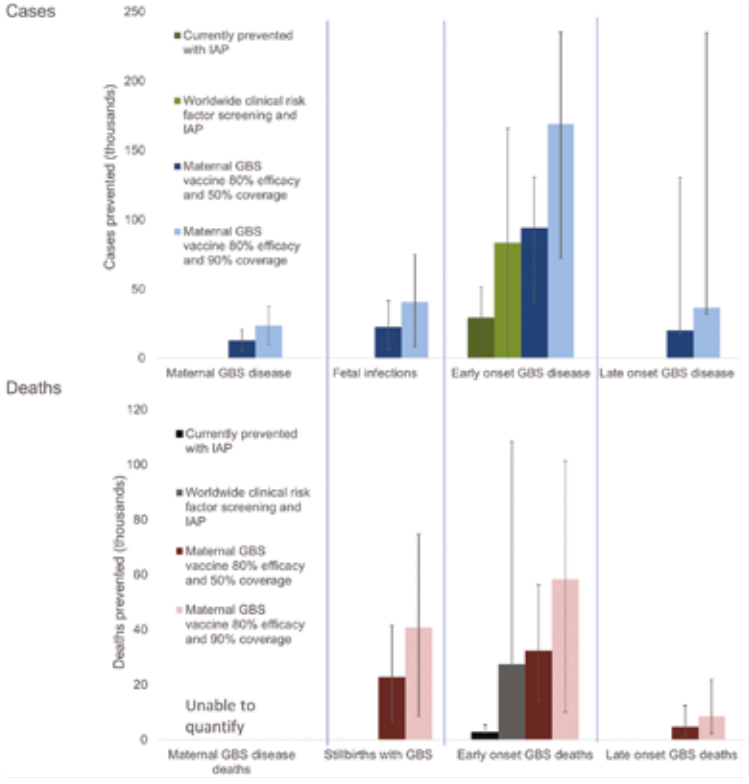


Figure 7. Scenarios of estimated cases of group B streptococcal (GBS) disease and deaths prevented with different intervention methods in a year. For worldwide clinical risk factor screening and intrapartum antibiotic prophylaxis (IAP) where microbiological screening was in place, this estimate was applied for that country. To facilitate comparison between the current situation and interventions, case fatality risks have been applied as at present (ie, a higher case fatality risk for deliveries without a skilled birth attendant).

LOGBS, which more frequently is meningitis, may be differentially reduced if cerebrospinal fluid sampling is infrequently undertaken, as is often the case in Asia [41].

In high-income contexts, reported incidence data are more reliable. In these countries incidence and trends can be monitored, and surveillance data show that GBS remains one of the most important neonatal and young infant pathogens. The United Kingdom, the Netherlands, and France recently reported increases in incidence of infant disease [42–44]. In the era of *Haemophilus influenzae* type b and pneumococcal conjugate vaccines, GBS is now the leading cause of bacterial meningitis in young children in the United Kingdom and the United States [45, 46]. In low- and middle-income contexts, reported incidence data are more subject to the biases in case ascertainment described. However, for Africa, our estimates of cases of infant disease and fetal infection or stillbirth, are consistent with recent reports of high incidence of GBS disease in facilities from Kenya and South Africa, where assessment and sampling recently have been systematic [47, 48]. For Asia, there is more uncertainty as to the burden of GBS disease. Until recently, the incidence of infant GBS disease was thought to be very low. In addition, there are no data on GBS disease in stillbirth from Asia. In our model, with a very high number of live births in Asia, absolute numbers of infants with EOGBS were high, despite the lower maternal colonization prevalence, suggesting that cases are currently underestimated. For stillbirths, if the prevalence of GBS disease in stillbirths is comparable to Africa, rather than high-income contexts, the total number of stillbirths with GBS disease worldwide would almost double. However, the compartmental model could overestimate invasive infant disease and/or stillbirth if there are biological differences, which it does not account for. There may be differences in virulence of GBS strains circulating in the region. GBS clonal complex 17 (ST17), strongly associated with serotype III, is hypervirulent [48, 49] and less frequently reported in Asia, both for maternal colonization [16] and neonatal disease [19].

