Osteoarticular infections: insights on bacteremic clinical forms and antimicrobial alternatives against *Pseudomonas aeruginosa* from a translational perspective

Joan Gómez Junyent

UNIVERSITAT DE BARCELONA

Facultad de Medicina

OSTEOARTICULAR INFECTIONS: INSIGHTS ON BACTERAEMIC CLINICAL FORMS AND ANTIMICROBIAL ALTERNATIVES AGAINST *PSEUDOMONAS AERUGINOSA* FROM A TRANSLATIONAL PERSPECTIVE

Memoria presentada por

JOAN GÓMEZ JUNYENT

para optar al grado de Doctor en Medicina

Barcelona, noviembre de 2020

El Dr. Javier Ariza Cardenal, catedrático de la Facultad de Medicina de la Universitat de Barcelona, y el Dr. Oscar Murillo Rubio, profesor de la Universitat de Barcelona y médico adjunto del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge, hacen constar que la tesis titulada

Osteoarticular infections: insights on bacteraemic clinical forms and antimicrobial alternatives against *Pseudomonas aeruginosa* from a translational perspective

que presenta el licenciado Joan Gómez Junyent, ha sido realizada bajo su dirección en el Campus de Bellvitge de la Facultad de Medicina, la consideran finalizada y autorizan su presentación para que sea defendida ante el tribunal que corresponda.

En Barcelona, noviembre de 2020

Dr. Javier Ariza Cardenal

Dr. Oscar Murillo Rubio

A Gara,

per tant, en tan poc temps

A Cinta i Cora,

junts som invencibles

I’ve learned that people will forget what you said,

people will forget what you did,

but people will never forget how you made them feel.

Maya Angelou

L’essentiel est invisible pour les yeux.

Antoine de Saint-Exupéry, Le petit prince

The research presented in this thesis has been carried out thanks to the

Formación de Profesorado Universitario (FPU)

Ministerio de Educación, Cultura y Deporte

Beca FPU 14/03124

Some studies have been supported by scientific projects awarded to the osteoarticular infections unit in which I have collaborated

FIS PI14/00511: Alternativas terapéuticas frente a la infección in vitro de cuerpo extraño producida por bacilos gram-negativos multiresistentes: estudios farmacodinámicos en monoterapia y en combinación. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge

Merck Investigator Studies Program IISP 54711 (Merck, Sharp & Dohme S.A.): Efficacy of Ceftolozane-tazobactam, alone and in combination, against *in vitro* foreign-body infection caused by multidrug-resistant *Pseudomonas aeruginosa*: a pharmacodynamic study. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge.

AGRAÏMENTS

Escriure els agraïments sempre és una part molt especial d'un moment tan únic, d'un procés llarg, cuinat a foc lent, amb molts instants i, el més important, amb moltes persones protagonistes en l'abans i el durant. Finalitza, a més, un procés de 5 anys enriquidor i intens, en una etapa única de la història recent de les malalties infeccioses, enmig d'una pandèmia per una malaltia nova. Un moment també per a reivindicar l'ofici de metge, que em va atraure tant dels meus directors de tesi quan els vaig conèixer durant la meva residència de Medicina Interna.

Em sento enormement afortunat d'haver tingut de directors de tesi al Xavier Ariza i l'Oscar Murillo. S'han convertit en amics i companys a la vida. Les estones compartides amb el Xavier al despatx, amb la foscor progressiva (divendres), parlant de malalts, de ciència, revisant una vegada i una altra les dades per arribar als petits detalls, han estat una meravella, així com les estones per compartir vida extra-hospitalària. Em va sorprendre de resident i em segueix sorprenent ara, amb força, passió i vitalitat, un gran sentit de la justícia, una clarividència extraordinària del que és important i sempre disposat a cuidar els malalts i els seus alumnes, en totes les dimensions. La porta sempre oberta, un gran referent per a la medicina i la vida. Gràcies.

Gràcies també a l'Oscar, que va acollir en el seu moment a un metge resident "pipiolin" i que l'ha acompanyat (i l'ha fet créixer) també durant tots aquests anys, amb entusiasme, constància i comprensió. He gaudit molt intensament (i m'he aprofitat) de la seva ment privilegiada, de les seves grans habilitats amb la literatura i els articles en construcció i també de molts moments en què compartir vida i temps, amb alegria, tot i les pedres al camí que ens hem anat trobant. Gràcies per sempre saber trobar el paper adient, de director quan calia, d'amic en d'altres quan era moment de celebrar.

L'essència del bone team ha donat encara més sentit a tot plegat, tant les generacions actuals com les passades. Afortunat he estat també de compartir camí amb l'Eva Benavent, per l'entusiasme des del primer dia i la disponibilitat, per alegrar-se com ningú dels meus èxits i a la vegada fer-me costat en els moments durs personals i professionals. La Cris El Haj em va acollir al laboratori amb comprensió i delicadesa, amb riures i somriures, i em va ajudar i ensenyar tot i més. Gràcies també a la Laura Soldevila, dolça en les formes i amant de la feina ben feta, preocupant-se constantment dels

malalts. Gràcies a l'Alba Ribera pels mesos que vam viure junts, per cuidar-me i orientar-me tant a la planta com al laboratori. Aún recuerdo con mucho cariño esa rotación por Trauma-Sépticos con Jaime Lora-Tamayo, sus momentos docentes, esas conversaciones tranquilas de todo (confitería), las risas y ese ir construyendo una amistad que sigue, pese a la distancia. Y a Gorane Euba, pese a no haber prácticamente concidido, nos abriste el camino a los demás. Finalmente, gracias a Ivan Pelegrín, miembro oficioso del bone team, excelente médico, persona excepcional, entregado y apasionado, que cuida a los enfermos como nadie, por estar ahí siempre.

Ha estat un plaer poder treballar amb l'equip de bacterièmies, Roman Pallarés i Imma Grau, sempre propers i acollidors. Igualment, aquesta tesi no hagués estat possible sense la participació còmplice de l'equip de Traumatologia Sèptics, Xavier Cabo, Salva Pedrero, Victor Casals i Kilian Fraga, amb qui hem fet bon equip i als qui guardo un gran afecte; he gaudit treballant amb vosaltres, amb els passes de planta, aprenent l'art de la traumatologia de sèptics. També el meu reconeixement i agraïment a la resta del Servei de Traumatologia, especialment a l'equip d'esquena, a tots els residents amb qui hem treballat a la unitat i també a la infermeria, pilar fonamental de la nostra feina. Gràcies també als pacients, veritable sentit de la nostra feina i recerca.

Gràcies al Servei de Malalties Infeccioses i de Medicina Interna, per tota la medicina que he aprés durant la residència i anys posteriors, m'hi he sentit molt ben acollit. Gràcies de forma especial a Carme Peña, Carme Cabellos, Miguel Santín, Núria Fernández-Sabé i Arkaitz Imaz. Un reconeixement i gratitud especials per tots els residents que han anat passant per la unitat, per la feina i la dedicació. Als companys becaris de Malalties Infeccioses (Sara Grillo, Júlia Laporte, Isabel Oriol, Cris Royo, Aina Gomila, Guille Cuervo, Alexander Rombauts i Adaia Albasanz), pel suport i les estones compartides durant aquests anys. També gràcies als becaris de generacions anteriors (Laura Muñoz, Marta Bodro, Uri Gasch, Silvia Gómez-Zorrilla), per tot l'aprenentatge en èpoques de resident.

La feina que hem fet durant aquests anys i la que fem en el dia a dia seria impossible sense la contribució del Servei de Microbiologia. Especialment, vull agrair a la Fe Tubau, per la seva tendresa, somriure incansable i estar sempre disponible a respondre tots els meus dubtes sobre antibiogrames, resistències i soques bacterianes. A la Dolors Garcia, per tota l'ajuda en revisar les mostres amb una feina metòdica i precisa. També a Yanik

Sierra per tot l'esforç, dedicació i bon ambient que portava al laboratori. Gràcies a Raul Rigo per la seva incansable col·laboració, sempre disponible i amb una gran predisposició per ajudar, reflexionar i buscar solucions. Igualment, gràcies al Benjamín Torrejón, per guiar-me entre microscopia confocal, trobar els plans perfectes i tantes tardes mirant les fotos de biofilm en un ambient tan distés i col·laborador.

I am very grateful to Jian Li and his team in Monash University, Melbourne. It was an amazing professional and personal experience and I was warmly welcomed the minute I arrived, they made me feel at home. Especially, I would like to acknowledge all the help and hard work done by Heidi Yu and Hasini Wickremasinghe, for great moments in the lab and in the city, our walks to Clayton station and coffee breaks. We built friendship there. Thanks also to Melbourne city, which shed some light on very dark times.

Aquesta tesi també és en part de la família que es tria, els amics que han acompanyat i sentit de la interminable tesi doctoral. Especialment, gràcies als meus CoR per moments únics, pel suport i per l'estima, per la connexió en la medicina i la vida. També als soso pool, i als seus membres més propers, pel viscut i el que ha de venir, per les nits de pilladors i per anar construint amistat. També als meus amics "oncòlegs", gent meravellosa, que han entès i acompanyat en tot el procés personal i de recerca. Gràcies amics de sempre, per mantenir l'amistat tot i passar el temps.

El suport i amor constants dels meus pares, Pilar i Álvaro, en totes les fases educatives i professionals de la meva vida ha estat també vital. Un suport i amor que s'han traduït en les seves eleccions a la vida, en posar sempre els fills per davant del seu benestar i plaer, per creure enormement en el valor de l'educació i la feina ben feta (no té fronteres), en un marc de seguretat i felicitat. Gràcies a la meva germana Mariona, per creure en mi de forma incondicional i viure la vida amb emoció i seny a la vegada. A la resta de les famílies Gómez i Junyent, per estar allà, especialment a la meva àvia Lola, exemple de vitalitat i energia. Gràcies també a la meva família tortosina (Tere, Fermín, Maite, Isma, Ismael, Ivan) i reusenca per acollir-me des del primer dia i fer-me sentir tan estimat.

Finalment, gràcies Cinta, per fer camí amb mi, per ser referent en tants moments i ser el pal de paller d'aquesta petita gran família que hem anat construint amb esforç, entrega i amor. Per ser una metgessa meravellosa i apassionada, científica convençuda,

que gaudeix enormement amb les coses grans i petites. Pel concepte “pedra gran, pedra petita”. La vida no ens ho ha posat fàcil però aquí estem, jums (amb m).... I a les meves filles, Gara i Cora, veritables regals a la vida, sempre presents en cos i ànima.

RESUMEN

1. Introducción

Las infecciones osteoarticulares son frecuentes en la práctica hospitalaria y tienen un impacto importante para el paciente y el sistema sanitario, al asociarse con múltiples cirugías y tratamientos antibióticos prolongados. Algunas de estas infecciones se asocian a la presencia de material ortopédico (infecciones de prótesis articular, osteomielitis asociadas a cuerpo extraño, infecciones de instrumentación de espalda) mientras que otras son nativas (artritis séptica, osteomielitis vertebral y periférica). Las infecciones osteoarticulares pueden estar asociadas a la presencia de bacteriemia, que puede considerarse como causa o consecuencia de las mismas.

Las infecciones osteoarticulares se encuentran dentro del grupo de infecciones de difícil tratamiento, fundamentalmente por la presencia de biofilm bacteriano. Estas estructuras complejas son difíciles de erradicar con tratamiento antibiótico convencional, ya que las bacterias se vuelven menos susceptibles a los antimicrobianos (tolerancia antibiótica). El biofilm es especialmente relevante en la patogenia y dificultad de tratamiento en las infecciones asociadas a cuerpo extraño y/o infecciones crónicas.

Desde el punto de vista clínico y, de forma general, las infecciones se dividen en agudas y crónicas. Las primeras incluyen infecciones de instauración brusca, ocasionalmente bacteriémicas, y se suelen presentar con signos y síntomas inflamatorios, dolor agudo, fiebre, supuración (en infecciones post-quirúrgicas) y/o derrame articular. Estas infecciones están causadas fundamentalmente por microorganismos virulentos, como *Staphylococcus aureus*, *Streptococcus* spp. y bacilos Gram negativos. En cambio, las infecciones crónicas se asocian a un cuadro clínico de instauración subaguda con signos y síntomas leves o moderados con dolor, limitación funcional y ocasionalmente derrame articular, con escasos signos inflamatorios. Estas infecciones suelen estar causadas por microorganismos poco virulentos, como estafilococos coagulasa-negativos o *Propionibacterium acnes*.

El diagnóstico incluye datos clínicos y analíticos además de pruebas de imagen y técnicas microbiológicas, tanto de hemocultivos como de muestras locales. El tratamiento global incluye frecuentemente la necesidad de desbridamiento quirúrgico. Las infecciones asociadas a cuerpo extraño pueden manejarse mediante desbridamiento, antibióticos y

retención del implante (DAIR), habitualmente en infecciones agudas post-quirúrgicas o hematógenas, o mediante explante del implante, habitualmente en infecciones crónicas post-quirúrgicas o cuando el DAIR fracasa. Las infecciones nativas también suelen manejarse con desbridamiento quirúrgico, con diferentes técnicas en función del tipo de infección.

El tratamiento antibiótico es otro componente fundamental en el manejo global de estas infecciones y se suele individualizar en función del tipo de infección, perfil de sensibilidad del microorganismo y características del paciente. Idealmente, debe ser un tratamiento antibiótico poco tóxico y que permita ser administrado durante largos periodos de tiempo, además de tener actividad frente a bacterias estacionarias e intracelulares.

En infecciones estafilocócicas, este tratamiento suele incluir la administración de rifampicina, que tiene un papel clave cuando el manejo incluye la retención del implante. En infecciones por bacilos Gram negativos, especialmente *Pseudomonas aeruginosa*, suelen recomendarse las quinolonas. Sin embargo, el incremento sustancial de la resistencia a fluoroquinolonas ha generado la necesidad de alternativas terapéuticas, que podría incluir el empleo de colistina en combinación con beta-lactámicos y la administración de los beta-lactámicos mediante infusión continua.

En los últimos años se ha progresado en el conocimiento de las infecciones osteoarticulares, aunque la mayoría de estudios son pequeños y con muestras heterogéneas. Hay, sin embargo, múltiples cuestiones no resueltas, como por ejemplo, particularidades de las infecciones bacteriémicas. Asimismo, en una época dominada por la multiresistencia y la ausencia de alternativas antibióticas, es esencial encontrar opciones terapéuticas que permitan mejorar el pronóstico de estos pacientes. En ese sentido, alternativas terapéuticas como el uso de beta-lactámicos en infusión continua o las combinaciones de los mismos con colistina podrían ser útiles. Asimismo, los datos obtenidos de estudios experimentales pueden ser útiles para diseñar estrategias de tratamiento antibiótico.

2. Objetivos

A. Impacto y pronóstico de la infección osteoarticular bacteriémica

A.1. Impacto de la infección osteoarticular bacteriémica

Objetivo 1 – Describir tendencias epidemiológicas y microbiológicas de infecciones osteoarticulares bacteriémicas a lo largo de las últimas décadas.

Objetivo 2 – Analizar las características de las infecciones osteoarticulares bacteriémicas asociadas a la presencia de endocarditis infecciosa.

Objetivo 3 – Comparar las características de los pacientes con artritis séptica bacteriémica, en relación con el sitio de adquisición.

A.2. Pronóstico de la infección osteoarticular bacteriémica

Objetivo 4 – Analizar la mortalidad y factores de riesgo asociados en pacientes con infecciones osteoarticulares bacteriémicas.

B. Tratamiento antibiótico de las infecciones osteoarticulares por *Pseudomonas aeruginosa*

B.1. Estudios clínicos

Objetivo 5 – Analizar la eficacia y la monitorización plasmática de los beta-lactámicos en infusión continua para las infecciones osteoarticulares por *Pseudomonas aeruginosa* resistente a fluoroquinolonas.

B.2. Estudios experimentales

Objetivo 6 – Evaluar la actividad de ceftolozano-tazobactam, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* multiresistente en un modelo dinámico *in vitro*.

Objetivo 7 – Evaluar las características farmacocinéticas y farmacodinámicas de ceftazidima en infusión continua, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* en un modelo dinámico *in vitro*.

3. Métodos

3.1. Investigación clínica

Los estudios clínicos incluidos en esta tesis se han desarrollado dentro del marco de la Unidad de Infección Osteoarticular del Hospital Universitari de Bellvitge. Esta unidad es de referencia nacional, habiendo sido reconocida como tal por el Ministerio de Sanidad (CSUR desde 2010 y renovado en 2019), y en ella se realiza un manejo multidisciplinar de las infecciones osteoarticulares. También ha participado el grupo de estudio de bacteriemias, con la recogida prospectiva de los casos. Los estudios clínicos realizados en esta unidad han contado con la colaboración del Laboratorio de Microbiología y de Bioquímica del hospital, donde se han realizado el procesamiento de las muestras de cultivo y el desarrollo y estandarización de los métodos de UHPLC-MS/MS para la determinación de concentraciones de antibiótico, respectivamente.

3.2. Investigación experimental

Hay dos estudios experimentales incluidos en la presente tesis y ambos son estudios *in vitro* que utilizan el modelo del CDC Biofilm Reactor de infección de cuerpo extraño. Uno de los estudios ha sido realizado en el Laboratorio de Infección Experimental de la Universitat de Barcelona (Campus Bellvitge), vinculado al Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge. Este estudio evalúa la actividad de ceftolozano-tazobactam, con y sin colisitina, frente a una infección de biofilm por *Pseudomonas aeruginosa* multiresistente.

El segundo estudio se realizó en el Monash Biomedicine Discovery Institute, en la Monash University (Melbourne, Australia), donde el doctorando se trasladó para la realización del proyecto en el grupo del Profesor Jian Li. Este estudio evalúa las características farmacocinéticas/farmacodinámicas de ceftazidima en infusión continua, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* sensible a ambos antibióticos.

4. Resultados por objetivos

Objetivo 1 – Describir tendencias epidemiológicas y microbiológicas de infecciones osteoarticulares bacteriémicas a lo largo de las últimas décadas.

Artículo 1 – *The changing epidemiology of bacteremic osteoarticular infections in the early 21st century.* O. Murillo, I. Grau, J. Lora-Tamayo, **J. Gómez-Junyent**, A. Ribera, F. Tubau, J. Ariza, R. Pallarés. Clinical Microbiology and Infection 2015; 21(3):254 e1-8.

En este estudio observacional y retrospectivo, se incluyeron todos los episodios de infecciones osteoarticulares bacteriémicas en el Hospital Universitari de Bellvitge entre 1985 y 2011. Se incluyeron 601 episodios, lo que representan un 1.8% del total de bacteriemias durante ese periodo. Las infecciones osteoarticulares eran artritis séptica (46%), osteomielitis vertebral (38%) y osteomielitis periférica (16%). Al comparar los periodos 1985-91 y 2007-11, la incidencia de infecciones osteoarticulares bacteriémicas aumentó de 2.34 a 5.78 episodios/100000 habitantes y año ($p<0.001$). Los episodios nosocomiales y asociados al ámbito sanitario aumentaron de un 18% a un 30% ($p<0.001$) y de un 10% a un 25% ($p<0.001$), respectivamente.

Las características de los pacientes también se modificaron, con un aumento de la edad media (49 a 65 años, $p<0.001$), presencia de comorbilidades (23% a 59%, $p<0.001$) e infecciones asociadas a material ortopédico (7% a 28%, $p<0.001$). El tipo de infecciones también se modificó, con un descenso de la proporción de artritis séptica respecto al total de casos (57% a 38%, $p<0.001$). La incidencia de artritis nativa descendió, con aumento paralelo en el número de infecciones protésicas ($p<0.001$), así como la incidencia de osteomielitis vertebral. La proporción de infecciones asociadas a material ortopédico aumentó del 7% al 28% ($p<0.001$).

Comparado con pacientes jóvenes, los mayores de 65 años presentaron más frecuentemente osteomielitis vertebral, infección de prótesis articular e infecciones enterocócicas. El porcentaje de infecciones osteoarticulares bacteriémicas causadas por cepas de *Staphylococcus aureus* meticilin-sensible descendió, mientras que los casos por *S. aureus* meticilin-resistente, estreptococos, enterococos y bacilos gram-negativos aumentaron.

Objetivo 2 – Analizar las características de las infecciones osteoarticulares bacteriémicas asociadas a la presencia de endocarditis infecciosa.

Artículo 2 – *Endocarditis associated with vertebral osteomyelitis and septic arthritis of the axial skeleton.* O. Murillo, I. Grau, **J. Gómez-Junyent**, C. Cabrera, A. Ribera, F. Tubau, C. Peña, J. Ariza, R. Pallarés. Infection 2018 Apr;46(2):245-251.

Se trata de un estudio observacional (1993-2014) que incluye dos cohortes: a) pacientes con endocarditis infecciosa (n=607) y b) pacientes con infecciones osteoarticulares bacteriémicas (n=458). Hubo 70 casos con endocarditis infecciosa e infección osteoarticular concomitante, representando el 11.5% de todas las endocarditis y el 15% de todas las infecciones osteoarticulares bacteriémicas.

De los casos con endocarditis infecciosa, la infección osteoarticular asociada afectaba principalmente al esqueleto axial (n=54, 77%): 43 eran osteomielitis vertebral (61%), mayormente causadas por microorganismos poco virulentos (estreptococos de los grupos viridans y bovis, enterococos y estafilococos coagulasa-negativos), y 15 eran artritis séptica del esqueleto axial (21%), principalmente causadas por *Staphylococcus aureus*.

La infección osteoarticular bacteriémica con afectación del esqueleto axial se asoció independientemente a la presencia de endocarditis infecciosa (OR ajustada=2.2; IC95% 1.1-4.3), tras ajustar por edad, sexo y microorganismo.

Objetivo 3 – Comparar las características de los pacientes con artritis séptica bacteriémica, en relación con el sitio de adquisición.

Artículo 3 – *Clinical findings of bacteremic septic arthritis according to the site of acquisition: the overlap between healthcare-related and community- and nosocomial-acquired cases.* O. Murillo, **J. Gómez-Junyent**, I. Grau, A. Ribera, C. Cabrera, S. Pedrero, F. Tubau, J. M. Nolla, J. Ariza, R. Pallarés. European Journal of Internal Medicine 2016 Mar;28:38-42.

En este estudio, evaluamos de forma retrospectiva todos los casos de artritis séptica bacteriémica en nuestro hospital entre 1985 y 2013 en relación al sitio de adquisición. Durante ese periodo, hubo 273 casos de artritis séptica bacteriémica, de las cuales el 51% eran comunitarias, el 31% eran nosocomiales y el 18% eran asociadas al ámbito sanitario.

Los casos nosocomiales y asociados al ámbito sanitario se presentaron más frecuentemente en individuos mayores y con más comorbilidades (tratamiento inmunosupresor y enfermedad renal crónica, principalmente). La artritis séptica periférica fue la presentación clínica más habitual. Las infecciones del esqueleto axial predominaron en los casos comunitarios y asociados al ámbito sanitario (24%), mientras que las infecciones de prótesis articular fueron más frecuentemente nosocomiales (44%).

En cuanto a la etiología, los casos por *Staphylococcus aureus* meticilin-resistente y *Pseudomonas aeruginosa* fueron más frecuentemente nosocomiales (21% y 6%, respectivamente) y asociados al ámbito sanitario (14% y 8%, respectivamente). Los casos causados por estreptococos fueron más habitualmente comunitarios (30%) y asociados al ámbito sanitario (28%).

Objetivo 4 – Analizar la mortalidad y factores de riesgo asociados en pacientes con infecciones osteoarticulares bacteriémicas.

Artículo 3 – *Clinical findings of bacteremic septic arthritis according to the site of acquisition: the overlap between healthcare-related and community- and nosocomial-acquired cases.* O. Murillo, **J. Gómez-Junyent**, I. Grau, A. Ribera, C. Cabrera, S. Pedrero, F. Tubau, J. M. Nolla, J. Ariza, R. Pallarés. European Journal of Internal Medicine 2016 Mar;28:38-42.

Artículo 4 – *Analysis of mortality in a cohort of 650 cases of bacteremic osteoarticular infections.* **J. Gómez-Junyent**, O. Murillo, I. Grau, E. Benavent, A. Ribera, X. Cabo, F. Tubau, J. Ariza, R. Pallarés. Seminars in Arthritis and Rheumatism 2018; 48(2):327-333.

En este trabajo, pretendimos analizar la mortalidad y factores de riesgo asociados en una cohorte de infecciones osteoarticulares bacteriémicas, entre 1985 y 2014. En 650 casos, la mortalidad fue del 12.2% (41.8% fallecieron en los primeros 7 días). Comparado con otros tipos de infecciones osteoarticulares, la artritis séptica periférica se asoció con mayor mortalidad (18.6% vs 8.3%, p<0.001). La mortalidad a los 30 días en las artritis sépticas fue mayor en los casos nosocomiales (26%), comparado con las adquiridas en el ámbito sanitario (18%) y comunitarias (7%)

La mortalidad por artritis séptica periférica fue mayor que por otros tipos de infección osteoarticular en todos los grupos de edad. La mortalidad asociada a las infecciones por *Staphylococcus aureus* fue del 14.9%, mayor en las cepas meticilin-resistentes que en las sensibles (26.7% vs 12.6%, p=0.005). Aunque la mortalidad asociada a *S. aureus* fue mayor en todos los tipos de infección osteoarticular, aquellos con artritis séptica periférica presentaron mayor mortalidad. En un modelo multivariado, la artritis séptica periférica (OR ajustada 2.12, IC95% 1.22–3.69, p=0.008) y la infección por *S. aureus* se asociaron a mayor mortalidad (OR ajustada 2.19, IC95% 1.23–3.90, p=0.006).

También evaluamos el impacto del desbridamiento quirúrgico en 239 pacientes con artritis séptica periférica; los pacientes tratados con cirugía no presentaron grandes diferencias comparados con los pacientes manejados de forma conservadora, a excepción de una mayor proporción de infección por *S. aureus*. La mortalidad fue menor en aquellos tratados con desbridamiento quirúrgico (14.7% vs 33.3%, p=0.003). El desbridamiento quirúrgico se asoció a una menor menor mortalidad en un análisis multivariado (OR ajustada 0.23, IC95% 0.09–0.57, p=0.002) y tras propensity-score matching (OR 0.81, 95%CI 0.68-0.96, p=0.014).

Objetivo 5 – Analizar la eficacia y la monitorización plasmática de los beta-lactámicos en infusión continua para las infecciones osteoarticulares por *Pseudomonas aeruginosa* resistente a fluoroquinolonas.

Artículo 5 – *Efficacy and therapeutic drug monitoring of continuous beta-lactams infusion for osteoarticular infections caused by fluoroquinolone-resistant Pseudomonas aeruginosa: a prospective cohort study.* **J. Gómez-Junyent**, R. Rigo-Bonnin, E. Benavent,

L. Soldevila, A. Padullés, X. Cabo, F. Tubau, J. Ariza, O. Murillo. Eur J Drug Metab Pharmacokinet. 2020 May 21. Online ahead of print.

Artículo 6 – *Measurement of ceftolozane and tazobactam concentrations in plasma by UHPLC-MS/MS. Clinical application in the management of difficult-to-treat osteoarticular infections.* R. Rigo-Bonnin, **J. Gómez-Junyent**, L. García-Tejada, E. Benavent, L. Soldevila, F. Tubau, O. Murillo. Clinica Chimica Acta 2019; 488:50-60.

Este estudio prospectivo realizado en el Hospital Universitari de Bellvitge, en el seno de un programa institucional para la optimización del tratamiento antimicrobiano, incluyó a los pacientes con infecciones osteoarticulares por *Pseudomonas aeruginosa* tratados con beta-lactámicos en perfusión continua. Durante el tratamiento, se realizó monitorización plasmática de concentraciones mediante UHPLC-MS/MS. Se incluyeron 52 pacientes, 19 (36.5%) con infecciones por cepas resistentes a fluoroquinolonas. Había 13 pacientes (68.4%) con cepas MDR o XDR y 11 pacientes (57.9%) tenían infecciones asociadas a material de osteosíntesis.

En los pacientes con infecciones osteoarticulares por *P. aeruginosa* resistente a fluoroquinolonas, la duración mediana de los beta-lactámicos en infusión continua fue de 36 días y 10 pacientes (52.6%) recibieron combinaciones con colistina. Se usaron dosis menores de beta-lactámico de las que se hubieran utilizado en caso de administrarse en bolus. Se recogieron 82 muestras plasmáticas y la mayoría de pacientes tuvieron concentraciones de mantenimiento entre 3 y 10 veces la CMI de la cepa aislada. Concentraciones mayores se obtuvieron mayoritariamente en pacientes con insuficiencia renal mientras que concentraciones menores fueron más habituales ante microorganismos con CMI altas. Hubo 17 ajustes de dosis; 8 pacientes necesitaron disminución de la dosis, 5 por insuficiencia renal crónica o aguda.

En los pacientes con infecciones osteoarticulares por *P. aeruginosa* sensible a fluoroquinolonas (n=33), la duración mediana de los beta-lactámicos en infusión continua fue significativamente menor (18 días) y el más utilizado fue ceftazidima. La mayoría recibió tratamiento de combinación con ciprofloxacino, previo a la secuenciación a fluoroquinolona en monoterapia. Se recogieron 110 muestras para

monitorización de concentraciones plasmáticas y se realizaron 19 ajustes de dosis, 10 descensos (5 por insuficiencia renal crónica o aguda).

Hubo 4 pacientes con infecciones por cepas resistentes a fluoroquinolonas que fracasaron (21.1%), similar a aquellos con cepas sensibles tratados con quinolonas (16.7%) (p=0.699). Los beta-lactámicos en infusión continua fueron bien tolerados y el efecto adverso más frecuente fue la insuficiencia renal aguda.

Objetivo 6 – Evaluar la actividad de ceftolozano-tazobactam, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* multiresistente en un modelo dinámico *in vitro*.

Artículo 7 – *Efficacy of ceftolozane/tazobactam, alone and in combination with colistin, against multidrug-resistant Pseudomonas aeruginosa in an in vitro biofilm pharmacodynamic model.* **J. Gómez-Junyent**, E. Benavent, Y. Sierra, C. El Haj, L. Soldevila, B. Torrejón, R. Rigo-Bonnin, F. Tubau, J. Ariza, O. Murillo. International Journal of Antimicrobial Agents 2019; 53(5): 612-619.

De cara a estudiar la actividad de ceftolozano-tazobactam, con y sin colistina, frente a biofilm de *Pseudomonas aeruginosa* multiresistente, se realizaron experimentos mediante un modelo dinámico *in vitro* basado en el CDC Biofilm Reactor. Se utilizaron 3 cepas, todas resistentes a ceftazidima y sensibles a colistina: MDR-HUB1, ceftolozano-tazobactam y meropenem sensibles; XDR-HUB2, ceftolozano-tazobactam sensible y meropenem resistente; y MDR-HUB3, ceftolozano-tazobactam resistente y meropenem sensible. Se compararon pautas de ceftolozano-tazobactam, meropenem y ceftazidima, con y sin colistina, además de monoterapia con colistina.

En cuanto a la actividad de monoterapias, ceftolozano-tazobactam fue poco eficaz pero no aparecieron resistencias, ceftazidima fue ineficaz y colistina fue inicialmente eficaz, pero hubo recrecimiento y aparición de cepas resistentes. Meropenem fue bactericida ante cepas sensibles a carbapenems.

En cuanto a la actividad de las combinaciones, ceftolozano-tazobactam con colistina fue el tratamiento más eficaz ante la cepa resistente a meropenem XDR-HUB2 (Δlog UFC/mL

54-0h = -4.42 vs -3.54 para meropenem-colistina; p=0.002), mientras que esta combinación para la cepa sensible MDR-HUB1 (-4.36) fue menos eficaz que meropenem con colistina (Δlog UFC/mL = -6.25; p<0.001). Ceftolozano-tazobactam con colistina fue ineficaz para la cepa resistente a este betalactámico (MDR-HUB3); meropenem con colistina fue el tratamiento más activo (Δlog UFC/mL = -6.37; p<0.001 vs otros tratamientos).

Las combinaciones de beta-lactámicos activos con colistina evitaron la aparición de cepas resistentes a colistina.

Objetivo 7 – Evaluar las características farmacocinéticas y farmacodinámicas de ceftazidima en infusión continua, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* en un modelo dinámico *in vitro*.

Artículo 8 – *In vitro* pharmacokinetics of ceftazidime and its combinations with colistin against *Pseudomonas aeruginosa* biofilm. **J. Gómez-Junyent**, O. Murillo, H. H. Yu, M. A. K. Azad, H. Wickremasinghe, R. Rigo-Bonnin, E. Benavent, J. Ariza, J. Li. Enviado a publicación.

De nuevo usando el modelo dinámico *in vitro* del CDC Biofilm Reactor, evaluamos la actividad anti-biofilm de concentraciones crecientes de ceftazidima en infusión continua (4, 10, 20 y 40 mg/L), con y sin colistina, frente a *Pseudomonas aeruginosa* sensible a ambos antibióticos. Se emplearon dos cepas, una fue la de referencia PAO1 y la segunda fue una cepa clínica (HUB8) causante de infección osteoarticular.

En cuanto a la actividad de las monoterapias, frente a PAO1 a las 54 horas, se observó una actividad anti-biofilm ligeramente superior con concentraciones de ceftazidima de 20 mg/L (Δlog UFC/mL = -2.84) y 40 mg/L (-3.05). En cambio, no se observaron diferencias frente a la cepa HUB8. Una mayor proporción de colonias resistentes a ceftazidima aparecieron en experimentos con concentraciones menores de este antibiótico. Colistina en monoterapia fue bactericida frente a HUB8 (Δlog UFC/mL = -3.07), pero tuvo una actividad menor frente a PAO1 (-1.12).

Las combinaciones de ceftazidima con colistina incrementaron la actividad de las monoterapias. Se observó mayor actividad anti-biofilm con combinaciones de colistina

y ceftazidima con concentraciones de 40 mg/L (Δlog UFC/mL = -4.19 PAO1; -4.71 HUB8) que con 4 mg/L (-3.10 PAO1; -3.44 HUB8). Las combinaciones evitaron la aparición de cepas resistentes a colistina o ceftazidima.

5. Discusión

5.1. Infecciones osteoarticulares bacteriémicas

5.1.1. La incidencia creciente de las infecciones osteoarticulares bacteriémicas.

Nuestros resultados sugieren una tendencia creciente en la incidencia de las infecciones osteoarticulares bacteriémicas, que se ha asociado a un incremento de la esperanza de vida y de las comorbilidades. Los cambios tecnológicos introducidos en la práctica médica han supuesto una mejora de la calidad de vida de individuos de todas las edades, pero también un incremento de la incidencia de infecciones asociadas a material ortopédico.

El patrón actual de estas infecciones se ha modificado por el impacto de las infecciones asociadas a implantes, que son prácticamente un tercio del total. Esto ha modificado asimismo el patrón de las infecciones nativas, con un incremento de la osteomielitis vertebral. De hecho, los cambios también parecen relacionarse con la edad, ya que la artritis séptica nativa y la osteomielitis periférica predominarían en los jóvenes, mientras que la infección protésica y la osteomielitis vertebral son más frecuentes en mayores.

Aunque *S. aureus* sigue siendo el microorganismo más habitual, su proporción en relación con otros microorganismos ha decaído, en relación con un incremento de casos por estreptococo y enterococo. La introducción de MRSA en los últimos años es asimismo un hecho destacable. Las infecciones por MRSA, estreptococo y enterococo también se han relacionado con una mayor edad.

5.1.2. La asociación de la endocarditis infecciosa con las infecciones osteoarticulares del esqueleto axial.

En este estudio, los diferentes microorganismos causantes de endocarditis infecciosa tuvieron capacidades distintas de producir tipos concretos de infecciones

osteoarticulares secundarias. Curiosamente, los microorganismos menos virulentos (*S. viridans*, *S. bovis*, enterococos y estafilococos coagulasa negativos) presentaron mayor frecuencia de osteomielitis vertebral.

La prevalencia de endocarditis infecciosa asociada a infecciones osteoarticulares varía del 5-10% al 25-30% en función del tipo de infección osteoarticular analizada. En nuestro estudio, el mayor porcentaje de endocarditis fue en aquellos con afectación del esqueleto axial y, de hecho, observamos que la mayoría de infecciones osteoarticulares asociadas con endocarditis infecciosa tuvieron especial tropismo por dichas localizaciones.

Aunque *S. aureus* fue el microorganismo más frecuentemente encontrado, la presencia de infección osteoarticular por los microorganismos menos virulentos se producía frecuentemente en contexto de endocarditis infecciosa. De hecho, observamos que estos microorganismos, en contexto de endocarditis infecciosa, causaron de forma casi exclusiva osteomielitis vertebral. En cambio, pese a que *S. aureus* es capaz de producir todos los tipos de infección osteoarticular, la presencia de artritis séptica del esqueleto axial por dicho microorganismo se produjo habitualmente en contexto de endocarditis infecciosa. En conjunto, la afectación del esqueleto axial (osteomielitis vertebral y/o artritis) en contexto de bacteriemia sugiere la necesidad de descartar endocarditis infecciosa y la realización de ecocardiograma.

5.1.3. La importancia del sitio de adquisición sobre las características y el pronóstico de las artritis sépticas bacteriémicas.

Aunque las diferencias entre infecciones nosocomiales y comunitarias son bien conocidas, en los últimos años han ganado protagonismo aquellas asociadas al ámbito sanitario. En nuestro estudio, hemos mostrado que los casos de infecciones osteoarticulares asociadas al ámbito sanitario comparten características con los casos comunitarios y nosocomiales.

Como era de esperar, los pacientes con infecciones nosocomiales y asociados al ámbito sanitario eran mayores y con más comorbilidades. En cuanto al patrón clínico, las infecciones nosocomiales mostraron predilección por la artritis periférica y la infección

protésica. Estos casos nosocomiales probablemente se relacionan con episodios post-quirúrgicos precoces, mientras que los episodios de infección protésica de adquisición comunitaria o en ámbito sanitario, se podrían relacionar con infecciones hematógenas.

Aunque *S. aureus* fue el principal microorganismo, el porcentaje de MRSA fue francamente elevado en episodios nosocomiales y asociados al ámbito sanitario y prácticamente inexistente en las de adquisición nosocomial. Los estreptococos fueron más frecuentemente causantes de infecciones comunitarias y asociadas al ámbito sanitario, mientras que los bacilos Gram negativos fueron más habituales de adquisición nosocomial y asociadas al ámbito sanitario.

En conjunto, la identificación de los casos relacionados con el ámbito sanitario, incluyendo los episodios nosocomiales, es de gran importancia en la práctica habitual, de cara al diseño del tratamiento antimicrobiano empírico y el manejo clínico global.

5.1.4. La notable mortalidad de las infecciones osteoarticulares bacteriémicas y el rol del desbridamiento quirúrgico en el manejo de las artritis sépticas periféricas.

La mortalidad es un factor pobremente estudiado en trabajos observacionales de infecciones osteoarticulares. En nuestro estudio, la mortalidad fue significativa, especialmente en aquellos con artritis de articulaciones periféricas y con infecciones causadas por *S. aureus*. De hecho, la mortalidad de la bacteriemia estafilocócica es relativamente alta y, especialmente, en casos de bacteriemia por cepas meticilin-resistente.

Estudios previos que incluyeron casos bacteriémicos y no bacteriémicos de artritis séptica han descrito cifras de mortalidad de alrededor del 10%. La alta mortalidad descrita en nuestra cohorte se explica por la inclusión de casos exclusivamente bacteriémicos y por el alto porcentaje de pacientes mayores y con enfermedades subyacentes. Sin embargo, las particularidades de la artritis séptica podrían jugar algún papel, especialmente en relación al acúmulo purulento en un espacio cerrado, el alto inóculo o la significativa respuesta inflamatoria. Los datos de mortalidad en artritis séptica sugieren mayor riesgo en episodios nosocomiales y relacionados con el ámbito

sanitario, especialmente en infección protésica, lo que ha sido descrito en estudios previos.

Nuestros resultados apoyan el desbridamiento quirúrgico para el manejo de pacientes con artritis séptica periférica. Esta maniobra permite evacuar el contenido purulento de la articulación, lo que podría mejorar la actividad antibiótica y reducir la respuesta inflamatoria. En aquellos que no están en condiciones de someterse a cirugía, el drenaje por artrocentesis debería considerarse a la espera de poder realizar el procedimiento quirúrgico.

5.2. Tratamiento antibiótico de las infecciones osteoarticulares por *Pseudomonas aeruginosa*

5.2.1. Los beta-láctámicos en infusión continua son una estrategia válida para las infecciones osteoarticulares por P. aeruginosa resistente a fluoroquinolonas.

Ante la incidencia creciente de infecciones osteoarticulares por *P. aeruginosa* resistente a fluoroquinolonas, son necesarias nuevas estrategias terapéuticas. Nuestros datos sugieren que el uso de beta-lactámicos en infusión continua, guiado por monitorización plasmática de concentraciones y usado frecuentemente en combinación con colistina, se asocia a un pronóstico similar al de los pacientes con infecciones por cepas sensibles y tratados con fluoroquinolonas. Además, su uso fue muy bien tolerado.

El uso de beta-lactámicos en infusión continua permite la optimización de su farmacodinamia, al obtener concentraciones por encima de la CMI durante el 100% del tiempo. En este contexto, la importancia de obtener concentraciones más allá de 4 veces la CMI no es bien conocida, pero podría tener un papel relevante en las infecciones asociadas a biofilm.

La monitorización plasmática de concentraciones de beta-lactámicos fue útil para el manejo clínico de los pacientes. Su uso fue especialmente relevante para aquellos pacientes en las que las concentraciones de mantenimiento (*f*Css) son menos predecibles o más variables (insuficiencia renal) así como en aquellos con infecciones por microorganismos con CMI altas, donde la obtención de *f*Css relevantes puede ser complicado. Obtuvimos concentraciones adecuadas de beta-lactámicos con dosis

menores que las administradas con bolus intermitente. Aunque es improbable que las concentraciones plasmáticas se correlacionen con las locales, el uso de beta-lactámicos en infusión continua probablemente asegura concentraciones locales libres de forma constante, lo que podría explicar en parte los buenos resultados obtenidos.

5.2.2. Colistina en combinación con ceftolozano-tazobactam como estrategia útil en las infecciones de biofilm por P. aeruginosa multiresistente.

El aislamiento de *P. aeruginosa* MDR/XDR limita de forma extraordinaria las opciones terapéuticas en infecciones de cuerpo extraño. La recuperación de colistina y la aparición de nuevos antibióticos, como ceftolozano-tazobactam, abre la posibilidad de nuevas opciones de tratamiento.

Ceftolozano-tazobactam en monoterapia tuvo una eficacia limitada frente a biofilm de *P. aeruginosa*, lo que contrasta con la sorprendente actividad anti-biofilm de meropenem ante cepas sensibles, por un mecanismo no claramente conocido. Algunos estudios que empleaban microscopía confocal habían sugerido buena actividad de meropenem, pero nuestro estudio es el primero en realizar estudios de recuentos bacterianos. Futuros estudios son necesarios para dilucidar los mecanismos de esta actividad anti-biofilm particular de meropenem.

Las combinaciones de beta-lactámicos con colistina incrementó de forma significativa la acción de las monoterapias ante biofilm de *P. aeruginosa*. Mientras que colistina podría actuar ante las capas más profundas del biofilm, dada su actividad preferente ante bacterias con menor metabolismo, los beta-lactámicos podrían actuar ante las bacterias más activas de las capas más superficiales. Ceftolozano-tazobactam con colistina sería la combinación más adecuada para las cepas resistentes a carbapenems (no productoras de carbapenemasas), mientras que meropenem con colistina tendría mayor actividad para las cepas sensibles.

Los beneficios de usar combinaciones de beta-lactámicos con colistina se extienden a minimizar la amplificación de subpoblaciones resistentes a colistina en cepas de *P. aeruginosa* heteroresistentes. Este hecho parece depender de la sensibilidad del beta-lactámico, siendo mayor la protección si la cepa es sensible. Esto contrasta con el uso

de colistina en monoterapia, que se asocia a un cambio poblacional en cepas heteroresistentes con el riesgo de desarrollo de resistencias.

5.2.3. La actividad concentración dependiente de los beta-lactámicos en infusión continua frente a infecciones de biofilm por P. aeruginosa.

El conocimiento de los parámetros PK/PD asociados con mayor eficacia antibacteriana es crucial para definir la dosis óptima para el manejo de las infecciones. Este estudio ha evaluado los datos PK/PD de ceftazidima en infusión continua ante biofilm de *P. aeruginosa* sensible a beta-lactámicos, que sugieren un efecto concentración dependiente del beta-lactámico, especialmente cuando se combina con colistina. Evaluamos concentraciones que se pueden obtener en práctica clínica, lo que puede ser útil para los clínicos.

Ceftazidima en monoterapia (en infusión continua) tuvo una eficacia anti-biofilm notable en ambas cepas de *P. aeruginosa*, pero se observó un ligero aumento de actividad con concentraciones altas ante la cepa PAO1. Asimismo, observamos mayor protección del desarrollo de resistencias a ceftazidima con mayores concentraciones. Este potencial efecto concentración dependiente se observó especialmente con las combinaciones con colistina; ante ambas cepas, altas concentraciones de ceftazidima con colistina fueron más eficaces que las bajas concentraciones con colistina.

Los mecanismos de este efecto concentración dependiente de ceftazidima ante células de biofilm de *P. aeruginosa* no son bien conocidos. Posiblemente, altas concentraciones permiten una mayor difusión de ceftazidima a través de la estructura heterogénea del biofilm. La adición de colistina lo facilitaría, al desestructurar el biofilm y permitir el acceso de beta-lactámicos a subpoblaciones de capas más profundas.

Nuestro estudio también subraya el rol de las combinaciones de ceftazidima y colistina para disminuir la aparición de cepas resistentes a colistina e incluso evitar el cambio poblacional en cepas de *P. aeruginosa* heteroresistentes.

6. Conclusiones (por objetivos)

A. Impacto y pronóstico de la infección osteoarticular

Objetivo 1 – Describir tendencias epidemiológicas y microbiológicas de infecciones osteoarticulares bacteriémicas a lo largo de las últimas décadas.

1.1. Las infecciones osteoarticulares bacteriémicas han aumentado a lo largo del tiempo, en contexto de una población de mayor edad y con más enfermedades crónicas. Este aumento se asocia a una mayor incidencia de bacteriemia, pero también a mayor frecuencia de afectación osteoarticular.

1.2. La osteomielitis vertebral y los casos asociados con cuerpos extraños han presentado un mayor incremento relativo, así como aquellos de adquisición nosocomial o relacionados con el ámbito sanitario.

1.3. *Staphylococcus aureus* es el microorganismo más frecuentemente asociado con las infecciones osteoarticulares bacteriémicas, pero los casos por cepas meticilin-resistentes o por estreptococos y enterococos han aumentado.

Objetivo 2 – Analizar las características epidemiológicas, clínicas y microbiológicas de las infecciones osteoarticulares bacteriémicas asociadas a la presencia de endocarditis infecciosa.

2.1. Las infecciones osteoarticulares bacteriémicas que afectan el esqueleto axial (osteomielitis vertebral y artritis séptica) parecen estar asociadas a endocarditis infecciosa.

2.2. Las endocarditis infecciosas por *Staphylococcus aureus* parecen asociarse a artritis séptica del esqueleto axial, mientras que las causadas por el grupo de microorganismos menos virulentos (*Streptococcus viridans*, *Streptococcus bovis*, enterococos y estafilococos coagulasa-negativo) a osteomielitis vertebral.

2.3. Debería considerarse realizar una ecocardiografía transesofágica en estos pacientes.

Objetivo 3 – Comparar las características y pronóstico de los pacientes con artritis séptica bacteriémica, en relación con el sitio de adquisición.

3.1. Los casos de artritis séptica bacteriémica relacionados con el ámbito sanitario presentan características clínicas similares con casos comunitarios y su etiología se solapa con los casos nosocomiales.

3.2. El sitio de adquisición de la artritis séptica bacteriémica debería considerarse al planificar procedimientos diagnósticos y terapéuticos.

Objetivo 4 – Analizar la mortalidad y factores de riesgo asociados en pacientes con infecciones osteoarticulares bacteriémicas.

4.1. Las infecciones osteoarticulares bacteriémicas presentan mortalidad significativa, especialmente en pacientes mayores, con artritis reumatoide o cirrosis hepática, casos nosocomiales y asociados al ámbito sanitario, y casos causados por *Staphylococcus aureus*.

4.2. La artritis séptica periférica se asocia con mayor mortalidad comparado con otras formas de infección osteoarticular bacteriémica.

4.3. El desbridamiento quirúrgico en la artritis séptica periférica se asocia a menor mortalidad, por lo que debería incorporarse de forma sistemática en el manejo global de estos pacientes.

B. Tratamiento antibiótico de las infecciones osteoarticulares por *Pseudomonas aeruginosa*

Objetivo 5 – Analizar la eficacia y la monitorización plasmática de los beta-lactámicos en infusión continua para las infecciones osteoarticulares por *Pseudomonas aeruginosa* resistente a fluoroquinolonas.

5.1. La administración de beta-lactámicos en infusión continua con monitorización plasmática de niveles es una estrategia terapéutica segura y útil, asegurando concentraciones plasmáticas deseadas y evitando toxicidad.

5.2. La administración optimizada de beta-lactámicos en infusión continua, con o sin colistina, es una estrategia terapéutica prometedora para el manejo de infecciones osteoarticulares por *Pseudomonas aeruginosa* resistente a fluoroquinolonas.

Objetivo 6 – Evaluar la actividad de ceftolozano-tazobactam, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* multiresistente en un modelo dinámico *in vitro*.

6.1. Ceftolozano-tazobactam en monoterapia muestra baja actividad ante cepas multiresistentes de *Pseudomonas aeruginosa* en un modelo dinámico *in vitro*, pero su combinación con colistina sería una alternativa adecuada para cepas resistentes a carbapenems, sensibles a este nuevo beta-lactámico.

6.2. Meropenem, con o sin colistina, muestra mayor eficacia que ceftolozano-tazobactam en este modelo ante la cepa sensible a ambos antibióticos.

6.3. La combinación de beta-lactámicos con colistina podría ser altamente eficaz y proteger frente al desarrollo de resistencias frente a infecciones de cuerpo extraño por *Pseudomonas aeruginosa* MDR/XDR.

Objetivo 7 – Evaluar las características farmacocinéticas y farmacodinámicas de ceftazidima en infusión continua, con y sin colistina, frente a una infección de biofilm por *Pseudomonas aeruginosa* en un modelo dinámico *in vitro*.

7.1. Altas concentraciones de beta-lactámicos en combinación con colistina se asocian con clara actividad anti-biofilm ante *Pseudomonas aeruginosa* en un modelo dinámico *in vitro* y protegen ante el desarrollo de resistencias en estas infecciones.

7.2. Los beta-lactámicos podrían tener actividad concentración dependiente frente biofilms de *Pseudomonas aeruginosa* sensible. Este fenómeno podría ser relevante y debería tenerse en cuenta en la práctica clínica habitual, mediante el uso de altas concentraciones en infusión continua.

SCIENTIFIC PRODUCTION

Most of the studies included in this thesis have been published in scientific journals and/or presented in national or international scientific congress.

Publications in scientific journals

1. The changing epidemiology of bacteremic osteoarticular infections in the early 21st century. O. Murillo, I. Grau, J. Lora-Tamayo, **J. Gómez-Junyent**, A. Ribera, F. Tubau, J. Ariza, R. Pallarés. Clinical Microbiology and Infection 2015; 21(3):254 e1-8. IF: 4.575. First decile under category "Infectious Diseases".

2. Endocarditis associated with vertebral osteomyelitis and septic arthritis of the axial skeleton. O. Murillo, I. Grau, **J. Gómez-Junyent**, C. Cabrera, A. Ribera, F. Tubau, C. Peña, J. Ariza, R. Pallarés. Infection 2018 Apr;46(2):245-251. IF: 2.927. Second quartile under category "Infectious Diseases".

3. Clinical findings of bacteremic septic arthritis according to the site of acquisition: the overlap between healthcare-related and community- and nosocomial-acquired cases. O. Murillo, **J. Gómez-Junyent**, I. Grau, A. Ribera, C. Cabrera, S. Pedrero, F. Tubau, J. M. Nolla, J. Ariza, R. Pallarés. European Journal of Internal Medicine 2016 Mar;28:38-42. IF: 2.960. Second quartile under category "Internal Medicine".

4. Analysis of mortality in a cohort of 650 cases of bacteremic osteoarticular infections. **J. Gómez-Junyent**, O. Murillo, I. Grau, E. Benavent, A. Ribera, X. Cabo, F. Tubau, J. Ariza, R. Pallarés. Seminars in Arthritis and Rheumatism 2018; 48(2):327-333. IF: 5.072. First decile under category "Rheumatology".

5. Efficacy and therapeutic drug monitoring of beta-lactams in continuous infusion for osteoarticular infections caused by fluoroquinolone-resistant *Pseudomonas aeruginosa*: a prospective cohort study. **J. Gómez-Junyent**, R. Rigo-Bonnin, E. Benavent, L. Soldevila, A. Padullés, X. Cabo, F. Tubau, J. Ariza, O. Murillo. Eur J Drug Metab Pharmacokinet. 2020 May 21. Online ahead of print. IF: 1.913. Eighth quartile under category "Pharmacology and Pharmacy".

6. Measurement of ceftolozane and tazobactam concentrations in plasma by UHPLC-MS/MS. Clinical application in the management of difficult-to-treat

osteoarticular infections. R. Rigo-Bonnin, **J. Gómez-Junyent**, L. García-Tejada, E. Benavent, L. Soldevila, F. Tubau, O. Murillo. Clinica Chimica Acta 2019; 488:50-60. IF: 2.615. First decile under category "Medical Laboratory Technology".

7. Efficacy of ceftolozane/tazobactam, alone and in combination with colistin, against multidrug-resistant *Pseudomonas aeruginosa* in an *in vitro* biofilm pharmacodynamic model. **J. Gómez-Junyent**, E. Benavent, Y. Sierra, C. El Haj, L. Soldevila, B. Torrejón, R. Rigo-Bonnin, F. Tubau, J. Ariza, O. Murillo. International Journal of Antimicrobial Agents 2019; 53(5): 612-619. IF: 4.621. First quartile under category "Infectious Diseases".

8. *In vitro* pharmacokinetics of ceftazidime and its combinations with colistin against *Pseudomonas aeruginosa* biofilm. **J. Gómez-Junyent**, O. Murillo, H. H. Yu, M. A. K. Azad, H. Wickremasinghe, R. Rigo-Bonnin, E. Benavent, J. Ariza, J. Li. Submitted for publication.

Papers at scientific conferences

1. Changing epidemiology of bacteremic osteoarticular infections in a teaching hospital in Barcelona. **J. Gómez-Junyent**, J. Lora-Tamayo, O. Murillo, I. Grau, M. Cisnal, J. Ariza, R. Pallares. 23th ECCMID, Berlin, Germany, 2013. Presentation number O286.

2. Infectious endocarditis among patients with bacteremic osteoarticular infections. C. Cabrera, O. Murillo, I. Grau, **J. Gómez-Junyent**, A. Ribera, C. Peña, F. Tubau, J. Ariza, R. Pallares. 25th ECCMID, Copenhague, Denmark, 2015. Presentation number P0007.

3. Cambios epidemiológicos de la artritis séptica bacteriémica en un hospital universitario (1985-2011). **J. Gómez-Junyent**, O. Murillo, I. Grau, J. Lora-Tamayo, M. Cisnal, J. Ariza, R. Pallarés. XVII SEIMC. Zaragoza, España, 2013. Presentation number P135.

4. Mortalidad y factores pronóstico en una cohorte de pacientes con bacteraemia e infecciones osteoarticulares. **J. Gómez-Junyent**, O. Murillo, I. Grau, C. Cabrera, X. Cabo, F. Tubau, J. Ariza, R. Pallarés. XX SEIMC, Barcelona, Spain, 2016. Presentation number O060.

5. Betalactámicos en infusión continua o extendida para el tratamiento de infecciones osteoarticulares por bacilos gram negativos un estudio piloto. **J. Gómez-Junyent**, L. Soldevila, E. Benavent, R. Rigo, A. Ribera, X. Cabo, F. Tubau, J. Ariza, O. Murillo. XXI SEIMC, Málaga, Spain, 2017. Presentation number O154.

6. Efficacy of ceftolozane-tazobactam alone and in combination with colistin against extensively drug-resistant *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic biofilm model. **J. Gómez-Junyent**, E. Benavent, Y. Sierra, F. Tubau, L. Soldevila, B. Torrejón, J. Ariza, O. Murillo. 28th ECCMID, Madrid, Spain, 2018. Presentation number O0078.

7. Eficacia de ceftolozano-tazobactam y su combinación con colistina frente a *Pseudomonas aeruginosa* multirresistente en un modelo farmacodinámico *in vitro* de cuerpo extraño. **J. Gómez-Junyent**, E. Benavent, Y. Sierra, F. Tubau, L. Soldevila, B. Torrejón, J. Ariza, O. Murillo. XXII SEIMC, Bilbao, Spain, 2018. Presentation number O0205.

8. Pharmacokinetics/Pharmacodynamics of ceftazidime with and without colistin against *Pseudomonas aeruginosa* biofilms using an *in vitro* model. **J. Gómez-Junyent**, O. Murillo, H. Yu, MH. Azad, H. Wickremasinghe, E. Benavent, C. El Haj, L. Soldevila, J. Ariza, O. Murillo. 29th ECCMID, Amsterdam, The Netherlands, 2019. Presentation number P0574.

TABLE OF CONTENTS

ABBREVIATIONS

95% CI: 95% Confidence Interval

AEMPS: Agencia Española del Medicamento y Productos Sanitarios

BL: Beta-lactam

BL-CI: Continuous Beta-lactam Infusion

CBR: CDC Biofilm Reactor

CI: Continuous Infusion

CLSI: Clinical and Laboratory Standards Institute

CLSM: Confocal Laser Scanning Microscopy

C_{max}: Peak concentration

CMS: Colistimethate sodium

CNS: Coagulase-negative Staphylococci

COPD: Chronic Obstructive Pulmonary Disease

Css: Steady-state Concentration

DAIR: Debridement, Antibiotics and Implant Retention

ESBL: Extended-Spectrum Beta-lactamase

EMA: European Medicines Agency

*f*Css: Free Steady-state Concentration

GEIO: Grupo Español de Infecciones Osteoarticulares

GNB: Gram-negative Bacilli

GPC: Gram-positive Cocci

HPLC: High-performance Liquid Chromatography

IB: Intermittent Bolus

IDUs: Intravenous Drug Users

IE: Infective Endocarditis

IQR: Interquartile Range

MBEC: Minimum Biofilm Eradication Concentration

MBIC: Minimum Biofilm Inhibitory Concentration

MDR: Multi-drug Resistance

MHA: Mueller Hinton Agar

MHB: Mueller Hinton Broth

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin-resistant *Staphylococcus aureus*

MSSA: Methicillin-susceptible *Staphylococcus aureus*

NA: Nutrient Agar

OAI: Osteoarticular Infection

OD: Optical Density

OR: Odds Ratio

PAP: Population Analysis Profile

PBS: Phosphate Buffered Saline

PJI: Prosthetic Joint Infection

PK/PD: Pharmacokinetics/Pharmacodynamics

PO: Peripheral Osteomyelitis

REIPI: Red Española de Investigación en Patología Infecciosa

SA: Septic Arthritis

SAT: Suppressive Antimicrobial Treatment

SD: Standard Deviation

SEIMC: Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica

$t_{1/2}$: Half-life

T>MIC: Time above MIC

TBC: Total Body Clearance

TDM: Therapeutic Drug Monitoring

TSA: Trypticase Soy Agar

TSB: Trypticase Soy Broth

UHPLC-MS/MS: Ultra-high Performance Liquid Chromatography with Tandem Mass Spectrometry

VO: Vertebral Osteomyelitis

XDR: Extensively Drug-resistant

INTRODUCTION

1. Osteoarticular infections

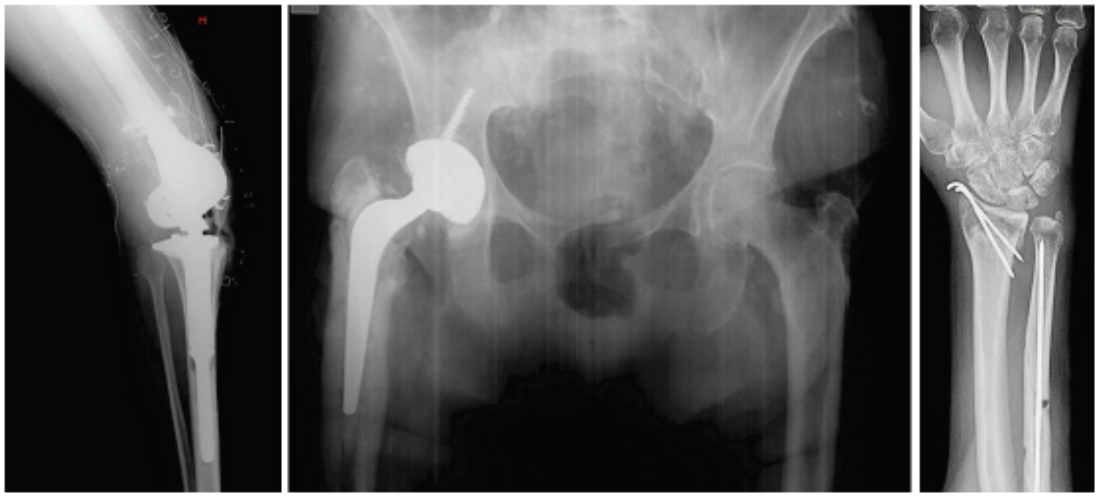
Osteoarticular infections (OAIs) include a wide range of infections affecting bone and joints, often with the participation of orthopaedic devices or implants. OAIs are often defined as difficult-to-treat infections and usually require a multidisciplinary approach involving orthopaedic surgeons, infectious diseases specialists and clinical microbiologists.

Due to ageing of population, the frequent management of orthopaedic diseases with surgery and the growing prevalence of individuals with multiple baseline conditions and immunosuppressive diseases or treatments, the incidence of OAIs seem to be increasing over time. Therefore, it should be considered as a first magnitude health problem.

1.1. Orthopaedic device-related infections – Prosthetic joint infections

As mentioned above, OAIs often involve an orthopaedic device, such as intramedullary nails, external-fixation pins, plates, screws… which are used for the fixation of fractures. Orthopaedic devices also include material used for the management of conditions of the vertebral spine, such as scoliosis, canal stenosis or disk herniation.

Figure 1. Radiographies of different orthopaedic devices.



The classical paradigm of device-related OAIs are prosthetic joint infections (PJI), which are a serious and feared complication of joint replacement. The implant of prosthetic

joints has significantly increased over the years (1) and has improved the quality of life of most individuals. Most prosthesis are implanted in patients with joint degenerative diseases, such as arthrosis, aseptic necrosis of the femoral head or rheumatoid arthritis. Other prostheses are placed in patients with certain types of fracture of the femoral head, especially among older patients. Most prostheses are placed in the hip or the knee, but other joint locations may also be replaced, such as the shoulder, the elbow or the ankle.

Prosthetic joints can involve all components of the joint (total prosthesis) or only a part, as in the case of hip hemiarthroplasties or unicompartmental prosthetic knee joints. Metallic components of the prostheses, which can be made of different materials, are frequently stabilized by cementing them to the bone. Prosthetic joints can either be primary (a first substitution of the native joint) or a revision arthroplasty (secondary and subsequent substitution of prosthetic joints).

1.2. Epidemiology and risk factors

OAIs are a growing problem in the infectious disease field, frequently seen in hospitals and emergency departments. However, the current prevalence and incidence of non-device related OAIs, such as native septic arthritis (SA) and osteomyelitis, are often difficult to precise. A retrospective study performed in the United States between 2009-2012 found that SA was responsible for more than 16,000 visits in Emergency Departments, representing 0.01% of all visits (2).

Regarding PJI, different studies suggest that the overall likelihood of infection is 0.5-4% (1, 3-7). Apparently, the rate of PJI is highest during the first two years after index arthroplasty, being 1.5%, compared to 0.5% after two years of prosthesis placement (8). Infections in the first 2 years may represent around 70% of all PJI (6).

Several risk factors have been described for OAIs, some of which are shared by the different entities. These include underlying baseline conditions, such as diabetes mellitus, rheumatoid arthritis, liver cirrhosis or chronic kidney disease. The use of immunosuppressive drugs, including steroids, have also been associated with OAIs (9). Regarding device-related infections, pre-operative factors, like obesity or ASA score, and

post-operative complications, such as hematoma, superficial surgical site infection, wound drainage and wound dehiscence, have been linked to a greater risk of infection. A revision prosthesis is also associated with a higher risk of PJI, compared to a primary prosthesis (5, 10-12).

Bacteraemia is often intimately associated to particular OAIs, like SA or vertebral osteomyelitis (VO). In contrast, device-related infections are mostly acquired in the post-operative phase, usually without concurrent bacteraemia. However, a haematogenous seeding to devices can also occasionally occur and represent a well-recognized form of infection. Actually, the risk of haematogenous seeding is also dependent on the microorganism causing the bacteraemia and has been found to be significantly higher with *Staphylococcus aureus* (30-40%) (13, 14).

1.3. The impact of osteoarticular infections

OAIs represent an important health problem in our current societies, with repercussions on patient's quality of life and also on health systems. At the individual level, OAIs can cause relevant morbidity and patients may require multiple surgeries, long antimicrobial treatments and sequelae with immobility, leading occasionally to the need of wheelchairs or even becoming bedridden. All these factors can also have a psychological impact on affected patients (15).

Although overall mortality rates may not be as significant as with other infections, this may be changing due to a shift in patients' characteristics. The ageing of the population and the more frequent coexistence of multiple medical comorbidities may be leading to an increase in mortality rates of patients with OAIs. Actually, OAIs may result in decompensation of underlying diseases in old patients. However, it should be considered that OAI-related mortality has received scarce attention in the literature.

Since patients with OAI usually require long hospital admissions and multiple interventions and antimicrobial treatments, it is clear that such infections may have an impact on already budget-constrained health systems. The global management of OAIs is costly, which includes not only the treatment of the infection itself, but also patients' rehabilitation, with further admissions at specialized centres (4, 16).

Therefore, it is important to increase our current knowledge on OAIs in order to improve the healthcare provided. Most studies of OAIs are observational, retrospective and unicentric in their design; moreover, they often include heterogeneous cohorts of patients with OAIs, caused by different microorganisms. There is a clear need of randomized clinical trials and large observational multicentre studies, in order to clarify issues concerning diagnosis, treatment and prognosis.

2. Pathogenesis and microbiological aspects

2.1. Aetiology of osteoarticular infections

The microbiology of OAIs depend on the type and source of infection and the characteristics of patients, including their medical baseline conditions. The site where the infection has been acquired may also play a role in defining the aetiology of the infections. In this line, nosocomial and healthcare-related infections, which have clearly emerged in the last decades, may be associated with the presence of specific microorganisms and, also, with particular patterns of antimicrobial resistance (17, 18).

Non-device associated infections, like SA and osteomyelitis, are mainly caused by *S. aureus*, representing more than 50% of all cases (19-24). Streptococci are the second most frequent aetiologic agent (30%) and may be more frequent among the elderly. Gram-negative bacilli (GNB) represent around 15-20% of all cases, occurring usually in older adults, immunosuppressed patients or intravenous drug users. The most frequent species are *Escherichia coli* and *Pseudomonas aeruginosa*. The occurrence of GNB is also frequent in patients with osteomyelitis associated with open fractures after trauma and such infections tend to be polymicrobial (25). Fungal infections are infrequent.

The aetiology of device-related infections, particularly PJI, is similar to OAIs without device, but some considerations should be made. Again, the microbiology is dependent on the type of infection (see below) and the patients' characteristics (7, 12). A large multicentre study in Spanish hospitals during 10 years including more than 2000 PJI showed that Gram-positive cocci (GPC) are predominant, accounting for almost 80% of all cases (26). Coagulase-negative staphylococci (CNS) are the most frequent GPC,

especially in chronic infections (27), whereas *S. aureus* represents around 30% of all cases, especially in early and haematogenous infections. Streptococci represent 8-10% of all cases, but more frequently in the context of haematogenous infections and older adults, whereas Enterococci represent 8% of PJI. PJI by GNB account for 25% of all cases and, again, *E. coli* and *P. aeruginosa* are the most frequent microorganisms. Anaerobic bacteria cause PJI in less than 7% of all cases, but the role of *Propionibacterium acnes* should be acknowledged, especially in shoulder infections (28, 29). Polymicrobial infections are also frequent in the setting of PJI, especially in early post-operative infections (27, 30).

It is important to consider that the microbiology of PJI may be changing over time, as suggested by Benito *et al* (26). The authors found that there was a trend towards a mild decline in cases by GPC, parallel to an increase in cases by GNB. Importantly, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) microorganisms is likely to change this scenario, with significant increases in cases caused by these resistant bacteria. As an example, methicillin-resistant *S. aureus* (MRSA) and MDR GNB may account for 8-14% and 4-7% of all cases, respectively.

2.2. The role of bacterial biofilm

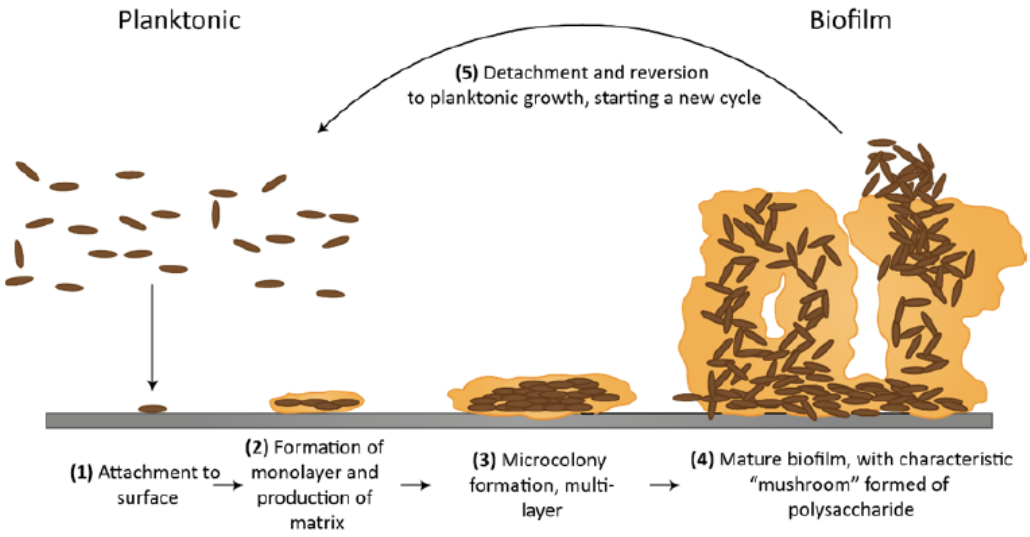
OAIs are generally considered difficult-to-treat infections, due to the presence of bacterial biofilms. This is especially true with device-related infections and chronic osteomyelitis, due to the presence of foreign or biologic inert material. The biofilm component of other acute OAIs, such as SA or VO, may also be present but less predominant. Bacterial biofilms represent a challenge for the antimicrobial management of these infections and are often the cause of clinical failure because they are very difficult to eradicate (7, 31).

Bacterial biofilms are defined as complex communities of bacterial cells enclosed in a hydrated extracellular matrix and generally adhered to inert or living surfaces (32). The extracellular matrix of biofilms contains polysaccharides, proteins and bacterial DNA and depends on the bacterial strain and the growth conditions (33). Channels within the

biofilm structure allow the flow of water and nutrients (34). Almost all bacteria organize themselves in such structures in order to survive in hostile environments.

The formation of biofilm begins when bacterial cells attach to surfaces (Figure 2). Then, cells can proliferate and initially form small microcolonies which can lead to large populations of aggregated cells. Actually, some studies have suggested that aggregates of free-floating bacteria may have advantage to attach to surfaces and form biofilm structures, compared to individual cells (35, 36).

Figure 2. Representation of biofilm formation and life cycle (37).

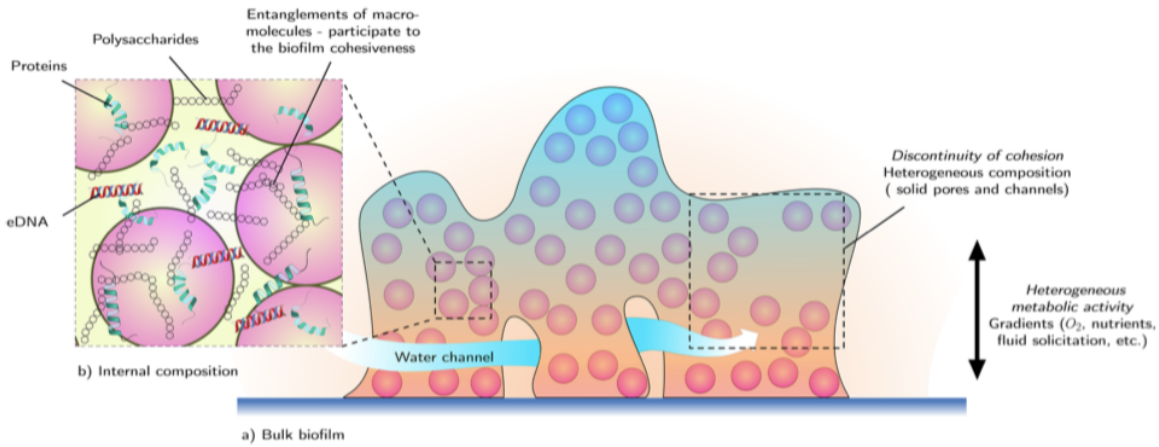


Then, bacteria excrete the extracellular matrix that will protect them from environmental challenges. In this stage, the biofilm is still unstable and susceptible to eradication. Maturation of the biofilm is a critical step in avoiding its eradication and is mediated by several physiological regulatory processes. Among such processes, quorum-sensing plays a key role. This is a cell-to-cell communication process which allows bacteria to adapt their phenotype in response to cell density and composition of the biofilm (38). The synthesis of signalling molecules called autoinducers usually parallels an increase in the cell population and can allow gene regulation by individual cells. As a result, biofilm-embedded cells undergo several metabolic and replicative changes which will transform the biofilm into a sessile structure and allow the survival

of the community (39). Within a mature biofilm, cells can disperse and modify their status to planktonic growth, colonizing close or distant sites, initiating the cycle and extending the biofilm (32, 40).

Biofilm structures are heterogeneous and nutrient and oxygen gradients are present according to the different layers. Outer or superficial layers of the biofilm tend to have greater concentration of nutrients, whereas cells in the deeper or inner layers of the biofilm survive in an anaerobic environment (41, 42). In this line, cells in the deeper biofilm layers usually reduce their metabolic and growth status, whereas cells in the outer layers may be more similar to cells in the planktonic state.

Figure 3. Physical heterogeneity of bacterial biofilms (41).



The biofilm structure largely explains why biofilm-associated infections are generally difficult to eradicate only with antibiotics. Several inherent resistance mechanisms have been defined for biofilm infections:

- Slow penetration of antimicrobial agents: Particular molecules may have difficulties in accessing the glycoproteic matrix. Glycopeptides may have special difficulty in diffusing within the biofilm (43).

- Inactivation within the biofilm: An increased concentration of extracellular beta-lactamases has been described within biofilms when exposed to beta-lactams (44-46) and aminoglycosides are usually inactive in acidic pH (47).

- Tolerance to antibiotics: This is a characteristic mechanism of resistance. Most antimicrobial agents are active when bacteria are in a growing state. Thus, given the altered growth rate of biofilm-embedded cells, susceptibility is dramatically modified as compared to the same microorganism in the planktonic state and higher antibiotic concentrations are usually needed (32).

- Formation of persister cells: These slow or non-dividing cells (dormant state) are less susceptible to antibiotics and can lead to infections when antibiotics are not present. They are usually a very low fraction within biofilm structures and are different from classical tolerant subpopulations (48).

It is also important to acknowledge that biofilms can develop protective mechanisms against host immune responses (49). Other acquired mechanisms of antimicrobial resistance have also been described, such as horizontal gene transmission and increased mutation frequency (50-52).

Some bacteria may also be present as intracellular microorganisms (53), which have been described in the setting of clinical and experimental foreign-body infections (54). These intracellular microorganisms may be less susceptible to the activity of the immune system (55) and some antimicrobials may have difficulties in accessing the intracellular space or may be inactivated once inside. Particular intracellular microorganisms, including *S. aureus*, may also be able to escape phagocytosis, avoiding the elimination of the pathogen (56, 57).

Taken together, all these phenomena make it very challenging and almost impossible to eradicate biofilm-associated infections only with antimicrobial agents. Indeed, in the setting of OAIs, a surgical approach is almost always necessary, together with an individualized antimicrobial treatment tailored to patients' characteristics and infection particularities.

2.3. Particular microorganisms

2.3.1. *Staphylococcus aureus*

S. aureus is a major cause of community-acquired and nosocomial infections and has a significant ability to attach to medical devices, causing biofilms and chronic infections. *S. aureus* has also a particular tropism for the osteoarticular tissue, especially necrotic or damaged tissue (58, 59). Several factors have been implicated in biofilm formation, such as the polysaccharide intercellular adhesion, surface-associated proteins or eDNA within the biofilm matrix (60). *S. aureus* biofilms also have dispersal strategies which will facilitate the dissemination of the infection and seem to be mediated by proteases that are regulated with the quorum sensing system *agr* (61, 62).

Although also present in other microorganisms, the existence of certain subpopulations of *S. aureus* presenting as small colony variants has been linked to infection persistence (63). These are phenotypically different colonies of *S. aureus* that can persist in host cells and resist intracellular elimination, causing recurrent infections (64). These small colony variants need to regulate their metabolism and growth to persist intracellularly and have been viewed as a means of survival for *S. aureus* inside cells (65).

2.3.2. *Pseudomonas aeruginosa*

P. aeruginosa is another well-known microorganism prone to biofilm formation and recurrent infections. Although the classical paradigm are respiratory infections in patients with cystic fibrosis or chronic respiratory conditions, it can cause biofilm-associated infections in multiple tissues (66). It has an ability to adapt to anaerobic environments, forming mucoid biofilms, and can also develop resistance mechanisms when challenged by antibiotic pressure. Actually, biofilm-forming *P. aeruginosa* may be more prone to mutation than planktonic cells (50). In this line, strains with high mutation rates have also been described, which may be more frequently present within biofilms (67).

3. Clinical aspects

3.1. Clinical presentation and classification

The clinical presentation of OAIs depend on the route of acquisition, the presence of a device and on the virulence of the causative microorganism. The anatomic location affected by a particular OAI also influences the clinical presentation.

In this line, SA can usually be divided into peripheral or axial, depending on the location of the affected joints. Patients with peripheral SA usually present acutely with a warm, swollen and painful joint together with fever and movement restriction of the affected joint (9, 21). Low virulent microorganisms can result in a subacute presentation. SA can either be monoarticular or polyarticular. The most frequently involved joints are the knee and the hip, followed by the shoulder and the ankle. Axial arthritis include those occurring on acromioclavicular, sternoclavicular, sternoocostal, pubic symphysis, interapophyseal, and sacroiliac joints and have been poorly described in the literature; such joints have particular characteristics (limited movement range, some have small joint spaces, some are cartilaginous joints) and septic arthritis in these locations may result in particular clinical presentations.

VO usually presents as back pain, which is exacerbated with physical activity and palpation of the affected area, motion limitation and fever (68). The most frequent sites are the lumbar spine, followed by the thoracic spine and the cervical spine (69). Neurological symptoms such as radicular pain, motor and sensory symptoms or even cord compression may occasionally be present and suggest the extension of the infection to the epidural space.

The clinical symptoms associated with PO will also depend on the route of acquisition (trauma, haematogenous seeding…) and whether its presentation is acute or chronic (70, 71). Acute PO usually presents with pain at the involved site, local findings (tenderness, warmth, erythema) and fever. Chronic PO may also present with pain, erythema or swelling and a sinus tract is often encountered.

Regarding implant-associated infections, the most available knowledge is based on research focused on PJI. Many extrapolate classifications on PJI for other implant-associated infections, although they are definitely not the same entities. Further studies

will necessarily deal with these issues and classify all implant-associated infections according to their particularities.

In the last decades, many classifications for PJI have been suggested, which mainly depend on the route of acquisition and the time to infection from index arthroplasty. The most frequently used classifications are those suggested by Tsukayama (72) and Zimmerli (7). In recent years, Zimmerli and colleagues have proposed a new classification (73):

- Acute haematogenous: Infection with a symptoms duration of 3 weeks after an uneventful postoperative period.
- Early post-interventional: Infection that manifests within 1 month after an invasive procedure (surgery or arthrocentesis).
- Chronic: Infection with symptoms duration >3 weeks, beyond the early post-interventional period.

Different causative microorganisms (with different virulence) probably explain the fact that these forms present differently according to time to infection or with limited symptoms.

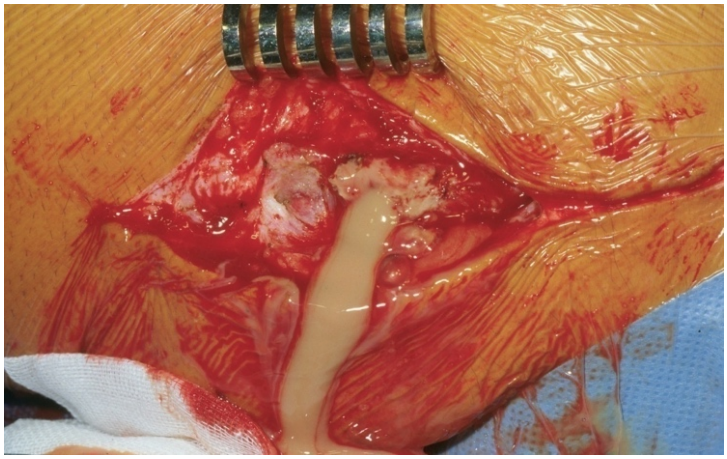
These classifications have been used to select those patients to be managed successfully with debridement, antibiotics and implant retention (DAIR), avoiding implant removal, although there is still discussion on this issue. Actually, the choice of DAIR over implant removal according to time to infection from arthroplasty is still unresolved and controversial in the most recent classifications or guidelines, such as the Second International Consensus Meeting on orthopaedic infections (74).

Early post-interventional and haematogenous PJI usually present as an acute event with warmth, swelling and erythema of the affected joint. Joint effusion is also a frequent symptom, as purulent discharge from the wound. Fever and bacteraemia (especially with haematogenous PJI) may be present, occasionally with shock; the collection of blood cultures is recommended. These forms are usually caused by virulent microorganisms, such as *S. aureus*, *Streptococcus* spp. and GNB.

Late chronic PJI usually presents with mild or moderate symptoms such as chronic pain, functional limitation and occasionally joint effusion. Low-grade warmth and erythema

may also be present. A sinus tract, although often missing, is highly suggestive of such infections. Less virulent microorganisms such as coagulase-negative staphylococci or *P. acnes* are the most common causative microorganisms.

Figure 4. Sinus tract with purulent discharge from a PJI.



3.2. The role of bacteraemia

Bloodstream infections have a close relationship with OAIs, either implant-associated or not. Actually, whether bacteraemia is the cause or consequence of an OAI is often difficult to elucidate. Certain OAIs are frequently associated with the concomitant presence of bacteraemia, such as native SA or VO (22-24). Implant-associated infections are most frequently post-surgical, where the occurrence of bacteraemia is usually uncommon. However, such infections can also be haematogenous, in the setting of a bacteraemia, either documented or not.

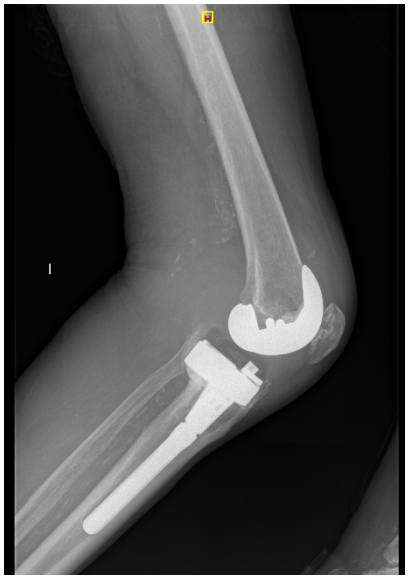
S. aureus is the paradigm of a microorganism presenting a haematogenous seeding to bone, joints and associated devices, although Streptococci may also cause such infections. The proportion of patients with metastatic infection of a joint prosthesis among those with staphylococcal bacteraemia can be as high as 34% (13). A study has suggested that the risk of a haematogenous seeding in patients with *S. aureus* bacteraemia may be higher if it is community-acquired (in contrast to nosocomial) and also associated with the number of arthroplasties (75).

3.3. Diagnosis of osteoarticular infections

The clinical diagnosis of OAIs depends on the presence of classical symptoms and signs, a compatible physical examination and the performance of complementary tests. Local inflammatory signs, fever, sinus tract and/or wound discharge may suggest an OAI. Late chronic implant-associated infections may be much more difficult to diagnose, since often the only symptom is chronic insidious pain, without local findings (3).

White cell count, C-reactive protein and erythrocyte sedimentation rate should be measured upon suspicion of an OAI. These parameters can support the presumptive diagnosis, although their sensitivity and specificity is usually low (7, 9, 21, 70, 76, 77). However, they are useful to monitor the response to treatment, although the preferred marker is C-reactive protein (78).

Figure 5. Radiologic signs of implant loosening in a patient with prosthetic joint infection.



Imaging is also a key part of the diagnostic evaluation of OAIs. Plain radiographs are an important first step, as they can reveal an alternative diagnosis and detect suggestive signs of infection. Although rarely useful for native SA diagnosis, they can be helpful for VO and PO; for the latter, it may reveal the presence of bone destruction or sequesters (71). Plain radiographs are also very useful for the diagnosis of PJI, especially with late chronic infections and studied serially over time (79). Typical radiologic signs of infection include peri-implant radiolucency, peri-implant osteolysis and prosthesis loosening or components migration. They can also help discriminate if the implant is fixed.

VO and PO usually require more complex imaging techniques, like CT-scan and MRI. CT-scan can discriminate between normal and abnormal tissue, provide excellent images of sequestered tissue and fair description of surrounding soft tissues. However, image artefacts in the presence of implants can limit their interpretability. Currently, the main

role of CT-scan is as an alternative when MRI cannot be performed and also to perform guided biopsies for microbiological sampling.

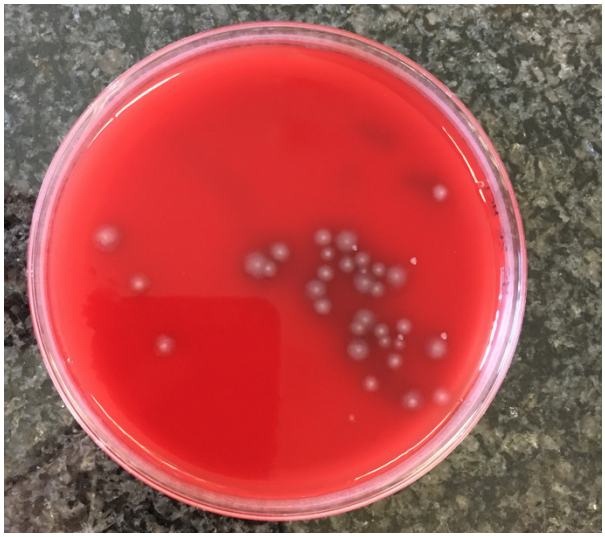
MRI currently represents the gold standard for the diagnosis of VO, although it is sometimes used for PO. Its main limitation is its contraindication in patients with incompatible implants. MRI is very sensitive and accurate for the diagnosis of VO, showing high intensity in the affected disk and two adjacent vertebral bodies (80). It has the advantage of providing accurate images of surrounding soft tissues, revealing the presence of abscesses, and is very helpful when the patient has neurological symptoms and signs, in order to rule out epidural/medullary complications. However, it should be noted that bone oedema or bone/disk findings may persist beyond end of treatment without representing therapeutic failure; thus, repeating MRI after treatment is probably unnecessary in the absence of symptoms/signs suggesting failure (81).