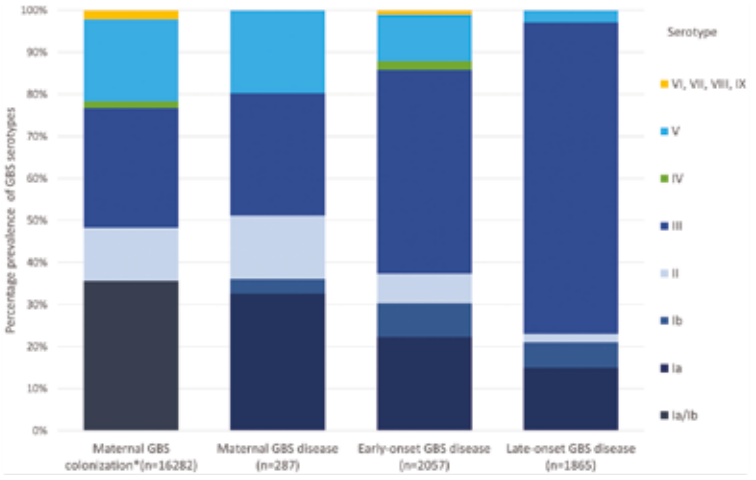


Figure 8. Group B streptococcal (GBS) serotypes colonizing mothers and causing disease in pregnant/postpartum women and infants. *Maternal colonization studies frequently reported Ia/Ib together, so these data are shown pooled. More details are shown in Supplementary Table 5.

Our estimates of maternal GBS disease, stillbirths, and infants with invasive GBS disease presenting with neonatal encephalopathy are all likely to be underestimates as they are all subject to similar challenges for case ascertainment as infant invasive disease, and we were not able to include these in the compartmental model. Invasive GBS disease in newborns presenting with neonatal encephalopathy is further underestimated as the data derive mainly from cooling trials in high-income contexts, with strict case definitions (Figure 2) [22]. In addition, we do not attempt to measure the burden of noninvasive in utero infection, which may sensitize the fetus and increase the risk of neonatal encephalopathy (Figure 9). The challenges of estimating noninvasive disease and the potential size of the unquantified burden are illustrated by the data on the attributable cases of preterm birth [21]. Even a small increase in risk of preterm birth attributable to GBS would account for many preterm births. For other pathogens, such as *Streptococcus pneumoniae*, invasive disease among children accounts for only 10% of all serious disease, with the majority (>80%) of deaths occurring from nonbacteremic pneumonia cases. Robust epidemiological data are critical

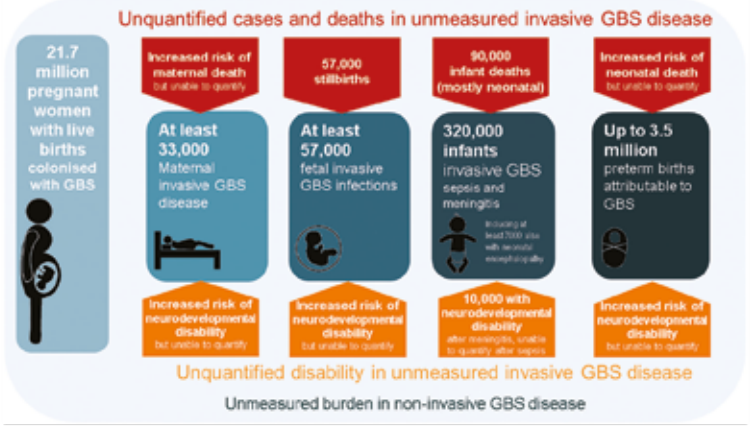


Figure 9. Summary of outcomes and measurement gaps in terms of deaths and disability from group B *Streptococcus* (GBS) in pregnant women, stillbirths, and infants worldwide in 2015. More details of cases and outcomes are shown in Supplementary Figures 4, 6, 7, 10–12.

Table 5. Maternal Group B *Streptococcus* Vaccines in Development With Data in the Public Domain

Vaccine Candidate	Manufacturer	Vaccine Construct	Phase				Program Status
			Discovery	Preclinical	Phase 1	Phase 2	
NA	Pfizer	Multivalent CPS conjugate		X			Clinical program start in 2017 [55]
GBS vaccine	Novartis/GSK	Trivalent CPS (serotypes Ia, IIb, III) conjugated to CRM_{197}, unadjuvanted				X	Completed safety and immunogenicity in pregnant women. Study completed [27, 56–61]
NA	GSK	Pentavalent (Ia, Ib, II, III, V) CPS-CRM_{197}		X			
NA	GSK	Pilus proteins		X			
NA	Biovac	Polyvalent CPS conjugate	X				Program start in 2017
GBS-NN vaccine/ MVX13211	Minervax	N-domains of Rib and Alpha C surface proteins, unadjuvanted or Alhydrogel-adjuvanted			X		Safety and immunogenicity in nonpregnant women. Study completed [26, 62, 63].