Nuclear medicine can also be used as an imaging technique, mainly for the study of presumptive PJI (82, 83). Gammagraphy with ^{111}In-marked leukocytes has traditionally been used, although it can be limited by a suboptimal sensitivity and specificity, especially with non-cemented prosthesis. In recent years, positron-emission tomography with 18-F-Fluoro-D-deoxyglucose has been incorporated in the diagnostic workout of OAIs (84, 85), although it is not widely available and systematically performed.

Microbiological studies are fundamental in OAIs diagnosis and characterization. Blood cultures should be collected in many cases, especially with an acute presentation of fever and local inflammatory signs. Due to the high percentage of bacteraemia in native SA and VO, it should be common clinical practice to obtain them before antibiotic treatment. The causative microorganism can be known with positive blood cultures and, frequently in the case of VO, they can spare the performance of invasive procedures.

Local samples are essential in most cases, especially when the infection is not associated with concomitant bacteraemia. Pre-operative samples, such as swabs from sinus tracts, usually have low predictive value and may often reveal the colonization of the sinus tract from the patient's skin flora. However, results from swabs may be relevant if virulent microorganisms such as *S. aureus* are isolated (3).

Figure 6. Trypticase soy agar plate showing colonies of *P. aeruginosa*.



Obtaining local samples prior to surgery should be part of the routine diagnostic workup, when possible. Clinicians should aim to obtain high-quality samples, such as synovial fluid, purulent material from abscesses or bone biopsy. Procedures to obtain such samples, such as arthrocentesis or imaging-guided bone biopsy, should be performed. Joint aspirate cell count may suggest infection (especially when >1700 leukocytes/mm^3 or if >65% neutrophils) (86) and may rule out crystal arthritis, which can mimic SA (87). Gram staining can occasionally reveal the potential aetiological agent (88) and all samples should be sent for culture, in order to identify the causative microorganism and susceptibility profile.

Positive intraoperative cultures are of high value and, thus, antibiotics should be withheld (if possible) until the collection of such samples, unless the microorganism has been previously identified. Regarding implant-associated infections, the number of samples that should be collected is not well defined and probably differs according to the type of infection (acute vs chronic). Some authors have recommended that several peri-implant samples (at least 5-6) should be taken, which is probably most useful for chronic post-operative infections. Such number of samples may facilitate ruling out the presence of contaminant microorganisms from the patient's skin flora, such as coagulase-negative staphylococci (CNS) or *P. acnes*. Isolating the same microorganism in ≥2 samples has >97% specificity (89), although isolating virulent microorganisms (*S. aureus*, GNB or virulent Streptococci) in ≥1 sample may be sufficient. Sonication of implants has also been suggested to improve the diagnostic yield, especially in chronic infections (90). Samples in both aerobic and anaerobic media should be incubated at least 7 days and a longer incubation may increase the rate of positive cultures, especially

for slow-growing bacteria (91). The incubation of samples in media for mycobacteria and fungi is also usually performed.

Histopathological findings can also support the diagnosis of OAI. The presence of 1-10 polymorphonuclear leukocytes in peri-implant samples defines acute inflammation and is suggestive of infection (92). Other findings may guide the causative agent, such as the presence of granulomas for *M. tuberculosis*.

4. Surgical management

The adequate management of OAIs is complex and requires the participation of multiple medical and surgical specialists. The reasons behind the need of a multidisciplinary approach rely on the frequent presence of biofilms, the multiple comorbidities affecting patients and the importance of preserving the functional mobility and tissues of the infected site. Surgery is central in the management of OAIs, by removing necrotic and infected tissue and the implant, if present, as well as reducing the bacteria inoculum, which facilitates the activity of the antimicrobial therapy. Given the tolerance within biofilms, it is unlikely that osteoarticular biofilm-associated infections may be cured only with antibiotics.

The decision on which surgical approach should be performed is often not obvious and a case-by-case approach is usually necessary. Despite this and especially regarding PJI, in the last decades, multiple authors have suggested algorithms to select the optimal management for each patient. Surgeon's and patient's preferences are also important factors in the management of OAIs.

4.1. Implant-associated infections

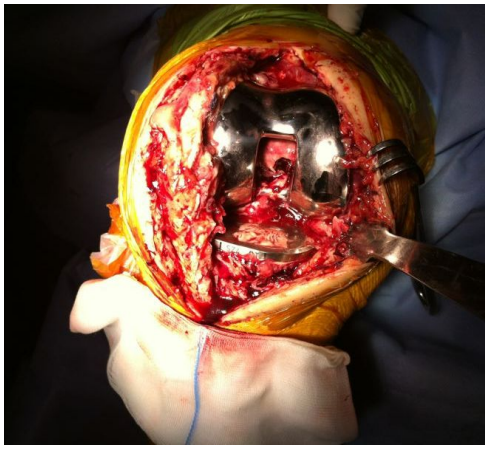
Whether implant-associated infections can be managed with implant retention or removal will depend on multiple factors. Often, the same criteria are followed for all implant-associated infections, although the most available knowledge is based on PJI. For the purpose of this section, the management of PJI will be illustrated, although it may be applicable to other implant-associated infections.

4.1.1. Debridement, antibiotics and implant retention (DAIR)

This treatment strategy allows the maintenance of the original prosthesis, while removing necrotic tissue, haematoma, abscesses and debris. A thorough debridement is recommended to increase the likelihood of cure and, in this line, the removal of mobile components of the prosthesis (e.g. polyethylene) is usually performed (93, 94). Actually, not removing such components is commonly associated with an increased failure risk (95).

Although DAIR has usually been associated with higher risk of failure compared to implant removal, it can be an optimal strategy for several reasons: it is less aggressive than implant removal, the original fixed prosthesis can be maintained, there is less bone stock loss and has economic benefits in comparison to implant removal (96-98). Moreover, DAIR failure may not represent a limitation for a further removal surgery.

Figure 7. Knee prosthetic joint infection managed with DAIR, with removal of polyethylene components.



A key decision in the approach to any patient with PJI is whether that patient qualifies for DAIR or not. In recent years, many authors have attempted to establish several criteria to select those patients that will be successfully managed with DAIR. Zimmerli's criteria is among the most commonly used (7). According to these criteria, DAIR should be recommended to patients with haematogenous or early post-surgical infections (<3 months), clinical symptoms duration ≤21 days, with a fixed implant, good condition of periprosthetic soft tissues and causative microorganisms susceptible to antimicrobials with activity against biofilms. It should be considered, though, that some of these criteria are rather arbitrary and not based on evidence. This is especially relevant for time to infection from arthroplasty and time from diagnosis to debridement, but generally reflect the difficulty in eradicating an infection when the biofilm is mature.

4.1.2. Implant removal

When DAIR is not indicated or has failed, implant removal is usually performed. Occasionally, the limb cannot be preserved and an amputation is needed to control the infection, but fortunately, this is a rare procedure in patients with PJI, representing less than 0.5% procedures in one study (99). In general, implant removal can be divided into 3 surgical strategies:

a) Two-stage exchange: It is the gold standard for PJI managed with implant removal. With this strategy, the success rate is between 85-90% (98, 100-102). This strategy involves two surgeries. In the first surgery, after obtaining cultures, debridement and removal of prosthesis and mobile components are performed, followed by the implantation of an antibiotic-loaded spacer during the same procedure. An antibiotic-free period of 6 weeks or greater is generally recommended. In a second surgery, cultures are obtained to confirm the sterility of the surgical site, the spacer is removed and a new arthroplasty or arthrodesis is implanted. Second-stage cultures can be positive in 6-20% of cases (103-105) being CNS the most frequent microorganism isolated.

b) One-stage exchange: Usually reserved for patients with good bone stock and condition of soft tissues, without many systemic compromising conditions and the absence of difficult-to-treat microorganisms (7, 106). Although there are some reports on knee PJI (107), it is usually performed in patients with hip PJI. In this procedure, an open arthrotomy is performed, followed by extensive debridement and removal of the prosthesis and associated cement. In the same surgical act, a new prosthesis is implanted, typically using antibiotic-impregnated cement to fix the prosthesis.

c) Resection arthroplasty: This procedure is usually performed as salvage surgery and reserved for patients with multiple prior surgeries in order to avoid amputation. In a single procedure, after obtaining cultures, debridement and removal of prosthesis and mobile components are performed, without implantation of spacer or new arthroplasty.

This is generally performed for hip PJI (Girdlestone procedure) (108). Arthrodesis may also be an elective procedure in patients undergoing removal of a knee arthroplasty.

4.2. Other infections

Non-implant associated infections commonly managed with surgery in adult patients are native SA and PO. Surgical treatment is usually not necessary for VO, although it may be needed in particular circumstances, especially when severe neurological symptoms are present.

The optimal management of native SA requires the removal of the intraarticular pus from the infected joint, which may facilitate the activity of antimicrobials. Methods include needle aspiration, arthroscopy and open arthrotomy, being the last two the most frequently performed. It is common that patients may be managed initially with less aggressive procedures (needle aspiration), requiring arthroscopy or arthrotomy based on the clinical course. Most centres, however, perform arthroscopy or arthrotomy for joint drainage. Several retrospective and prospective studies have suggested that there are similar outcomes with both procedures, but arthroscopy seems to be associated with a faster recovery, less complications and reduced costs (109, 110). A randomized trial in a single centre including 25 patients with knee septic arthritis found similar results (111).

Debridement is usually a key step in the global treatment of PO (70, 71). The removal of sequesters, dead bone and necrotic tissue down to living bone is essential to increase the likelihood of cure. Occasionally, extensive bone resection needs to be performed, leading to dead spaces which need to be filled with spacers or beads, impregnated with antibiotics. Bone transports can be carried out in a second stage and the collection of samples for culture in subsequent surgeries after debridement is recommended to confirm the sterility of the surgical site. Bone grafting and myocutaneous flaps may also be needed, which involves the participation of plastic surgeons.

5. Antimicrobial treatment

5.1. General principles

Antimicrobial treatment is another key component of the management of OAIs. Although guidelines and recommendations have been extensively published, antibiotics should be tailored to each clinical case, taking into account the patient's circumstances and comorbidities, the duration of treatment and the type of infection. In this line, the presence of biofilm in most OAIs, especially when associated to implants, represents a challenge in defining the most appropriate antimicrobial treatment. Most antibiotics that are commonly used in the clinical practice of infectious diseases are aimed to or licensed for infections involving planktonic bacteria. Such antibiotics are not necessarily the most active drugs against biofilm-embedded bacteria.

As mentioned above, tolerance within biofilms is responsible for the lower activity observed with most antibiotics in this setting. The pharmacokinetics/pharmacodynamics (PK/PD) parameters defined for antimicrobials are based on studies involving planktonic bacteria and not necessarily correlate with PK/PD parameters for biofilm-embedded bacteria. Similarly, the *in vitro* parameters that we commonly use to define susceptibility to antimicrobials, such as the minimum inhibitory concentration (MIC) or the minimum bactericidal concentration, are not applicable. Some authors have defined alternative parameters, such as the minimum biofilm inhibitory concentration (MBIC) or the minimum biofilm eradication concentration (MBEC) (112), but the feasibility and usefulness of these are controversial (113). As a general rule, the use of high doses of antimicrobials, often in combination, and for longer period of times, has been recommended (3, 114).

Given all these considerations, the selection of the antibiotic is an important point. The optimal antibiotic for biofilm-associated infections should have good diffusion in bone tissue and biofilm, activity against intracellular and/or biofilm-embedded bacteria and a low probability of causing adverse events if administered over long periods of time. As an example, rifampin may have excellent anti-biofilm activity in infections by staphylococci (95, 115, 116). The ability of antibiotics to diffuse within bone tissue is critical and, overall, there is insufficient data on this issue, since most studies have

included samples obtained from uninfected bone tissue. Fluoroquinolones, macrolides and linezolid seem to have good diffusion in bone (ratio bone/plasma concentration 0.30-1.2), followed by cephalosporins, glycopeptides and penicillins (ratio 0.10-0.30) (117, 118).

The duration of therapy is also controversial and should be tailored to each clinical case. As mentioned, long treatments are often needed; peripheral native SA is generally treated between 3-6 weeks, according to the causative microorganism. Antimicrobial treatment for VO is usually extended for 6-8 weeks (119), although some authors may recommend longer periods, especially if the patient has abscesses that could not be drained (68). A randomized clinical trial found that 6 weeks of antimicrobial treatment resulted in similar cure rates as 12 weeks of therapy in patients with VO (120).

Regarding PJI, the IDSA guidelines recommend that the antimicrobial treatment should be given between 3-6 months after surgery (114), but this is not supported by other scientific communities or authors (3, 7). These groups suggest that an optimal treatment can be limited to 6-8 weeks, depending on the surgical approach; patients managed with DAIR may receive treatment for longer duration (8 weeks). Some studies have found good outcomes with shorter treatment duration (121-123).

The route of antibiotic administration has also been a matter of discussion. Although some antibiotics may have excellent bioavailability by oral administration, there has been a general belief to prioritize the intravenous route for the management of patients with OAIs, with potential associated complications (124). Recently, a randomized trial compared oral vs intravenous administration of antibiotics for OAIs in adult patients and found that there were not statistically significant differences in failure rates. As expected, catheter-related complications and a longer hospital stay were more frequent in patients assigned to the intravenous arm (125).

Overall, there is paucity of data on antimicrobial treatment for biofilm-related, implant-associated OAIs. Prospective observational studies and randomized trials, although not impossible, are often difficult to perform or may be limited by the inclusion of heterogeneous populations or different treatment approaches between participating centres. Most studies were not designed to evaluate the particular efficacy of antibiotics

and, therefore, conclusions may be misleading. Most recommendations are based on empirical experiences and/or expert opinions, thus reducing the quality of the available evidence.

In this context, translational research including *in vitro* and *in vivo* studies may contribute to the overall understanding of the antimicrobial activity against biofilms, although such studies are certainly limited by their experimental design. Several biofilm models have been described, such as the tissue cage (54), osteomyelitis (126) or PJI models (127). Thus, translational research may result in a bench-to-bedside circuit, by raising clinical questions which may be partially answered in the laboratory and guide therapeutic tools and prompt further clinical research.

5.2. Antimicrobial therapy against *Pseudomonas aeruginosa*

Fluoroquinolones are the mainstay of the antimicrobial treatment of OAIs caused by GNB, including *P. aeruginosa*. It has been suggested that these antibiotics have good anti-biofilm activity and good diffusion within tissues (128). Retrospective studies have found a better outcome in patients with PJI by GNB managed with DAIR when treated with fluoroquinolones compared to alternative therapies (129-131). A large multicentre study found that treatment with fluoroquinolones was an independent protective factor in these patients (aHR 0.23, 95%CI 0.13-0.40); in this study, the beneficial effect of fluoroquinolones was present for all GNB-PJI but also for those caused by *P. aeruginosa* (132).

Other antimicrobials are usually associated with worse outcomes, but these may be needed in some circumstances, particularly due to growing fluoroquinolone resistance among GNB, even in the field of OAIs (26). In this line, the activity of beta-lactams (BLs) in this setting has been questioned, due to biofilm tolerance (133). The best antimicrobial treatment for OAIs by fluoroquinolone-resistant GNB is unclear. Actually, fluoroquinolone resistance among GNB can also be associated with resistance to many antimicrobials, including BLs and aminoglycosides (134). In recent years, unfortunately, infectious diseases clinicians and clinical microbiologists have become familiar with the emergence of MDR and XDR GNB, which limits the available therapeutic options, given

the lack of new antimicrobials (135). This has renewed the interest for other alternatives, such as old antibiotics (polymixins), or the optimization of antibiotics administration (continuous infusion of BLs).

5.2.1. Colistin

Polymixins are peptide antibiotics which became available for clinical use in the 1960s, but were abandoned shortly after due to toxicity. In recent years, due to the emergence of MDR and XDR strains with resistance to many antimicrobial families, polymixins have emerged as a last-line therapy for the treatment of infections by these microorganisms. Two polymixins have been classically used in clinical practice: polymixin B and polymixin E (colistin), which is most commonly employed (136).

Colistin has a wide antimicrobial spectrum, including GNB and *P. aeruginosa*, and is a bactericidal concentration-dependent antibiotic, with the ratio AUC/MIC being the best PK/PD parameter that predicts its efficacy. Colistin is administered as an inactive pro-drug, colistimethate, which is mostly cleared by the kidney. A fraction of colistimethate is converted into colistin, which has predominant non-renal clearance (137). These metabolic pathways result in low plasma concentrations of colistin, despite high doses of colistimethate being administered.

Actually, population PK/PD analyses, mostly performed among patients in intensive care units (ICUs), have found high inter-individual variability in colistin plasma concentrations. In a study performed among 214 critically-ill patients, median average steady-state plasma colistin concentrations were 2.35 mg/L, ranging from 0.24-9.92 mg/L (138). These results illustrate important interpatient variation, even among those with similar renal function. In addition, adequate colistin plasma concentrations can increase slowly after parenteral administration of colistimethate and a loading dose has been suggested (139). All these PK data translate the challenges in administrating colistimethate and obtaining adequate colistin plasma concentrations. This is relevant since colistin MIC90 for most microorganisms is 1mg/L and protein binding is approximately 50% (140). Following the current available knowledge on colistin PK,

efforts have been made to provide recommendations on optimal dosing and monitoring (141).

The clinical use of colistin has some inconveniences. Firstly, heteroresistance among strains of *P. aeruginosa*, *Klebsiella* spp or *Acinetobacter* spp has been described (142-144). This translates a potential for resistance emergence if resistant subpopulations are exposed to suboptimal concentrations. Secondly, nephrotoxicity limits the administration of colistin and a minimum plasma concentration >2.4 mg/L has been associated with the risk of renal function deterioration (145, 146). Nephrotoxicity seems to be mediated by the accumulation of polymixins in tubular cells (147).

Given these drawbacks, combination therapy with another antibiotic has been recommended when using colistin. A synergistic effect is aimed, to increase antibacterial activity and minimize the emergence of resistance (148). Combination therapy could result in subpopulation synergy, as different antimicrobials may target different subpopulations. A mechanistic synergy effect has also been proposed: colistin, which is positively charged, acts by targeting the negatively-charged bacterial membrane, enhancing its uptake and that of other molecules (149). The resulting permeabilisation of the cell membrane may facilitate the penetration of other antimicrobials (150).

Combination therapy has been evaluated in experimental models of planktonic bacteria (151-153) and there are also clinical studies showing good results against infections caused by MDR microorganisms (154, 155). Regarding biofilm-embedded bacteria, colistin has shown remarkable activity against *P. aeruginosa*. Actually, studies using confocal laser scanning microscopy have shown that colistin may target predominantly deeper layers of *P. aeruginosa* biofilm, in contrast with other antibiotics such as BLs or ciprofloxacin (156). This may be linked to colistin's activity being independent of the hydroxyl radicals' formation and, thus, being especially bactericidal in anaerobic conditions (157, 158).

PK/PD studies using an *in vitro* biofilm model have shown that the combination of carbapenems and colistin are synergistic and bactericidal against *P. aeruginosa*, including MDR and carbapenem-producing isolates (159). There are no data, however, on other subclasses of BLs. Clinical experience of colistin with/or without other

antibiotics against OAIs is scarce. One study found that combination therapy of BLs plus colistin was associated with better outcomes than any monotherapy against OAIs by MDR/XDR *P. aeruginosa* (160).

5.2.2. Ceftolozane-tazobactam

In recent years, new BLs have been developed to tackle the crisis on antimicrobial resistance and the lack of active drugs. Ceftolozane-tazobactam and ceftazidime-avibactam are two cephalosporins in combination with beta-lactamases, with activity against most MDR and/or XDR GNB.

Ceftolozane is a novel cephalosporin with activity against most GNB and some Streptococci, but limited against Staphylococci (161). It has a potent activity against *P. aeruginosa* and is stable against AmpC mediated hydrolysis (162). Its activity is enhanced by the combination with tazobactam, expanding its spectrum of activity against extended spectrum beta-lactamase (ESBL)-producers GNB. However, it should be reminded that it lacks activity against carbapenemase-producers GNB (163).

Ceftolozane-tazobactam has been approved for the treatment of complicated urinary tract and intraabdominal infections, after results from randomized clinical trials (164, 165). From its approval, clinical experience, mostly from retrospective case-series and including several types of infections, has been reported (166, 167). Resistance emergence has also been reported in some of these studies, although cure rates were generally favourable (around 70%).

Experience with ceftolozane-tazobactam against biofilm-associated infections is scarce and is limited to small case-series, either alone or in combination (168). Indeed, ceftolozane-tazobactam *in vitro* activity against biofilm-embedded MDR *P. aeruginosa* has not been extensively evaluated, including the potential role of combination therapy.

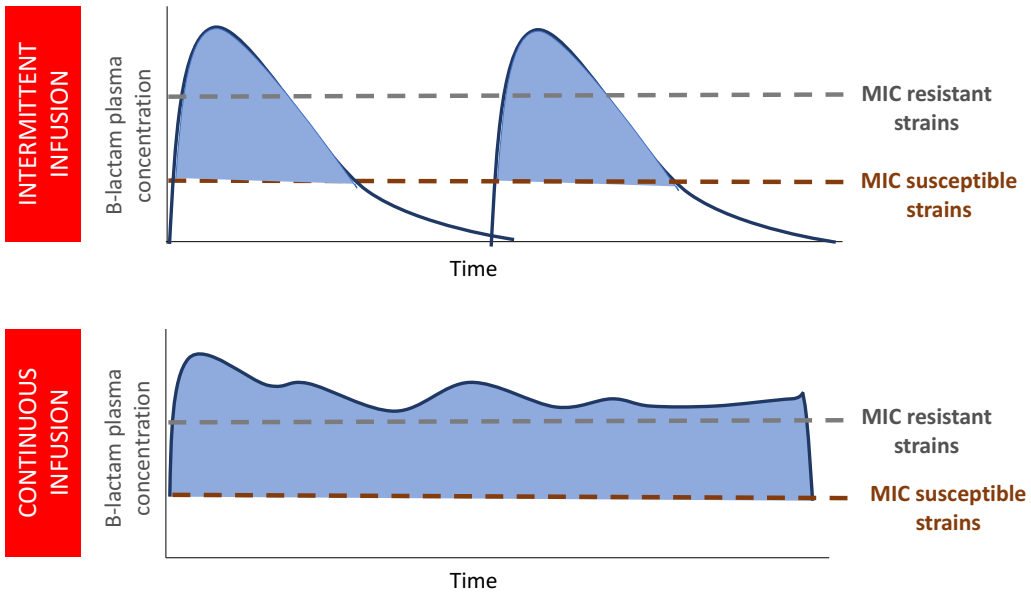
5.2.3. The use of continuous beta-lactam infusion

BLs are generally administered several times a day by intermittent bolus (IB), because they have short half-lives (47). Their activity is time-dependent; this means that their

efficacy depends on the time that the free concentration of BL is above the MIC (%T>MIC), which needs to be between 40-60%. However, higher %T>MIC rates may be needed in particular scenarios to manage difficult-to-treat infections, for example in critically ill patients or OAIs, where biofilm tolerance is frequent (169, 170).

Pharmacodynamic data suggest that continuous infusion (CI) may be more effective than IB, because it maintains the antibiotic concentration above the MIC for longer (ensuring %T>MIC≈100%). This may also be beneficial for preventing the emergence of resistant strains (171-173). There are clinical studies, including randomized trials, which have suggested that CI may result in better outcomes compared to IB, in particular scenarios (174-176). The use of continuous BL infusion may be particularly interesting in the setting of infections by resistant microorganisms with high MIC to BL, as these may recover their antimicrobial activity if given in CI, in contrast to IB (Figure 8).

Figure 8. Differences in PK/PD by using beta-lactams in intermittent or continuous infusion.



Experimental studies in planktonic bacteria have also shown that maximum killing rates are obtained at concentrations of free drug 4 times the MIC (47, 173) and thus, increasing the concentration above this ratio does not result, apparently, in a higher killing. However, the usual IB administration achieves peak concentrations well above

this target in order to be able to ensure an appropriate T>MIC value, due to the short half-life of most BLs.

In the particular field of biofilm-related OAIs, in which the bactericidal effectiveness of BL has been questioned (130, 132), the required levels of BL are unclear but it seems reasonable that a higher plasma concentration at a longer %T>MIC could reflect higher BL levels at the site of infection, improve clinical outcomes, and also result in a lower risk of selecting resistant strains or even reduce health costs. Moreover, in the presence of fastidious microorganisms such as *P. aeruginosa* or MDR GNB, an optimized BL administration (usually in combination) is advisable.

Overall, CI of BL is a promising alternative for the management of complex OAIs by GNB and *P. aeruginosa*, but there is scarce data on its role in the literature.

HYPOTHESIS AND JUSTIFICATION OF THE DOCTORAL PROJECT

OAIs and those affecting orthopaedic devices are health problems of first magnitude in our societies. These infections have been increasing in recent years and it is highly likely that their incidence will continue to grow, mainly due to the ageing of populations, who frequently undergo orthopaedic procedures, with the risk of post-operative infections. The presence of bacterial biofilms makes OAIs very complex and challenging diseases. This characteristic is crucial to understand the pathogenesis, diagnostics and treatment options.

In order to provide the best possible management for these difficult-to-treat infections, it is essential to undertake well-designed studies. The lack of clinical trials in the setting of bone and joint infections is worrisome, although it should be acknowledged that these studies are often difficult and expensive to perform. In recent years, however, large multicentre studies have been performed, providing insight in the management and outcome of these infections, mainly PJI. Most of these studies are observational, retrospective or include small heterogeneous samples, which may limit their generalizability and applicability.

Although progress has been made in the last decades, there are several clinical questions that are still unresolved. The objectives of this thesis are to address common clinical problems of OAIs and potential therapeutic options from a perspective of an infectious disease specialist. Specifically, the role of bacteraemia in OAIs has been poorly described in the literature, including particular clinical presentations, the association with endocarditis and its impact on mortality.

Recently, we have become used to managing infections caused by MDR microorganisms and this is especially difficult in biofilm-associated infections. In this setting, it is essential to find treatment alternatives which can result in good outcomes. Given the difficulties in performing large multicentre studies, including clinical trials, to evaluate the role of particular antimicrobials, experimental models have been developed to provide a practical approximation to clinical therapeutics. Actually, translational research has become relevant in recent years to provide valuable information on the potential activity of antimicrobials against particular microorganisms or settings, such as biofilm-embedded bacteria. Findings from these studies can support clinical practice and provide ideas for future research.

In this line, the role of new antimicrobials against biofilm infections by *P. aeruginosa*, such as ceftolozane-tazobactam, with or without colisitin, has not been addressed. Other alternatives that have been suggested, such as the optimization of BLs by administering them in CI, have not been explored in the setting of OAIs. Actually, the PK/PD of BLs in biofilm infections are not well understood.

This thesis has been conceptualized as a continuation of several research projects previously performed by the Osteoarticular Infections Unit of Hospital Universitari de Bellvitge. This group is integrated by the directors of this thesis, who are both professors in the Faculty of Medicine of Universitat de Barcelona. In the last years, this group has developed an academic line of research on osteoarticular and biofilm-associated infections, from clinical and experimental backgrounds. As a result, multiple doctoral projects with recognized results have been carried out, with several publications of high impact. Also, new hypothesis and questions have arisen during these years, which we attempt to answer in the following pages.

The thesis directors and the doctoral candidate have aimed to deepen in the available current knowledge of two fundamental issues of OAIs. Firstly, the epidemiological, microbiological and prognosis aspects of bacteraemic clinical forms. These projects began when the doctoral candidate was finishing his specialty training in Internal Medicine and Infectious Diseases, in collaboration with infectious disease specialists performing surveillance of bloodstream infections in the hospital. These studies are based on databases including data on bloodstream infections during >30 years. As a result of that data collection and the subsequent analyses, several communications and manuscripts were performed. The doctoral candidate was intimately and progressively involved in that process, which was especially evident when he was incorporated as a pre-doctoral fellow. Secondly, given the importance of *P. aeruginosa* causing OAIs and the emergence of MDR in this field, we aimed to provide some evidence on antimicrobial alternatives for such infections, namely the use of continuous beta-lactam infusion (clinical research) and colistin and beta-lactam combinations (experimental/translational research).

The Osteoarticular Infection Unit at Hospital Universitari de Bellvitge provides an excellent framework to develop clinical studies and attempt to answer some of these

clinical questions. This research activity is integrated within the platforms of the Spanish Network for Research in Infectious Diseases (REIPI) and Grupo Español de Infecciones Osteoarticulares (GEIO) of Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). The ostearticular infections unit, led by Dr Javier Cabo, includes a multidisciplinary team of different medical and surgical specialists in the management of OAIs. The doctoral candidate has been part of this team and participated daily on the clinical work of the unit, also creating and updating clinical databases that have been used for the clinical studies.

In Hospital Universitari de Bellvitge, an institutional program has been implemented in the Osteoarticular Infection Unit to systematically use continuous beta-lactam infusion for OAIs caused by GNB. Plasma samples have been prospectively collected to monitor target achievement and prevent toxicity, which have been measured in the Clinical Laboratory Department. Data collected from this clinical experience has been used to evaluate its potential role against fluoroquinolone-resistant *P. aeruginosa* and also standardize the measurement of ceftolozane-tazobactam concentration in human plasma.

Finally, experimental studies have also been performed for this thesis, evaluating the role of ceftolozane-tazobactam and colistin combinations against biofilm-embedded MDR *P. aeruginosa*, and exploring PK/PD of BLs against biofilm-embedded *P. aeruginosa*. Both studies have given the doctoral candidate the opportunity to deepen the knowledge on clinical microbiology and understand the importance of performing translational research. The former study was performed in the Laboratory of Experimental Infection (Universitat de Barcelona, Campus Bellvitge), also integrated within the platform REIPI. The latter study was performed in collaboration with Jian Li's group at the Monash Biomedicine Discovery Institute (Melbourne, Australia), which was also a fantastic professional experience.

During these years of doctoral research, several public and private competitive grants have been obtained and have enabled sufficient funding to undertake some of these studies. The investigations presented in this thesis provide new and relevant information on clinical and therapeutic aspects of OAIs, including basic research.

AIMS

A. Impact and prognosis of bacteraemic osteoarticular infections

A.1 Impact of bacteraemic osteoarticular infections

Aim 1 – To describe epidemiological and microbiological trends of bacteraemic osteoarticular infections across time.

Aim 2 – To analyse the characteristics of bacteraemic osteoarticular infections associated with the presence of infective endocarditis.

Aim 3 – To compare the characteristics of bacteraemic septic arthritis, according to the site of acquisition.

A.2 Prognosis of bacteraemic osteoarticular infections

Aim 4 – To analyse the mortality and associated risk factors in patients with bacteraemic osteoarticular infections.

B. Antimicrobial treatment against infections by *Pseudomonas aeruginosa*

B.1 Clinical studies

Aim 5 – To analyse the efficacy and therapeutic drug monitoring of continuous beta-lactam infusion for osteoarticular infections by fluoroquinolone-resistant *Pseudomonas aeruginosa*.

B.2 Experimental studies

Aim 6 – To evaluate the activity of ceftolozane-tazobactam, with and without colistin, against a biofilm infection by multidrug-resistant *Pseudomonas aeruginosa* in a dynamic *in vitro* model.

Aim 7 – To evaluate the pharmacokinetics and pharmacodynamics of ceftazidime in continuous infusion, with or without colistin, against a biofilm infection by *Pseudomonas aeruginosa* in a dynamic *in vitro* model.

MATERIALS AND METHODS

1. Clinical Research

1.1. Setting

The Osteoarticular Infection Unit at Hospital Universitari de Bellvitge

Hospital Universitari de Bellvitge is a 700-bed, tertiary-care teaching hospital in Barcelona, Spain, with a dedicated multidisciplinary unit for bone and joint infections. This unit has been recognized by the Spanish Ministry of Health as a reference centre within the National Health System for the management of difficult-to-treat OAIs.

Specialists in the management of such infections include infectious disease specialists, orthopaedic surgeons, microbiologists, rheumatologists and radiologists. A specialized nurse team also ensures excellent care of infected and contaminated wounds. In the unit, standard sterility measures are applied, together with strict hand-washing policies. Patients colonized with MDR microorganisms (e.g. MRSA, MDR *P. aeruginosa* or ESBL-producers *Enterobacteriaceae*) are isolated in airlock-provided rooms, which also have a double-door entry system to prevent further resistant strains dissemination.

Patients with a wide variety of OAIs are hospitalized in this unit, including native SA, VO and PO, diabetic foot infections, complicated skin and soft tissue infections and device-associated orthopaedic infections, such as PJI and implant-associated osteomyelitis.

Daily, orthopaedic surgeons, infectious disease specialists and nurses assess together the clinical situation (including medical and surgical problems) of admitted patients. Decisions about the surgical and medical management, such as wound dressings or antibiotic treatment, are made. Then, microbiologists and infectious diseases specialists have a meeting to evaluate and discuss the microbiology results of available samples from those patients. The laboratory of microbiology is key for a specialized management of OAIs in our unit, supporting the clinical practice. Patients are also followed-up in the outpatient clinic by the same orthopaedic surgeons and infectious diseases specialists; the outpatient clinic for both specialists is on the same weekday, which enables joint assessment of the patient.

The leaders of the Osteoarticular Infections Unit are the orthopaedic surgeon Prof. Javier Cabo and the infectious disease specialist Prof. Javier Ariza, who is also one of the directors of this thesis.

Figure 1. The PhD student (last on the right), PhD directors (third and sixth from left to right) and infectious disease specialists in charge of the Osteoarticular Infection Unit.

Bloodstream infections surveillance at Hospital Universitari de Bellvitge

Since early 1980s, infectious disease specialists have been prospectively collecting all cases of bloodstream infections. Working daily in close collaboration with the Department of Microbiology, these specialists fill a case report form containing data on demographics, clinical characteristics, source of bacteraemia, microbiology and mortality data at 30 and 90 days since the episode. Such large database has been used to provide data on epidemiological trends and publish several studies on such infections.

Clinical Research Platforms

The Osteoarticular Infection Unit at Hospital Universitari de Bellvitge undertakes research projects in collaboration and within the framework of research platforms. One platform is called “Difficult to Treat Infections and Antimicrobians Use” from Bellvitge Biomedical Research Institute (IDIBELL), which is coordinated by Oscar Murillo, director

of this thesis. This group aims to optimise the antimicrobial medications of difficult-to-treat infections, including their mechanism of action and their ability to reach the pathogenic organisms.

Another platform is The Spanish Network for Research in Infectiosus Diseases (REIPI) (www.reipi.org), which was funded by Instituto de Salud Carlos III and represents an attempt to perform multicentre studies within Spanish teaching hospitals in order to deepen on the available knowledge of infectious diseases, including diagnostics and therapeutic management. The Department of Infectious Diseases of Hospital Universitari de Bellvitge is part of this network. There is a specific research line of OAIs, integrated within the work-package "Management of other complex infections", which is, in part, coordinated by Oscar Murillo, director of this thesis. Several publications and diagnostic and therapeutic guidelines performed within this group are the result of such a multicentric research effort.

Finally, the Grupo de Estudio de Infección Osteoarticular (GEIO) from Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC) was created in 2015 as a joint network of specialists on clinical microbiology and infectious diseases, with a particular interest on OAIs. This group is currently headed by Dra. Natividad de Benito (Infectious Diseases specialist at Hospital Santa Creu i Sant Pau, Barcelona, Spain) and Dr. Oscar Murillo, director of this thesis. The main objectives of this group are designing updated protocols and guidelines, participating in multicentre studies and sharing opinions between experts. As a result, several meetings have been held and research projects have been performed or are under way.

Clinical Laboratory Department at Hospital Universitari de Bellvitge

This department includes specialists in different fields, such as clinical biochemistry, clinical molecular biology, haematology and immunology. These specialists are responsible for the clinical and teaching activities of the hospital, facilitating the diagnosis, prevention and follow-up of several diseases. All these activities need to be approved by the Departament de Sanitat i Seguretat Social de la Generalitat de Catalunya (Decret 7/1995). Within this laboratory, several research projects are also undertaken, related to the fields mentioned above.

The Clinical Laboratory Department participates actively in the therapeutic drug monitoring of BL for patients with OAIs, admitted in the hospital. Currently, there is an institutional program for the optimization of antimicrobials for such infections, which includes the administration of BL in continuous infusion. This has required the implementation of ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) for the simultaneous measurement of BL concentrations in human plasma. Such methods have been standardized and validated by Dr Raul Rigo-Bonnin, a clinical biochemist, who has also reported his findings and methodology in prestigious journals from the field (177, 178).

1.2. Clinical approach to osteoarticular infections and definitions

1.2.1. *Clinical and microbiological diagnosis of osteoarticular infections*

All cases included in the studies fulfilled the main diagnostic criteria for each type of OAI. Patients with a short history of a warm, swollen and tender joint were considered to have SA until proven otherwise. SA included native cases and PJI. An arthrocentesis is routinely performed to obtain synovial fluid samples for microbiological analyses and a pair of blood cultures is taken; in cases in which the process affects joints with difficult access (ie., the axial skeleton) only blood cultures are initially taken. Non-infectious aetiologies of acute arthritis are also ruled out prior to establishing the diagnosis of SA. Joint involvement was classified as ‘peripheral’ (i.e., joints of the appendicular skeleton, which were either native or PJI) and ‘axial’ (i.e., the axial skeleton, including the acromioclavicular, sternoclavicular, sternocostal, pubic symphysis, inter-apophyseal, and sacroiliac joints).

PJI was defined according to current guidelines (114) by the isolation of a pathogenic microorganism from two or more surgical, joint-aspirated or blood cultures, or by one such positive culture plus the presence of typical signs and clinical symptoms (inflammatory signs, the presence of a sinus tract or purulence around the prosthesis during surgery).

VO, with or without spine arthrodesis, was defined by the presence of back pain, motion limitation, spinal tenderness, and/or macroscopic pus through the surgical wound,

together with characteristic imaging findings (computed tomography or magnetic resonance imaging) (68). PO was defined by the presence of typical signs and symptoms such as a draining fistula, bone tenderness, and/or local swelling, and characteristic imaging findings (bone radiograph, computed tomography or magnetic resonance imaging) (70) and included cases with or without an orthopaedic device.

Due to their particular characteristics, we usually categorize OAIs into three groups:

a) PJI
b) Osteoarthritis with device (vertebral or peripheral osteomyelitis associated with the presence of a device).
c) Osteoarthritis without device (native septic arthritis and vertebral or peripheral osteomyelitis not associated with the presence of a device).

Some patients with OAIs, especially with concomitant bacteraemia, also present with infective endocarditis (IE). This is more frequent with Gram-positive microorganisms, such as *S. aureus* or *Streptococcus* spp. In these cases, IE is ruled out with transthoracic or transoesophageal echocardiography. Cases of IE are diagnosed when fulfil Duke criteria (Table 1) (179).

1.2.2. *Surgical management*

Patients are usually managed with surgery according to current guidelines and recommendations (7, 9, 21, 70, 71). However, the ultimate decision on performing surgery or the final surgical option relies on the treating clinician, based on patient's circumstances and condition. Generally, this decision is taken within a multidisciplinary team of orthopaedic surgeons and infectious diseases specialists.

Acute orthopaedic-associated infections (early acute post-surgical PJI, haematogenous PJI and device-associated osteoarthritis) are commonly managed with DAIR, according to current recommendations. Stable devices and soft tissues in good conditions are usually also required for performing DAIR; this consists of an extensive debridement or necrotic tissues, pus and/or hematoma, together with exchange of mobile components, when feasible.

Table 1. Definition of terms used in the modified Duke criteria for the diagnosis of infective endocarditis.

Major criteria	Blood cultures positive for IE
	Typical microorganisms consistent with IE from 2 separate blood cultures in the absence of a primary focus, or microorganisms consistent with IE from persistently positive blood cultures defined as follows: at least 2 positive cultures of blood samples drawn >12 hours apart or all 3 or a majority of ≥4 separate cultures of blood (with first and last sample drawn at least 1 hour apart)[1]
	Single positive blood culture for *Coxiella burnetii* or anti-phase 1 IgG antibody titer ≥1:800
	Evidence of endocardial involvement
	Echocardiogram positive for IE[2]
Minor criteria	Predisposing heart condition or IDU
	Fever
	Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhages, and Janeway lesions
	Immunological phenomena: glomerulonephritis, Osler nodes, Roth spots, and rheumatoid factor
	Microbiological evidence: positive blood culture but does not meet a major criterion (excluding single positive culture for coagulase-negative staphylococci and organisms that do not cause endocarditis) or serological evidence of active infection with microorganism consistent with IE

A definitive diagnosis of IE is based on pathological criteria (microorganisms demonstrated by culture or histological examination of a vegetation, a vegetation that has embolized, or an intracardiac abscess specimen; or pathological lesions; vegetation or intracardiac abscess confirmed by histological examination showing active endocarditis) or clinical criteria (2 major criteria, 1 major criterion and 3 minor criteria, or 5 minor criteria). A possible diagnosis of IE is based on 1 major criterion and 1 minor criterion, or 3 minor criteria). [1]Viridans streptococci, *Streptococcus bovis*, HACEK group, *Staphylococcus aureus* or community-acquired enterococci. [2]Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material in the absence of an alternative anatomic explanation; abscess; or new partial dehiscence of prosthetic valve or new valvular regurgitation.
IE: Infective endocarditis. IDU: Injection drug user. HACEK: *Haemophilus* species, *Aggregatibacter* species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species.

Patients with chronic device-associated infections (chronic post-surgical PJI and device-associated osteoarthritis) are commonly managed with implant removal. Patients with acute infections who failed after DAIR often require implant removal. For PJI, implant removal has been classified into three surgical approaches, which have been described in the Introduction section (12):

a) Two-stage exchange: Patients are considered under the two-stage exchange scheme if the intention is to implant a new prosthesis or arthrodesis, irrespective if this second stage is finally performed.
b) One-stage exchange
c) Hip resection arthroplasty

PO is usually managed with debridement and occasionally requires a radical resection of sequestered bone, which may leave dead spaces. In such cases, antibiotic-impregnated spacers are also used to fill the space and provide local antibiotic activity. These patients often require further reconstructive surgeries of the bone and soft tissues, which can include bone grafting or plastic surgery. If osteomyelitis occurs on non-consolidated fractures, local external fixation is sometimes needed to provide mechanical stability.

VO usually is managed without surgery, only with medical treatment. Associated complications, such as spinal cord compression or epidural abscesses, occasionally need surgery.

Peripheral SA usually requires joint drainage which, in our hospital, is mostly performed with an arthrotomy. Extensive debridement and irrigation is performed to eliminate all purulent content and debris, rapidly decompressing the joint. Occasionally, some patients can be managed with arthroscopic debridement or single or repeated needle aspiration (arthrocentesis). SA of the axial skeleton is often only treated with antibiotics, although eventually some patients may require needle aspiration.

1.2.3. *Medical management*

Surgical treatment is complemented with an antimicrobial therapy, specifically selected for each patient. Antibiotics are usually withheld until valuable surgical samples are taken, provided that the microorganism is unknown or if permitted by the patient's condition. An empirical broad-spectrum combination of antimicrobials (i.e. vancomycin plus ceftazidime) is initiated until microorganisms are identified and the antimicrobial susceptibility profile is known.

Then, a tailored antimicrobial therapy is administered for a variable time period, usually ranging between 6 and 8 weeks, according to patient's particularities and infection characteristics. SA is usually treated for a shorter period of time, being 3-4 weeks for staphylococcal and streptococcal infections and around 6 weeks for GNB infections.

The treatment scheme of OAIs is usually divided in two phases. In an initial phase, which lasts for a few days or weeks, antibiotics with activity against planktonic bacteria are administered, targeting rapidly growing bacteria which may be present next to the surgical site, especially after the procedure. Intravenous BLs, lipopeptides or glycopeptides are usually given. In a second phase, which represents the majority of treatment duration, antimicrobials targeting biofilm-embedded bacteria are added. During this phase, staphylococci are usually treated with a rifampin-based combination, either with levofloxacin (if methicillin-susceptible) or linezolid (if methicillin-resistant). Streptococci and enterococci are usually treated with BLs, although levofloxacin with or without rifampin can be used for the former. Ciprofloxacin is usually the drug of choice for infections by GNB.

For the particular case of *P. aeruginosa*, a combination therapy with BL and another antipseudomonal agent is often administered. For fluoroquinolone-susceptible *P. aeruginosa*, oral ciprofloxacin (750-1000mg twice daily but adjusted according to renal function) is combined with a BL for at least 7-14 days, before sequencing to ciprofloxacin alone. For fluoroquinolone-resistant *P. aeruginosa*, we combine BL with colistin, which is started at 2 million IU (MIU) every 8 hours (without loading dose) when renal function is normal, but adjusted in those with chronic kidney disease (CKD) (138). In order to prevent potential toxicity, colistin is generally not considered in fragile patients (those aged $\geq$75 years and/or with comorbidities), especially if they have CKD (180) and/or are managed with implant removal or bone resection. The antipseudomonal BL is generally ceftazidime except in cases of MDR/XDR strains when the BL with the lowest MIC is usually chosen. Doses of BL and combination agents are also adjusted if acute kidney injury (AKI) occurred during therapy (181).

Actually, since 2015, in Hospital Universitari de Bellvitge, there is an institutional program to promote the administration of continuous BL infusion (BL-CI) for OAIs by GNB. The daily dosage of BL-CI is based on previously described formula and our own

experience (173, 182), according to BL total body clearance (TBC) and desired target concentrations.

- Daily dose (mg) = 24 (h) x TBC (L/h) x *f*Css (mg/L)

TBC: Total Body Clearance.

*f*Css: The target free steady-state concentration

Since ceftazidime, cefepime and ceftolozane are almost completely cleared by glomerular filtration, creatinine clearance (calculated using the Cockcroft-Gault formula) (183) is used to estimate the antibiotic's TBC. For piperacillin and aztreonam, given their combined renal (glomerular filtration and active tubular secretion) and non-renal clearance, TBC values are used from previously reported studies (184, 185). A loading dose is commonly administered to rapidly achieve target steady-state concentrations (Css). Loading doses of BL are 2 g (ceftazidime, cefepime, aztreonam), 2/1 g (ceftolozane-tazobactam), and 4/0.5 g (piperacillin-tazobactam).

In each patient, the first blood samples to obtain Css are extracted at least 24h after BL-CI initiation. Then, samples are extracted to monitor Css at the clinicians' discretion, weekly if possible. Clinicians are encouraged to obtain a new sample at least 24-48h after modifying the daily BL dose. Once extracted, samples are immediately centrifuged and frozen at -80ºC until analysis to avoid stability disturbances.

In general, we aim to optimize the most relevant PK/ PD target for BL by ensuring that T > MIC = 100% and that *f*Css is at least 3 or 4 times the MIC (3–4xMIC), since this concentration has been correlated with the maximum killing effect of BL (47, 173). However, as higher concentrations can also be desirable in OAIs due to poor bone penetration or biofilm-associated BL tolerance in these infections, we use *f*Css values of between 3–4xMIC and 10xMIC. The daily dosage is adjusted based on the measured BL concentrations in plasma; our protocol suggests that dose adjustments (increases or decreases) should be 50% of the initial dose. Dose reductions are usually performed when concentrations are 10–15xMIC, higher than 100 mg/L (186), or when toxicity occurs. However, the criteria for adjusting the dosage is ultimately decided by the clinicians, and the decision to adjust the dose is taken by consensus after a

multidisciplinary discussion. For isolates with high MICs, 3–4xMIC is often difficult to achieve, so lower levels above the MIC (≥ 1.5–2xMIC) are considered acceptable. Free BL fractions are estimated from reported protein binding in healthy subjects (184, 187-190).

1.2.4. *Outcome and follow-up*

Follow-up after discharge takes place in the outpatient clinic, shared by orthopaedic surgeons and infectious disease specialists. Patients are assessed at months 1, 3, 6 and 12 after the episode and often follow-up extends to 24 months.

In general, failure is usually defined as a composite endpoint of mortality and local failure. Local failure is evaluated in patients not presenting mortality and only if related to persistence/relapse, excluding superinfection or orthopaedic reasons as a cause for extra surgeries. Therefore, failure consists of:

- Death related to the infection
- Local failure
    - Managed with DAIR or debridement
        - Implant removal due to infection persistence/relapse, in patients with device.
        - New debridements >30 days since the first, due to persistence/relapse.
        - Relapsing symptoms during follow-up.
        - Long-term suppressive antimicrobial therapy (SAT)
    - Managed with implant removal
        - Symptom persistence beginning within 30 days after implant removal, leading to SAT and/or new surgeries, irrespective of when these are performed.
        - Relapsing symptoms in asymptomatic patients initially considered cured after implant removal.
        - Positive cultures in asymptomatic patients undergoing a second-stage surgery.

Mortality is a key component of failure and has deserved special attention in our studies. For the studies on bacteraemic OAIs, mortality was recorded if death occurred within 30 days from the diagnosis, and early mortality if death occurred within 7 days.

1.3. Complementary tests

1.3.1. *Microbiological process*

Microbiological samples are collected during the diagnosis workup and occasionally during follow-up. These include blood cultures and local samples, such as tissue specimens, joint aspirates and surgical samples (synovial tissues, peri-prosthetic bone and soft tissue, synovial fluid and prosthetic cement). Sonication of prosthetic components is not routinely performed in our centre.

Blood samples are cultured following standard criteria by the automated BACTEC™ method, using both aerobic and anaerobic media. We use the following BACTEC™ systems (Becton-Dickinson Microbiology Systems, Franklin Lakes, NJ, USA): the BACTEC™ NR-860 in the 1990s, the BACTEC™ 9240 in the 2000s, and the BACTEC™ FX in the 2010s. Identification of microorganisms and their antibiotic susceptibility have been typically performed by standard biochemical reaction, disc diffusion or microdilution, and the MicroScan system (Dade Behring, West Sacramento, CA, USA). Recently, the MALDI-TOF Biotyper™ measurement system (Bruker, Billerica, MA, USA) has been incorporated for microorganisms' identification. Antimicrobial susceptibility is defined according to Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing (191, 192).

Surgical samples are seeded in liquid (thioglycolate) and solid media (5% sheep blood, chocolate and MacConkey agar) and incubated for 10 days. Liquid cultures are routinely plated on solid media every 48 hours or whenever they become turbid. Specific media are also used for investigations of anaerobic microorganisms, fungi and mycobacteria. A single valuable sample with a virulent microorganism, such as *S. aureus*, *Streptococcus*, *P. aeruginosa* or *Enterobacteriaceae*, is considered relevant. For low-virulence microorganisms that can be skin-flora contaminants (i.e. CNS, *P. acnes*), two or more

positive samples yielding the same microorganism are usually needed to consider it the causative agent of the infection.

1.3.2. *Sequential procedures for the development of the UHPLC-MS/MS method used for the measurement of ceftolozane-tazobactam concentration in human plasma*

Following the implementation of a program for the use of BL-CI in our hospital, the standardization of a UHPLC-MS/MS methodology for measuring BL concentrations has been developed. The ideal sample for measuring BL concentrations is plasma. The methodology for the simultaneous measurement of amoxicillin, ampicillin, cloxacillin, piperacillin, cefepime, ceftazidime, cefuroxime, aztreonam and meropenem, in addition to two β–lactamase inhibitors (clavulanat and tazobactam), has been described elsewhere (177, 178). Recently, our group also standardized the measurement of ceftolozane and tazobactam in human plasma and the methodology is depicted below.

- *Preparation of calibration, control and internal standards (IS)*

For ceftolozane (donated by Merck, Sharp & Dohme S.A., Barcelona, Spain) and tazobactam (purchased from United States Pharmacopeia, Rockville, MD, USA), two stock solutions of each one from independent weighing were prepared at a concentration of 2.00 g/L. For each drug, several working standards were prepared, representing growing concentrations (from 10 to 1250 mg/L). These solutions were stored light-protected at (−75 ± 3)°C. On the day of analysis, 100 μL of calibration materials (from 1 to 125 mg/L) were also prepared and also four plasma quality controls.

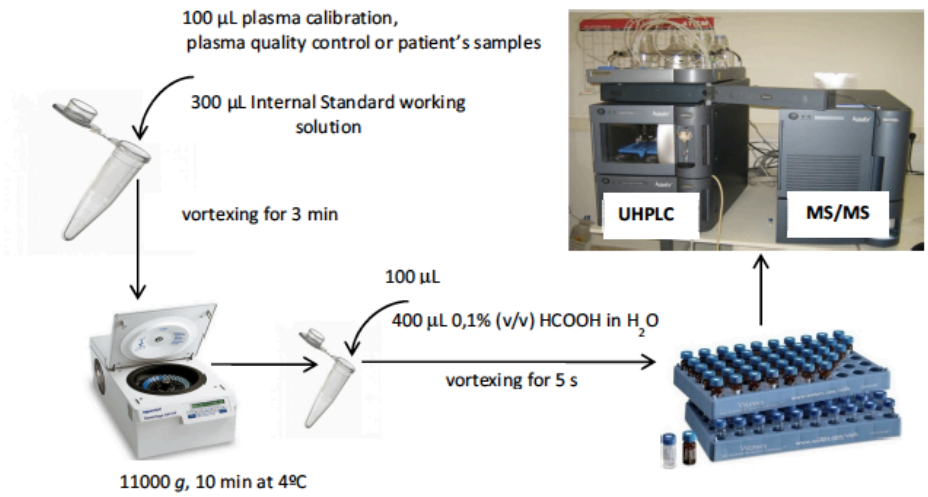
Stock solutions of ceftazidime-D5 and sulbactam in 1:10 proportion were prepared. These solutions were stored at (−75 ± 3) °C. A working solution of IS was prepared freshly for 20 samples analysis by adding 150 μL of each stock solution to 5.70 mL of acetonitrile.

- *Sample preparation*

One hundred μL of calibration, quality control or plasma samples from patients were transferred to 1.50 mL-polypropilene microcentrifuge tubes and 300 μL of internal

standard were added for protein precipitation. Tubes were vortexed for 3 minutes, subsequently centrifuged for 10 minutes and 100 µL of supernatant transferred to new microcentrifuge tubes containing 400 µL of 0.1% (v/v) formic acid in water. The tubes were vortexed for ten seconds and the full volume was transferred into specific screw-neck glass vials with silicon septa caps and placed in the autosampler for injection.

Figure 2. Sequential procedure for measurement of BL concentrations from plasma.



- *Instrumentation and equipment*

Analyses were conducted using an Acquity® UPLC® chromatographic system coupled to an Acquity® TQD® tandem-quadrupole mass spectrometer (Waters, Milford, MA, USA). The mass spectrometer operated in multiple reaction monitoring and in positive and negative electrospray ionization modes. Two transitions were followed, one for quantification and the other for identification or confirmation.

- *Validation of the method*

Validations were carried out according to the European Medicines Agency (EMA), CLSI and EUROLAB guidelines. The developed procedure was validated in terms of selectivity, carry-over, lower limit of quantification, imprecision, bias, dilution integrity, recovery, matrix effect, stability and measurement uncertainty.

- *Applicability of the method*

We evaluated the applicability of the UHPLC-MS/MS procedures by processing plasma samples from patients with OAIs treated with ceftolozane-tazobactam in CI. Blood samples were obtained at least 72 hours after the beginning of ceftolozane-tazobactam in order to assure that it represented concentrations at the steady-state condition. Samples were collected in BD Vacutainer® lithium-heparin tube (Becton Dickinson, Franklin Lakes, NJ, USA) and immediately refrigerated at (2–8) °C. Finally, they were then centrifuged at 2000g for 10 min at (4 ± 1) °C, aliquoted, and stored at (−75 ± 3) °C until analysis.

1.4. Study design and statistical analysis

The clinical studies included in this thesis are all observational; four of them are retrospective analysis of prospectively collected data and the remaining study (the use of BL-CI for OAI by *P. aeruginosa*) is a prospective study. Data has been collected and introduced, after a critical revision, in Microsoft Access databases. The observational prospective study on BL-CI had a specific protocol which was approved by the hospital's Ethics Committee and authorized by the Spanish Agency of Drugs and Sanitary Products (AEMPS); all patients signed an informed consent before entering into the study (Annex 1).

Data were analysed with Stata 13.1 (Stata Corporation, USA). Categorical variables were described by counts and percentages, while median and interquartile range (IQR) were used to summarize continuous variables. Comparisons between groups were performed with the chi-square test or Fisher exact test for categorical variables and the t-test or Mann–Whitney test for continuous variables. Changing trends in categorical or continuous variables across studies' periods were analysed with non-parametric tests.

Risk factors for a defined outcome were analysed by univariate and multivariate logistic regression, obtaining unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (95% CI). Kaplan-Meier survival curves were used to evaluate the probability of success during follow-up and the log-rank test analysed differences between groups.

The likelihood ratio test was used to obtain p values in regression modelling. A p value ≤0.05 was considered statistically significant.

Given the observational design of our studies, to evaluate the impact of interventions on certain outcomes, propensity score matching analyses were performed. Clinically relevant variables were introduced in the propensity model, together with baseline characteristics found to have a univariate association with the intervention being evaluated ($p < 0.1$). Nearest neighbour matching with replacement was performed with 0.1 calliper. Mean standardized differences for covariates between matched groups were checked prior to treatment effects estimation.

When evaluating the efficacy of antimicrobials, in order to avoid survivor's bias, the influence of the antimicrobial on the outcome was only considered in patients with the same possibilities of receiving this antibiotic; thus, cases failing before a specified time cut-off, and thus having received antimicrobials for a short period of time, were excluded for this analysis.

2. Experimental Research

2.1. Setting

2.1.1. *Laboratory of Experimental Infection at the School of Medicine (Universitat de Barcelona, Campus Bellvitge)*

The Osteoarticular Infection Unit from the Department of Infectious Diseases has traditionally attempted to develop translational research, allowing a continuum between the clinical bedside and the laboratory. This interest comes from the need to find therapeutic alternatives for the management of complex, foreign-body infections in an era of MDR and a lack of new antimicrobials. This translational research is an excellent platform to provide relevant answers to questions that arise in the clinical field and apply such answers to the clinical practice and research. This research is also

performed within the framework of REIPI, which include several *in vitro* and *in vivo* experimental models of infectious diseases.

This laboratory is located in the Faculty of Medicine of Universitat de Barcelona, Campus Bellvitge, and is also linked to Idibell, the research foundation associated with Hospital Universitari de Bellvitge. Our group has led several projects on the *in vitro* and *in vivo* efficacy of different antimicrobials against foreign-body infections. Initially, *in vivo* models using the tissue cage in rats were developed to evaluate the activity of antimicrobials and combinations against methicillin-susceptible and resistant *S. aureus*, leading to several publications in recognized international journals (54, 193-196). In recent years, *in vitro* experiments have been added, with a dynamic biofilm model (CDC Biofilm Reactor [CBR]), to investigate the efficacy of antimicrobials and combinations against *P. aeruginosa* and *K. pneumoniae*, which have also been published (159, 197).

2.1.2. *Monash Biomedicine Institute, Monash University*

The Monash Biomedicine Discovery Institute (Monash University, Melbourne, Australia, www.monash.edu) is a large world-leading institution with more than 120 research teams from multiple disciplines into six priority areas. The Department of Microbiology is part of this renowned institute and include several research lines, such as Antimicrobial Systems Pharmacology Laboratory, headed by Prof. Jian Li. Given the lack of new antibiotics for Gram-negative superbugs in the near future, Prof. Li has dedicated the majority of his research career to optimize the clinical use of polymixins and develop novel and safer polymixins.

This research, which has also been done in partnership with the Monash Institute of Pharmaceutical Sciences, has resulted in reducing the knowledge gap on colistin and polymixin B, which are currently considered last-line therapies for MDR and XDR GNB. Over the years, Prof. Li, in collaboration with others, has contributed to develop a simple method to assay colistimethate sodium (CMS) and colistin by high performance liquid chromatography (HPLC), has described the PK of CMS and colistin in patients, guiding dosage regimens, has evaluated the PK/PD of colistin and polymixin B, alone or in combination with other antimicrobials, against several MDR GNB and has deepen the knowledge on polymixins toxicology.

Figure 3. The Monash Biomedicine Discovery Institute (Monash University, Melbourne, Australia) and the PhD student working in the laboratory.

Prof. Li's group and our research group collaborated in the past in evaluating the efficacy of colistin, with or without doripenem, against a biofilm infection by MDR *P. aeruginosa*, using the CBR (159). Such enriching collaboration, given the common interest on polymixins, resulted in a new joint project on the PK/PD of ceftazidime in CI, with or without colistin, against a biofilm infection by *P. aeruginosa*. The doctoral candidate moved to Melbourne (Australia) to perform this project, under the supervision of Prof. Li.

2.2. Bacterial strains

All strains were subcultured from a frozen stock on nutrient trypticase soy agar plates with 5% sheep blood (TSA; Becton Dickinson, Madrid) and preserved in cryotubes at -80.C for subsequent use.

First study - Efficacy of ceftolozane/tazobactam, alone and in combination with colistin, against multidrug-resistant Pseudomonas aeruginosa in an in vitro biofilm pharmacodynamic model.

Three clinical isolates of *P. aeruginosa* from Hospital Universitari de Bellvitge (HUB), all colistin-susceptible but ceftazidime-resistant strains, were used: HUB1, a ceftolozane/tazobactam- and meropenem-susceptible MDR strain (ST308); HUB2, a ceftolozane/tazobactam-susceptible and meropenem-resistant XDR strain (ST175); and HUB3, a ceftolozane/tazobactam-resistant and meropenem-susceptible MDR strain

(ST274). The three strains are worldwide disseminated and considered high-risk clones (198); mechanisms of resistance were AmpC hyperproduction for all strains (MDR-HUB3 having the AmpR mutation G154R), plus OprD porin deletion in XDR-HUB2.

Second study - In vitro pharmacokinetics of ceftazidime and its combinations with colistin against Pseudomonas aeruginosa biofilm.

Initially, four strains of *P. aeruginosa* were evaluated. One was the reference strain PAO1 (American Type Culture Collection, Rockville, MD, USA) and the rest were clinical isolates causing biofilm-related osteoarticular infections in HUB. All strains were susceptible to ceftazidime and colistin. Finally, two strains of *P. aeruginosa* were employed in experiments with the CBR; one was PAO1 and the other was a clinical isolate (HUB8), chosen based on preliminary studies.

2.3. Preliminary studies

2.3.1. *Susceptibility studies*

Susceptibility studies included the determination of MIC, MBIC and MBEC. MICs of BL and colistin (sulphate) were determined by broth microdilution in Mueller-Hinton broth (MHB) (Becton Dickinson, Madrid; Oxoid, Basingstoke, UK). The susceptibility profile to both antibiotics in *P. aeruginosa* was defined according to the EUCAST breakpoints (192).

MBIC and MBEC were determined using an MBEC™ device (Innovotech Inc., Edmond, Canada), also with MHB. The methodology has been previously standardized (199, 200). Briefly, colonies recovered from a subculture were incubated at 37ºC in a gyratory shaker in Tryptone Soy Broth (TSB) to create an inoculum that matched 1 McFarland concentration. This solution was diluted in MHB 1:30 and added to the MBEC™ device, which was then incubated for 24h at 37ºC.

Later, the pegs were rinsed in phosphate buffer solution (PBS) and introduced into a new 96-well microtiter plate that contained increasing concentrations of antibiotic (challenge plate) and incubated for 24h at 37ºC. After rinsing again, the pegs were transferred to a new microtiter plate containing fresh sterile MHB (recovery plate) and

sonicated (Branson Ultrasonics, USA). Then, the peg lid from the recovery plate was replaced by a lid of a microtiter plate and further incubated 24h at 37ºC. To determine MBIC and MBEC value, we used a microtiter plate reader (Tecan Trading AG, Switzerland) to obtain optical density measurements at 650nm (OD650). The MBIC was defined based on OD650 measurements at 0 and 6h, as the lowest concentration that resulted in an OD650 difference at or below 10% of the mean of two positive controls. MBEC was determined after 24h incubation and clear wells (OD650 < 0.1) were taken as evidence of biofilm eradication.

2.3.2. *Generation times*

We compared growth rates between strains that were going to be investigated in the CBR. To do this, we incubated a bacterial inoculum of 10^5 cfu/mL in MHB at 37ºC in a shaker during 24 hours. Samples were collected at different timepoints, serially diluted with sterile saline and plated on drug-free TSA or nutrient agar (NA). Absolute bacterial counts were obtained after incubation at 37ºC during 24 hours.

The generation time was calculated as:

$g = \text{Ln } 2/\mu$; where μ is the growth rate

$\mu = (\text{Ln } N - \text{Ln } N0)/(t - t0)$; where N is the number of bacteria at time t, and N0 is the number of bacteria at time t0.

2.3.3. *Population Analysis Profiles of Colistin*

Baseline heteroresistance to colistin was examined by performing population analysis profiles (PAPs) of the *P. aeruginosa* strains, as reported elsewehere (142, 151). The presence of subpopulations able to grow in the presence of ≥2, 4, 8, and 16mg/L of colistin was screened.

Briefly, one colony previously incubated in TSA or NA was incubated in 10 mL of TSB overnight at 37ºC in a shaker. Bacterial suspensions were serially diluted with sterile saline and plated on Mueller Hinton Agar (MHA) or NA impregnated with colistin at

concentrations mentioned above. Colonies were counted after 24 hours of incubation (48 hours for small colonies) at 37ºC.

2.3.4. *Biofilm formation assays*

The ability of the different strains to form biofilm was evaluated with a method previously described by Stepanovic *et al* (201), based on using crystal violet (CV) to stain biofilms. A colony subcultured in NA media was selected, grown in 10mL of TSB for 24h at 37ºC and further diluted 1:100. Then, 200µL of this dilution were poured on each well in a 96-well microtiter plate, together with negative controls (200µL of sterile TSB), and incubated for 24h at 37 ºC. Wells were emptied, washed three times in PBS and heat-fixed at 60ºC for 60min. Then, 200µL of 2% CV were added to each well to stain the biofilm for 15min, followed by washing three times in PBS. Finally, the biofilm was solubilized by adding 200µL of 30% acetic acid to each well for 30min.

The OD was then read at 570nm. The cut-off value (ODc) was established by the following equation: ODc = Mean OD of negative controls + (3 x standard deviation of negative controls). The mean OD of the different isolates was then compared to ODc, defining the ability of each isolate to form biofilm, as follows:

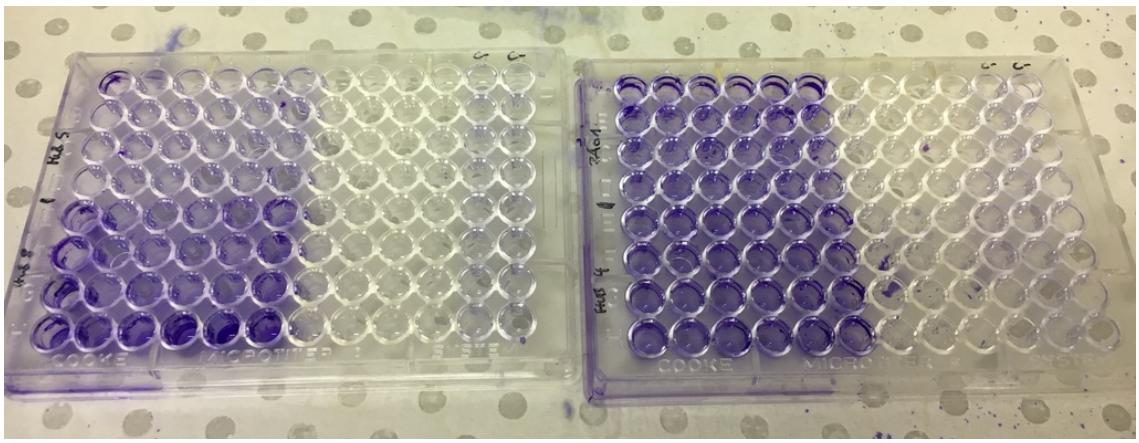
$OD \leq ODc$ = No biofilm producer

$ODc < OD \leq 2xODc$ = Weak biofilm producer

$2xODc < OD \leq 4xODc$ = Moderate biofilm producer

$4xODc < OD$ = Strong biofilm producer

Figure 4. Biofilm formation assays based on crystal violet staining in 96-well plates.



2.3.5. *Time-kill curves*

Time-kill curves were performed in the second study to evaluate the killing profile of several concentrations of ceftazidime against the strains that would be studied in the CBR. Ceftazidime concentrations evaluated were 1x, 2x, 4x, 8x, 16x and 32xMIC, together with controls.

Isolates were subcultured on NA and incubated overnight at 37ºC. One colony was then grown in 10 mL MHB at 37ºC; after that, an early log-phase culture was obtained. Ceftazidime at the appropriate concentration was then added to 20 mL of a log-phase broth culture of 10^6, and incubated at 37ºC for 24 hours. Samples were taken at different points, serially diluted and plated onto NA. Plates were incubated at 37ºC for 24 hours for viable counting.

2.4. A dynamic *in vitro* biofilm model

2.4.1. *Components of the CBR*

In vitro dynamic experiments were performed with a standardized and validated model, the CBR (Biosurface Technologies, Montana, USA). This model has been previously used by several authors to perform basic research on biofilms, allowing for PK/PD analysis of antimicrobial efficacy (202). Our group has previously worked with this model and standardized the procedure to obtain the best conditions for biofilm formation and antimicrobial challenge (159, 197, 203).

The CBR consists of a one-litre glass vessel with an effluent spout giving place to an operational volume of 350 mL. Continuous mixing of the broth is provided by a magnetic baffled stir bar. Antibiotics and media can be added through the ports in the top lid of the reactor, from where 8 rods descend, each housing 3 removable Teflon coupons (biofilm growth surfaces), for a total of 24 sampling opportunities throughout the experiment.

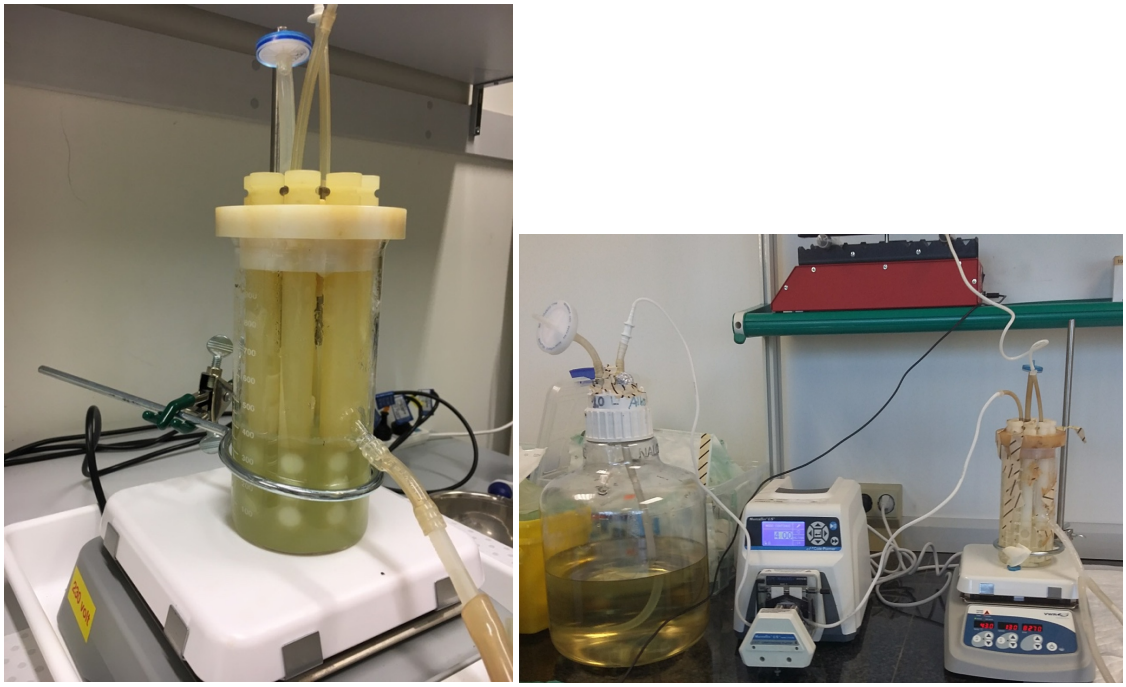
The CBR is placed on a hot magnetic stir plate, allowing to adjust the stirring to 130rpm and temperatures of 37ºC. Media is placed on 10-20L autoclavable polycarbonate carboys (Thermo Scientific-Nalgene) and pumped into the reactor with a peristaltic pump (Masterflex, Cole-Parmer, USA) at the desired rate. Antibiotics can be

administered into the reactor in continuous infusion (with the peristaltic pump, mixed with the media) or in intermittent infusion with a syringe pump (NE-1000 Syringe Pump, New Era Pump Systems Inc, USA).

2.4.2. *Operating procedure*

Our protocol followed previously reported methods (159, 203), and consisted of a biofilm conditioning phase, in which the biofilm was formed for 48h, followed by a therapeutic phase. Isolates were initially subcultured onto TSA or NA plates and incubated at 37ºC for 24 hours. One colony was selected and incubated overnight in 10 mL of TSB at 37ºC for 24 hours. The biofilm conditioning phase started with bacteria (from the overnight culture) inoculation into the reactor (initial inoculum of 7 log cfu/mL), followed by a 24h batch culture at 37º in drug-free 20%-TSB. Then, fresh sterile 20%-TSB was infused into the model for 24h using a peristaltic pump (Masterflex, Cole-Parmer, USA), to achieve a bacterial residence time within the reactor shorter than the generation time for the suspended bacteria. Once the biofilm was formed, the therapeutic phase started (time zero, 0h), which was prolonged for 54 hours.

Figure 5. The CDC Biofilm Reactor after the 24h-static phase (left) and during the 24h-dynamic phase (right).



2.4.3. *Antibiotic regimens*

Antibiotic regimens were administered into the reactor after the biofilm conditioning phase and could be given in IB or CI, as mentioned above. Independently of the antibiotic infusion mode, media was continuously infused into the reactor at a flow-rate reproducing the respective antibiotic half-life ($t_{1/2}$).

In the first study, regimens evaluated were ceftazidime, meropenem, ceftolozane/tazobactam and colistin, as monotherapies, the respective BL in combination with colistin, and controls (no antibiotic). For the three beta-lactam regimens, a bolus dose was injected into the model every 8h to achieve the desired free-drug peak concentration (C_{max}) (fC_{max}; in accordance with the protein binding for each drug). Pharmacokinetic parameters simulated for BL were: ceftazidime, 2g every 8h (fC_{max} 134mg/L, half-life [$t_{1/2}$] 2h, flow rate 2mL/min, protein binding considered 16%); meropenem, 2g every 8h (fC_{max} 90mg/L, $t_{1/2}$ 1h, flow rate 4mL/min, protein binding considered 10%); ceftolozane/tazobactam, 2g/1g every 8h (fC_{max} 111mg/L, $t_{1/2}$ 2.5h, flow rate 1.61mL/min, protein binding considered 21% / fC_{max} 25mg/L, $t_{1/2}$ 2.5h, flow rate 1.61mL/min, protein binding considered 30%, respectively) (187, 188, 204). For the particular case of ceftolozane/tazobactam combination, with different $t_{1/2}$ (2.5h and 1h, respectively), we reproduced the $t_{1/2}$ of ceftolozane and assumed that tazobactam would be eliminated at the same $t_{1/2}$, this providing tazobactam concentrations during the whole 8h-period always in adequate proportion with ceftolozane (at least 2:1). In all cases, flow rates were calibrated prior to each experiment and monitored throughout to ensure the system was performing optimally.

Colistin was pumped into the CBR as a CI at 3.50mg/L, which mimicked the plasma steady-state concentration observed in humans by 6-9MU colistin every 24h (139, 205). This was achieved by bolus administration at 0h followed by infused medium with colistin at the appropriate concentration.

In the second study, regimens evaluated were ceftazidime in CI at clinically achievable concentrations (4, 10, 20 and 40 mg/L), colistin in CI, combinations of colistin plus 4 or 40 mg/L ceftazidime and controls. For ceftazidime regimens, a bolus dose was injected at 0h and infused medium with ceftazidime at the appropriate concentration was

administered. The flow-rate reproduced ceftazidime $t_{1/2}$ (2h, flow rate 2mL/min). Colistin was administered the same way as in the first study.

2.4.4. *Pharmacodynamic analysis*

Samples from media (free-floating bacteria) and at least three coupons from a rod (biofilm-embedded bacteria) were collected at 0, 6, 24, 30, 48, and 54 hours (two extra coupons were collected at 54h).

Coupons were rinsed twice in PBS for 3 minutes each time, to remove non-adherent cells. Then, each coupon was placed in a 50-mL centrifuge tube containing 10 mL of PBS. Biofilm-embedded bacteria were recovered by three alternating cycles of vortexing and sonication, 1 minute each, followed by a final 1 minute of vortexing (Branson Ultrasonics, USA). Biofilm-embedded and free-floating cells were serially diluted with sterile saline and plated onto drug-free TSA or NA. Colonies were counted after 24 hours of incubation at 35ºC and 48 hours for the plates with small colonies.

Bacterial counts were expressed as $\log_{10}$ cfu/mL (means and standard deviations [SD]). The efficacy of a therapeutic regimen was evaluated against biofilm-embedded and free-floating bacteria using the log change method from hour 0 to each timepoint ($\Delta\log_{10}$ cfu/mL Xh-0h). Treatments were considered to be bactericidal (99.9% kill) when they led to a ≥3 $\log_{10}$ cfu/mL reduction, compared with the corresponding counts at zero time. Monotherapy or combination regimens causing a reduction of ≥1 $\log_{10}$ cfu/mL at a specified time were considered active. Synergy was defined as ≥2 $\log_{10}$ cfu/mL killing for the combination relative to the most active corresponding monotherapy at a specified time; additivity was defined as 1 to 2 $\log_{10}$ cfu/mL greater killing for the combination.

2.4.5. *Pharmacokinetic studies*

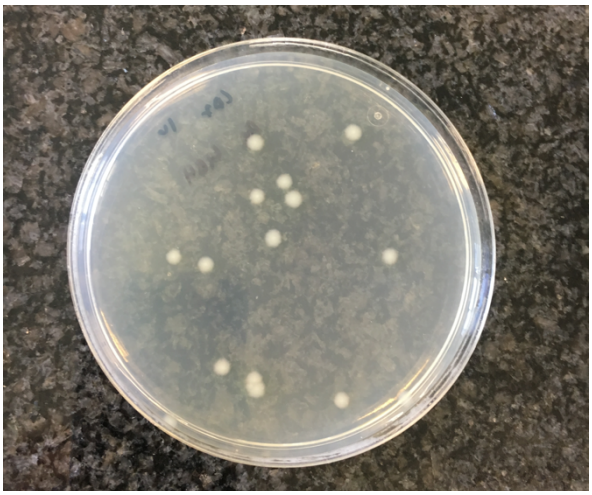
Samples (1 mL) collected in duplicate from the model at different timepoints were placed in 1.5 mL microcentrifuge tubes and immediately stored at -80ºC. Concentrations of colistin and BL were measured using HPLC as previously described (177, 178, 206).

2.4.6. *Resistance studies*

The emergence of resistance to ceftolozane-tazobactam and colistin (first study) and ceftazidime and colistin (second study) was evaluated throughout the treatment phase, when these antibiotics were administered.

MHA plates were prepared with a concentration at or above the EUCAST breakpoint of resistance. Antbiotic concentrations evaluated with this method were: ceftolozane-tazobactam 4-4 mg/L and colistin 2 mg/L (first study); and ceftazidime 16 mg/L and colistin 4 mg/L (second study). Samples (100 μL) of biofilm-embedded and free-floating bacteria were plated and incubated at 37ºC for 24 hours (or 48 hours for small colonies).

Figure 6. Agar plates with antibiotics (ceftazidime 16 mg/L) with *P. aeruginosa*-resistant strains.



Additionally, for experiments containing colistin in monotherapy or combination, PAPs of biofilm-embedded and free-floating cells recovered after 54 hours were performed. Changes between baseline and after treatment populations were examined. The methodology for PAPs has been described above.

2.4.7. *Confocal laser scanning microscopy (CLSM)*

In both experimental studies, coupons were evaluated by CLSM to confirm biofilm infection (0h) and treatment activity (54h). Images of the biofilms stained with LIVE/DEAD BacLight Bacterial Viability Kit (ThermoFisher Scientific, USA) were acquired. The microscopes employed differed in both studies:

- First Study: Leica TCS-SL filter-free spectral confocal laser scanning microscope (Leica Microsystems, Germany) equipped with a 488nm argon laser and 543nm He/Ne laser (Centres Científics i Tecnològics, Universitat Barcelona, Spain) using a 63x oil immersion objective (1.4 numerical aperture). Different image stacks

were acquired with a a 0.5 microns' distance between planes and pinhole size at 1AU.

- Second study: Nikon Eclipse Ti confocal laser scanning microscope (Nikon Instruments Inc., Japan) equipped with a 488nm argon laser and 561nm He/Ne laser (Monash Micro Imaging, Monash University, Australia) using a 20x dry objective (0.75 numerical aperture) for the second study. Different image stacks were acquired with 3 microns' distance between planes and pinhole size at 1.2AU.

The number of total planes was calculated according with the thickness of each biofilm. Different stacks were obtained randomly of each coupon. Selected fields were acquired with image resolution of 1024x1024 pixels. The images obtained were processed with IMARIS software (Bitplane AG, Switzerland).

2.4.8. *Statistical analysis*

Data were analysed using Stata 13.1 (Stata Corporation, USA). An analysis of variance with Tukey´s post hoc test was performed for each treatment regimen to evaluate changes in the log cfu/mL for free-floating and biofilm-embedded bacteria. A p value of ≤0.05 was considered statistically significant.

3. Funding and grants

The doctoral candidate received the following grants and funding during his research process:

- Grant FPU from the Spanish Ministry of Education (FPU 14/03124).
- Travel grant from the Spanish Ministry of Education (EST 17/00672).
- Travel grant from Universitat de Barcelona.

In addition, some studies were supported with the following grants:

- **FIS PI14/00511:** *Alternativas terapéuticas frente a la infección in vitro de cuerpo extraño producida por bacilos gram-negativos multiresistentes: estudios*

farmacodinámicos en monoterapia y en combinación. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge.

- **Merck Investigator Studies Program IISP 54711 (Merck, Sharp & Dohme S.A.):** *Efficacy of Ceftolozane-tazobactam, alone and in combination, against in vitro foreign-body infection caused by multidrug-resistant Pseudomonas aeruginosa: a pharmacodynamic study. Lead researcher: Oscar Murillo from Hospital Universitari de Bellvitge.*

RESULTS

A. IMPACT AND PROGNOSIS OF BACTERAEMIC OSTEOARTICULAR INFECTIONS

A.1 Impact of bacteraemic osteoarticular infections

In the last years, our group has aimed to analyse several aspects of bacteraemic OAIs, including epidemiological trends, associations with infective endocarditis (IE) and changes in patterns of SA. To do this, we analysed our hospital's database on bloodstream infections and added some specific variables on OAIs. As years went by, we included more cases and enlarged the database for the subsequent manuscripts.

Aim 1 – To describe epidemiological and microbiological trends of bacteraemic osteoarticular infections across time

Article 1 – *The changing epidemiology of bacteremic osteoarticular infections in the early 21st century.* O. Murillo, I. Grau, J. Lora-Tamayo, **J. Gómez-Junyent**, A. Ribera, F. Tubau, J. Ariza, R. Pallarés. Clinical Microbiology and Infection 2015; 21(3):254 e1-8.

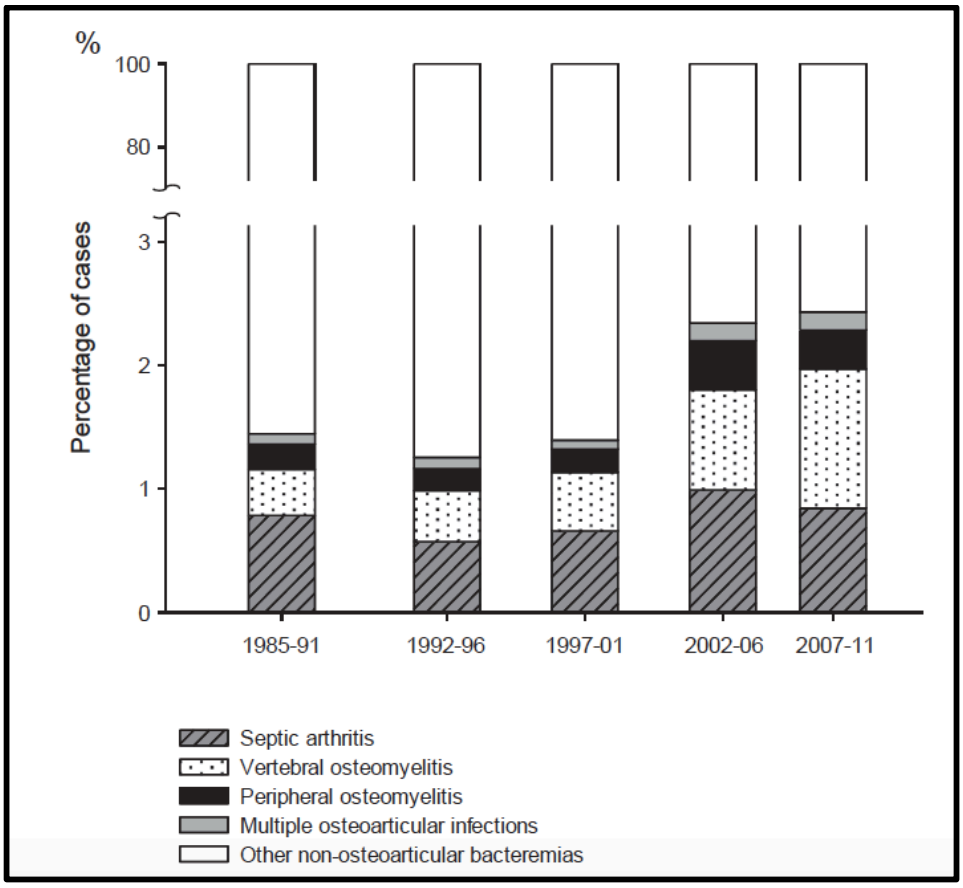
Communication 1 - *Changing epidemiology of bacteremic osteoarticular infections in a teaching hospital in Barcelona.* **J. Gómez-Junyent**, J. Lora-Tamayo, O. Murillo, I. Grau, M. Cisnal, J. Ariza, R. Pallares. 23th ECCMID, Berlin, Germany, 2013. Presentation number O286.

In this study, we aimed to determine changes over time in the types and characteristics of OAIs, the microorganisms causing these infections, associated comorbidities and other patient characteristics. We retrospectively analysed a cohort of bacteremic OAIs from a large database of bacteraemia episodes collected from 1985 to 2011. To assess the epidemiological changes over time, the bacteraemic episodes were grouped into five periods: 1985–91 (P1), 1992–96 (P2), 1997–2001 (P3), 2002–06 (P4) and 2007–11 (P5). Data on this population were obtained from the public website page of the Official Statistics in Catalonia (207): 2991146 inhabitants (P1), 2468378 (P2), 2740127 (P3), 3021176 (P4) and 3185079 (P5).

1.1 Characteristics of the general cohort

A total of 32727 episodes of clinically significant bacteraemia were registered during the period of study. Of these, 601 episodes (1.8%) had a concomitant OAI. There was an increasing incidence of bacteraemic OAIs in five periods (P1 to P5), rising from 2.34 to 5.78 episodes/100000 inhabitants per year ($p<0.001$); this was also the case for the incidence of total bacteraemia in these periods, rising from 162 to 238 episodes/100000 inhabitants per year ($p<0.001$). The proportion of bacteraemic OAI in relation to all bloodstream infections increased, from 1.45% in P1 to 2.43% in P5 ($p<0.001$; Figure 1.1.).

Figure 1.1. Proportion of bacteraemic osteoarticular infections compared with the total cases of bacteremia.