Abbreviations: CPS, capsular polysaccharide; GBS, group B Streptococcus; NA, not available.

to strengthen the investment case for a GBS vaccine and to firmly establish the true global burden of disease [50]. Vaccine probe studies during phase 3 maternal vaccine trials, or postlicensure, could also be used to contribute to our understanding of the total disease burden, including noninvasive disease [51].

The current mainstay of prevention against infant GBS disease is IAP, which prevented an estimated 29 000 (UR, 0–51 000) cases of EOGBS in 2015, mainly in high-income contexts [19]. IAP implementation is more common, and more feasible, in high-income contexts [23], because it requires a continuum of care, including a skilled birth attendant able to administer antibiotics intravenously, and with access to laboratories for a microbiological approach, and/or careful assessment for a clinical risk factor–based approach. There is low implementation, or no IAP national policy where health systems have limited infrastructure. In addition, IAP does not target the 204 000 (UR, 69 000–481 000) cases of maternal, fetal, and late-onset infant invasive infection (7–89 days). It is possible that there is some coincident reduction in disease with IAP, particularly in pregnant women [17], but it is likely administered too late in the context of stillbirth [18]. In addition, IAP does not reduce maternal colonization, so there is no reduction in infant exposure and colonization, and consequent late-onset infant disease [19].

Maternal vaccination has the advantage over IAP in that it could leverage off existing antenatal care platforms, as successfully used in high-burden countries to reduce neonatal tetanus, where high coverage has been achieved [52]. It would also be expected to reduce adverse outcomes for invasive disease in pregnant and postpartum women, fetuses/stillbirths, and infants. A maternal GBS vaccine with 80% efficacy and 50% coverage would prevent 127 000 (63 000–282 000) infant and maternal GBS cases, and 60 000 (UR, 22 000–110 000) stillbirths and infant deaths. If coverage were increased to 90%, 229 000 (UR, 114 000–507 000) infant and maternal GBS cases and 108 000 (UR, 20 000–198 000) fetal and infant deaths could be prevented. Maternal GBS vaccination could also reduce the unquantified burden from noninvasive

Table 6. Comparison of Annual Estimates of Infectious Etiologies Causing Stillbirth, Infant Disease, and Death Worldwide, Including Those Where Maternal Vaccination Is Used or Could Be Used to Reduce This Burden

Disease	Stillbirths	No. of Neonatal or Other Relevant Deaths Related to Maternal Infection or Nonimmunity	No. of Neonatal/Infant Cases per Year
Group B *Streptococcus*	57 000 (12 000–103 000)	90 000[a] (41 000–185 000)	319 000 (119 000–417 000)
Respiratory syncytial virus	Not applicable	27 300[b] (20 700–36 200) [64]	NA
Pertussis	NA	2700[c,d] [65]	NA
Syphilis	200 000 [7]	62 000[c] [66]	102 000 [66]
Tetanus	Not applicable	34 000[c] (18 000–84 000) [67]	NA
HIV/AIDS	9 000 [7]	86 000[e] (76 000–101 000) [67]	NA
Malaria	213 000 [7]	NA	NA

Data are presented as estimate (uncertainty range).

Abbreviations: HIV, human immunodeficiency virus; NA, no relevant estimate available.

[a] Young infants (0–89 days).

[b] Infants (0–6 months).

[c] Neonates (0–27 days).

[d] World Health Organization modelling-based estimates approximately 56 700 pertussis deaths in children <5 years of age in 2015 from which the neonatal component is derived [65]. Other work suggests the burden could be higher, with 160 700 deaths (range, 38 000–670 000 with sensitivity analyses) in children <5 years of age [68].

[e] Children <5 years of age but due to mother-to-child transmission.

Table 7. Data Inputs Into the Compartmental Model by Step, Considering the Main Biases

		Care and Measurement Gap and Resultant Biases				
Compartmental model	Parameter	Lack of Access to Care	Poor Quality of Care and Lack of Clinical Assessment	Failure to Take Appropriate Samples due to Lack of Protocols, Skilled Personnel, or Supplies	Poor Quality of Laboratory Methods to Support Pathogen Detection	Model Data Input Used
Step 1 Colonization	Infants exposed to maternal GBS at birth [16]	Maternal colonization prevalence measured in facilities: could increase or decrease prevalence dependent on risk factors for maternal GBS colonization		Sample-taking can reduce maternal colonization eg, taking a high vaginal swab. This was adjusted for in prevalence data included.	Culture methods such as broth enrichment increase detection of GBS. Where these were not used, we adjusted the prevalence data included.	Maternal colonization prevalence adjusted for swab sample site and culture methods
Step 2: Cases	IAP policy	IAP only where care accessed	Intrapartum antibiotics could be given inappropriately with overuse or underuse			IAP policy applied nationally with estimated coverage
	Risk of EOGBS	EOGBS underestimated where care access low	EOGBS underestimated if clinical assessment limited	EOGBS underestimated if sampling limited	EOGBS underestimated if laboratory methods insensitive	Risks based on IAP policy in counrtry and estimated coverage
	Ratio of EOGBS to LOGBS	May appear lower with lack of access to care especially at the time of birth	Will likely decrease EOGBS and LOGBS but less change to ratio	Will likely decrease EOGBS and LOGBS but less change to ratio	Will likely decrease EOGBS and LOGBS but less change to ratio	Ratio from regional data due to differences in IAP policies and potential for true differences in EOGBS and LOGBS disease incidence; no adjustments made
	Ratio of meningitis to sepsis cases in EOGBS	Ratio may be higher with lack of access to care, if CFR lower in meningitis	Ratio may be lower with poor quality of care and lack of assessment if meningitis not recognized	Ratio may be lower due to insufficient CSF sampling	Ratio may be higher where blood culture detection more difficult than detection in cerebrospinal fluid	Ratio from worldwide data, may be increased or decreased in either direction; no adjustment made
	Ratio of meningitis to sepsis cases in LOGBS	Ratio may be higher with lack of access to care if CFR lower in meningitis	Ratio may be lower with poor quality of care and lack of assessment if meningitis not recognized	Ratio may be lower due to insufficient CSF sampling	Ratio may be higher where blood culture detection more difficult than detection in CSF	Ratio from worldwide data, may be increased or decreased in either direction; no adjustment made
Step 3: Deaths	Case fatality risk in EOGBS	Reduced with low access to care	Increased with lack of appropriate assessment	Reduced if samples not taken from sickest infants		CFR adjusted to increase where access to care reduced (lack of skilled birth attendant).
	Case fatality risk in LOGBS	Reduced with low access to care but likely less of effect than for EOGBS	Increased with lack of appropriate assessment	Reduced if samples not taken from sickest infants		CFR not adjusted for LOGBS, this likely underestimates death.
Step 4: Disability	NDI risk in infant meningitis (EOGBS and LOGBS)	May decrease NDI if more deaths, but may increase if NDI not detected, eg, through premature death	Underdetection of NDI			NDI incidence at 18 months of age all from developed countries, this likely underestimates cases in the rest of world

Abbreviations: CFR, case fatality risk; CSF, cerebrospinal fluid; EOGBS, early-onset group B Streptococcus; GBS, group B Streptococcus; IAP, intrapartum antibiotic prophylaxis; LOGBS, late-onset group B Streptococcus; NDI, neurodevelopmental impairment.

Table 8. Key Findings and Implications

What's new about this?
- These are the first systematic estimates of the worldwide burden of GBS and we include outcomes for pregnant and postpartum women, stillbirth, and infants, with later impairment. For infants this includes invasive disease, overlapping with neonatal encephalopathy, and also noting preterm birth–associated GBS as a pathway resulting in deaths and disability.
- Data gaps remain a challenge, but the compartmental model includes more national data and is less susceptible to underestimating the burden through low ascertainment of clinical cases, especially in low-income contexts. We have followed international estimation guidelines and data inputs and code are available online [28, 29].

What are the main findings?
- Cases: 319 000 (UR, 119 000–417 000) infant and 33 000 (UR, 13 000–52 000) maternal cases of GBS disease; 7000 (UR, 300–19 000) infant cases also had neonatal encephalopathy. Fetal infections would be at least the 57 000 (UR, 12 000–104 000) stillbirths.
- Deaths: 57 000 (UR, 12 000–104 000) stillbirths and 90 000 (UR, 36 000–169 000) infant deaths, which is more than the total number of deaths from HIV (mother to child transmission), or more than the combined neonatal deaths from tetanus, pertussis, and RSV (Table 6).
- Disability: >10 000 (UR, 3000–27 000) new cases of neurodevelopmental impairment per year due to infant GBS meningitis.
- Other outcomes: Up to 3.5 million cases of preterm birth attributable to GBS.

How can the data be improved?
- Geographic: more data are needed worldwide, but especially from Asia.
- Outcomes: particular gaps include maternal disease, stillbirth, impairment after infant GBS sepsis, and comorbidity with neonatal encephalopathy. Inclusion of GBS assessments in maternal and neonatal cause-of-death studies should be enhanced.
- Economic: cost-effectiveness modeling based on these estimates, and translation to DALYs would be a further step before undertaking economic modeling.
- Vaccine trials: standardized definitions of vaccine endpoints also enabling comparison of observational data, and informing program monitoring and evaluation.