The bacteraemic OAI were considered to be 'primary' (456 cases, 76%) or 'secondary' (145, 24%). Among the latter, the most frequent distant foci of infection were vascular catheter-related infections (n = 49, 8%) and IE (n = 42, 7%). The prevalence of

nosocomial episodes of bacteraemic OAIs significantly increased during the study period, whereas community-acquired infections diminished (P1 to P5, 18% and 72% to 30% and 45%, respectively; p<0.001). Concurrently, we observed the progressive appearance of healthcare-associated episodes (from 10% to 25%; p<0.001).

Table 1.1. shows the most important characteristics of the patients with bacteraemic OAIs, and the changes observed over time. The median age increased significantly throughout the period studied; this was also the case when intravenous drug users were excluded (data not shown) taking into account that the proportion of this population decreased over time (p<0.001). Concurrently, we observed an increasing percentage of patients suffering from comorbid conditions (p<0.001).

Out of 601 cases of bacteraemic OAIs, 36 (6%) had multiple OAIs. Overall, SA was the most frequent (n = 291, 46%), followed by VO (n = 241, 38%) and PO (n = 105, 16%). Changes over time for different types of OAI are presented in Table 1. Although the number of episodes of SA increased over the period, its frequency with respect to all OAIs significantly decreased (57% in P1 and 38% in P5; p<0.001). The type of SA changed between the first (P1) and the last (P5) period of the study, with a lower incidence of native arthritis and an increase in the number of PJI (p<0.001). Although the incidence of PO remained stable throughout the period studied, the number of VO increased significantly. In fact, the number of all foreign body-related infections increased from 7% in P1 to 28% in P5 (p<0.001).

1.2. Microbiology of bacteraemic OAI

Gram-positive cocci caused 80% of OAI. The proportion of polymicrobial and anaerobic OAI was very low (2.4% and 1%, respectively) (Table 1.2.). *S. aureus* was by far the most frequent cause of episodes of all types of OAIs, whereas other microorganisms showed more affinity for a particular infection (Table 1.3.). In comparison with *S. aureus*, the viridans streptococci and *E. faecalis* groups produced more VO (56% and 57%, respectively), whereas pyogenic streptococci species caused SA (67%). Among cases of bacteraemic OAI caused by *S. aureus*, MRSA strains were more commonly involved in cases of PJI than MSSA strains (p<0.05).

Table 1.1. Characteristics of patients and episodes of bacteraemic osteoarticular infections and their changes over time.

	All OAI (n = 601)	Periods of study					p value[a]
		1985-1991 (n = 70)	1992-1996 (n = 70)	1997-2001 (n = 97)	2002-2006 (n = 181)	2007-2011 (n = 183)	
Age (median, IQR)	63 (50-74)	49 (24-64)	58 (32-68)	64 (50-74)	64 (53-74)	65 (53-77)	<0.001
Male	366 (61)	39 (56)	42 (60)	68 (70)	114 (63)	103 (56)	0.2
Underlying diseases							
One or more	307 (51)	16 (23)	22 (31)	51 (13)	111 (61)	107 (59)	<0.001
Intravenous drug users	57 (10)	17 (24)	9 (13)	13 (13)	11 (6)	7 (4)	<0.001
Diabetes mellitus	153 (26)	11 (16)	13 (19)	24 (25)	55 (30)	50 (27)	0.013
Neoplasm	71 (12)	1 (1)	3 (4)	15 (15)	30 (16)	22 (12)	0.006
Cardiopathy	88 (15)	1 (1)	2 (3)	14 (14)	29 (16)	42 (23)	<0.001
Chronic kidney disease	44 (7)	0	2 (3)	5 (5)	17 (9)	20 (11)	<0.001
Immunosuppressive therapy	88 (15)	6 (9)	4 (6)	14 (14)	34 (19)	30 (16)	0.01
Rheumatoid arthritis	30 (5)	5 (7)	0	7 (7)	10 (6)	8 (4)	0.2
Systemic autoimmune disease	16 (3)	1 (1)	2 (3)	3 (3)	3 (2)	7 (1)	0.4
Type of OAI							

Septic arthritis[b]	291 (46)	41 (57)	37 (49)	50 (49)	88 (45)	75 (38)	0.006
Native[c]	228 (78)	37 (90)	32 (86)	40 (80)	70 (79)	49 (65)	
Prosthetic[c]	63 (22)	4 (10)	5 (14)	10 (20)	18 (21)	26 (35)	0.001
Vertebral osteomyelitis[b]	241 (38)	18 (25)	26 (35)	37 (36)	71 (37)	89 (46)	0.001
Native[c]	206 (86)	18 (100)	25 (96)	37 (100)	60 (85)	66 (74)	
Spine instrumentation[c]	35 (14)	0	1 (4)	0	11 (15)	23 (26)	<0.001
Peripheral osteomyelitis[b]	105 (16)	13 (18)	12 (16)	15 (15)	34 (18)	31 (16)	0.9
With osteosynthesis[c]	16 (15)	1 (7)	3 (25)	1 (7)	8 (23)	3 (10)	0.3
Device-related infections[d]	114 (19)	5 (7)	9 (13)	11 (11)	37 (20)	52 (28)	<0.001

Data presented as numbers of cases (percentage), unless stated otherwise.

[a]p value represents the changing trends over periods of the study (Jonckheere–Terpstra test).

[b]Total: 637 OAI types in 601 cases of bacteraemic OAI, with 36 cases that presented more than one concurrent infection (6%).

[c]Percentages of episodes are calculated with respect to total episodes of septic arthritis, vertebral osteomyelitis or peripheral osteomyelitis, respectively.

[d]Device-related infections include prosthetic joint infections and osteosynthesis hardware.

Table 1.2. Microbiology of the 601 episodes of bacteraemic osteoarticular infections.

Gram-positive microorganisms			492 (80%)
Staphylococcus spp.			386 (62%)
	S. aureus[a]	368 (59%)	
	Coagulase-negative	18 (3%)	
Streptococcus spp.			86 (17%)
	S. pneumoniae	14 (2%)	
	S. pyogenes	12 (%)	
	S. agalactiae	23 (4%)	
	S. anginosus group	7 (1%)	
	S. bovis[b]	7 (1%)	
	Other group viridans streptococci[c]	15 (2%)	
	Other Streptococcus spp.[d]	8 (1%)	
Enterococcus spp.			15 (2%)
	E. faecalis	5 (2%)	
	E. faecium	1 (0.2%)	
Other Gram-positive microorganisms[e]			5 (0.8%)
Gram-negative microorganisms			126 (20%)
Enterobacteriaceae			87 (14%)
	E. coli	58 (9%)	
	K. pneumoniae	8 (1%)	
	P. mirabilis	8 (1%)	
	S. enteritidis	5 (0.8%)	
	M. morganii	3 (0.4%)	
	E. cloacae	2 (0.3%)	
	Citrobacter spp.	2 (0.3%)	
	S. marcescens	1 (0.2%)	
Non-fermenting Gram-negative bacilli			13 (2%)
	P. aeruginosa	11 (2%)	
	A. hydrophila	1 (0.2%)	
	A. baumanii	1 (0.2%)	

Other Gram-negative microorganisms			18 (3%)
	N. meningitidis	8 (1%)	
	Haemophilus spp.	4 (0.6%)	
	Veillonella spp.	2 (0.3%)	
	E. corrodens	2 (0.3%)	
	K. kingae	1 (0.2%)	
	Campylobacter spp.	1 (0.2%)	
Anaerobic Gram-negative microorganisms			8 (1%)
	Bacteroides spp.	7 (1%)	
	Porphyromonas spp.	1 (0.2%)	

Total: 618 isolates in 601 episodes of bacteraemic osteoarticular infections, with 15 episodes (2.4%) of polymicrobial bacteraemia.
[a]This included 49 isolates (13%) of methicillin-resistant *S. aureus*.
[b]*S. bovis* type I (5 isolates) and *S. bovis* type II (2 isolates).
[c]*S. sanguis* (6 isolates), *S. mitis* (6 isolates), *S. mutans* (2 isolates) and *S. salivarius* (1 isolate).
[d]*S. equi* group C (3 isolates), β-haemolyticus *Streptococcus* of group G (4 isolates) and β-haemolyticus *Streptococcus* of group F (1 isolate).
[e]*Abiotrofia* spp. (1 isolate), *Aerococcus viridans* (1 isolate), *Corynebacterium* spp. (1 isolate), *Eubacterium lentum* (1 isolate) and *Arcanobacterium* spp. (1 isolate).

Figure 1.2. illustrates the increasing incidence of the more frequent aetiologies in the study period. The number of episodes of OAIs caused by *S. aureus* increased throughout the period of study, but its global proportion with respect to other microorganisms fell from 71% in P1 to 57% in P5 (p=0.1). Significant changes were observed in the proportion of episodes caused by MSSA (a decrease from 71% in P1 to 45% in P5; p<0.001) and those by MRSA (an increase from 0% to 12%, respectively; p<0.001).

The episodes of OAI caused by *Streptococcus* spp. and *Enterococcus* spp. showed a trend towards an increase (from 10% to 19%; p=0.141). The frequency of *Enterobacteriaceae* and *P. aeruginosa* bacteraemia remained more stable (17% in P1 and 21% in P5). The number of ESBL-producing *Enterobacteriaceae* and MDR *P. aeruginosa* was very low (two and three episodes, respectively), but they increased in the last two periods (from none in the three first periods to two cases in P4 and three cases in P5; p=0.082).

Table 1.3. Association between the most frequent microorganisms and type of osteoarticular infections.

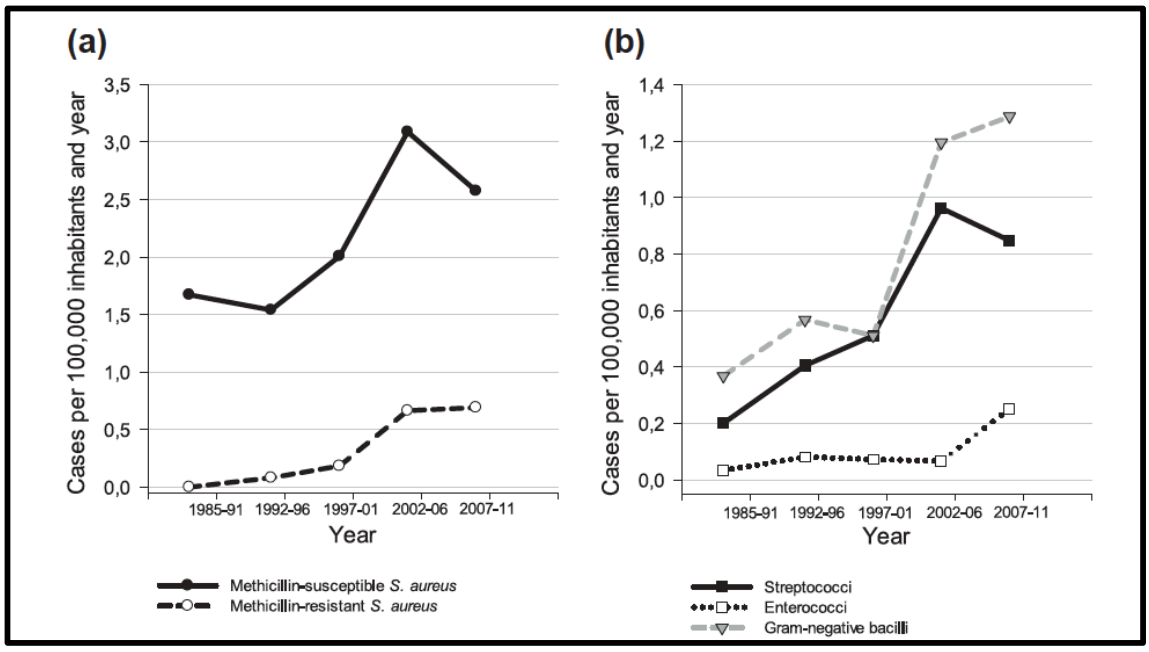
	Arthritis			Vertebral osteomyelitis			Peripheral osteomyelitis (POM)			
	All	Native	Prosthetic	All	Native	Spine	All	DM Foot	POM-no OS	OS
S. aureus (n = 384)	182 (47)	141 (36)	41 (11)	131 (35)	109 (29)	22 (6)	71 (18)	16 (4)	42 (11)	13 (3)
MSSA (n = 333)[a]	156 (47)	126 (38)	30 (9)	120 (36)	100 (30)	20 (6)	57 (17)	11 (3)	35 (11)	11 (3)
MRSA (n = 51)	26 (51)	15 (29)	11 (22)	11 (22)	9 (19)	2 (3)	14 (27)	5 (10)	7 (14)	2 (3)
Pyogenic streptococci (n = 57)[b]	38 (67)	32 (56)	6 (11)	15 (26)	15 (26)	0	4 (7)	3 (5)	1 (2)	0
Viridans streptococci (n = 41)[c]	12 (29)	10 (24)	2 (5)	23 (56)	23 (56)	1 (2)	6 (15)	0	6 (15)	0
Enterococcus (n = 14)[d]	5 (36)	3 (22)	2 (14)	8 (57)	8 (57)	1 (7)	1 (7)	0	0	1 (7)
GNB (n = 118)[e]	44 (37)	34 (28)	10 (9)	51 (44)	51 (44)	11 (9)	23 (19)	11 (9)	10 (8)	2 (2)

Note: Data presented are number of cases (percentage); cases with multiple OAI (n = 36) are included.

Abbreviations: POM, peripheral osteomyelitis; DM foot, diabetic foot infections; POM-no OS, peripheral osteomyelitis without osteosynthesis; OS, osteosynthesis; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; pyogenic streptococci, *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*; GNB, Gram-negative bacilli.

[a]Differences between MSSA and MRSA, p=0.03 (chi-square); [b]Differences between *S. aureus* (All) and pyogenic streptococci (All), p=0.02 (chi-square); [c]Differences between *S. aureus* (All) and viridans streptococci (All), p=0.02 (chi-square); [d]No significant differences between S. aureus (All) and *Enterococcus* sp. (All); *e*No significant differences between *S. aureus* (All) and GNB (All).

Figure 1.2. Incidence of the more frequent microorganisms responsible for bacteraemic osteaorticular infections in the study period. (a) Incidence of cases caused by methicillin-susceptible and -resistant *Staphylococcus aureus*. (b) Incidence of cases caused by *Streptococcus* sp., *Enterococcus* sp. and Gram-negative bacilli.



1.3. Characteristics of bacteraemic osteoarticular infections according to patients' age

Table 1.4. shows the main characteristics of episodes of OAIs and the differences between age groups (group 1, patients aged 49 or less, group 2, patients aged 50 to 64, and group 3, patients aged 65 or over).

In comparison with younger patients (group 1), patients in groups 2 and 3 had more underlying diseases, and OAI were more frequently hospital-acquired or healthcare-related. PO was more frequent in younger patients, whereas there were more episodes of VO in groups 2 and 3 ($p<0.05$). There were no differences in the total number of episodes of arthritis, but older patients (groups 2 and 3) had significantly more PJI.

Table 1.4. Clinical and microbiological characteristics of cases of osteoarticular infections according to patients' age: Group 1 (age≤49 years), Group 2 (age 50–64 years), and Group 3 (age≥65 years).

	Group 1 n = 149	Group 2 n = 178	p value (1 vs 2)	Group 3 n =274	p value (2 vs 3)	p value (1 vs 3)
One or more underlying diseases	24 (16)	106 (60)	<0.001	177 (65)	0.3	<0.001
Intravenous drug users	56 (38)	1 (1)	<0.001	0	0.2	<0.001
Place of acquisition						
Community	108 (72)	92 (52)		134 (49)		
Healthcare	10 (7)	35 (20)		54 (20)		
Nosocomial	31 (21)	51 (29)	<0.001	85 (31)	0.8	<0.001
Device-related OAI[a]	19 (13)	26 (15)	0.6	68 (25)	0.009	0.003
Septic arthritis	76 (51)	82 (46)	0.3	133 (48)	0.6	0.6
Native	74 (50)	72 (40)	0.09	82 (30)	0.02	<0.001
Prosthetic	2 (1)	10 (6)	0.04	51 (18)	<0.001	<0.001
Vertebral osteomyelitis	37 (25)	66 (37)	0.02	102 (37)	0.9	0.01
Spine instrumentation	9 (6)	16 (9)	0.3	10 (4)	0.02	0.2
Peripheral osteomyelitis	37 (25)	27 (15)	0.03	40 (15)	0.9	0.009
S. aureus	111 (75)	104 (58)	0.002	153 (56)	0.6	<0.001
MSSA	108 (73)	90 (50)	<0.001	120 (44)	0.2	<0.001
MRSA	3 (2)	14 (8)	0.02	32 (12)	0.2	0.001
Pyogenic streptococci	8 (5)	13 (7)	0.4	28 (10)	0.3	0.09
Viridans streptococci	3 (2)	16 (9)	0.007	20 (7)	0.5	0.02
Enterococcus spp.	2 (1)	1 (1)	0.4	12 (4)	0.02	0.09
Gram-negative bacilli	20 (13)	39 (22)	0.047	53 (19)	0.5	0.1

[a]Device-related OAI include infections of prosthetic joint, spine instrumentation and osteomyelitis with osteo-synthesis hardware.

OAI, osteoarticular infection; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus.*

Regarding microbiology, *S. aureus* was involved in a greater number of episodes of OAIs in younger patients than in the older ones (p=0.002); when these staphylococcal OAIs occurred, MSSA strains were responsible for almost all episodes in group 1, whereas MRSA strains were significantly more frequent in patients in groups 2 and 3. Streptococcal infections (either pyogenic or viridans species) increased in older patients, and enterococcal OAIs were significantly related with the oldest patients (group 3). OAIs caused by GNB were less common among younger patients (group 1).

The main differences between groups 2 and 3 were that the oldest group presented significantly less native SA and more PJI, and a higher frequency of enterococcal infections.

Additionally, we compared the median age of patients with bacteraemic OAIs during the study period by types of infection and microbiology (Table 1.5.). Of note, the median age of all SA significantly increased from 48 years in P1 to 68 years in P5 (p<0.001); this was mainly due to the rise in the number of PJI cases, which occurred in an older population that did not significantly change over time. In a similar way, a significant increase in the age of patients was observed for staphylococcal OAIs, this being related to the rise in the number of episodes caused by MRSA strains in older patients. All of these differences were maintained when the young population of intravenous drug users were excluded. In contrast, no significant changes in the median age of patients over time were observed for episodes of VO and for episodes of OAI caused by other relevant microorganisms.

Table 1.5. Changes in the age of patients with bacteraemic osteoarticular infections during the study period according to type of infection and microbiology.

	All study period	1985-1991	1992-1996	1997-2001	2002-2006	2007-2011	p value
Type of OAI							
Septic arthritis	63 (49-75)	48 (25-63)	53 (30-69)	63 (51-73)	67 (52-76)	68 (54-78)	0.001
Native	58 (43-71)	45 (24-60)	52 (29-66)	58 (48-70)	62 (48-74)	60 (50-75)	<0.001
Prosthetic	75 (68-79)	69 (61-74)	73 (60-75)	74 (69-77)	76 (67-84)	77 (74-82)	0.1
Vertebral osteomyelitis	64 (53-74)	58 (25-73)	62 (51-66)	65 (46-76)	64 (55-74)	65 (54-74)	0.3
Native	65 (54-74)	58 (25-73)	63 (51-66)	65 (46-76)	66 (55-74)	67 (56-76)	0.09
Spine instrumentation	59 (48-66)	-	-	-	60 (51-64)	58 (42-68)	0.9
Peripheral osteomyelitis	59 (39-59)	28 (21-54)	33 (28-57)	66 (53-74)	58 (41-68)	65 (52-72)	0.001
Native	59 (40-68)	27 (21-57)	30 (28-55)	65 (51-71)	60 (46-66)	64 (51-71)	0.001
With osteosynthesis	58 (35-81)	-	37 (30-61)	-	44 (33-75)	83 (78-86)	0.3
Microbiology							
S. aureus	61 (44-73)	40 (23-62)	51 (28-66)	63 (48-73)	62 (49-74)	65 (65-80)	0.001
MSSA	58 (42-71)	40 (23-62)	51 (28-66)	62 (47-73)	61 (45-73)	63 (49-74)	0.001
MRSA	72 (62-77)	-	64 (64-65)	70 (60-75)	62 (62-76)	73 (58-78)	0.8
Pyogenic streptococci	66 (53-73)	56 (45-67)	73 (62-79)	53 (26-70)	67 (57-74)	66 (62-73)	0.4
Viridans streptococci	66 (59-73)	49 (25-60)	66 (61-73)	69 (61-77)	69 (57-77)	64 (59-72)	0.1
Enterococcus spp.	71 (68-80)	-	52 (37-68)	78 (74-82)	75 (71-80)	69 (65-78)	0.2
Gram-negative bacilli	64 (51-76)	64 (50-75)	56 (46-67)	69 (51-81)	64 (51-73)	66 (54-78)	0.2

Median age (IQR) is presented when there were at least three cases in the study period.

OAI, osteoarticular infection; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus.*

Aim 2 – To analyse the characteristics of bacteraemic osteoarticular infections associated with the presence of infective endocarditis

Article 2 – *Endocarditis associated with vertebral osteomyelitis and septic arthritis of the axial skeleton.* O. Murillo, I. Grau, **J. Gómez-Junyent**, C. Cabrera, A. Ribera, F. Tubau, C. Peña, J. Ariza, R. Pallarés. Infection 2018 Apr;46(2):245-251.

Communication 2 - *Infectious endocarditis among patients with bacteremic osteoarticular infections.* C. Cabrera, O. Murillo, I. Grau, **J. Gómez-Junyent**, A. Ribera, C. Peña, F. Tubau, J. Ariza, R. Pallares. 25th ECCMID, Copenhague, Denmark, 2015. Presentation number P0007.

The aim of this study, which includes a large number of patients (without IDUs), was to analyse the clinical, epidemiological and microbiological characteristics of bacteraemic OAIs associated with the presence of IE.

In this study, we retrospectively analysed cases with IE (group 1) between 1993 and 2014, with a special focus on metastatic OAIs. We also analysed cases with bacteraemic OAIs (group 2) during the same period. Patients were excluded if they were younger than 18 years old or IDUs. Cases caused by *Neisseria* spp. were also excluded in order to avoid including cases with reactive rather than SA.

2.1 Cases with infective endocarditis and association with osteoarticular infections

During the period of study (1993–2014), there were 607 cases of IE. The most frequent aetiologies were: *S. viridans* group (n = 181, 30%), *S. aureus* (n = 123, 20%; 20 cases caused by methicillin-resistant strains), CNS (n = 96, 16%), enterococci (n = 94, 15%), *S. bovis* group (n = 64, 10%), and others (n = 49, 9%). The proportion of metastatic OAIs (70 cases) among patients with IE was 11.5% (70/607 cases); the microorganisms responsible were *S. aureus* (27/123, 22%), enterococci (10/94, 11%), *S. viridans* (16/181, 9%), *S. bovis* group (6/64, 9%), and CNS (6/96, 6%). The type of OAI observed in these patients (see below) was involvement of the axial skeleton in 77% of cases (VO, SA or both). Patients with IE and associated OAIs, compared with those without OAIs, were older, had less frequently cardiac predisposing factors, the aortic valve was less

commonly affected, had fewer prosthetic valves, and were caused more often by *S. aureus* (Table 2.1.).

Table 2.1. Characteristics of patients with infective endocarditis comparing those with and without osteoarticular infections.

	IE with OAIs (n = 70)	IE without OAIs (n = 537)	p value
Age (median, IQR)	68 (59-74)	65 (54-74)	0.10
Sex (male)	50 (71)	347 (65)	0.90
Cardiac predisposing factor for IE[a]	30 (43)	333 (62)	0.001
Additional emboli[b]	14 (20)	134 (25)	0.30
Valvular location			
Mitral valve	42 (60)	270 (50)	0.10
Aortic valve	29 (41)	301 (56)	0.02
Tricuspid valve	5 (7)	17 (3)	0.10
Prosthetic valve	11 (16)	167 (31)	0.002
Presence of vegetation	46 (66)	331 (62)	0.40
Positive blood cultures (≥4)	41 (59)	305 (57)	0.20
Microorganism			
S. aureus	27 (39)	96 (18)	<0.001
Coagulase-negative staphylococci	6 (9)	90 (17)	0.10
S. viridans	16 (23)	165 (31)	0.50
S. bovis	6 (9)	58 (11)	0.80
***Enterococcus* spp**	10 (14)	84 (16)	0.70
Pyogenic streptococci[c]	3 (4)	20 (4)	0.90
Others	2 (3)	24 (4)	0.90

Data expressed as No. (%), if not stated otherwise. IE: Infective endocarditis. OAI: Osteoarticular infection. [a]Includes degenerative or rheumatic valvulopathy, mitral prolapse, bivalve aorta, congenital valvulopathy, previous IE or prosthetic valve. [b]Includes emboli to the brain, spleen, vascular periphery artery or kidneys, or presence of Roth spots. [c]Pyogenic streptococci: *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*.

2.2 Description of bacteraemic osteoarticular infections with or without infective endocarditis

During the same period, there were 458 cases of bacteraemic OAIs (36 had multiple OAIs): VO (n = 202), peripheral SA (n = 175), SA of the axial skeleton (n = 67), and PO (n = 50). Seventy out of 458 OAIs (15%) were associated with IE. This proportion differed according to the type of OAI: 22% (15/67) for SA of the axial skeleton, 21% (43/202) for VO, 11% (19/175) for peripheral SA, and 4% (2/50) for PO. Table 2.2. shows the microorganisms causing OAIs and the proportion with concomitant IE.

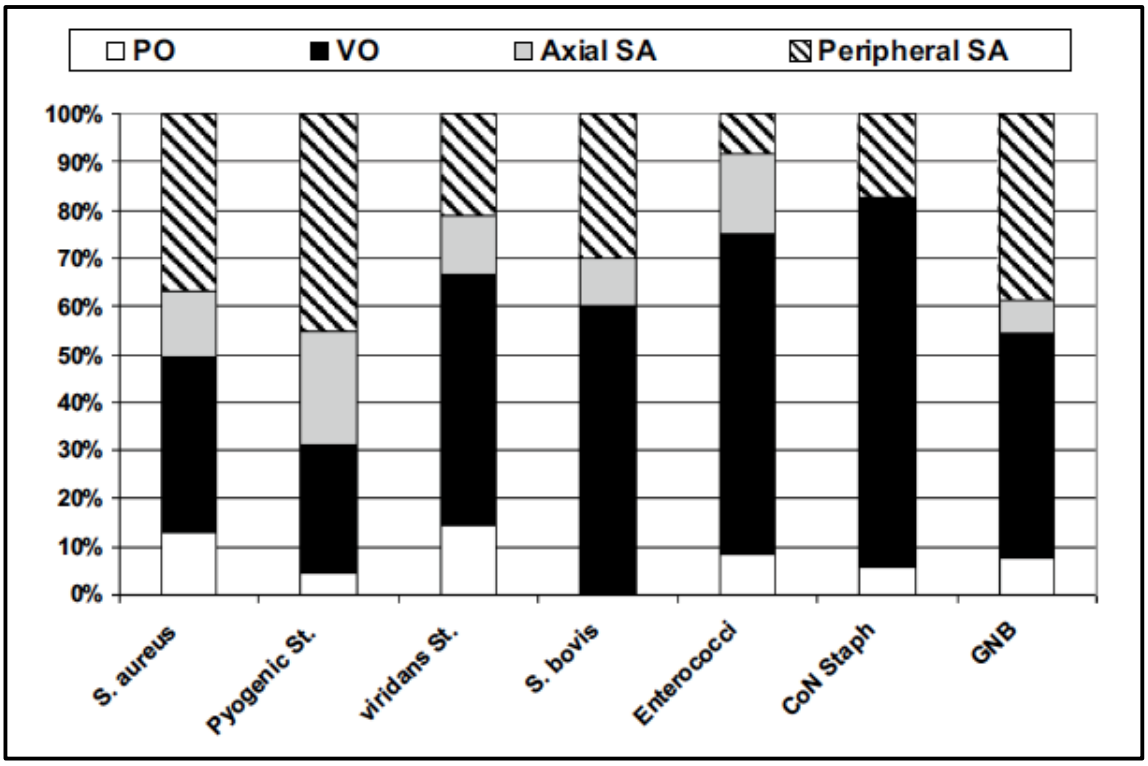
Table 2.2. Comparison between the presence or not of infective endocarditis among cases of bacteraemic osteoarticular infections according to causative microorganisms.

Microorganisms	Presence of IE		p value
	Yes	No	
S. aureus **(n = 254)**	27 (11)	227 (89)	0.002
Coagulase-negative staphylococci (n = 17)	6 (35)	11 (65)	0.02
S. viridans **group (n = 37)**	16 (43)	21 (56)	0.001
S. bovis **(n = 9)**	6 (67)	3 (33)	<0.001
Enterococcus **spp. (n = 11)**	10 (91)	1 (9)	<0.001
Pyogenic streptococci[a] (n = 56)	3 (5)	52 (95)	0.03
Gram-negative bacilli (n = 74)	1 (1)	73 (99)	<0.001

Data expressed as No. (%). IE: Infective endocarditis. [a]Pyogenic streptococci: *S. pneumoniae, S. pyogenes, S. agalactiae.*

Moreover, Figure 2.1. shows the proportion of different OAIs for each microorganism. The ratio of VO vs peripheral SA for each microorganism was as follows: 0.6 for pyogenic streptococci, 0.99 for *S. aureus*, 1.2 for Gram-negative bacilli, 2 *S. bovis*, 2.4 viridans streptococci, 4.3 coagulase-negative staphylococci, and 8 enterococci.

Figure 2.1. Proportion of different types of bacteremic osteoarticular infection according to causative microorganism.



PO, Peripheral osteomielitis; VO, Vertebral osteomielitis; SA, Septic arthritis; CoN Staph, Coagulase-negative staphylococci; GNB, Gram-negative bacilli

Analysing the types of OAIs and their relationship with the presence of IE (Table 2.3.), we observed that *S. aureus* was the main agent responsible for the association with SA of the axial skeleton, whereas viridans streptococci, *S. bovis*, enterococci and CNS were mainly responsible for the association with VO. Particularly, there were 17 cases of bacteraemic OAIs caused by CNS (13 VO, 76%; and 4 SA, one PJI); 6 of them had IE (5 native and 1 prosthetic valve) and in 9 cases, it was associated with a peripheral or central venous catheter infection. Pyogenic streptococci also caused a large number of SA of the axial skeleton (15/67, 22%), but only a few cases of IE (3/607, 0.5%, all these cases had involvement of the axial skeleton).

Table 2.3. Types of bacteraemic osteoarticular infections (OAIs) caused by *S. aureus* compared with *S. viridans* group, *S. bovis*, enterococci and coagulase-negative staphylococci according to the presence or absence of infective endocarditis (IE)

Bacteremic OAI	***S. aureus* (n = 254)**			***S. viridans*, *S. bovis*, *Enterococcus* spp. and coagulase negative staphylococci (n = 73)**		
	With IE (n = 27)	**Without IE (n = 227)**	**p value**	**With IE (n = 38)**	**Without IE (n = 35)**	**p value**
SA, all	16 (59)	120 (53)	0.5	11 (29)	11 (31)	0.8
Axial SA	8 (30)	29 (13)	0.02	5 (13)	3 (9)	0.5
Peripheral SA	11 (41)	89 (39)	0.9	7 (18)	9 (26)	0.4
VO	10 (37)	89 (39)	0.8	30 (79)	19 (54)	0.02
PO	2 (7)	33 (14)	0.3	0	8 (23)	0.002

OAI, Osteoarticular infection; IE, Infective endocarditis; SA, Septic arthritis; VO, Vertebral osteomielitis; PO, Peripheral osteomielitis.

A comparative analysis of bacteraemic OAI cases with or without IE (univariate analysis) is presented in Table 2.4. The main parameters associated with IE were: OAIs with involvement of the axial skeleton (OR 3.1), VO (OR 2.3), and several aetiologies such as CNS (OR 3.2), *S. viridans* group (OR 5.4), and enterococci and *S. bovis* (OR 28.4). An OAI with involvement of the axial skeleton was associated with IE (adjusted OR = 2.2; 95% CI 1.1–4.3) after adjusting for age, sex and microorganisms.

Table 2.4. Comparison between bacteraemic osteoarticular infection (OAI) cases with or without concomitant infective endocarditis (IE).

	OAI with IE (n = 70)	OAI without IE (n = 388)	p value	Unadjusted OR (95% CI)
Age (median, IQR)	68 (59-74)	67 (55-77)	0.8	
Male sex	50 (71)	236 (61)	0.09	
Type and location of OAI[a]				
All OAIs of axial skeleton	54 (77)	203 (52)	<0.001	3.1 (1.7-5.6)
Vertebral osteomyelitis	43 (61)	159 (41)	0.002	2.3 (1.4-3.9)
SA of axial skeleton	15 (21)	52 (13)	0.08	1.8 (0.9-3.3)
Peripheral SA	19 (27)	156 (40)	0.04	0.6 (0.3-1)
Peripheral osteomyelitis	2 (3)	48 (12)	0.02	0.2 (0.05-0.9)
Microbiology				
S. aureus	27 (39)	227 (58)	0.002	0.4 (0.3-0.7)
CoNS	6 (9)	11 (3)	0.02	3.2 (1.1-9)
S. viridans	16 (23)	20 (5)	<0.001	5.4 (2.7-11.2)
***S. bovis* and enterococci**	16 (23)	4 (1)	<0.001	28.4 (9.2-88.2)
Pyogenic streptococci	3 (4)	52 (13)	0.03	0.3 (0.1-0.9)
Gram-negative bacilli	1 (1)	72 (19)	<0.001	0.06 (0.01-0.5)
30-day mortality	9 (13)	44 (11)	0.7	

OAI, Ostearticular infection; IE, Infective endocarditis; OR, Odds ratio; 95% CI, 95% confidence interval; IQR, Interquartile range; SA, Septic arthritis; CoNS, Coagulase-negative staphylococci.

[a]Among OAI cases with IE, five presented with concomitant vertebral osteomyelitis and septic arthritis (two septic arthritis of the axial skeleton + peripheral septic arthritis; two septic arthritis of the axial skeleton; one peripheral septic arthritis); three cases of septic arthritis presented concomitant involvement of the peripheral and axial skeletons.

Aim 3 – To compare the characteristics of bacteraemic septic arthritis, according to the site of acquisition

Article 3 – *Clinical findings of bacteremic septic arthritis according to the site of acquisition: the overlap between healthcare-related and community- and nosocomial-acquired cases.* O. Murillo, **J. Gómez-Junyent**, I. Grau, A. Ribera, C. Cabrera, S. Pedrero, F. Tubau, J. M. Nolla, J. Ariza, R. Pallarés. European Journal of Internal Medicine 2016 Mar;28:38-42.

Communication 3 - *Cambios epidemiológicos de la artritis séptica bacteriémica en un hospital universitario (1985-2011).* **J. Gómez-Junyent**, O. Murillo, I. Grau, J. Lora-Tamayo, M. Cisnal, J. Ariza, R. Pallarés. XVII SEIMC. Zaragoza, España, 2013. Presentation number P135.

In this study, we analysed a large cohort presenting bacteraemic SA (native or prosthetic) over the last 3 decades, excluding IDUs. Cases were considered to be nosocomial-acquired, healthcare-related or community-acquired in accordance with the definitions provided by Friedman et al (17).

A total of 35250 episodes of clinically significant bacteraemia were recorded during the period of study. Among these, 273 cases (0.8%) had a concomitant SA; the source of bacteraemia was considered “primary” in 200 cases (73%), and “secondary” in the remaining 73 cases (27%). Among the latter, the most frequent initial origins of bacteraemia were vascular-catheter infection (n = 27, 10%), IE (n = 20, 7%), and soft tissue infections (n = 13, 5%).

The site of acquisition of SA was classified as: community-acquired (n=139, 51%), nosocomial (n=84, 31%), and healthcare-related (n = 50, 18%). Differences in the source of bacteraemia regarding the site of acquisition were observed between primary and vascular catheter foci (which represented 81% and 0% of community-acquired cases, 69% and 21% of nosocomial-acquired cases, and 62% and 18% of healthcare-related cases respectively; $p<0.001$ and $p=0.05$).

SA occurred more frequently in male patients (56%), and the median age was 67 years (IQR 55–77). The most frequent baseline conditions are presented in Table 3.1. Older

and more fragile patients were more likely to have nosocomial or healthcare-related sites of acquisition. Nosocomial and healthcare-related cases were more likely than community-acquired cases to present relevant risk factors for SA such as immunosuppressive therapy, chronic renal insufficiency or prosthesis infection.

The location of SA also differed depending on the site of acquisition: while in community-acquired and healthcare-related cases the location was similar (peripheral joints in 76% and the axial skeleton in 24%), in nosocomial cases it was mainly the peripheral joints (92% vs 8% for the axial skeleton; p=0.003 and p=0.01, respectively) (Table 3.1). The higher number of PJIs acquired in the hospital environment was responsible for the differences in the overall percentage of peripheral joint SA between nosocomial and community-acquired or healthcare-related cases (p<0.001 and p=0.005, respectively; Table 3.1).

There were 70 episodes (26%) of PJI, all of them monoarticular and mostly affecting the hip (n = 41) and the knee (n = 26). The main differences between PJI and native SA cases were that PJIs occurred more frequently in older patients (median age, IQR: 76, 71–81 vs 64, 52–74; p<0.001) and had more infections caused by MRSA and *Enterobacteriaceae* strains (17% vs 8%, and 20% vs 10%; p=0.03 and p=0.04, respectively). With regard to the site of acquisition, native SA and PJI were broken down as follows: nosocomial-acquired, 23% and 53% (p<0.001); healthcare-related, 20% and 14% (p=0.3); and community-acquired, 57% and 33% (p=0.001).

The microorganisms responsible for bacteraemic SA are presented in Table 3.2. These episodes were mainly caused by Gram-positive bacteria (81%), and only five cases (2%) were polymicrobial. *S. aureus* was by far the most frequent microorganism (n = 157, 57%), the strains being MSSA in 82% of the cases and MRSA in 18%. *Streptococcus* spp. caused 21% (n = 59) of SA cases, with *S. agalactiae* predominating (n = 21). Among GNB, *Escherichia coli* (n = 19) and *Pseudomonas aeruginosa* (n = 9) were the most frequent isolates.

Table 3.1. Patients' characteristics, location, microbiology and mortality of septic arthritis cases according to the site of acquisition.

	All (n = 273)	Community-acquired (n = 139)	Nosocomial (n = 84)	p value (C vs N)	Healthcare-related (n =50)	p value (N vs H)	p value (C vs H)
Age (median, IQR)	67 (55-77)	65 (52-76)	73 (58-79)	0.03	66 (60-76)	0.3	0.2
Male	153 (56)	79 (57)	41 (49)	0.2	32 (64)	0.09	0.4
Underlying diseases							
One or more	167 (61)	74 (54)	53 (63)	0.2	40 (80)	0.04	0.001
Diabetes mellitus	74 (27)	39 (28)	21 (25)	0.6	14 (28)	0.7	0.9
Cancer	37 (14)	9 (6)	14 (17)	0.02	14 (28)	0.1	<0.001
Immunosuppressive therapy	67 (25)	28 (20)	17 (20)	0.9	22 (44)	0.003	0.001
Cardiopathy	51 (19)	21 (15)	19 (23)	0.2	11 (22)	0.9	0.3
Chronic kidney disease	20 (7)	0	3 (4)	0.02	17 (34)	<0.001	<0.001
Rheumatoid arthritis	27 (10)	13 (9)	7 (8)	0.8	7 (14)	0.3	0.4
Location of SA							
Axial	53 (19)	33 (24)	7 (8)	0.003	12 (24)	0.01	0.9
Peripheral	220 (81)	105 (76)	77 (92)	0.003	38 (76)	0.01	0.9
Native	150 (55)	81 (59)	40 (48)	0.1	28 (56)	0.3	0.7
Prosthesis	70 (26)	24 (17)	37 (44)	<0.001	10 (20)	0.005	0.6

Microbiology							
MSSA	129 (47)	75 (54)	37 (44)	0.1	17 (34)	0.2	0.01
MRSA	27 (10)	2 (1)	18 (21)	<0.001	7 (14)	0.3	<0.001
***Streptococcus* spp.**	59 (22)	41 (30)	4 (5)	<0.001	14 (28)	<0.001	0.8
Pyogenic streptococci[1]	43 (16)	32 (23)	2 (2)	<0.001	9 (18)	0.001	0.5
Other streptococci	16 (6)	9 (6)	2 (2)	0.2	5 (10)	0.05	0.4
***Enterococcus* spp.**	5 (2)	1 (1)	2 (2)	0.3	2 (4)	0.6	0.1
GNB	49 (18)	19 (14)	21 (25)	0.03	9 (18)	0.3	0.5
Escherichia coli	19 (7)	13 (9)	4 (5)	0.2	2 (4)	0.8	0.2
Pseudomonas aeruginosa	9 (3)	0	5 (6)	0.004	4 (8)	0.6	0.001

Data expressed as No. (%), if not stated otherwise.

Abbreviations: C, community-acquired; N, nosocomial-acquired; H, healthcare-related; SA, septic arthritis; MSSA, methicillin-susceptible Stahylococcus aureus; MRSA, methicillin-resistant S. aureus; GNB, Gram-negative bacilli.

[1]Pyogenic streptococci: S. pyogenes, S. agalactiae and S. pneumoniae.

Table 3.2. Microorganisms responsible for all cases of septic arthritis.

Gram-positive microorganisms			226 (81%)
Staphylococcus spp.			160 (58%)
	S. aureus	157 (57%)	
	MSSA	129 (47%)	
	MRSA	28 (10%)	
	Coagulase-negative	3 (1%)	
Streptococcus spp.			59 (21%)
	S. agalactiae	21 (7%)	
	S. pneumoniae	13 (5%)	
	S. pyogenes	9 (3%)	
	Other[a]	16 (5%)	
Enterococcus spp.			5 (2%)
	E. faecalis	5 (2%)	
Other Gram-positive microorganisms			2 (0.7%)
	Corynebacterium spp.	1 (0.4%)	
	Gemella spp.	1 (0.4%)	
Gram-negative microorganisms			52 (19%)
Enterobacteriaceae			35 (12%)
	E. coli	19 (7%)	
	K. pneumoniae	6 (2%)	
	S. enteritidis	3 (1%)	
	P. mirabilis	2 (0.8%)	
	E. cloacae	2 (0.8%)	
	M. morganii	2 (0.8%)	
	S. marcescens	1 (0.4%)	
Non-fermenting Gram-negative bacilli			9 (4%)
	P. aeruginosa	9 (4%)	
Other Gram-negative microorganisms			8 (3%)
	B. fragilis	4 (1.5%)	
	Haemophilus spp.	2 (0.8%)	
	Eubacterium spp.	1 (0.4%)	

	A. hydrophila	1 (0.4%)

Total: 278 isolates in 273 cases of bacteremic SA, with 5 cases of polymicrobial bacteremia.
[a]Other *Streptococcus* groups included: *Streptococcus equi* (n=3), *Streptococcus* group G (3), *Streptococcus* bovis (3), *Streptococcus intermedius* (2), *Streptococcus mitis* (2), *Streptococcus sanguis* (1), *Streptococcus salivarius* (1), and *Streptococcus* group F (1).

Significant differences in the aetiology of SA with regard to the site of acquisition are shown in Table 3.1. *S. aureus* was the most frequently involved microorganism in all cases; MSSA strains were less common than other aetiologies in healthcare-related cases than in the others. In addition, the presence of MRSA strains was significantly higher in nosocomial-acquired and healthcare-related cases (33% and 29% of all *S. aureus* respectively) than in community-acquired cases (1%; $p<0.001$). The proportion of streptococcal SA was significantly higher among community-acquired and healthcare-related cases than among nosocomial-acquired cases, especially in those caused by the pyogenic *Streptococcus* species. Infections caused by GNB were more frequently nosocomial than community-acquired ($p = 0.03$); all episodes of SA caused by *P. aeruginosa* were nosocomial-acquired or healthcare-related. Microorganisms that were more typically related to PJI than to native SA were MRSA and *Enterobacteriaceae* strains ($p = 0.03$ and $p = 0.04$ respectively).