What does it mean for policy and programs?
- Current provision of IAP prevents an estimated 29 000 (UR, 0–51 000) cases of EOGBS disease.
- Maternal vaccination: With 80% efficacy and 90% coverage could prevent 229 000 (UR, 114 000–507 000) infant and maternal GBS cases, 41 000 (UR, 8000–75 000) stillbirths, and 67 000 (UR, 12 000–123 000) infant deaths.

Abbreviations: DALY, disability-adjusted life-year; EOGBS, early-onset group B Streptococcus; GBS, group B Streptococcus; HIV, human immunodeficiency virus; IAP, intrapartum antibiotic prophylaxis; RSV, respiratory syncytial virus; UR, uncertainty range.

disease, including, but not limited to, preterm birth. Several GBS vaccine candidates are now in active development [26] and these must be subject to appropriate safety and efficacy tests, but vaccine manufacturers are increasingly committed to investing in a GBS vaccine (Table 5).

There are key public health and economic considerations, to which these estimates contribute. These include (1) the estimated vaccine-preventable mortality burden; (2) the estimated scope, size and cost of a licensure trial; and (3) the cost-effectiveness of a maternal GBS vaccine, to inform policy recommendations, vaccine demand, and financing [51, 53, 54]. Cost-effectiveness models for a maternal GBS vaccine thus far have primarily considered GBS sepsis and meningitis as avertable causes of neonatal mortality [53]. Our estimates of the burden of GBS disease in pregnant and postpartum women and stillbirths, as well as infant disease, suggest they may be additional endpoints worthy of inclusion in a GBS vaccine trial.

GBS is a leading cause of invasive infection in infants, but GBS disease in pregnant and postpartum women and stillbirths is also important worldwide. GBS accounts for a far higher burden of young infant mortality than other infectious diseases for which maternal vaccines are under development or in use, such as respiratory syncytial virus, pertussis, or tetanus (Table 6). Despite GBS accounting for only a small proportion of all stillbirths, the absolute number is equal to a quarter of stillbirths attributed to syphilis, for which there is already a screening program. An effective maternal GBS vaccine offers an all-encompassing approach to reducing GBS disease, and, as vaccination strategies can achieve high coverage in even the most challenging settings, it is likely to be a more equitable intervention than IAP. Maternal GBS vaccination has the potential to reduce this disease burden worldwide, within the next generation and including the poorest families (Table 8).

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. The concept of the estimates and the technical oversight of the series were led by J. E. L. and A. C. S. The analyses and first draft of the paper were undertaken by A. C. S. with F. B. and J. E. L. The multiple regression analyses were done by F. B.; H. B. and S. C. advised on analyses and contributed to revisions of drafts. N. R., M. K., C. T., J. H., and M. M. input data for the analyses. The GBS Estimates Expert Advisory Group (C. J. B., L. B., M. G., P. T. H., S. A. M., M. I., K. L. D., C. R., S. K. S., S. J. S., A. S., J. V.) contributed to the conceptual process throughout, notably on the disease schema and data inputs. All the authors reviewed and gave input to the manuscript.

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References

1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet **2015**; 385:430–40.
2. Blencowe H, Cousens S, Jassir FB, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. Lancet Glob Health **2016**; 4: e98–108.
3. Lawn JE, Blencowe H, Oza S, et al. Every Newborn: progress, priorities, and potential beyond survival. Lancet **2014**. doi:10.1016/S0140-6736(08)61345–8.
4. Graham W, Woodd S, Byass P, et al. Diversity and divergence: the dynamic burden of poor maternal health. Lancet **2016**; 388:2164–75.
5. Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet **2013**; 380: 2197–223.
6. Goldenberg RL, McClure EM, Saleem S, Reddy UM. Infection-related stillbirths. Lancet **2010**; 375:1482–90.
7. Lawn JE, Blencowe H, Waiswa P, et al. Stillbirths: rates, risk factors, and acceleration towards 2030. Lancet **2016**; 387:587–603.
8. Seale AC, Blencowe H, Zaidi A, et al. Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. Pediatr Res **2013**; 74(suppl 1): 73–85.
9. Blencowe H, Vos T, Lozano R, Cousens S, Lawn JE. Beyond survival: estimates of neonatal morbidities and long term disabilities at regional and global level for 2010—introduction, overview of methods and relevant Global Burden of Disease findings. Pediatr Res **2013**.
10. Blencowe H, Lee AC, Cousens S, et al. Preterm birth-associated neurodevelopmental impairment estimates at regional and global levels for 2010. Pediatr Res **2013**; 74(suppl 1): 17–34.
11. Lee AC, Koruki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res **2013**; 74(suppl 1): 50–72.
12. Seale AC, Mwaniki M, Newton CR, Berkley JA. Maternal and early onset neonatal bacterial sepsis: burden and strategies for prevention in sub-Saharan Africa. Lancet Infect Dis **2009**; 9:428–38.
13. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet **2012**; 379:547–56.
14. Nan C, Dangor Z, Cutland CL, Edwards MS, Madhi SA, Cunnington MC. Maternal group B *Streptococcus*-related stillbirth: a systematic review. BJOG **2015**; 122:1437–45.
15. Lawn JE, Bianchi-Jassir F, Russell NJ, et al. Group B streptococcal disease worldwide for pregnant women, stillbirths, and children: why, what, and how to undertake estimates? Clin Infect Dis **2017**; 65(suppl 2):S89–99.
16. Russell N, Seale AC, O'Driscoll M, et al. Maternal colonization with group B *Streptococcus* and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S100–11.
17. Hall J, Hack Adams N, Bartlett L, et al. Maternal disease with group B *Streptococcus* and serotype distribution worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S112–24.
18. Seale AC, Blencowe H, Bianchi-Jassir F, et al. Stillbirth with group B streptococcal disease worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S125–32.
19. Madrid L, Seale AC, Kohli-Lynch M, et al. Infant group B streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S160–72.
20. Kohli-Lynch M, Russell N, Seale AC, et al. Neurodevelopmental impairment in children after group B streptococcal disease worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S190–9.
21. Bianchi-Jassir F, Seale AC, Kohli-Lynch M, et al. Preterm birth associated with group B *Streptococcus* maternal colonization worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S133–42.
22. Tann CJ, Martinello K, Sadoo S, et al. Neonatal encephalopathy with group B streptococcal disease worldwide: systematic review, investigator group datasets, and meta-analysis. Clin Infect Dis **2017**; 65(suppl 2):S173–89.
23. Le Doare K, O'Driscoll M, Turner K, et al. Intrapartum antibiotic chemoprophylaxis policies for the prevention of group B streptococcal disease worldwide: systematic review. Clin Infect Dis **2017**; 65(suppl 2):S143–51.
24. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. N Engl J Med **2000**; 342:15–20.
25. Centers for Disease Control and Prevention. Perinatal group B streptococcal disease after universal screening recommendations—United States, 2003–2005. MMWR Morb Mortal Wkly Rep **2007**; 56:701–5.
26. Heath PT. Status of vaccine research and development of vaccines for GBS. Vaccine **2016**; 34:2876–9.
27. Heyderman RS, Madhi SA, French N, et al. Group B *Streptococcus* vaccination in pregnant women with or without HIV in Africa: a non-randomised phase 2, open-label, multicentre trial. Lancet Infect Dis **2016**; 16:546–55.
28. Stevens GA, Alkema L, Black RE, et al; GATHER Working Group. Guidelines for accurate and transparent health estimates reporting: the GATHER statement. Lancet **2016**; 388:e19–23.
29. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ **2009**; 339:b2700.
30. Russell N, Seale AC, O'Sullivan C, et al. Risk of early-onset neonatal group B streptococcal disease with maternal colonization worldwide: systematic review and meta-analyses. Clin Infect Dis **2017**; 65(suppl 2):S152–9.
31. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials **1986**; 7:177–88.
32. United Nations Population Division (UNPD). World Population Prospects. Available at: http://esa.un.org/wpp/. Accessed 27 August 2017.
33. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet **2012**; 379: 2162–72.
34. Institute for Health Metrics and Evaluation. IHME global data exchange. Available at: http://ghdx.healthdata.org/ihme_data. Accessed 5 May 2017.
35. World Health Organization. WHO global health observatory. Available at: http://www.who.int/gho/en/. Accessed 5 May 2017.
36. The World Bank. World Bank indicators. Available at: http://data.worldbank.org/indicator. Accessed 5 May 2017.
37. United Nations Interagency Group for Child Mortality Estimation (IGME). Child mortality estimates. Available at: http://www.childmortality.org. Accessed 5 May 2017.
38. World Bank. Data from MICS/DHS surveys and CRVS data. Available at: http://data.worldbank.org/indicator. Accessed 5 May 2017.
39. United Nations. Maternal mortality estimation inter-agency group (MMEIG). Available at: http://www.un.org/en/development/desa/population/publications/mortality/maternal-mortality-report-2015.shtml. Accessed 21 May 2017.
40. Newman L, Kamb M, Hawkes S, et al. Global estimates of syphilis in pregnancy and associated adverse outcomes: analysis of multinational antenatal surveillance data. PLoS Med **2013**; 10:e1001396.
41. Knoll MD, Moïsi JC, Muhib FB, et al; PneumoADIP-Sponsored Surveillance Investigators. Standardizing surveillance of pneumococcal disease. Clin Infect Dis **2009**; 48(suppl 2):S37–48.
42. Bekker V, Bijlsma MW, van de Beek D, Kuijpers TW, van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis **2014**; 14:1083–9.