A.2 Prognosis of bacteraemic osteoarticular infections

Aim 4 – To analyse the mortality and associated risk factors in patients with bacteraemic osteoarticular infections

Article 3 – *Clinical findings of bacteremic septic arthritis according to the site of acquisition: the overlap between healthcare-related and community- and nosocomial-acquired cases.* O. Murillo, **J. Gómez-Junyent**, I. Grau, A. Ribera, C. Cabrera, S. Pedrero, F. Tubau, J. M. Nolla, J. Ariza, R. Pallarés. European Journal of Internal Medicine 2016 Mar;28:38-42.

Article 4 – *Analysis of mortality in a cohort of 650 cases of bacteremic osteoarticular infections.* **J. Gómez-Junyent**, O. Murillo, I. Grau, E. Benavent, A. Ribera, X. Cabo, F. Tubau, J. Ariza, R. Pallarés. Seminars in Arthritis and Rheumatism 2018; 48(2):327-333.

Communication 4 - *Mortalidad y factores pronóstico en una cohorte de pacientes con bacteraemia e infecciones osteoarticulares.* **J. Gómez-Junyent**, O. Murillo, I. Grau, C. Cabrera, X. Cabo, F. Tubau, J. Ariza, R. Pallarés. XX SEIMC, Barcelona, Spain, 2016. Presentation number O060.

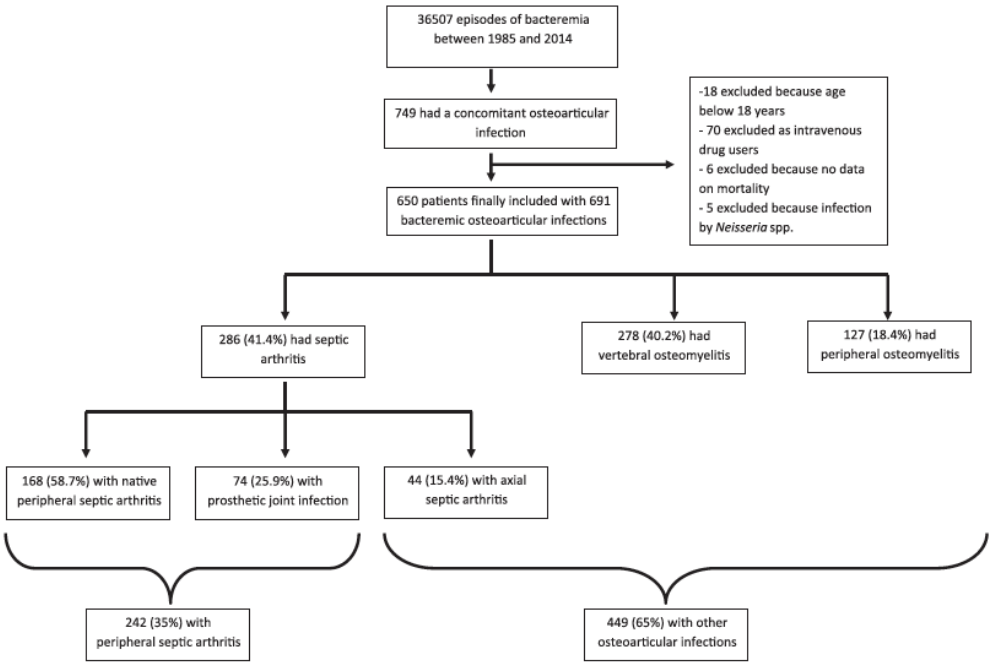
In this study, our aim was to analyse the mortality (30-day case-fatality rate) among a large cohort of patients with bacteraemic OAIs, and to investigate the host, microbiological, and interventional factors that may influence prognosis.

We performed a retrospective study in a large teaching hospital (Hospital Universitari de Bellvitge) including all cases of bacteraemic OAIs between 1985 and 2014. Patients were excluded if they were younger than 18 years old or intravenous drug users. Cases caused by *Neisseria* spp. were also excluded in order to avoid including cases with reactive rather than SA. Mortality was recorded if death occurred within 30 days from the diagnosis, and early mortality if death occurred within 7 days. Moreover, we also include mortality data from Article 3, especially evaluating the impact of site acquisition on prognosis.

4.1 Description of the global cohort

Between 1985 and 2014, a total of 36507 episodes of bacteraemia were recorded in our institution, of which 749 (2.1%) had a concomitant OAI. Of these, we excluded 18 that occurred in patients < 18 years, 70 that occurred in IVDU, 5 that were caused by *Neisseria spp.*, and 6 that lacked data on mortality. Finally, 650 episodes were therefore analysed (Figure 4.1.).

Figure 4.1. Flowchart of participants into the study.



The median age of the 650 included patients was 66 years (IQR 54–75), of which 59.7% were males. The most frequent baseline medical conditions were diabetes mellitus (31.3%), immunosuppressive therapy (16%) and cancer (13.4%). Of note, there were 36 patients with rheumatoid arthritis, representing 25% of cases in the whole cohort of bacteraemia (36/144 patients), in contrast with those without rheumatoid arthritis (614/36363; 1.7%) ($p<0.001$).

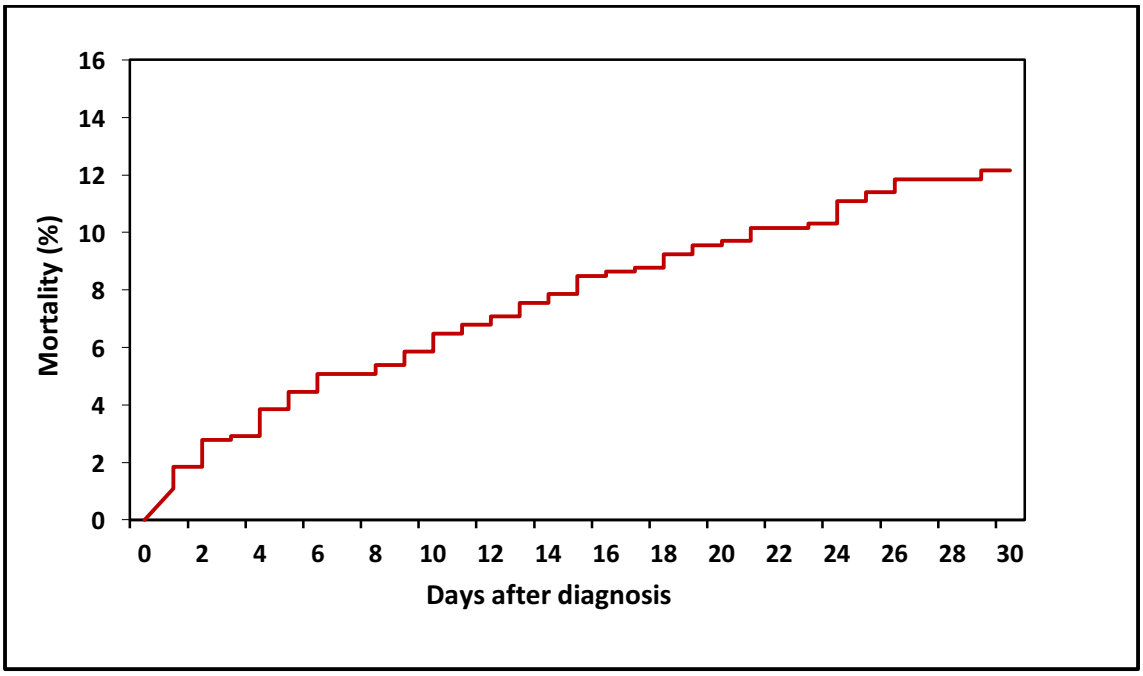
These 650 patients presented with 691 OAIs, which were 286 SA (41.4%), 278 VO (40.2%) and 127 PO (18.4%). Among the 286 cases of SA, axial arthritis was present in 44 (15.4%), native peripheral arthritis in 168 (58.7%) and PJI in 74 (25.9%). Thus, 242

patients (84.6%) presented with peripheral SA. The main microorganism involved was *S. aureus* (56.8%), of which 16.3% of strains were methicillin-resistant. Cases in those with rheumatoid arthritis were mainly caused by *S. aureus* (80.6%). Other groups of microorganisms were: GNB (22.2%), with *E. coli* accounting for more than 10% of all cases, pyogenic streptococci (9.1%) and viridans-group streptococci (6.5%).

4.2 Analysis of mortality

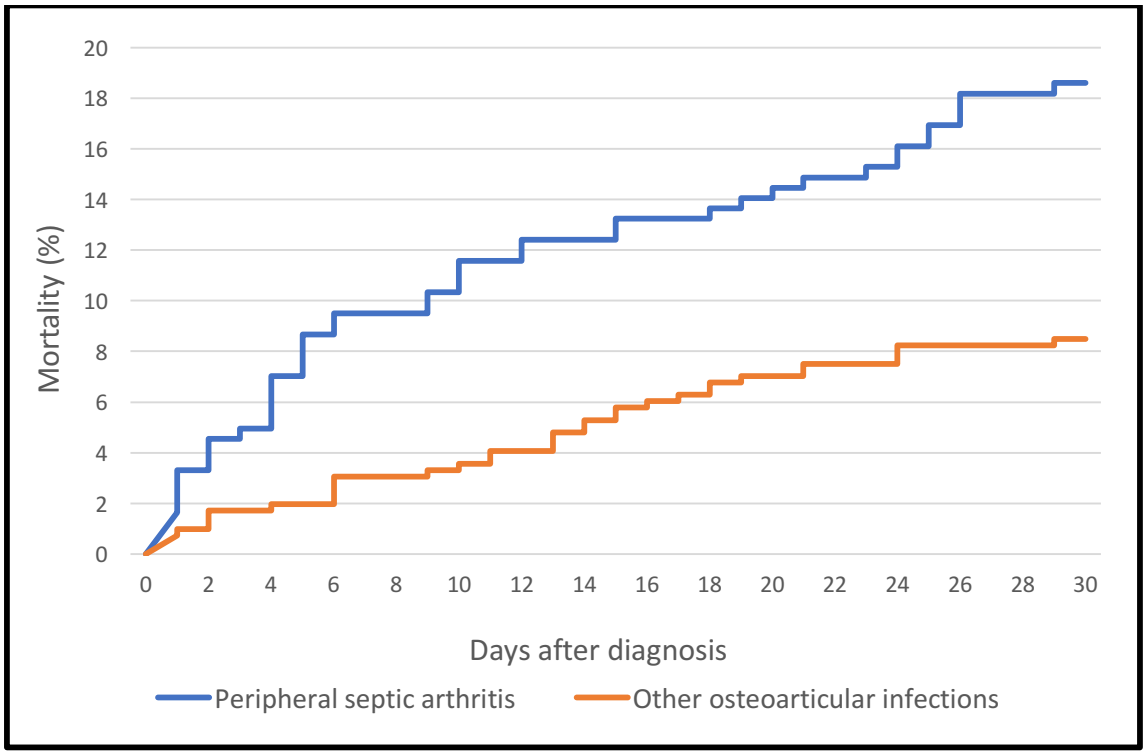
Among 650 patients with bacteraemic OAIs, mortality (30-day case-fatality rate) was observed in 79 cases (12.2%), without significant differences across the study periods: 7/71 (9.9%) in 1985-1994, 34/226 (15%) in 1995-2004 and 38/353 (10.8%) in 2005-2014 (p=0.564). Early mortality (≤7 days) was 5.1% (33 cases), representing 41.8% of patients who died (Figure 4.2.).

Figure 4.2. Cumulative mortality among all 650 cases with bacteraemic osteoarticular infections.



Mortality was greater in cases with PJI (19/74; 25.7%) and native SA (26/168; 15.5%), compared with axial SA (2/44; 4.6%), VO (22/278; 7.9%), and PO (11/127; 8.7%). Thus, overall mortality was greater in those with peripheral SA, including native SA and PJI, (45/242; 18.6%) than in those with other OAIs (34/408; 8.3%) ($p<0.001$). Early mortality was also greater in cases with peripheral SA (22/242; 9.1%) than in cases with other OAIs (11/408; 2.7%) ($p<0.001$). Figure 4.3. shows the cumulative mortality in patients with peripheral SA and other OAIs. No significant differences in overall mortality were found between primary and metastatic OAIs (11.2% vs 15.09%; $p=0.192$) and primary and metastatic peripheral SA (17.6% vs 21.7%; $p=0.481$).

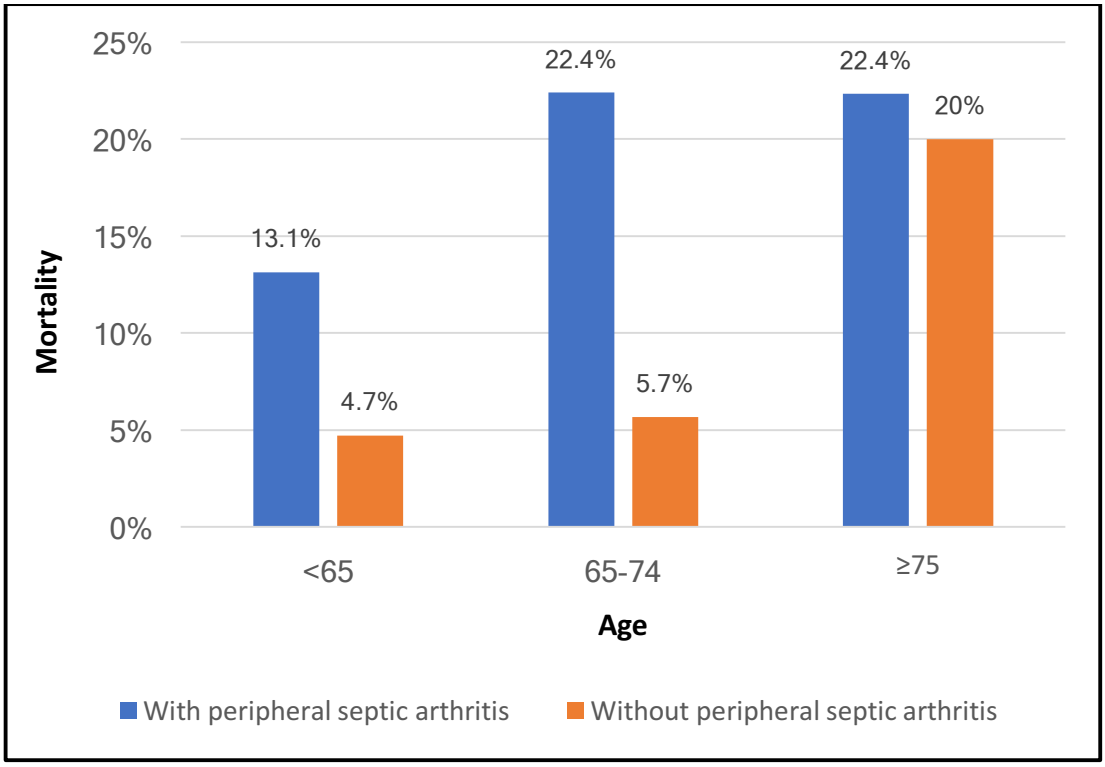
Figure 4.3. Cumulative mortality in patients with peripheral septic arthritis compared with other bacteraemic osteoarticular infections.



The 30-day mortality for each covariate, together with the unadjusted ORs, are summarized in Table 4.1. Patients older than 65 years were more likely to die than younger patients (17.2% vs 7.1%; $p<0.001$). In fact, mortality increased clearly with age, though patients with peripheral SA had higher mortality in all age groups (Figure 4.4).

Liver cirrhosis and rheumatoid arthritis were associated with greater mortality (deaths/total): 13/38 [34.2%] in patients with liver cirrhosis vs 66/612 [10.8%] in those without (p<0.001); 10/36 [27.8%] in patients with rheumatoid arthritis vs 69/614 [11.2%] in those without (p=0.003). These higher mortality rates were also observed in patients from the whole cohort of bacteraemia cases: 872/2965 [29.4%] in patients with liver cirrhosis vs 6005/33542 [17.9%] in those without (p<0.001); 43/144 [29.9%] in patients with rheumatoid arthritis vs 8145/36363 [22.4%] in those without (p=0.032). Thus, no significant differences in mortality rates were found in patients with these comorbidities when they had OAIs or bacteraemia from other sources. It should be noted that patients with rheumatoid arthritis had more often peripheral SA (77.8% vs 34.9%; p<0.001) and, among the 10 patients who died, 9 had peripheral SA.

Figure 4.4. Mortality by age group and presence or absence of peripheral septic arthritis.

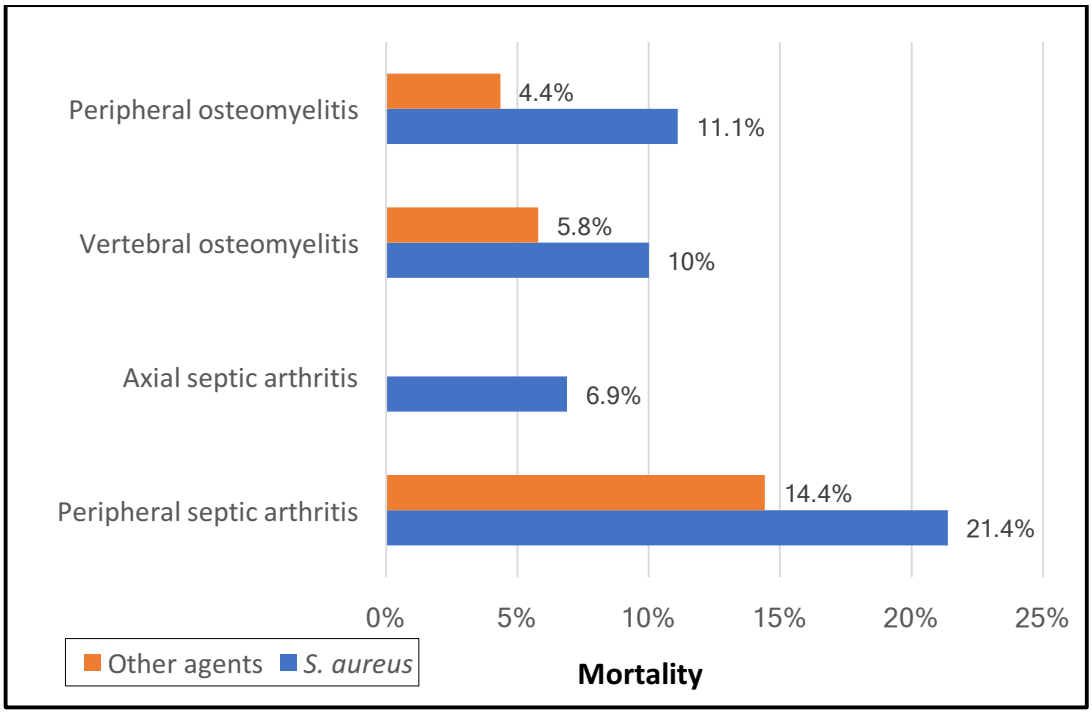


Bacteraemic OAIs caused by *S. aureus* was associated with a mortality rate of 14.9% (55/361), which was greater in cases of MRSA than MSSA (26.7% vs 12.6%; p=0.005). *S. aureus* was the causative agent in 69.6% of patients who died. Although mortality associated with *S. aureus* was higher in all OAIs, those associated with peripheral SA had

the greatest mortality (Figure 4.5.). Mortality was 5.1% (3/59) for patients with pyogenic *streptococci*, whereas it was 11.9% (5/42) for those with viridans *streptococci,* and 7.6% (11/144) for those with GNB.

Adjustment in a multivariate model (Table 4.1.), which included all statistically significant variables from the univariate analysis, indicated that peripheral SA was associated with a two-fold increased odds of mortality (adjusted OR 2.12; 95% CI 1.22–3.69; p=0.008). Other variables significantly associated with mortality in the multivariate model were age older than 65 years, liver cirrhosis, rheumatoid arthritis, the McCabe and Jackson score, and *S. aureus* infection.

Figure 4.5. Mortality rates by the type of osteoarticular infection and whether *Staphylococcus aureus* was the causative agent.



In Article 3, we also evaluated the mortality of SA within 30 days of diagnosis, which occurred to 41/273 patients (15%). In 19 out of 41 cases (46%) death occurred within a week of diagnosis. Healthcare-related cases presented higher mortality than community-acquired (18% vs 7%, p = 0.03) but lower than nosocomial-acquired (26%, p<0.001 vs community-acquired; p = 0.3 vs healthcare-related cases).

Table 4.1. Risk factors for 30-day mortality in patients with bacteraemic osteoarticular infections (n = 650).

Variable		Number of individuals	Dead within 30 days (%)	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age	≤65 years	325	23 (7.1)	1		1	
	> 65 years	325	56 (17.2)	2.73 (1.64–4.56)	<0.001	2.51 (1.41–4.49)	0.001
Sex	Female	262	33 (12.6)	1			
	Male	388	46 (11.9)	0.93 (0.58–1.50)	0.777		
Cancer	No	562	66 (11.7)	1			
	Yes	87	13 (14.9)	1.32 (0.69–2.51)	0.408		
Diabetes mellitus	No	446	54 (12.1)	1			
	Yes	203	25 (12.3)	1.02 (0.61–1.69)	0.940		
Liver cirrhosis	No	612	66 (10.8)	1		1	
	Yes	38	13 (34.2)	4.30 (2.10–8.81)	<0.001	3.10 (1.29–7.46)	0.014
Chronic kidney disease	No	587	69 (11.8)	1			
	Yes	63	10 (15.9)	1.42 (0.69–2.91)	0.359		
Rheumatoid arthritis	No	614	69 (11.2)	1		1	
	Yes	36	10 (27.8)	3.04 (1.41–6.57)	0.009	3.02 (1.20–7.55)	0.024

McCabe & Jackson score	I	528	34 (6.4)	1		1	
	II–III	122	45 (36.9)	8.49 (5.12–14.08)	<0.001	8.28 (4.71–14.56)	<0.001
Peripheral septic arthritis	No	408	34 (8.3)	1		1	
	Yes	242	45 (18.6)	2.51 (1.56–4.05)	<0.001	2.12 (1.22–3.69)	0.008
S. aureus infection	No	281	24 (8.5)	1		1	
	Yes	369	55 (14.9)	1.88 (1.13–3.11)	0.012	2.19 (1.23–3.90)	0.006

OR: Odds ratio. CI: Confidence interval. McCabe & Jackson score (I = nonfatal disease; II = ultimately fatal disease, III = rapidly fatal disease).

4.3 Impact of debridement in peripheral septic arthritis

We evaluated the impact of surgical debridement on mortality in 239 patients with peripheral SA (3 patients had no information available). There were not significant differences between those treated with surgical debridement (191; 79.9%) compared to those who were not (48; 20.1%), according to age, comorbidities, type of OAI and McCabe and Jackson score (Table 4.2.). *S. aureus* infection was more frequent among those treated with surgical debridement (62.8% vs 45.8%; p=0.032).

Table 4.2. Baseline characteristics of 239 patients with bacteraemic peripheral septic arthritis, according to receiving or not surgical debridement.

	No surgical debridement (n = 48)	**Surgical debridement (n = 191)**	**p value**
Age (median, IQR)	67 (54-77)	70 (58-78)	0.262
Age>65 years	25 (52.1)	114 (59.7)	0.340
Male	22 (45.8)	103 (53.9)	0.316
One or more underlying diseases	37 (77.1)	141 (73.8)	0.643
Diabetes mellitus	9 (18.8)	56 (29.3)	0.141
Neoplasm	9 (18.8)	25 (13.1)	0.316
Cardiopathy	7 (14.6)	25 (13.1)	0.786
Liver cirrhosis	5 (10.4)	9 (4.7)	0.132
Chronic kidney disease	3 (6.3)	14 (7.3)	0.795
Immunosuppressive therapy	10 (20.8)	47 (24.6)	0.583
Rheumatoid arthritis	6 (12.5)	21 (11.0)	0.768
McCabe-Jackson score I-II	12 (25.0)	37 (19.4)	0.388
Prosthetic joint infection	10 (20.8)	63 (33.0)	0.102
Post-surgical infections	5 (10.4)	28 (14.7)	0.446
***S. aureus* infection**	22 (45.8)	120 (62.8)	0.032

Data expressed as No. (%), if not stated otherwise. McCabe & Jackson score (I = nonfatal disease; II = ultimately fatal disease, III = rapidly fatal disease). IQR: Interquartile Range.

Importantly, mortality was lower among patients treated with surgical debridement compared with those who were not (28/191 [14.7%] vs 16/48 [33.3%]; p=0.003); in particular, early mortality was significantly lower among patients who underwent surgical debridement compared with those who did not (9/191 [4.7%] vs 12/48 [25%]; p<0.001). After adjusting for potential confounders (age, liver cirrhosis, rheumatoid arthritis, McCabe and Jackson score, PJI, and *S. aureus* infection) surgical debridement remained significantly associated with reduced mortality (adjusted OR 0.23; 95% CI 0.09–0.57; p=0.002). After propensity score matching (including age, McCabe and Jackson score, shock upon admission, PJI and *S. aureus*), surgical debridement was still associated with decreased mortality in patients with bacteraemic SA (OR 0.81; 95%CI 0.68-0.96; p=0.014).

B. ANTIMICROBIAL TREATMENT AGAINST INFECTIONS BY *PSEUDOMONAS AERUGINOSA*

B.1 Clinical studies

Aim 5 – To analyse the efficacy and therapeutic drug monitoring of continuous beta-lactam infusion for osteoarticular infections by fluoroquinolone-resistant *P. aeruginosa*

Article 5 – *Efficacy and therapeutic drug monitoring of continuous beta-lactam infusion for osteoarticular infections caused by fluoroquinolone-resistant Pseudomonas aeruginosa: a prospective cohort study.* **J. Gómez-Junyent**, R. Rigo-Bonnin, E. Benavent, L. Soldevila, A. Padullés, X. Cabo, F. Tubau, J. Ariza, O. Murillo. Eur J Drug Metab Pharmacokinet. 2020 May 21. Online ahead of print.

Article 6 – *Measurement of ceftolozane and tazobactam concentrations in plasma by UHPLC-MS/MS. Clinical application in the management of difficult-to-treat osteoarticular infections.* R. Rigo-Bonnin, **J. Gómez-Junyent**, L. García-Tejada, E. Benavent, L. Soldevila, F. Tubau, O. Murillo. Clinica Chimica Acta 2019; 488:50-60.

Communication 5 – *Betalactámicos en infusión continua o extendida para el tratamiento de infecciones osteoarticulares por bacilos gram negativos: un estudio piloto.* **J. Gómez-Junyent**, L. Soldevila, E. Benavent, R. Rigo, A. Ribera, X. Cabo, F. Tubau, J. Ariza, O. Murillo. XXI SEIMC, Málaga, Spain, 2017. Presentation number O154.

GNB and *Pseudomonas aeruginosa* are frequent causative microorganisms of OAIs. Fluoroquinolones are the mainstay of the antimicrobial therapy of patients with GNB-OAIs. The increasing resistance emergence to fluoroquinolones among GNB is worrisome, also because it is associated with the appearance of MDR and XDR strains, frequently resistant to most antibiotics *in vitro*. Colistin, which is often the only available option and has shown good anti-biofilm activity (156), has been recommended in combination with BL against MDR/XDR *P. aeruginosa* OIs (159). Nevertheless, in this scenario, new therapeutic alternatives are clearly needed. The use of continuous BL

infusion (BL-CI) could optimize their pharmacodynamics (173) and be useful to improve the outcome of patients with OAIs by *P. aeruginosa*.

We performed a prospective study to evaluate the use of BL-CI and therapeutic drug monitoring (TDM) and analyse the outcome of patients with OAIs by fluoroquinolone-resistant *P. aeruginosa* managed with an optimized therapeutic strategy in comparison to the outcome of patients who could receive fluoroquinolones. We also developed UHPLC-MS/MS procedures for the measurement of ceftolozane and tazobactam mass concentrations in plasma and apply it to treatment guidance in cases of OAIs.

During the study period, 61 patients with OAIs caused by *P. aeruginosa* were identified, of whom nine were excluded (one had a diabetic foot infection, three were treated with meropenem, and five patients each provided only one sample for TDM). Therefore, 52 patients were ultimately included in the analysis, 19 (36.5%) of whom had OAIs caused by fluoroquinolone-resistant *P. aeruginosa*.

5.1. Patients with OAIs caused by fluoroquinolone-resistant *P. aeruginosa*

5.1.1. Characteristics of the patients and infections

The main characteristics of the cases are summarized in Table 5.1. The majority were female (11, 57.9%) and the median age was 67 years (IQR 55-76). Most patients had at least one comorbidity. Three patients (15.8%) had CKD, but none were on haemodialysis. Device-related infections accounted for more than half of all OAIs (osteoarthritis, n=6, 31.6%; PJI, n=5, 26.3%). Most infections were caused by MDR or XDR strains (13, 68.4%) and 11 (57.9%) were carbapenem-resistant. Almost one third of all OAIs were polymicrobial. More than half of the patients had post-surgical OAIs and the OAI was catalogued as a superinfection in two patients (10.0%).

5.1.2. Management of infections

All but two patients were treated with surgery. Most (7, 63.6%) of those who had device-associated infections managed with surgery were treated with implant removal. The median duration of BL-CI was 36 days (IQR 28–39). Only one patient was initiated on BL-CI immediately; the rest received intermittent infusion for a median of 6 days (IQR 4–7)

prior to CI. The median global duration of antibiotic therapy was 42 days (IQR 30–46). Ten patients (52.6%) received combinations of BL with colistin.

Table 5.1. Baseline and clinical characteristics of 52 patients with osteoarticular infections by *Pseudomonas aeruginosa* and treated with continuous beta-lactam infusion.

Characteristic	All patients (n = 52)	Patients with fluoroquinolone-resistant strains (n = 19)	Patients with fluoroquinolone-susceptible strains (n = 33)	p value
DEMOGRAPHICS				
Age (median, IQR)	68 (55-75)	67 (55-76)	69 (55-74)	0.985
Male sex	27 (51.9)	8 (42.1)	19 (57.6)	0.282
Any comorbidity	31 (59.6)	13 (68.4)	18 (54.6)	0.326
Diabetes mellitus	12 (23.1)	6 (31.6)	6 (18.2)	0.270
Chronic heart disease	14 (26.9)	7 (36.8)	7 (21.2)	0.221
Chronic lung disease	10 (19.2)	5 (26.3)	5 (15.2)	0.325
Malignancy	1 (1.9)	0	1 (3.0)	0.444
Chronic kidney disease	8 (15.4)	3 (15.8)	5 (15.2)	0.951
Rheumatologic autoimmune disease	7 (13.5)	3 (15.8)	4 (12.1)	0.324
Immunosuppressive therapy	6 (11.5)	3 (15.8)	3 (9.1)	0.467
Chronic steroid therapy	6 (11.5)	4 (21.1)	2 (6.1)	0.103
CLINICAL DATA				
Type of infection				
Prosthetic joint infection	13 (25.0)	5 (26.3)	8 (24.2)	
Osteoarthritis (without device)	23 (44.2)	8 (42.1)	15 (45.5)	
Osteoarthritis (with device)	16 (30.8)	6 (31.6)	10 (30.3)	0.972
Device-related infections	29 (55.8)	11 (57.9)	18 (54.6)	0.815
Post-surgical infections	26 (50.0)	10 (52.6)	16 (48.5)	0.773
MICROBIOLOGICAL DATA				
Bacteremia	4 (7.7)	1 (5.3)	3 (9.1)	0.618

Polymicrobial infection	19 (36.5)	6 (31.6)	13 (39.4)	0.573
Superinfection	6 (11.5)	2 (10.5)	4 (12.1)	0.862
MDR or XDR strains	13 (25.0)	13 (68.4)	0	<0.001
Carbapenem-resistant strains	11 (21.2)	11 (57.9)	0	<0.001
ANTIBIOTIC THERAPY				
Duration of therapy, days	45 (42-56)	42 (30-46)	55 (42-59)	0.002
Duration of antibiotic therapy in CI, days	21 (14-36)	36 (28-39)	18 (13-23)	<0.001
Combination therapy[a]	39 (75.0)	10 (52.6)	29 (87.9)	0.004
Duration of combination therapy, days[b]	14 (10-21)	35 (20-36)	13 (10-15)	0.006
SURGICAL THERAPY				
None	3 (5.8)	2 (10.5)	1 (3.0)	
Debridement[c]	20 (38.5)	6 (31.6)	14 (42.4)	
DAIR	13 (25.0)	4 (21.1)	9 (27.3)	
Implant removal	16 (30.8)	7 (36.8)	9 (27.3)	0.315
OUTCOME				
Failure[d]	9 (18.4)	4 (21.1)	5 (16.7)	0.699
Adverse events	11 (21.2)	5 (26.3)	6 (18.2)	0.489

All data are expressed as number (percentage) unless stated otherwise.
IQR: Interquartile range. MDR: Multidrug-resistant. XDR: Extensively drug-resistant. DAIR: Debridement, antibiotics and implant retention.
[a]Combnation therapy included colistin for those with fluoroquinolone-resistant strains or ciprofloxacin for those with fluoroquinolone-susceptible strains. [b]Among those who received antibiotics in combination, in days. [c]Individuals without associated device. [d]Analysis made in 49 individuals (three individuals were excluded from the fluoroquinolone-susceptible group; two were not treated with ciprofloxacin and one received ciprofloxacin <21 days).

MIC values for each BL and doses used in BL-CI are summarized in Table 5.2. In general, patients were treated with median BL doses that were lower than the doses they may have received with intermittent infusion, according to product data sheets. BLs were chosen according to susceptibility, but two cases were treated with ceftazidime with a MIC of 16 mg/L. This was the lowest BL MIC available upon testing; alternatives such as ceftolozane-tazobactam or ceftazidime-avibactam were not yet available in our hospital.

Table 5.2. The median MIC, median dose, and estimated free concentration in plasma of each continuously infused beta-lactam used to treat fluoroquinolone-resistant *Pseudomonas aeruginosa* OAIs in the study.

Beta-lactam (number of plasma samples)[a]	MIC (range)	Dose (IQR)	Estimated free beta-lactam concentration (IQR)[b]	Estimated free beta-lactam concentration xMIC (IQR)
Aztreonam (n=19)	4 (2-8)	3 (1.5-4)	12.1 (9.1-18.2)	2.3 (2.0-3.9)
Ceftazidime (n=49)	2 (1-16)	2 (2-4)	18.2 (11.9-31.8)	9.1 (7.0-18.9)
Cefepime (n=10)	6 (4-8)	2 (2-2.5)	21.6 (14.2-30.4)	3.7 (3.5-4.5)
Ceftolozane-tazobactam (n=4)	4	5 (4-6)	16.7 (15.4-21.9)[c]	4.2 (3.9-5.5)[c]

MIC: Minimum inhibitory concentration. IQR: Interquartile range. MIC and concentrations expressed in mg/L, doses expressed in g per day.
[a]Plasma samples were collected from 4 patients treated with aztreonam, 12 with ceftazidime, 2 with cefepime and 1 with ceftolozane-tazobactam. [b]Median free beta-lactam concentrations based on patient samples obtained on various occasions; these concentrations reflect a mix of inter- and intraindividual variability. [c]Only ceftolozane concentrations showed here.

5.1.3. Therapeutic drug monitoring

Measurement standardization of concentrations of ceftolozane and tazobactam from human plasma was performed using UHPLC-MS/MS. Satisfactory results were obtained from validation results. The full description of results can be found in the article at the end of this book. This methodology was also employed to measure concentrations of other BLs.

Overall, 82 plasma samples were taken from the patients in order to monitor BL concentrations (a median of five per patient, IQR 3–6) during CI (Table 5.2). The median *f*Css expressed in mg/L and as multiples of the respective *P. aeruginosa* MIC in each patient is shown in Table 5.3. Most patients had a median *f*Css of between 3 and 10xMIC. Median *f*Css values of less than 3xMIC were mainly found in patients with OAIs caused by *P. aeruginosa* strains with MICs of 8–16 mg/L (cases 4, 14, 15, and 16), whereas four of the five patients with a *f*Css above 10xMIC had CKD or AKI (cases 2, 3, 7, and 8).

A total of 17 dose adjustments were performed in 12 patients (63.2%) during BL-CI (nine patients had one adjustment, one had two adjustments, and two had three

adjustments). The median time from sample collection to dose adjustment was 2 days (IQR 1–3). Three patients did not initially achieve a *f*Css of at least 3xMIC, two of whom had OAIs by *P. aeruginosa* with MIC of 8–16 mg/L to the BL used. The other patient, despite dose adjustment, did not meet that PK/PD target on subsequent samples. TDM values prompted ten dose decreases in eight patients; three of those patients presented AKI (cases 8, 9, and 17), two had CKD (cases 2 and 3), and two also received colistin (cases 5 and 10). Seven dose increases were performed in six patients, three of whom (cases 16, 17, and 19) had OAIs caused by strains with a BL MIC of 4–8 mg/L.

5.2. Patients with OAIs caused by fluoroquinolone-susceptible *P. aeruginosa*

Thirty-three patients had OAIs caused by fluoroquinolone-susceptible *P. aeruginosa* that were treated with BL-CI; those patients had similar characteristics to the patients infected with fluoroquinolone-resistant strains (Table 5.1.).

The median duration of BL-CI was significantly shorter (18 days, IQR 13–23), and the most frequently used BL was ceftazidime (Table 5.4). Again, patients received lower median BL doses than those they may have received with intermittent infusion, according to product data sheets. The majority received combination therapy with ciprofloxacin for a median of 13 days (IQR 10–15). Ciprofloxacin was used after BL discontinuation in 31 patients (median 31 days, IQR 22–37); two patients with a fluoroquinolone allergy were treated with BL-CI only.

Regarding the TDM, a total of 110 plasma samples were taken for BL concentration analysis (a median of three per patient, IQR 2–4) (Table 5.4). A total of 19 dose adjustments were performed in 15 patients (45.5%) during therapy (12 had one adjustment, 2 had two adjustments, and 1 had three adjustments). The median time from sample collection to dose adjustment was 1 day (IQR 0–3). Only four patients did not initially achieve a *f*Css of at least 3xMIC; those patients had OAIs by *P. aeruginosa* strains with a BL MIC of 4–8 mg/L. Three of those patients achieved the PK/PD target after dose adjustment, and the remaining patient had an OAI by *P. aeruginosa* with MIC of 8 mg/L to the BL used (piperacillin-tazobactam). Dose decreases were needed after receiving the results for ten of the samples during TDM. Five of those decreases were performed in patients with CKD/AKI.

Table 5.3. Baseline characteristics, treatment details and outcome of patients with osteoarticular infections by fluoroquinolone-resistant *Pseudomonas aeruginosa* who were treated with continuous beta-lactams infusion.

Case number	Age / Sex	CKD / AKI while on therapy	Type of infection	Surgical management	MDR or XDR	BL	MIC to BL used[a]	Median dose of BL[b] (range[c])	Median BL *f*conc[c] (range)	Median times BL *f*conc above MIC (range)	Colistin	Cured
1	62 / M	No / No	PJI	Implant retention	No	Ceftazidime	1	2 (1-3)	8.8 (3.9-15.9)	8.8 (3.9-15.9)	Yes	Yes
2	75 / F	Yes / No	PJI	Implant retention	No	Ceftazidime	1	1.5 (1-2)	33.9 (17.8-42.6)	33.9 (17.8-42.6)	No	No
3	76 / F	Yes / No	PJI	Implant removal	No	Ceftazidime	1	1.5 (1-2)	13.0 (8.1-42.7)	13.0 (8.1-42.7)	No	Yes
4	63 / M	No / No	Osteoarthritis with device	Implant removal	MDR	Ceftazidime	16	6	25.8 (24.3-27.4)	1.6 (1.5-1.7)	Yes	Yes
5	75 / F	No / No	Osteoarthritis with device	Implant retention	XDR	Ceftazidime	8	5.5 (5-6)	36.4 (32.2-40.7)	4.6 (4.0-5.1)	Yes	No
6	55 / F	No / No	Osteoarthritis with device	Implant removal	MDR	Ceftazidime	2	2	12.5 (11.7-13.3)	6.2 (5.8-6.6)	No	Yes
7	69 / M	No / Yes	Osteoarthritis without device	Debridement	No	Ceftazidime	1	2	24.0 (18.9-29.5)	24.0 (18.9-29.5)	Yes	Yes
8	80 / M	No / Yes	Osteoarthritis without device	Debridement	MDR	Ceftazidime	2	2 (1-4)	29.7 (15.4-49.8)	14.8 (7.7-24.9)	Yes	No
9	68 / M	No / Yes	Osteoarthritis without device	Debridement	XDR	Ceftazidime	16	4.5 (2-7)	132.3 (124.3-169.1)	8.3 (7.8-10.6)	Yes	Yes

10	49 / M	No / No	Osteoarthritis without device	Debridement – Bone resection	MDR	Ceftazidime	2	5.5 (4-7)	44.5 (22.3-56.3)	22.2 (11.2-28.2)	Yes	Yes
11	63 / F	No / No	Osteoarthritis without device	Debridement	No	Ceftazidime	2	3	15.0 (11.9 – 16.6)	7.5 (6-8.3)	Yes	Yes
12	40 / M	No / No	Osteoarthritis without device	Debridement – Bone resection	MDR	Ceftazidime	1.5	2 (1.5-2)	8.8 (4.2-12.7)	5.8 (2.8-8.5)	No	Yes
13	67 / M	No / No	PJI	Implant removal	XDR	Aztreonam	2	2	9.7 (7.7-10.9)	4.3 (3.9-5.4)	Yes	Yes
14	81 / F	Yes / No	PJI	Implant removal	XDR	Aztreonam	2	1.25 (1-1.5)	4.1 (3.9-6.7)	2.0 (1.9-3.3)	No	Yes
15	90 / F	No / No	Osteoarthritis with device	None	MDR	Aztreonam	8	4	18.2 (11.6-25)	2.3 (1.4-3.1)	No	Yes
16	80 / F	No / No	Osteoarthritis without device	None	XDR	Aztreonam	8	4.5 (4-5)	17.6 (12.1-20.6)	2.2 (1.5-2.6)	No	Yes
17	46 / F	No / Yes	Osteoarthritis with device	Implant removal	MDR	Cefepime	8	2 (1-3)	29.2 (14.2-49.8)	3.7 (1.8-6.2)	No	Yes
18	68 / F	No / No	Osteoarthritis without device	Debridement – Bone resection	No	Cefepime	4	2.5	16.0 (14.1-17.9)	4.0 (3.5-4.5)	No	Yes
19	40 / F	No / No	Osteoarthritis with device	Implant retention	XDR	Ceftolozane-tazobactam	4	5 (4-6)	16.7 (14.8-26.3)	4.2 (3.7-6.6)	Yes	No

CKD: Chronic Kidney Disease. AKI: Acute Kidney Failure. MDR: Multidrug-resistant. XDR: Extensively drug-resistant. BL: Beta-lactam. *f*conc: Free concentration. M: Male. F: Female. PJI: Prosthetic Joint Infection.

[a]Expressed in mg/L. [b]Expressed in grams. [c]Only for those with dose changes during treatment.

Table 5.4. The median MIC, median dose, and estimated free concentration in plasma of each continuously infused beta-lactam used to treat fluoroquinolone-susceptible *Pseudomonas aeruginosa* OAIs in the study.