43. Joubrel C, Tazi A, Six A, et al. Group B *Streptococcus* neonatal invasive infections, France 2007–2012. Clin Microbiol Infect **2015**; 21:910–6.
44. British Paediatric Surveillance Unit. British Paediatric Surveillance Unit annual report 2015–16. Available at: http://www.rcpch.ac.uk/system/files/protected/page/BPSU_AR1516_Web_0.pdf. Accessed 11 May 2017.
45. Okike IO, Johnson AP, Henderson KL, et al; neoMen Study Group. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clin Infect Dis **2014**; 59:e150–7.
46. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998–2007. N Engl J Med **2011**; 364: 2016–25.
47. Madhi SA, Radebe K, Crewe-Brown H, et al. High burden of invasive *Streptococcus agalactiae* disease in South African infants. Ann Trop Paediatr **2003**; 23:15–23.
48. Seale AC, Koech AC, Sheppard AE, et al. Maternal colonization with *Streptococcus agalactiae* and associated stillbirth and neonatal disease in coastal Kenya. Nat Microbiol **2016**; 1:16067.
49. Jones N, Bohnsack JF, Takahashi S, et al. Multilocus sequence typing system for group B *Streptococcus*. J Clin Microbiol **2003**; 41:2530–6.
50. Schrag SJ; Global Group B Streptococcal Vaccine Working Group. Group B streptococcal vaccine for resource-poor countries. Lancet **2011**; 378:11–2.
51. Madhi SA, Dangor Z, Heath PT, et al. Considerations for a phase-III trial to evaluate a group B *Streptococcus* polysaccharide-protein conjugate vaccine in pregnant women for the prevention of early- and late-onset invasive disease in young-infants. Vaccine **2013**; 31(suppl 4):D52–7.
52. Roper MH, Vandelaer JH, Gasse FL. Maternal and neonatal tetanus. Lancet **2007**; 370:1947–59.
53. Kim SY, Russell LB, Park J, et al. Cost-effectiveness of a potential group B streptococcal vaccine program for pregnant women in South Africa. Vaccine **2014**; 32:1954–63.
54. Kobayashi M, Schrag SJ, Alderson MR, et al. WHO consultation on group B *Streptococcus* vaccine development: report from a meeting held on 27–28 April 2016. Vaccine **2016**. doi:10.1016/j.vaccine.2016.12.029.
55. Pfizer. Press release: Pfizer awarded grant to evaluate vaccine to protect newborns against group B *Streptococcus* infection. Available at: http://www.pfizer.com/news/press-release/press-release-detail/pfizer_awarded_grant_to_evaluate_vaccine_to_protect_newborns_against_group_b_streptococcus_infection-0. Accessed 24 March 2017.
56. Novartis/GSK. Safety and immunogenicity of a trivalent group B *Streptococcus* vaccine in healthy pregnant women. Available at: https://clinicaltrials.gov/ct2/show/NCT02046148. Accessed 28 March 2017.
57. Madhi SA, Cutland CL, Jose L, et al. Safety and immunogenicity of an investigational maternal trivalent group B *Streptococcus* vaccine in healthy women and their infants: a randomised phase 1b/2 trial. Lancet Infect Dis **2016**; 16:923–34.
58. Baker CJ, Paoletti LC, Wessels MR, et al. Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. J Infect Dis **1999**; 179:142–50.
59. Baker CJ, Paoletti LC, Rench MA, Guttormsen HK, Edwards MS, Kasper DL. Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines. J Infect Dis **2004**; 189:1103–12.
60. Baker CJ, Rench MA, McInnes P. Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. Vaccine **2003**; 21:3468–72.
61. Baker CJ, Rench MA, Fernandez M, Paoletti LC, Kasper DL, Edwards MS. Safety and immunogenicity of a bivalent group B streptococcal conjugate vaccine for serotypes II and III. J Infect Dis **2003**; 188:66–73.
62. Minervax. Safety and immunogenicity of a group B *Streptococcus* vaccine in non-pregnant women 18–40 years of age (MVX13211). Available at: https://clinicaltrials.gov/ct2/show/NCT02459262. Accessed 28 March 2017.
63. Minervax. MinervaX is pursuing the development of a novel vaccine candidate against group B streptococci (GBS). Available at: http://minervax.com/. Accessed 10 May 2017.
64. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet **2017**. doi:10.1016/S0140-6736(17)30938-8.
65. Sobanjo-Ter Meulen A, Duclos P, McIntyre P, et al. Assessing the evidence for maternal pertussis immunization: a report from the Bill & Melinda Gates Foundation symposium on pertussis infant disease burden in low- and lower-middle-income countries. Clin Infect Dis **2016**; 63:123–33.
66. Wijesooriya NS, Rochat RW, Kamb ML, et al. Global burden of maternal and congenital syphilis in 2008 and 2012: a health systems modelling study. Lancet Glob Health **2016**; 4: e525–33.
67. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet **2016**; 388:3027–35.
68. Yeung KHT, Duclos P, Nelson EAS, Hutubessy RCW. An update of the global burden of pertussis in children younger than 5 years: a modelling study. Lancet Infect Dis **2017**. doi:10.1016/S1473-3099(17)30390-0.