Beta-lactam (number of plasma samples)[a]	MIC (range)	Dose (IQR)	Estimated free beta-lactam concentration (IQR)[b]	Estimated free beta-lactam concentration xMIC (IQR)
Ceftazidime (n=84)	1 (0.25-8)	2 (1-4)	12.1 (8.1-19.0)	9.2 (5.8-13.8)
Cefepime (n=17)	1 (1-2)	2 (1-2.5)	19.2 (11.4-26.0)	11.0 (8.9-13)
Piperacillin-tazobactam (n=9)	2 (1-4)	12 (10-12)	13.2 (11.5-17.5)[c]	2.3 (2.0-3.3)[c]

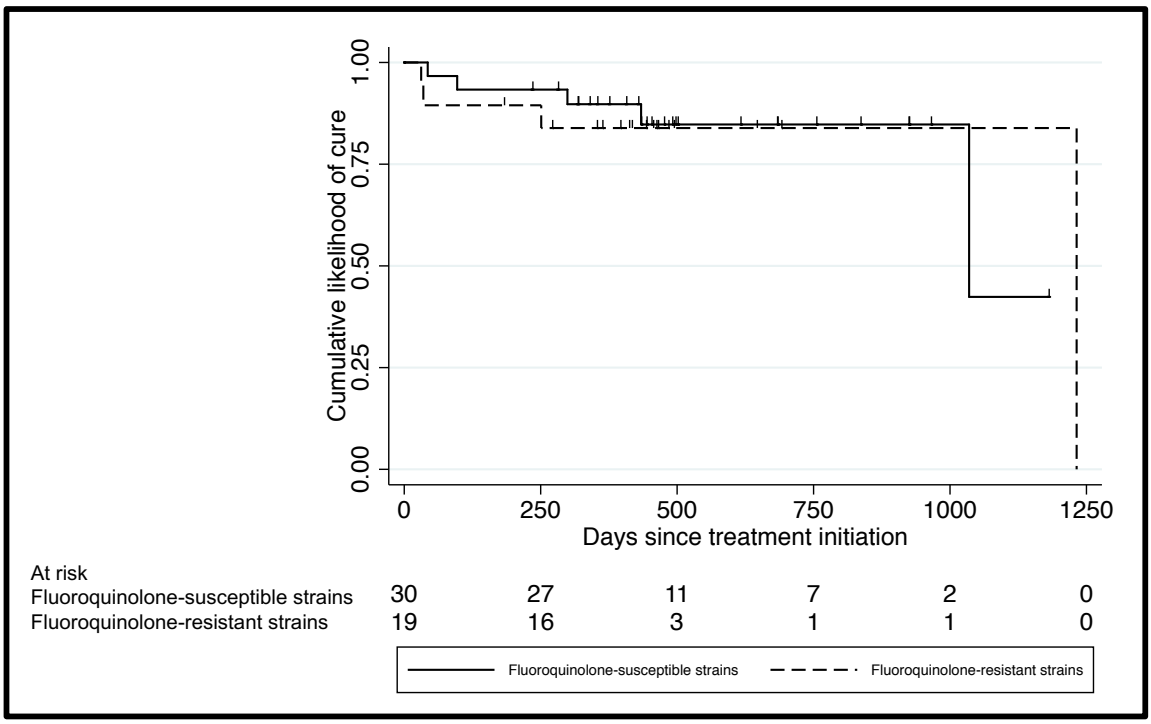
MIC: Minimum inhibitory concentration. IQR: Interquartile range. MIC and concentrations expressed in mg/L, doses expressed in g per day.
[a]Plasma samples were collected in 25 patients treated with ceftazidime, 6 with cefepime and 2 with piperacillin-tazobactam. [b]Median free beta-lactam concentrations based on patient samples obtained on various occasions; these concentrations reflect a mix of inter- and intraindividual variability. [c]Only piperacillin concentrations showed here.

5.3. Comparison of outcomes and adverse events

We evaluated treatment failure rates for 49 patients; three of the patients with fluoroquinolone-susceptible strains were excluded from this analysis because they were not treated with fluoroquinolones (two patients) or they had received ciprofloxacin for < 21 days before failure (one patient).

After a median follow-up of 444 days (IQR 338–617), the treatment had failed in nine patients (18.4%). There was no significant difference in failure rate between the patients with fluoroquinolone-resistant strains and those with susceptible strains (21.1% [4/19] vs 16.7% [5/30], respectively; p = 0.699) (Figure 5.1.). There was also no significant difference in baseline characteristics or in the achievement of the PK/PD target (a median *f*Css of at least 3xMIC) between the patients in whom the treatment failed and those in whom it did not fail (Table 5.5). Among the patients in whom treatment failed, one had a PJI and eight had osteoarthritis (four of whom had a device and four did not). In the patients with device-related OAIs, 4/5 (80%) patients in whom treatment failed were managed with implant retention, compared to 8/22 (36.4%) patients in whom treatment succeeded (p = 0.049).

Figure 5.1. Kaplan-Meier curves of cure likelihood among patients with osteoarticular infections by fluoroquinolone-susceptible (continuous line) and fluoroquinolone-resistant (dashed line) strains of *Pseudomonas aeruginosa*.



Adverse events occurred in five (26.3%) and six (18.2%) patients with fluoroquinolone-resistant and –susceptible strains, respectively (p = 0.489), at a median of 17 days (IQR 8–20) following BL-CI initiation. In patients with OAIs caused by fluoroquinolone-resistant *P. aeruginosa*, the adverse event was AKI in four (three of whom also received colistin) and diarrhoea in one (not associated with *Clostridium difficile*). Among those with susceptible strains, the adverse events were AKI (n = 3), encephalopathy (1), BL-related fever (1), and candidemia (1). All of the patients recovered aside from one (with AKI) who died due to a worsening of their baseline medical condition.

Table 5.5. Comparative analysis of patients in whom treatment of an osteoarticular infection caused by *Pseudomonas aeruginosa* failed or did not fail.

Characteristics	Patients in whom treatment failed (n = 9)	Patients in whom treatment did not fail (n = 40)	p value
DEMOGRAPHICS			
Age (median, IQR)	73 (65-75)	67 (55-75)	0.416
Female sex	5 (55.6)	19 (47.5)	0.662
Any comorbidity	4 (44.4)	26 (65.0)	0.253
Diabetes mellitus	2 (22.2)	10 (25.0)	0.861
Chronic heart disease	1 (11.1)	12 (30.0)	0.246
Chronic lung disease	1 (11.1)	9 (22.5)	0.444
Malignancy	0	1 (2.5)	0.632
Chronic kidney disease	2 (22.2)	5 (12.5)	0.451
Rheumatologic autoimmune disease	2 (22.2)	5 (12.5)	0.226
Immunosuppressive therapy	1 (11.1)	5 (12.5)	0.909
Chronic steroid therapy	1 (11.1)	5 (12.5)	0.909
CLINICAL DATA			
Type of infection			
Prosthetic joint infection	1 (11.1)	10 (25.0)	
Osteoarthritis (without device)	4 (44.4)	18 (45.0)	
Osteoarthritis (with device)	4 (44.4)	12 (30.0)	0.577
Device-related infections	5 (55.6)	22 (55.0)	0.976
Post-surgical infections	5 (55.6)	19 (47.5)	0.662
MICROBIOLOGICAL DATA			
Bacteremia	1 (11.1)	3 (7.5)	0.721
Polymicrobial infection	2 (22.2)	15 (37.5)	0.384
Superinfection	1 (11.1)	5 (12.5)	0.909
MDR or XDR strains	3 (33.3)	10 (25.0)	0.593
Carbapenem-resistant strains	2 (22.2)	9 (22.5)	0.763
TREATMENT DATA			
Achievement of PK/PD target[a]	8 (88.9)	36 (90.0)	0.921
Implant retention[a]	4 (80%)	8 (36.4%)	0.049

IQR: Interquartile range. MDR: Multidrug-resistant. XDR: Extensively drug-resistant. PK/PD: Pharmacokinetics/Pharmacodynamics.

[a]Number (%) of patients who had an estimated median free BL concentration of at least 3xMIC.

[b]27 patients with device-related infections were considered (5 failed, 22 did not fail; 2 patients not eligible for failure analysis).

B.2 Experimental studies

Aim 6 – To evaluate the activity of ceftolozane-tazobactam, with and without colistin, against a biofilm infection by multidrug-resistant *P. aeruginosa* in a dynamic *in vitro* model

Article 7 – *Efficacy of ceftolozane/tazobactam, alone and in combination with colistin, against multidrug-resistant Pseudomonas aeruginosa in an in vitro biofilm pharmacodynamic model.* **J. Gómez-Junyent**, E. Benavent, Y. Sierra, C. El Haj, L. Soldevila, B. Torrejón, R. Rigo-Bonnin, F. Tubau, J. Ariza, O. Murillo. International Journal of Antimicrobial Agents 2019; 53(5): 612-619.

Communication 6 – *Efficacy of ceftolozane-tazobactam alone and in combination with colistin against extensively drug-resistant Pseudomonas aeruginosa in an in vitro pharmacodynamic biofilm model.* **J. Gómez-Junyent**, E. Benavent, Y. Sierra, F. Tubau, L. Soldevila, B. Torrejón, J. Ariza, O. Murillo. 28th ECCMID, Madrid, Spain, 2018. Presentation number O0078.

Communication 7 - *Eficacia de ceftolozano-tazobactam y su combinación con colistina frente a Pseudomonas aeruginosa multirresistente en un modelo farmacodinámico in vitro de cuerpo extraño*. **J. Gómez-Junyent**, E. Benavent, Y. Sierra, F. Tubau, L. Soldevila, B. Torrejón, J. Ariza, O. Murillo. XXII SEIMC, Bilbao, Spain, 2018. Presentation number O0205.

The emergence of MDR GNB and particularly, the global spread of MDR *P. aeruginosa* is worrisome in the setting of device-related infections due to the presence of biofilms. Bacterial biofilms impair the activity of most antibiotics (208, 209), and very limited options exist for the treatment of these MDR *P. aeruginosa* strains, which are commonly resistant to fluoroquinolones and have a decreased susceptibility to BLs (210). Colistin is often the only active drug against these MDR microorganisms and may have notable anti-biofilm effect, especially against cells present within inner layers. However, its administration in combination with other antimicrobials has been advised, in order to minimize the emergence of resistance and obtain a synergistic effect. In recent years, the appearance of ceftolozane-tazobactam represents a promising opportunity for the

treatment of serious infections by MDR *P. aeruginosa*, but its anti-biofilm activity is unknown.

With this study, we aimed to evaluate the activity of ceftolozane/tazobactam, in comparison with that of meropenem and ceftazidime, alone and in combination with colistin against MDR and XDR *P. aeruginosa* in an *in vitro* pharmacodynamic biofilm model. We used a validated *in vitro* biofilm pharmacodynamic model, the CDC Biofilm Reactor (CBR). Simulated regimens of ceftolozane/tazobactam (2g/1g every 8h), meropenem (2g every 8h) and ceftazidime (2g every 8h), alone and in combination with colistin (continuous infusion) were evaluated against three colistin-susceptible and ceftazidime-resistant strains: MDR-HUB1, ceftolozane/tazobactam- and meropenem-susceptible; XDR-HUB2, ceftolozane/tazobactam-susceptible and meropenem-resistant; MDR-HUB3, ceftolozane/tazobactam-resistant and meropenem-susceptible. Colistin PAPs were performed to describe the presence of heteroresistant subpopulations (Figure 6.1) and susceptibility data is summarized in Table 6.1.

Figure 6.1. Baseline Population Analysis Profiles of the three strains of *P. aeruginosa* at an initial inoculum of 10^9 cfu/mL.

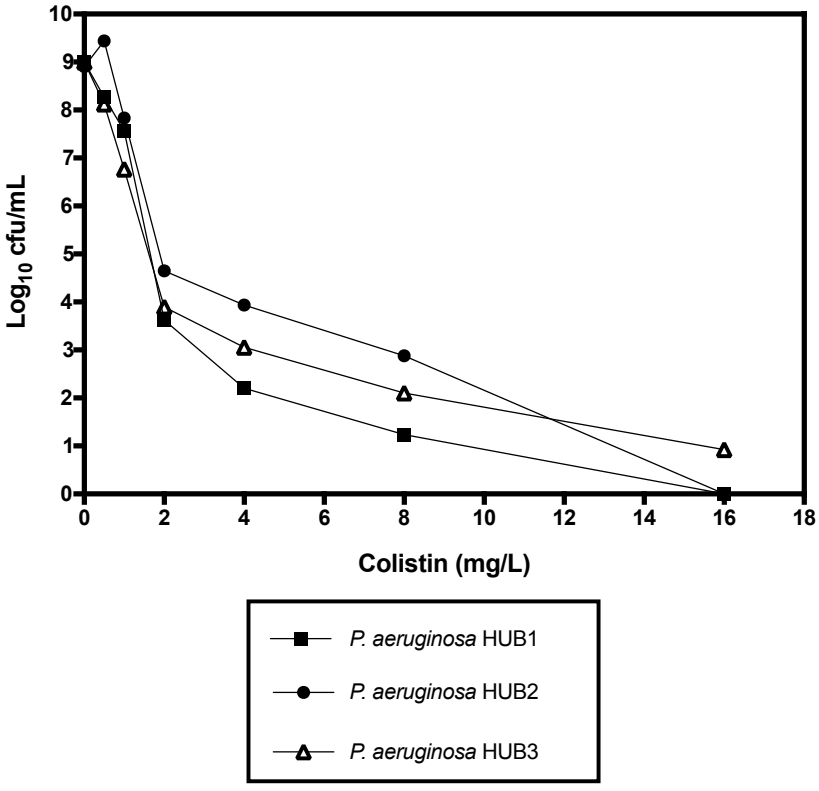


Table 6.1 Minimum inhibitory concentrations, minimum bactericidal concentrations, minimum biofilm inhibitory concentrations and minimum eradication concentrations for the different antibiotics among all *P. aeruginosa* strains.

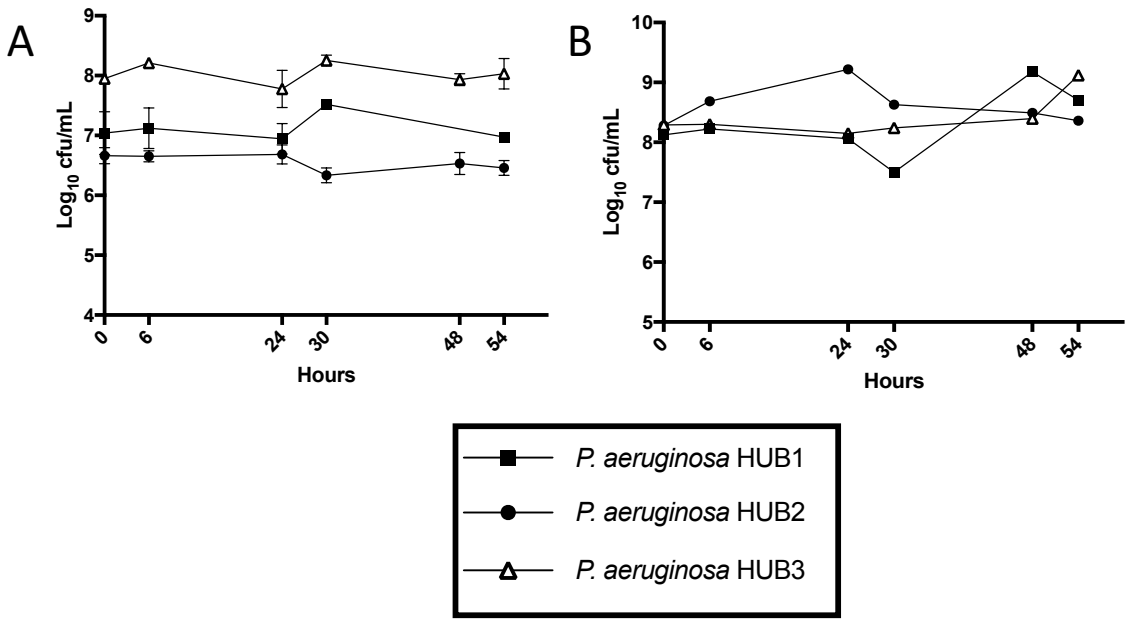
Antibiotics	**MDR-HUB1**				**XDR-HUB2**				**MDR-HUB3**			
	MIC	MBC	MBIC	MBEC	MIC	MBC	MBIC	MBEC	MIC	MBC	MBIC	MBEC
CST	1	4	8	>64	2	2	8	64	2	2	8	>64
CAZ	64	128	>256	>256	32	32	>256	>256	64	>256	>256	>256
MEM	2	4	2	>256	16	16	16	>256	2	4	2	>256
TOL/TZB	2/4	4/4	8/4	>256/4	4/4	4/4	16/4	>256/4	8/4	8/4	16/4	>256/4

MIC: Minimum inhibitory concentration. MBC: Minimum bactericidal concentration. MBIC: Minimum biofilm inhibitory concentration. MBEC: Minimum biofilm eradication concentration. CST: Colistin. CAZ: Ceftazidime. MEM: Meropenem. TOL/TZB: Ceftolozane/tazobactam.

6.1 Microbiological response

Bacterial counts in the absence of antibiotics throughout experiments for biofilm-embedded and free-floating bacteria are illustrated in Figure 6.2. Mean inoculums for biofilm-embedded cells at 0h were higher for MDR strains (HUB1 and HUB3) than for XDR-HUB2.

Figure 6.2. Bacterial growth in the absence of antibiotics for biofilm-embedded (A) and free-floating (B) cells for the three strains of *P. aeruginosa*. Time on the x-axis begins immediately after the 48h conditioning phase. Data presented as means±SD (A) or means (B).



Bacterial counts (log changes) of biofilm-embedded in the presence of antibiotics throughout the experiments are shown in Figure 6.3 and Table 6.2. Among monotherapies, at 54h, ceftolozane/tazobactam achieved a low killing only against susceptible strains (MDR-HUB1 and XDR-HUB2), which was only greater than controls for MDR-HUB1 (Δlog CFU/mL=-0.91; p=0.002), whereas ceftazidime was ineffective in all strains. Colistin therapy, overall, resulted in an initial killing against all strains but regrowth appeared later in a different manner (Δlog CFU/mL at 54h=-1.33 in MDR-HUB1, -1.85 in XDR-HUB2, and -2.07 in MDR-HUB3), this leading colistin to be the only effective monotherapy at 54h against XDR-HUB2 (p<0.001 vs controls and other

monotherapies). Of interest, meropenem alone was the most effective monotherapy and the only bactericidal regimen at 54h against both carbapenem-susceptible strains (Δlog CFU/mL= -4.55 in MDR-HUB1 and -3.96 in MDR-HUB3; p<0.001 vs controls and other monotherapies).

Regarding drug combinations, the addition of colistin to ceftolozane/tazobactam significantly increased the activity of monotherapies against both ceftolozane/tazobactam-susceptible strains (MDR-HUB1 and XDR-HUB2) at 54h (p<0.001), this leading to a bactericidal and synergistic effect in both cases. Ceftolozane/tazobactam plus colistin was the most effective combination against the meropenem-resistant XDR-HUB2 strain (Δlog CFU/mL=-4.42 vs -3.54 for meropenem-colistin; p=0.002); whereas this combination against MDR-HUB1 (Δlog CFU/mL= -4.36) was less effective than meropenem-colistin (-6.25; p<0.001) and showed similar efficacy as meropenem monotherapy (p=0.964). In contrast, the combination ceftolozane/tazobactam-colistin was ineffective against the ceftolozane/tazobactam-resistant strain (MDR-HUB3), being meropenem plus colistin the most bactericidal therapy (Δlog CFU/mL=-6.37; P<0.001 versus other regimens). The combination ceftazidime-colistin was slightly effective against MDR-HUB1 and MDR-HUB3 (no synergism nor bactericidal effect), but it achieved a bactericidal effect against XDR-HUB2 (Δlog CFU/mL=-3.10).

Overall, low non-bactericidal activity was observed among free-floating cells of the three strains of *P. aeruginosa* (mean inoculums at 0h around 8 log CFU/mL). Only meropenem and its combination with colistin showed activity at 54h against MDR-HUB1 strain (Δlog CFU/mL= -2.67 and -2.23, respectively).

Figure 6.3. Bacterial killing by monotherapies with colistin, ceftazidime, meropenem and ceftolozane-tazobactam and the combination of colistin with beta-lactams against biofilm-embedded cells of three different *P. aeruginosa* strains. Results are expressed using the log change method. Data presented as means±SD.

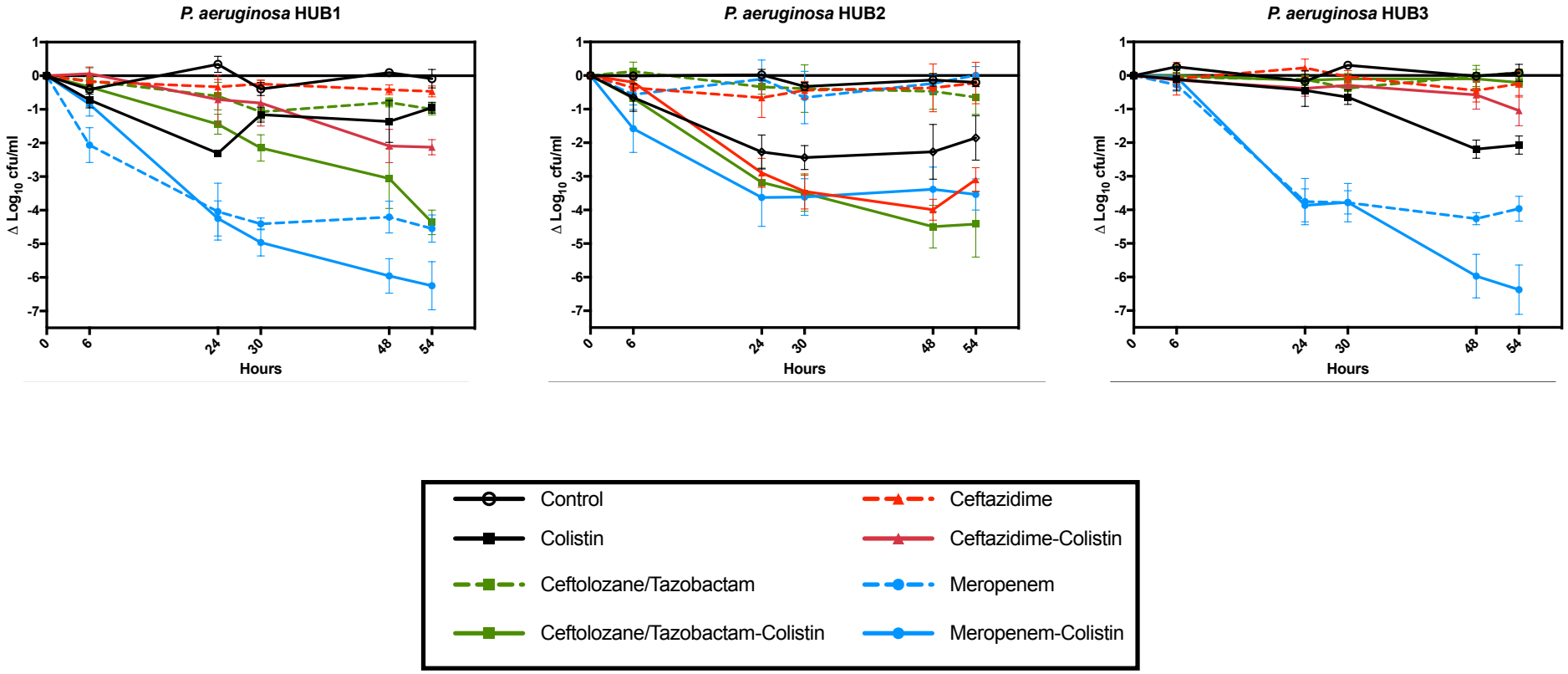


Table 6.2. Mean log changes of biofilm-embedded bacterial counts throughout the study.

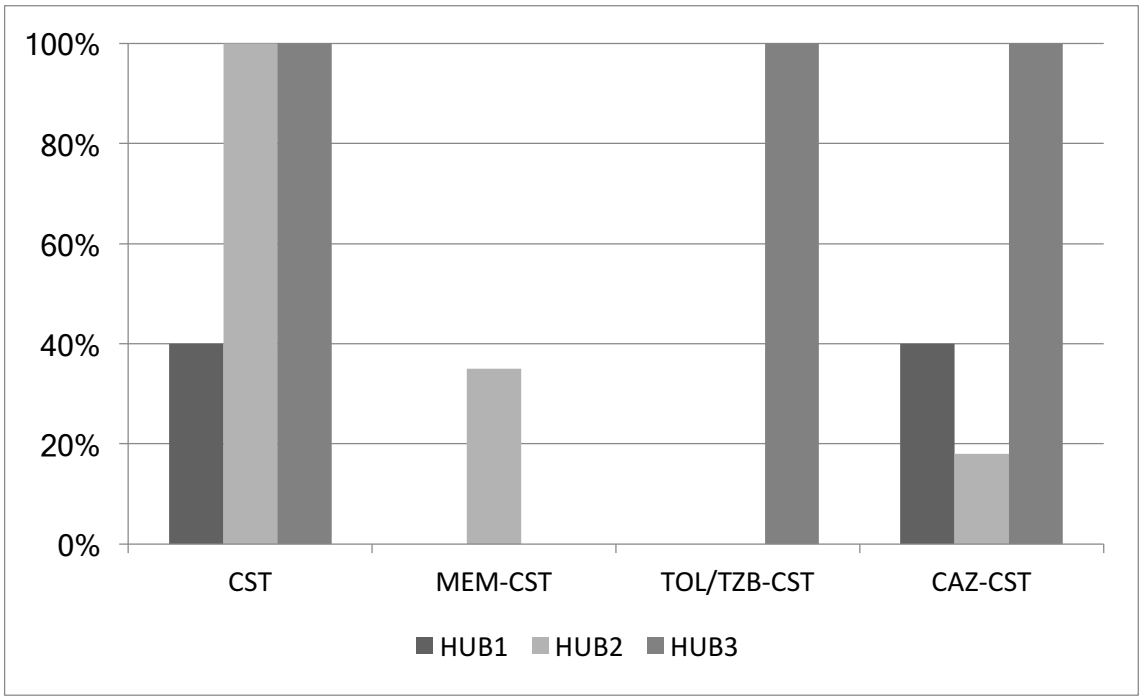
		Monotherapies				Combinations		
Strain	**Hour**	CST	CAZ	MEM	TOL/TZ	CAZ + CST	MEM + CST	TOL/TZ + CST
HUB1	6	-0.63	-0.17	-2.06	-0.15	+0.06	-0.85	-0.33
	24	-1.94	-0.33	-4.04	-0.60	-0.71	-4.25	-1.44
	30	-1.24	-0.25	-4.41	-1.07	-0.81	-4.96	-2.15
	48	-1.44	-0.41	-4.20	-0.79	-2.09	-5.96	-3.06
	54	-1.33	-0.47	-4.55	-0.99	-2.12	-6.25	-4.36
HUB2	6	-0.66	-0.37	-0.56	+0.13	-0.20	-1.58	-0.68
	24	-2.27	-0.66	-0.11	-0.34	-2.90	-3.63	-3.18
	30	-2.44	-0.44	-0.65	-0.39	-3.45	-3.61	-3.50
	48	-2.27	-0.36	-0.23	-0.47	-3.99	-3.38	-4.50
	54	-1.85	-0.22	-0.01	-0.64	-3.10	-3.54	-4.42
HUB3	6	-0.11	-0.10	-0.28	-0.05	-0.14	-0.04	-0.01
	24	-0.44	+0.23	-3.76	-0.12	-0.38	-3.87	-0.15
	30	-0.64	-0.04	-3.78	-0.38	-0.29	-3.78	-0.10
	48	-2.19	-0.45	-4.26	-0.02	-0.58	-5.97	-0.10
	54	-2.07	-0.25	-3.96	+0.09	-1.04	-6.37	-0.21

CST: Colistin; CAZ: Ceftazidime; MEM: Meropenem; TOL/TZ: Ceftolozane/tazobactam. Among monotherapies, a grey background represents a decrease ≥ 1 log cfu/mL whereas a blue background highlights a bactericidal effect. Among combinations, orange and green backgrounds represent additivity (decrease >1 and <2 log cfu/mL with the combination compared to its most active component) or synergy (decrease ≥ 2 log cfu/mL with the combination compared to its most active component), respectively.

6.2. Resistance studies

Resistant strains to ceftolozane/tazobactam among biofilm-embedded cells were not detected with any treatment (monotherapy or combination) in ceftolozane/tazobactam-susceptible strains. Regarding colistin resistance, at the end of treatment (Figure 6.4.), colistin monotherapy led to the emergence of resistant subpopulations at 54h among all strains. The combination of an active BL and colistin prevented from the emergence of resistant subpopulations, in contrast with what occurred when the BL was non-active *in vitro*. For the XDR-HUB2 strain, the PAPs of cells recovered at the end of treatments with meropenem-colistin and ceftazidime-colistin showed the same proportion of colistin-resistant subpopulations than that obtained at baseline; in contrast, this proportion increased with colistin monotherapy (until 10^{-2} CFU/ml).

Figure 6.4. Emergence of resistant colistin subpopulations among biofilm-embedded cells of the three *P. aeruginosa* strains according to the treatment regimen at 54 hours. Data expressed as proportion of samples with colonies growing at colistin concentration of 2mg/L among all tested.

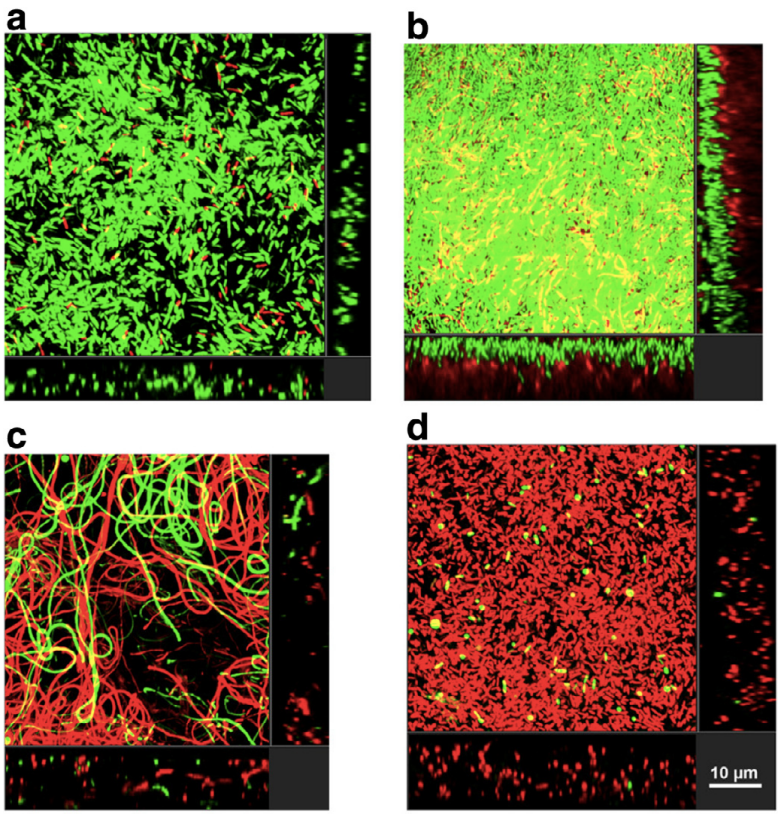


CST: Colistin. MEM: Meropenem. TOL/TZB: Ceftolozane-Tazobactam. CAZ: Ceftazidime.

6.3. Confocal laser scanning microscopy images

Well-formed biofilms prior to start the therapeutic experiments were observed in all strains. Treatment with BLs altered the shape of isolates in both live and dead cells. Colistin in monotherapy mainly had activity within deeper layers of the biofilm structure, whereas BLs plus colistin mainly resulted in activity against all the biofilm structure. Figure 6.5. shows some CLSM images of the biofilm-embedded cells of *P. aeruginosa* HUB2 according to treatment regimens.

Figure 6.5. Confocal laser scanning microscopy images of biofilm-embedded cells of *Pseudomonas aeruginosa* HUB2 at 0 hours (a), at 54 hours for the treatment of colistin in monotherapy (b), ceftolozane/tazobactam in monotherapy (c), and the combination of ceftolozane/tazobactam and colistin (d). Live cells are green due to staining with Syto 9, whereas dead cells appear red due to staining with propidium iodide. Maximum intensity projection of confocal images of total biofilm thickness is represented as central image. Rectangle images below and to the right of the projection correspond to XZ and YZ planes, respectively.



Aim 7 – To evaluate the pharmacokinetics and pharmacodynamics of ceftazidime in continuous infusion, with or without colistin, against a biofilm infection by *P. aeruginosa* in a dynamic *in vitro* model

Article 8 – *In vitro* pharmacokinetics of ceftazidime and its combinations with colistin against *Pseudomonas aeruginosa* biofim. **J. Gómez-Junyent**, O. Murillo, H. H. Yu, M. A. K. Azad, H. Wickremasinghe, R. Rigo-Bonnin, E. Benavent, J. Ariza, J. Li. Submitted for publication.

Communication 8 - *Pharmacokinetics/Pharmacodynamics of ceftazidime with and without colistin against Pseudomonas aeruginosa biofilms using an in vitro model.* **J. Gómez-Junyent**, O. Murillo, H. Yu, MH. Azad, H. Wickremasinghe, E. Benavent, C. El Haj, L. Soldevila, J. Ariza, J. Li. 29th ECCMID, Amsterdam, The Netherlands, 2019. Presentation number P0574.

In line with finding potential alternatives for the management of foreign-body associated infections by fluoroquinolone-resistant GNB and *P. aeruginosa*, the administration of BL-CI has been suggested, in order to optimize their PK/PD. With CI, BL concentrations for 100% of the time above MIC (T>MIC≈100%) can be achieved, which may be useful in foreign-body infections. Moreover, maximum killing rates in experimental studies are obtained when BL concentrations are at 3-4 times the MIC (3-4xMIC). However, our current knowledge of BLs PK/PD is based on studies performed with planktonic bacteria, which may not necessarily reflect what occurs with biofilm-embedded bacteria. For instance, whether BL may show concentration-dependent killing against *P. aeruginosa* biofilms is currently unknown. Given the potential benefit of combinations of colistin plus BLs, there is also interest in clarifying whether the addition of colistin can modify the PK/PD parameters of BL in biofilm-embedded bacteria. In this line, it is unclear if the concentration of BL is a relevant parameter when combined with colistin against biofilms by *P. aeruginosa*.

In this study, we aimed to evaluate the activity of several concentrations of CI ceftazidime against a biofilm infection by *P. aeruginosa* using the CBR and investigated if there was a differential activity of colistin combinations with higher or lower ceftazidime concentrations. Two strains of *P. aeruginosa* were employed, both

susceptible to ceftazidime and colistin; one was the reference isolate PAO1 and the other was the clinical isolate HUB8.

The determination of MIC, MBIC, MBEC and biofilm formation assays for both strains is summarized in Table 7.1. Both strains were strong biofilm formers and had high MBECs of ceftazidime and colistin. Baseline PAPs for both strains are illustrated in Figure 7.1.; heteroresistance to colistin was evident prior to colistin treatment.

Regimens evaluated were CI ceftazidime at clinically achievable concentrations (4, 10, 20 and 40 mg/L) and colistin in CI (3.5 mg/L). Therapeutic regimens evaluated were monotherapies of ceftazidime and colistin, 4 and 40 mg/L ceftazidime (CI) in combination with colistin, and controls (no antibiotic). In CBR experiments, achieved colistin concentrations (mean±SD) were 3.03±1.66 mg/L. Measured ceftazidime concentrations and ratios to MIC and MBIC are shown in Table 7.2.

Table 7.1. MICs, MBICs, MBECs and biofilm formation assays for the *Pseudomonas aeruginosa* strains examined in this study.

Isolates	MIC		MBIC		MBEC		Biofilm formation assays	
	CAZ	CST	CAZ	CST	CAZ	CST	Index (OD/ODc)	Category
PAO1	2	1	8	32	>512	>512	5.20	Strong
HUB8	1	1	2	32	>512	128	13.04	Strong

MIC, MBIC and MBEC are expressed in mg/L. Index and category of biofilm formation assays are based on the methodology described by Stepanovic *et al* (201). MIC: Minimum inhibitory concentration. MBIC: Minimum biofilm inhibitory concentration. MBEC: Minimum biofilm eradication concentration. CAZ: Ceftazidime. CST: Colistin.

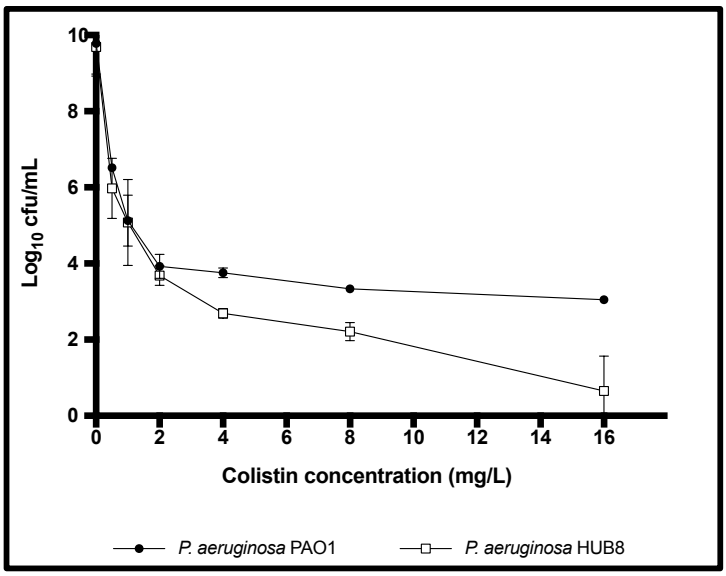
Table 7.2. Observed mean ceftazidime concentrations and ratios to MIC and MBIC during the CDC Biofilm Reactor experiments and emergence of ceftazidime resistance at 54h.

Therapeutic groups	PAO1				HUB8			
	CAZ concentration[1]	Ratio CAZ concentration/MIC	Ratio CAZ concentration/MBIC	CAZ Resistance (%)[2,3]	CAZ concentration[1]	Ratio CAZ concentration/MIC	Ratio CAZ concentration/MBIC	CAZ Resistance (%)[2,3]
CAZ4	4.55±0.6	2.27	0.57	Yes (66)	4.01±1.1	4.01	2	Yes (40)
CAZ10	9.51±0.5	4.77	1.18	Yes (66)	10.3±2.8	10.3	5.15	No (0)
CAZ20	18.03±3.0	9.01	2.25	Yes (40)	18.84±1.1	18.84	9.42	No (0)
CAZ40	34.48±5.0	17.24	4.31	Yes (26)	35.17±4.8	35.17	17.5	No (0)
CAZ4 + CST	3.33±0.2	1.67	0.42	No (0)	3.26±0.9	3.26	1.63	No (0)
CAZ40 + CST	38.65±4.1	19.33	4.83	No (0)	40.13±3.8	40.13	20.07	No (0)

[1]All measurements are expressed in mg/L as means ± standard deviation. [2]Percentage of plates containing ceftazidime 16 mg/L at 54h showing the presence of *P. aeruginosa* colonies. [3]At 54h, the proportion of ceftazidime-resistant isolates increased from $1x10^{-3}$ in CAZ20 and CAZ40 experiments to $1x10^{-1}$ – $1x10^{-2}$ in CAZ4 and CAZ10 experiments against PAO1, and was $1x10^{-2}$ – $1x10^{-3}$ in CAZ4 experiments against HUB8.

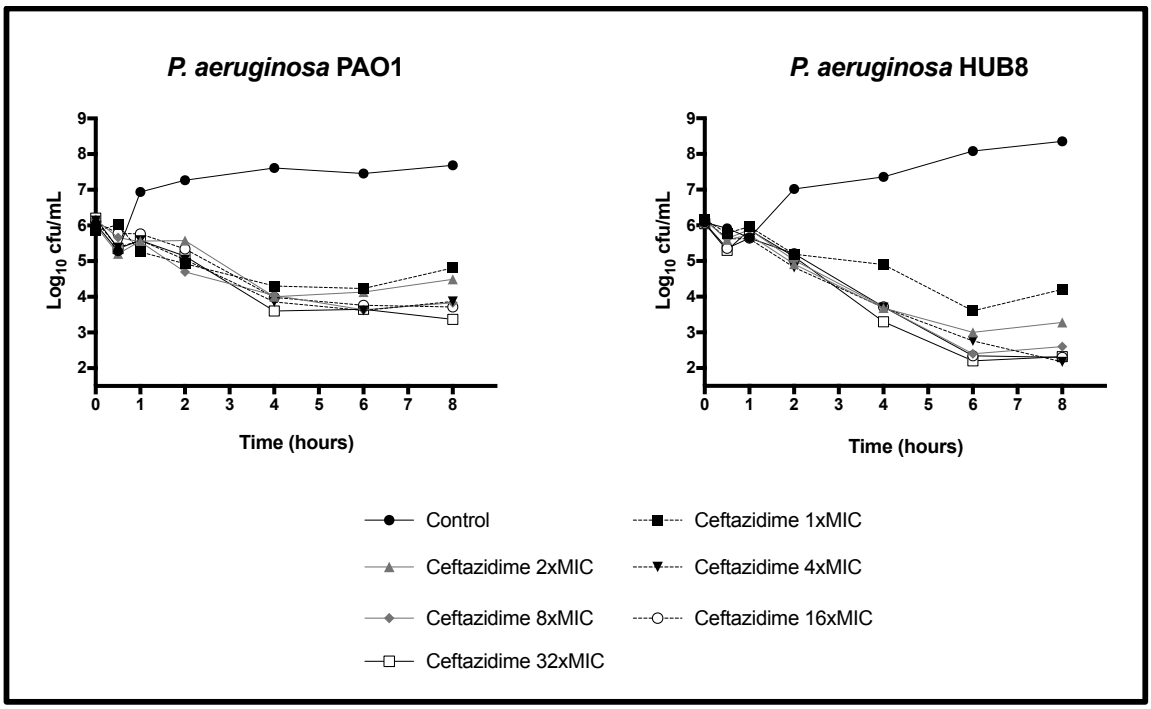
MIC: Minimum inhibitory concentration. MBIC: Minimum biofilm inhibitory concentration. CAZ: Ceftazidime. CST: Colistin. CAZ4: Ceftazidime 4 mg/L. CAZ10: Ceftazidime 10 mg/L. CAZ20: Ceftazidime 20 mg/L. CAZ40: Ceftazidime 40 mg/L.

Figure 7.1. Baseline Population Analysis Profiles of the strains of *Pseudomonas aeruginosa* evaluated in this study. Data presented as means ±SD.



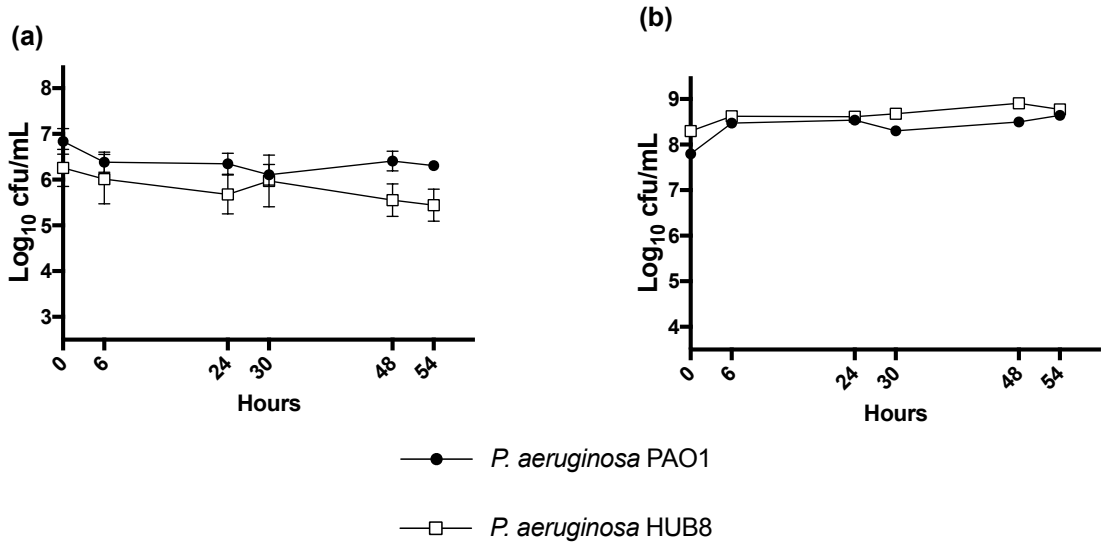
Static time-kill assays showed that concentrations of ceftazidime 4xMIC and above did not result in a greater killing (Figure 7.2.).

Figure 7.2. Time-kill curves of strains of *Pseudomonas aeruginosa* evaluated in the CDC Biofilm reactor exposed to several concentrations of ceftazidime. MICs are 2 mg/L for PAO1 and 1 mg/L for HUB8.