Appendix: Multiple Regression Model for Maternal GBS Colonization Prevalence

Purpose: Multiple regression models are commonly used to estimate the burden of diseases, particularly for a single parameter, eg, stillbirth rate or preterm birth rate. In the case of GBS, there are usually multiple outcomes that can be better estimated using a stable compartmental model as discussed elsewhere in this supplement [15]. However, we wanted to assess other approaches including whether a multiple regression model could be used to predict the national prevalence of maternal GBS colonization, using national-level covariates. Maternal GBS colonization prevalence is the parameter with the most data available to which a multiple regression model can be applied.

Inputs: Maternal GBS colonization prevalence data (n = 74) were obtained from published and unpublished literature on GBS worldwide, as described elsewhere in this supplement [16]. Fifteen national-level covariates plausibly associated with GBS colonization prevalence were selected for possible inclusion in the model as predictor variables (full details in Supplementary Table 3):

log adult female obesity [34], *skilled attendant at birth (SBA)* [35], *log antenatal care (4 visits), mean years female education* [32], *gross national income (GNI)* [36], *log neonatal mortality rate (NMR)* [37], *protected at birth against tetanus (PAB), low birthweight rate (LBW)* [38], *log general fertility rate (GFR)* [39], *log GINI coefficient* [36], *proportion cesarean delivery, syphilis index* [40], *United Nations region, United Nations subregion, and percentage population urban*.

The association between potential covariates and maternal GBS colonization prevalence was examined using scatterplots (Supplementary Figure 1). Univariable analyses were undertaken to quantify the relationship between GBS maternal colonization prevalence, and continuous variables to assess if these predictors performed better when log transformed. Predictors were then assessed for retention in the model by removing one predictor at a time from the model (starting with the predictor with largest Bayesian information criterion [BIC] on univariate analysis), and dropping the predictor if the model was improved (ie, lower BIC compared to the model containing the predictor).

Model equation and fit: The equation of the final model resulting from the process described above was:

$$\begin{aligned} Log\left(GBS\ prevalence_{ij}\right) = a + b\left(LBW_{ij}\right) + c\left(Log\left(GINI_{ij}\right)\right) \\ + d\left(GNI_{ij}\right) + e\left(SBA_{ij}\right) \\ + f\left(UNregion_{ij}\right) + u_j + e_{ij} \end{aligned}$$

b(), c(), d(), and e() represent functions each involving 2 parameters, f() indicates a 5 parameter function associated with 5 dummy variables representing different United

Nations regions, u_j represents country-specific random effects, assumed to be independent normally distributed with constant variance, and e_{ij} represents individual data point-level residuals, assumed to be independent normally distributed with constant variance.

We evaluated the model fit by running a series of diagnostic plots including analyses of regression residuals. The results from the diagnostic plots (Supplementary Figure 2A) and the scatterplot of observed vs predicted data (Supplementary Figure 2B) showed evidence that the model fits the data poorly.

Conclusions: This modeling approach was hampered by the limited number of data inputs and particularly by the lack of a strong relationship between available covariates and maternal GBS colonization.

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