The bacterial growth of biofilm-embedded and free-floating bacteria from CBR experiments in the absence of antibiotics is shown in Figure 7.3. Log changes in the presence of antibiotics for biofilm-embedded bacteria are illustrated in Figure 7.4A. and shown in Table 7.3. Log changes for free-floating bacteria are illustrated in Figure 7.4B. For planktonic bacterial cells at 54h, non-bactericidal killing was observed for all the treatments evaluated. The combination of colistin plus 40 mg/L ceftazidime was the most active treatment against PAO1 (Δlog_{10}cfu/mL=-2.61) and HUB8 (Δlog_{10}cfu/mL=-2.06).

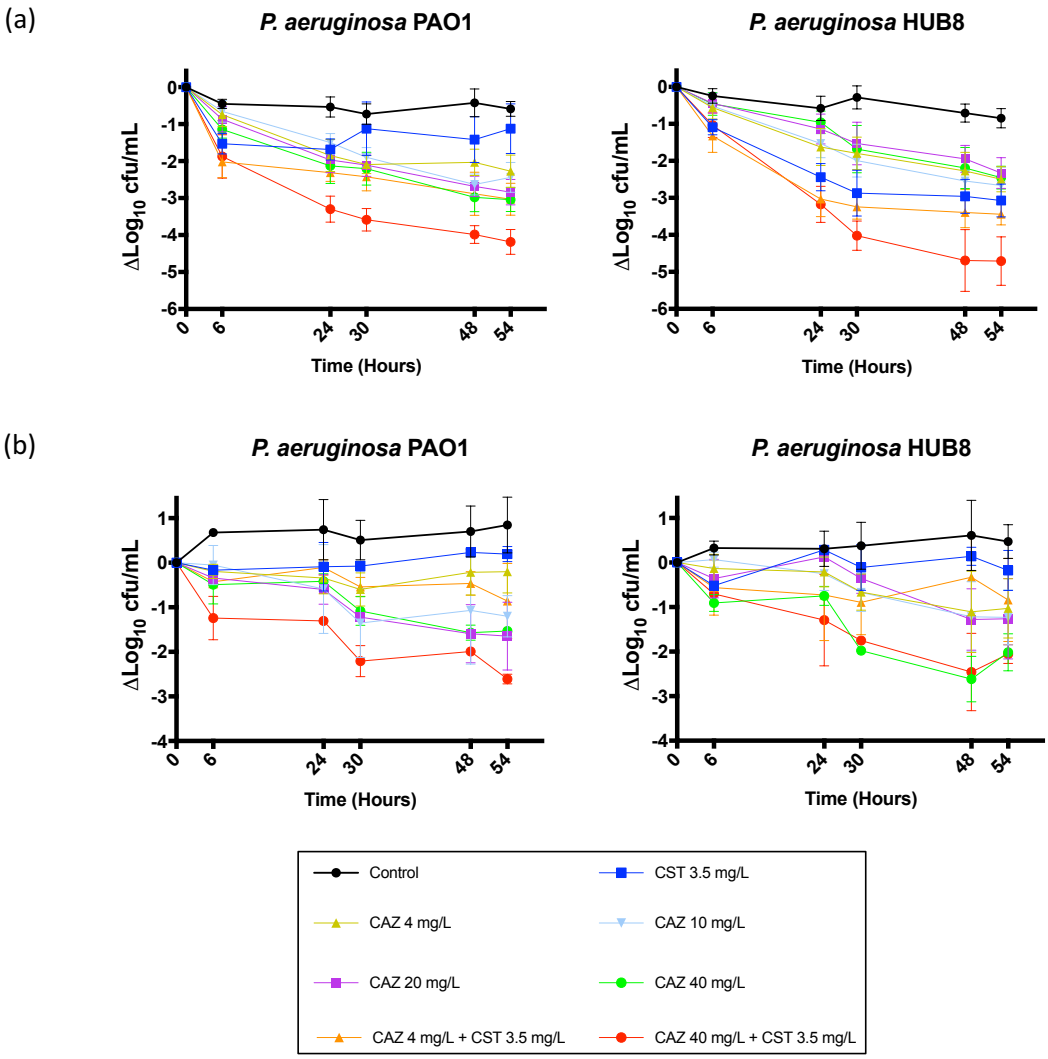
Figure 7.3. Bacterial growth in the absence of antibiotics for biofilm-embedded (a) and free-floating (b) cells for the strains of *Pseudomonas aeruginosa* evaluated with the CDC Biofilm Reactor. Time on the x -axis begins immediately after the 48-hour conditioning phase. Data presented as means ±SD



Regarding the activity against biofilm-embedded bacterial cells of PAO1, colistin monotherapy resulted in rapid initial killing, but regrowth was observed after 24h, with mild efficacy at 54h (Δlog_{10}cfu/mL=-1.12). Rapid killing was also observed at 24h against HUB8, but no regrowth occurred, and colistin was the only bactericidal monotherapy (Δlog_{10}cfu/mL=-3.07). Biofilm-embedded bacteria growing on 4 mg/L colistin plates were observed during experiments with colistin monotherapy against both strains; PAPs

of bacteria recovered at 54h after this treatment showed significantly greater proportions of colonies able to grow at concentrations of ≥2 mg/L colistin, compared to baseline (Figure 7.5.).

Figure 7.4. Bacterial killing by the treatment regimens against biofilm-embedded (a) and free-floating (b) cells of the *Pseudomonas aeruginosa* strains evaluated with the CDC Biofilm Reactor. Results are expressed using the log change method. Data presented as means ±SD.



P. aeruginosa: *Pseudomonas aeruginosa*. CST: Colistin. CAZ4: Ceftazidime 4 mg/L. CAZ10: Ceftazidime 10 mg/L. CAZ20: Ceftazidime 20 mg/L. CAZ40: Ceftazidime 40 mg/L.

Table 7.3. Mean log changes of biofilm-embedded bacterial counts throughout the study.

		Monotherapies					Combinations	
Strain	**Hour**	CST	CAZ4	CAZ10	CAZ20	CAZ40	CAZ4 + CST	CAZ40 + CST
PAO1	6	-1.53	-0.74	-0.66	-0.87	-1.14	-2.02	-1.87
	24	-1.69	-1.85	-1.50	-1.97	-2.13	-2.32	-3.30
	30	-1.12	-2.10	-1.90	-2.11	-2.20	-2.43	-3.59
	48	-1.42	-2.03	-2.63	-2.68	-2.98	-2.88	-3.98
	54	-1.04	-2.27	-2.44	-2.84	-3.05	-3.10	-4.19
HUB8	6	-1.09	-0.51	-0.52	-0.48	-0.46	-1.33	-1.04
	24	-2.44	-1.63	-1.53	-1.14	-0.96	-3.03	-3.18
	30	-2.87	-1.80	-1.99	-1.53	-1.68	-3.24	-4.02
	48	-2.96	-2.27	-2.54	-1.94	-2.19	-3.40	-4.69
	54	-3.07	-2.47	-2.66	-2.33	-2.45	-3.44	-4.71

CST: Colistin; CAZ4: Ceftazidime 4mg/L; CAZ4: Ceftazidime 10mg/L; CAZ20: Ceftazidime 20mg/L; CAZ4: Ceftazidime 40mg/L. Among monotherapies, a grey background represents a decrease ≥ 1 log cfu/mL whereas a blue background highlights a bactericidal effect. Among combinations, orange and green backgrounds represent additivity (decrease >1 and <2 log cfu/mL with the combination compared to its most active component) or synergy (decrease ≥ 2 log cfu/mL with the combination compared to its most active component), respectively.

Ceftazidime monotherapy resulted in >2 log_{10} reduction at 54h. Against PAO1, greater activities were observed with higher concentrations, being bactericidal with 40 mg/L ceftazidime (Δlog_{10}cfu/mL=-3.05) and almost bactericidal with 20 mg/L ceftazidime (Δlog_{10}cfu/mL=-2.84). Actually, higher concentrations (20-40 mg/L) of ceftazidime were significantly more active than lower concentrations (4-10 mg/L) against PAO1 ($p<0.001$). Against HUB8, no significant differences were observed when comparing higher and lower ceftazidime concentrations ($p=0.424$).

Well-formed biofilms prior to treatment were observed with CLSM for both strains (Figure 7.6). Monotherapies with 4 mg/L or 40 mg/L ceftazidime and colistin resulted in the appearance of a mixed staining in green and red fluorescence (Figure 7.7), showing a predominance of yellow images and reflecting the presence of live and damaged bacteria within the biofilm structure. Monotherapies with ceftazidime also altered the cell shape.

Resistance to ceftazidime among biofilm-embedded bacteria from both strains emerged in 4 mg/L ceftazidime experiments as soon as 24-30h after treatment. Ceftazidime resistance also emerged at 54h against PAO1 with 10, 20 and 40 mg/L ceftazidime, but not against HUB8. Ceftazidime MICs of resistant isolates ranged from 32 to 128 mg/L. Ratios of ceftazidime concentrations to MBIC above 5 protected against the emergence of ceftazidime resistance in HUB8 at 54h, which was achieved with concentrations higher than 4 mg/L. Ratios of ceftazidime concentrations to MBIC above 5 were not achieved in PAO1 with any concentration evaluated, but there was a lower percentage of ceftazidime-resistant isolates recovered at 54h after treatment with high ceftazidime concentrations (20 and 40 mg/L) than low concentrations (4 and 10 mg/L) (Table 7.2).

The addition of colistin to 4 mg/L or 40 mg/L ceftazidime increased the killing activity of monotherapies at 54h against biofilm-embedded bacteria of both isolates. The combination of colistin plus 4 mg/L ceftazidime resulted in significantly greater bactericidal activity than 4 mg/L ceftazidime monotherapy against PAO1 (-3.10 vs -2.27; $p<0.001$) and HUB8 (-3.44 vs -2.50; $p<0.001$). This combination was synergistic compared to colistin monotherapy against PAO1 ($p<0.001$), but not significantly more effective against HUB8 ($p=0.344$). The activity of colistin plus 40 mg/L ceftazidime resulted in the highest killing against both strains at 54h (Δlog_{10}cfu/mL=-4.19 against

PAO1; Δlog_{10}cfu/mL=-4.71 against HUB8) ($p<0.001$ in all comparisons). Colistin plus 40 mg/L ceftazidime resulted in a >1 log_{10} reduction at 54h compared to the combination of colistin plus 4 mg/L ceftazidime against both isolates ($p<0.001$).

No colistin- or ceftazidime-resistant strains emerged with either combination therapy. Colistin PAPs of recovered bacteria at 54h showed that combinations of colistin with 4 mg/L or 40 mg/L ceftazidime resulted in a lower proportion of heteroresistant colonies, compared to baseline (Figure 7.5). CLSM images showed a greater red fluorescence and higher presence of damaged bacteria with combination therapies of ceftazidime and colistin, which seemed more evident with 40 mg/L ceftazidime (Figure 7.7).

Figure 7.5. Population Analysis Profiles of biofilm-embedded cells of *Pseudomonas aeruginosa* evaluated with the CDC Biofilm Reactor after 54h of treatment regimens including colistin. Data presented as means ±SD. *P. aeruginosa*: *Pseudomonas aeruginosa*. CST: Colistin. CAZ: Ceftazidime.

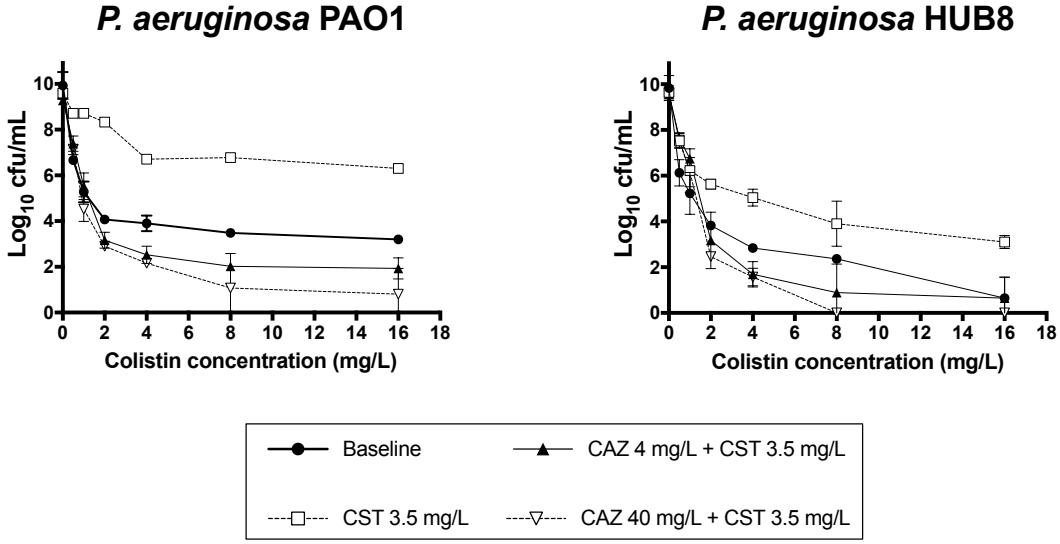


Figure 7.6. Three-dimensional representations of biofilm structures of strains of *Pseudomonas aeruginosa* evaluated with the CDC Biofilm Reactor at 0 hours, before treatment initiation.

***P. aeruginosa* PAO1** ***P. aeruginosa* HUB8**

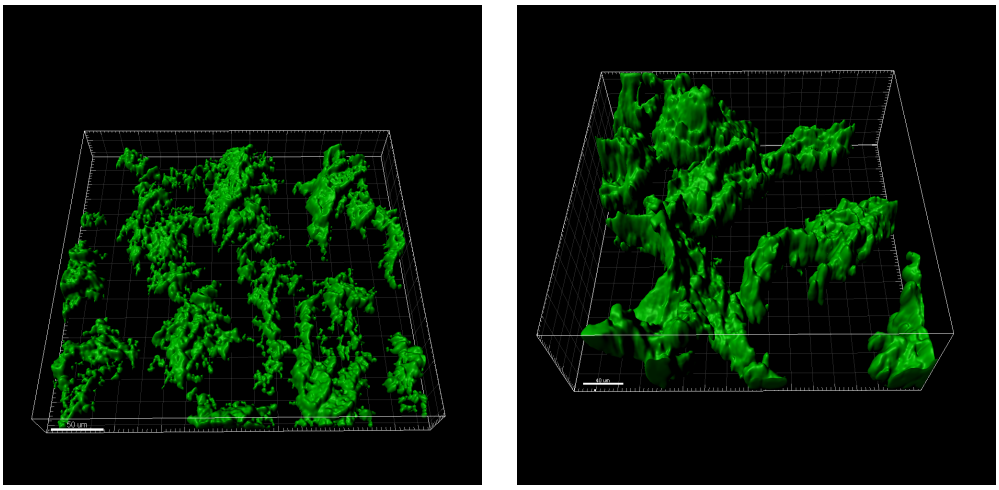
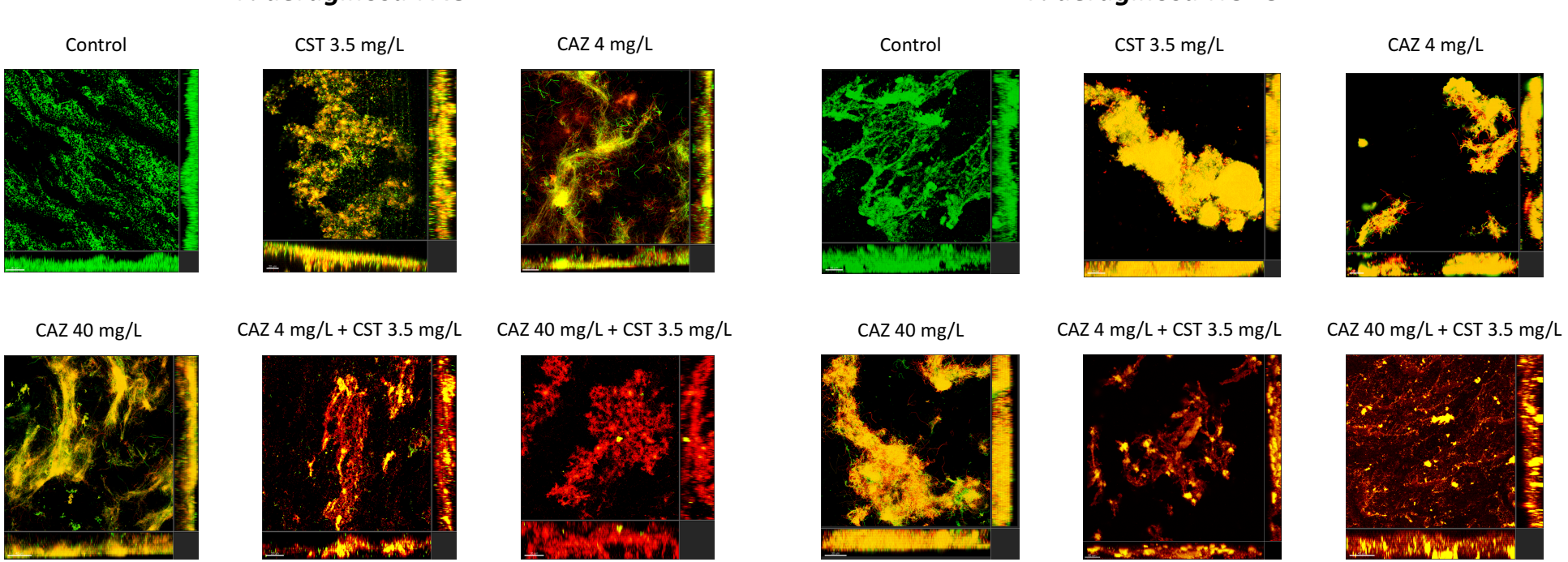


Figure 7.7. Confocal laser scanning microscopy images of biofilm-embedded cells of *Pseudomonas aeruginosa* evaluated with the CDC Biofilm Reactor after 54 hours of treatment. Live cells are green due to staining with Syto 9, whereas dead cells appear red due to staining with propidium iodide; yellow represents a mixture of live and dead cells. Maximum intensity projection of confocal images of total biofilm thickness is represented as central image. Rectangle images below and to the right of the projection correspond to XZ and YZ planes, respectively.



P. aeruginosa: *Pseudomonas aeruginosa*. CST: Colistin. CAZ: Ceftazidime.

DISCUSSION

1. Impact and prognosis of bacteraemic osteoarticular infections

1.1. Impact of bacteraemic osteoarticular infections

1.1.1. The increasing incidence of bacteraemic osteoarticular infections

We have described a progressive increase in the incidence of bacteraemic OAIs in our area during the last 3 decades, including all types of OAIs, as has been previously reported for VO (20).

The increased rates of OAIs have been linked with a longer life expectancy and the growing frequency of chronic medical conditions (20, 70, 211). In line with previous studies, we also observed a significant increase in the age of individuals with OAIs. Previous studies highlighted an association between OAIs and patients with intravenous drug abuse, who are usually younger (212-214). Our results suggest that this harmful social habit has been progressively abandoned in our area, and that it no longer has an effect on the current pattern of bacteraemic OAIs.

In connection with an increased life expectancy, the growing prevalence of chronic conditions observed in our cohort may be ageing-related. In addition, modern medical advances have generated new technologies that can be offered to a higher number of older patients (215). In this line, the occurrence of device-related infections clearly increased over time. Actually, the implantation and revision of joint prostheses is a frequent procedure in tertiary-care hospitals nowadays (216, 217), resulting in an increase of the absolute number of infections (7, 215).

The current pattern of bacteraemic OAI has been modified by the impact of these implant-associated infections, which represented 28% of all infections in the most recent period. While the incidence of PJI and osteosynthesis-related infections all rose, among non-device associated OAIs, VO was the only one to increase. The pattern of non-device associated OAIs also changed, due to population's age increases. While the presence of SA did not differ between patients of different ages, PO was predominant among patients under 50 years of age and VO was more frequent in older patients. Overall, the pattern of OAIs in younger patients reflected the predominance of native SA and PO, whereas older patients had more PJI and VO. Accordingly, the increase in the

median age of patients observed in some OAIs (i.e. SA) was mainly related to the rise in the number of episodes that occurred in older patients (i.e. PJI).

Although *S. aureus* was the most frequent microorganism causing OAIs, as has been extensively reported (21, 68, 70), we observed a trend towards a decline in its proportion in comparison to other agents. The significant emergence of MRSA in recent years has completely changed the picture of staphylococcal OAIs, occurring more frequently among older adults, and resulting in a decrease in cases by MSSA, which is especially predominant in young patients. The presence of *Streptococcus* and *Enterococcus* species is also notable and, together with staphylococcal cases, highlight the major role of gram-positive cocci in this setting; streptococcal and enterococcal cases were also more frequent among older individuals (218-220).

Regarding the worldwide emergence of MDR GNB (221, 222), we have witnessed only anecdotal episodes in the last 10 years. Drug-resistant GNB infections, including bacteraemia, have been reported in our country and elsewhere (222, 223). Indeed, an increase of PJI caused by MDR microorganisms has been described in our country (26). Such infections by MDR bacteria occur probably in the post-operative period after device placement and may be less likely to arise in the setting of bacteraemia or as a metastatic complication, due to MDR microorganisms' loss of virulence and fitness. Since we only included cases with concurrent bacteraemia, we may have missed cases caused by MDR microorganisms. Similarly, the low rate of polymicrobial and anaerobic OAIs may be explained by the inclusion only of bacteraemic cases.

1.1.2. The association of infective endocarditis with osteoarticular infections of the axial skeleton

The association of IE with OAI has been reported previously, but several questions remain to be clarified. The prevalence of IE as a source of bacteraemic OAI has been reported to range from ratios as low as <5–10% to as high as 25–30% when analysing a particular OAI such as VO (24, 224, 225). In a large series of all types of bacteraemic OAIs reported here, the presence of a concomitant diagnosis of IE was 15%, but it clearly differed between types of OAIs. The highest incidence of IE was among OAIs with involvement of the axial skeleton (SA of the axial skeleton in 22% of cases and VO in

20%). In fact, we observed that most OAIs associated with IE had involvement of the axial skeleton (77%).

Regarding microbiology, *S. aureus* was the most frequent aetiology of metastatic OAI associated with IE, but the whole group of "less virulent" microorganisms that included *S. viridans*, *S. bovis*, enterococci and coagulase-negative staphylococci strains caused a great percentage of these cases. Actually, this group of "less virulent" microorganisms were between two and eight times more likely to cause VO. Previous studies have reported the association between VO and IE, especially in the case of streptococcal aetiology (224, 225). Our results confirmed that this group of less virulent microorganisms was responsible for this association. In fact, we showed that when these microorganisms caused a bacteraemic OAI, they almost exclusively produced VO, and this was mainly caused by the existence of IE that may facilitate continuous bacteraemia. In fact, we consider that these streptococcal, enterococcal and coagulase-negative staphylococcal microorganisms, all of which have affinity for causing IE, also have a great ability for causing VO for reasons that are not well understood.

S. aureus can cause all types of bacteraemic OAIs. It is known that this microorganism has several virulence factors that facilitate either the adherence or the invasion of several tissues (226, 227). Therefore, *S aureus* is able to cause IE, like the group of less virulent microorganisms mentioned, but also caused all types of OAIs regardless of the presence or absence of IE. In fact, bacteraemic OAIs caused by *S. aureus* were significantly more frequent in the absence of concomitant IE. Overall, the contrast between OAIs caused by *S. aureus* and less virulent microorganisms illustrates that particular types of bacteraemic OAIs are less likely to be caused by some bacteria.

Interestingly, our results suggest that the main relationship between staphylococcal OAIs and IE was established with the presence of SA of axial skeleton. In our experience, *S. aureus*, together with pyogenic streptococci, are responsible for most cases of SA involving the axial skeleton (23, 225). While *S. aureus* caused a large number of both OAIs and IE, pyogenic streptococci only rarely produced IE, but in all these cases the OAI involved the axial skeleton. There is little previous information about SA of the axial skeleton, and the few studies available have reported its higher predominance among intravenous drug users (213, 228, 229). The results of our study (which did not include

intravenous drug users) suggest that, in the presence of staphylococcal bacteraemia affecting the axial skeleton (especially in the case of SA of the axial skeleton) the concomitant presence of IE should be ruled out.

The association of IE and OAIs with involvement of the axial skeleton was observed regardless of the microorganisms responsible or the sex and age of patients. It seems that some particular features of bone and joints that change throughout life, the characteristics of the continuous low-grade bacteraemia of IE, and the differences observed in the osteoarticular tropism of microorganisms may explain this association. Overall, we recommend that in the presence of bacteraemic OAIs involving the axial skeleton, IE should be ruled out and a transesophageal echocardiography performed.

1.1.3. The differential characteristics of bacteraemic septic arthritis according to the site of acquisition

In recent years, the site of acquisition of several infections has changed (17). While the distinction between nosocomial- and community-acquired infections is well known, several recent articles have reported the particularities of infections in the healthcare environment (17, 18, 230). In our first study, we showed a progressive increase in nosocomial episodes, the emergence of healthcare-related infections, and a decrease in the number of strictly community-acquired OAI. To our knowledge, no previous studies have focused on the site of infection in the setting of SA; the results of our third study show that healthcare-related cases share some features with community-acquired cases and other with nosocomial-acquired.

The characteristics of patients with nosocomial-acquired and healthcare-related SA were alike; these patients were older and had more frequently underlying medical conditions than patients with community-acquired SA. In contrast, the clinical pattern of SA with regard to the joints involved showed a clear predominance of peripheral joints (92%) among nosocomial-acquired cases, with a significant association with PJI. In contrast, the axial skeleton was involved in almost 25% of health care-related and community-acquired cases.

Although these differences observed between native SA and PJI had been previously reported (7, 231), our results highlight other interesting epidemiological points. As already known, PJI are usually classified into acute and late chronic according to the time elapsed since prosthesis implantation (1-3 months vs longer) (7). The clinical presentation of these two kinds of PJI reveal clear differences: while acute PJI may present with bacteraemia, this is extremely uncommon in late chronic infections. Thus, the high rates of PJI in nosocomial cases of SA may be related to the predominance of post-surgical acute infections. Less frequently, haematogenous seeding of microorganisms to the prosthesis may occur over time, and this may be reflected in the community and healthcare-related cases of PJI.

The presence of MRSA was clearly associated with nosocomial-acquired and healthcare-related sites, and was practically non-existent among community-acquired sites (1%). These results seem to be in agreement with others from elsewhere in Europe which have reported a lower frequency of community-acquired MRSA infections than in the USA (232-234). The association between nosocomial acquisition and PJI cases may partially explain the higher presence of MRSA infections at this site.

Moreover, our results showed that almost all streptococcal infections were acquired in the community and the healthcare environment, and were extremely rare among nosocomial-acquired cases. The great variety of *Streptococcus* species makes it hard to draw firm conclusions, but the viridans group and the pyogenic species seem to be more related to community-acquired infections. In this regard, our results for streptococcal SA underline the similarities between the healthcare-related and community-acquired populations. Finally, GNB was a more common aetiology of SA in the hospital environment, and was also frequent in the healthcare-related setting. *P. aeruginosa* was observed only at these sites, and *Enterobacteriaceae* strains were significantly more frequent as causes of PJI.

Overall, identification of health care-related SA appears to be important in clinical practice. The particular features of its aetiology, as well as the greater fragility of patients (who tend to be older and present more comorbid conditions), make an early distinction between healthcare-related and community-acquired SA mandatory in order

to plan the emergency indications of empirical antibiotic therapy and surgical procedures.

Our studies are inherently limited by its observational design, but in any case, they offer a global perspective of a great number of OAIs over a long period of time. It should be noted that our studies refer only to the incidence of bacteraemic OAI and we do not know the overall incidence of OAI, including bacteraemic and non-bacteraemic cases; therefore, in the present report, the incidence of overall OAIs has been underestimated. Indeed, the increased incidence of bacteraemic OAI in our studies could be due to either a greater number of patients with OAI or to a higher prevalence of bacteraemia among patients with OAI.

1.2. The significant mortality of bacteraemic osteoarticular infections and the role of surgical debridement for the management of bacteraemic peripheral septic arthritis

Mortality is classically an unrecognized outcome among studies evaluating the prognosis of OAIs. It is usually part of composite endpoints which include other outcomes, such as multiple surgeries, relapse or superinfection. In this line, the role of bacteraemia is relevant in most OAIs and has been recognized as a risk factor for failure in some studies (24, 95, 235). Thus, there was interest in evaluating the mortality figures and associated risk factors of bacteraemic OAIs.

Our findings regarding mortality of bacteremic OAIs were: first, mortality in patients with bactearemic OAIs was greater if the patient was elderly or had peripheral SA; second, early mortality (≤7 days) accounted for more than 40% of all deaths; third, underlying conditions like rheumatoid arthritis and liver cirrhosis were important risk factors for mortality; fourth, *S. aureus* was associated with high mortality; and fifth, surgical debridement was associated with decreased mortality in patients with peripheral SA.

Patients with rheumatoid arthritis and liver cirrhosis, in comparison with the general population, are considered to have an increased risk of mortality and greater susceptibility to infection, including bacteraemia, SA and osteomyelitis (236, 237). Our data reinforce this concept, showing that rheumatoid arthritis was a major risk factor

for increased mortality. Most patients with rheumatoid arthritis also presented with peripheral SA, this being the most frequent OAI among those who died. In current clinical practice, the increasing use and availability of biological therapies and steroids for patients with rheumatoid arthritis may be associated with this increased likelihood of severe infections (238, 239).

OAIs are one of the most frequent and serious complication of *S. aureus* bacteraemia (13, 240). We found that *S. aureus* was a predictor of 30-day mortality in the whole cohort. Indeed, *S. aureus* bacteraemia is associated with significant mortality rates (241) which are usually higher with methicillin-resistant *S. aureus* bacteraemia (242, 243).

Mortality was highest in cases of peripheral SA, among all types of OAIs (18.6%), with the lower mortality observed in patients with axial SA. In accordance with previous reports (213, 228, 229), the latter pattern of SA has been highlighted in young people and intravenous drug users, but is also present in other populations. According to our data, we confirmed that mortality was clearly different when associated with SA of the peripheral joints or of the axial skeleton, supporting the argument that they should be considered two separate entities of SA. Importantly, healthcare-related SA presented a higher risk for mortality than community-acquired cases but a lower risk than nosocomial-acquired cases. In this line, mortality associated with PJIs was significant, which has been suggested by previous reports (7, 30).

Previous reports that included bactearemic and non-bacteraemic cases of SA had shown mortality rates around 10% (244, 245), possibly representing a midpoint between our rates for peripheral and axial SA. The higher mortality of peripheral SA may partly be explained by the inclusion only of bacteraemic cases and partly by the patients' characteristics. However, the features of peripheral SA itself may play a major role, especially regarding the accumulation of purulent collections in enclosed spaces, the high inoculum associated with the infection, or the enhanced inflammatory response. Thus, bacteraemic peripheral SA should be considered an emergency, and physicians should manage it accordingly.

We recommend that surgical debridement be considered for any patient presenting with peripheral SA, especially if large joints are affected. This approach has also been recommended for native SA (21) and PJI (7). We showed that surgical debridement

reduced the risk of mortality, especially early mortality. In this context, we believe that surgery should ensure the removal of the purulent content in the joint, and that this may improve the effectiveness of antibiotics and reduce the inflammatory response. In turn, these factors affect the mortality observed in these patients. In those who may not be fit enough for surgery or transfer to the operation theatre, physicians may consider joint drainage by arthrocentesis, even repeated if needed, and defer the debridement until the patient has been stabilized.

Although we evaluated the role of surgical debridement in those with bacteraemic SA using a multivariate regression model and a propensity-score matching analysis, it is still possible that other variables may have affected our results and selection bias may still have occurred.

2. Antimicrobial treatment against infections by *Pseudomonas aeruginosa*

Clinical studies

2.1. Continuous beta-lactam infusion is a valid therapeutic alternative for osteoarticular infections by fluoroquinolone-resistant *P. aeruginosa*

Treatment with fluoroquinolones is considered the first-line antimicrobial therapy for OAIs caused by *P. aeruginosa* (7, 31). Some fluoroquinolone-resistant *P. aeruginosa* strains are MDR/XDR, which limits the probability of success even further and thus demands new therapeutic strategies. When we evaluated the role of BL-CI against OAI by fluoroquinolone-resistant *P. aeruginosa*, we found that an optimized treatment strategy of TDM-supported BL-CI, often used in combination with colistin, led to similar patient outcomes to those attained when OAIs caused by fluoroquinolone-susceptible *P. aeruginosa* were managed with ciprofloxacin.

CI has been suggested as a method that could optimize the pharmacodynamics of BL by ensuring *f*Css for T>MIC ≈ 100%. Extending the T>MIC may be appropriate in challenging scenarios (such as for critically ill patients or biofilm-related infections), as it has been linked to better outcomes (174-176). BL-CI may also recover the activities of BL against

MDR/XDR strains (which usually exhibit high BL MICs otherwise), and may optimize PK/PD indices (T>MIC = 100%) (186).

Colistin has shown remarkable anti-biofilm activity against *P. aeruginosa* in previous experimental models and a few clinical studies, mainly when used in combination with BL against MDR/XDR *P. aeruginosa* (156, 159, 160). However, when treating OAIs caused by fluoroquinolone-resistant *P. aeruginosa*, we avoided using colistin in older fragile patients, especially if they were managed by removing the implant or performing extensive bone resection, in order to prevent potential toxicity. Even in this situation, most patients were cured, further supporting the efficacy of BL-CI and suggesting that it is an effective therapeutic option for improving the outcomes of patients with OAIs caused by *P. aeruginosa* when fluoroquinolones cannot be used.

Previous studies in this area have shown that significantly poorer outcomes result when OAIs caused by GNB (including *P. aeruginosa*) are treated with regimens that do not include a quinolone—usually BL administered via intermittent infusion or orally (130, 132). Interestingly, Grossi *et al*. emphasized the importance of optimizing the intravenous administration of BL to improve the outcomes of orthopaedic device-related infections caused by fluoroquinolone-resistant GNB (246). Although we also included other OAIs aside from PJI, the results we obtained with BL-CI (with or without colistin) support the role of this therapeutic strategy in such a setting.

When optimizing BL pharmacodynamics through the use of CI (T>MIC = 100%), as we did, the importance of achieving BL concentrations exceeding four times the MIC in cases of biofilm-related infection is unclear. In contrast to previous assumptions inferred from studies involving planktonic bacteria (173), results from a limited number of studies using high bacterial inoculums or biofilm experimental models have suggested that BL may exhibit concentration dependent activity against *P. aeruginosa* (169, 247-249).

TDM was found to be useful for guiding an optimized BL-CI therapy for difficult-to-treat infections. We employed an easy-to-use method to calculate the appropriate initial BL-CI dosing regimen for each clinical case, based on the MIC and antibiotic clearance. This method, despite being a practical approximation for bedside use, shows poor correlation between observed and predicted BL concentrations in patients with CKD or

weight > 75 kg, and for BL that are not exclusively cleared renally (182). In our experience, most patients had a *f*Css of 3–10xMIC. Patients with a *f*Css exceeding 10xMIC generally had CKD/AKI, whereas patients with a *f*Css of less than 3xMIC had OAIs by *P. aeruginosa* with high MIC. This suggests that TDM may be especially relevant in cases where the BL *f*Css may be less predictable or highly variable (CKD/AKI) and in cases where it may be difficult to achieve a relevant *f*Css, such as cases with a high MIC (250). Although our approach is feasible and practical for clinical use, truly individualized treatments may require population pharmacokinetics models for an OAI setting, as they should provide more reliable dose adjustments. As reported before, the UHPLC-MS/MS was an appropriate procedure to be used in routine clinical practice for TDM of antibiotics.

The target BL *f*Css was generally obtained with lower doses compared to standard intermittent infusion, without affecting the outcome. It is unclear whether BL concentrations in plasma correlate with BL concentrations at the infection site (118), but CI probably maintains free (and active) local concentrations of BL, which may partly explain the good clinical courses observed in this study. However, despite their limitations, most pharmacokinetics studies that have evaluated BL bone concentrations have found bone-to-serum BL concentration ratios of less than one (117). Therefore, the local BL concentrations in our study may have been significantly lower than those obtained in plasma samples.

Due to variability in protein binding and the potential effect of hypoalbuminemia (251, 252), the real free BL concentrations may have differed from those estimated here, especially for highly protein-bound BL (i.e., aztreonam). Despite all these considerations, we believe that our results support the use of BL-CI for the management of OIs caused by fluoroquinolone-resistant *P. aeruginosa*.

Experimental studies

2.2. Colistin in combination with ceftolozane/tazobactam as a therapeutic alternative for biofilm-related infections by multidrug-resistant *P. aeruginosa*

The best treatment for osteoarticular and orthopaedic device-related infections caused by MDR *P. aeruginosa* is currently unknown. We have already mentioned the problem of biofilm tolerance to antibiotics, such as BLs, which act against processes occurring in growing bacteria (208, 209). In this setting, the occurrence of MDR/XDR *P. aeruginosa* isolates dramatically limits the therapeutic alternatives since these usually have a decreased susceptibility to BLs and are often only susceptible to colistin.

The appearance of ceftolozane/tazobactam in recent years has represented a clear alternative for the management of such difficult-to-treat infections. However, there is scarce experience in treating OAIs by MDR *P. aeruginosa* and published clinical cases have found contradictory results (168, 253, 254). In our model, ceftolozane/tazobactam in monotherapy showed low anti-biofilm efficacy against susceptible strains and it was ineffective against a ceftolozane/tazobactam-resistant strain (MDR-HUB3). Emergence of ceftolozane/tazobactam-resistant strains was non-existent throughout treatment, in line with the low frequency of spontaneous resistant mutants previously reported (255). Overall, given the contradictory results between our results and other publications, clinicians should be cautious when considering monotherapy with ceftolozane/tazobactam for OAIs by MDR/XDR *P. aeruginosa*.

When evaluating the comparative efficacy of other BLs, it was interesting to observe that meropenem alone achieved bactericidal activity against the two meropenem-susceptible strains, this suggesting a differential anti-biofilm activity by an unknown mechanism in comparison with other BLs, such as cephalosporins. In this line, Haagensen *et al*. used a dynamic biofilm model with flow cell technology and CLSM and showed that meropenem initially targeted *P. aeruginosa* subpopulations present at the periphery of the biofilm structure but repeated doses resulted in the progressive killing of cells in deeper layers (256, 257). Our results also confirmed the potential anti-biofilm effect of colistin, with greater activity in anaerobic conditions and may act as a biofilm destabilizer. CLSM pictures from our experiments showed how colistin has higher affinity for the killing of bacteria within inner layers of the biofilm population.

Our results highlight the greater activity of combining colistin with BLs in comparison with monotherapies, which has also been previously reported against planktonic bacteria (151, 258, 259). Our data also suggest that the anti-biofilm benefits of this combination extend to other subfamilies of BLs, apart from carbapenems, but the efficacy of each BL plus colistin combination may differ significantly according to its prior activity and the strains' variability. In this line, ceftolozane/tazobactam plus colistin would be the most appropriate combination for biofilm infections by XDR *P. aeruginosa*, whereas meropenem plus colistin would have greater activity for carbapenem-susceptible strains. Interestingly, meropenem plus colistin had also a significant activity against carbapenem-resistant strains, which our group had also previously reported in experiments using strains with different mechanisms of resistance (159).

The synergy observed with the BL and colistin combination has been previously associated with mechanistic and subpopulation synergy effects (258), which may also be applied to biofilm-related infections by targeting different subpopulations. Whereas colistin may target subpopulations with low metabolic activity within inner layers of the biofilm (156-158), BLs may act upon more metabolically active subpopulations, present at the periphery of the biofilm structure (256, 257). In the particular setting of biofilm-related osteoarticular and orthopaedic device-related infections, clinical data have also emphasized the benefits of using colistin in combination, especially against *P. aeruginosa* isolates (160).

The benefits of combining BLs with colistin in the management of biofilm-associated infections by MDR/XDR *P. aeruginosa* also extend to preventing the amplification of colistin-resistant subpopulations among heteroresistant strains. This clearly depended on the strain's susceptibility to BLs; the more likely the protection if the strain is susceptible. However, our analysis of colistin PAPs with XDR-HUB2 strain at the end of treatment showed a similar proportion of heteroresistant population with the combined treatments compared to PAPs at baseline, thus suggesting a stochastic expression of resistance rather than the emergence of real mutants. In contrast, this proportion of heteroresistant strains did change at the end of treatment with colistin alone.

Limitations of our results are based on the use of an *in vitro* dynamic model, since biofilm structures may be different *in vivo* and host-pathogen interactions are not taken into

account. Antibiotic concentrations near the biofilms may differ depending on the biofilm location *in vivo*. Finally, the use of a small number of *P. aeruginosa* strains is also a limitation and certainly, the use of more strains may provide a deeper understanding of the anti-biofilm activity of the treatments.

2.3. The concentration-dependent effect of continuous beta-lactam infusion against biofilm-related infections by *P. aeruginosa*

In an era of rapidly increasing antibiotic resistance, it is crucial to optimize treatment strategies to achieve the best possible outcomes in these difficult-to-treat scenarios (135). BLs are still one of the most frequently used antibiotics for the treatment of foreign-body associated infections by *P. aeruginosa*, although their anti-biofilm activity has been questioned (133). As traditional PK/PD principles are mainly based on the killing against planktonic bacterial cells, understanding the PK/PD parameters associated with the anti-biofilm efficacy is key in optimising the treatment for biofilm-related infections.

Using a biofilm PK/PD model with infection by *P. aeruginosa,* we examined several dosage regimens of CI ceftazidime, which optimized the time-dependent activity of beta-lactams by achieving T>MIC≈100% (173), while also allowed the evaluation of their potential concentration-dependent killing. Our findings may be of particular interest to clinicians, as we investigated clinically achievable concentrations of both ceftazidime and colistin.

Ceftazidime monotherapy at several concentrations in CI was associated with a notable efficacy after 54h of treatment (killing >2 $\log_{10}$cfu/ml). Our results suggest the importance of administering high concentrations of ceftazidime for longer periods of time in order to improve its anti-biofilm activity against *P. aeruginosa*. In our study, the %T>MIC during CI ceftazidime was optimized to maximum in all experiments (%T>MIC=100%) and we observed that higher concentrations of ceftazidime were associated with greater anti-biofilm activity against PAO1. This was not so evident against HUB8, with similar efficacy between ceftazidime regimens.

To our knowledge, no previous *in vitro* studies have evaluated the strategy of CI ceftazidime against *P. aeruginosa* biofilms or have compared its efficacy with that of

intermittent infusion. Our findings are consistent with previous studies by Hengzhuang *et al*. which employed *in vitro* and *in vivo* PK/PD models of biofilm infection by *P. aeruginosa* to show that the time-dependent killing of beta-lactams on planktonic bacteria was complemented with a dose-dependent killing against biofilms (247, 248, 260). Initially, they showed that imipenem exhibited time-dependent activity against biofilm by *P. aeruginosa* PAO1 and its isogenic mucoid variant strain, but higher concentrations for longer treatment periods were needed against biofilms, in comparison with those required for planktonic cells (247). To further validate their initial results, the authors used a neutropenic mouse model of biofilm lung infection by PAO1 *P. aeruginosa* strain and evaluated the effect of different PK/PD indices of imipenem and colistin monotherapies (248). The authors showed a concentration-dependent killing of colistin against biofilms and confirmed the time-dependent killing of imipenem; however, they noted that the AUC/MIC index of imipenem correlated well with anti-biofilm efficacy. Finally, they investigated the PK/PD of ceftazidime and imipenem in three different *in vitro* models of biofilm infection by PAO1 *P. aeruginosa* and its beta-lactamase overproducing mutant (260). In accordance with their previous works, similar results regarding PK/PD for imipenem were observed; however, a concentration-dependent killing of ceftazidime was observed against the beta-lactamase overproducing mutant, which the authors associated to beta-lactamases potential accumulation within biofilms. Overall, it was proposed that the importance of biofilm-related PK/PD parameters, such as the time exceeding the MBIC (%T>MBIC), AUC/MBIC or Cmax/MBIC complementing %T>MIC, can be optimized by the administration of CI beta-lactams for the treatment of biofilm-related infections by *P. aeruginosa*.

According to our results, the efficacy of ceftazidime monotherapies at 54h might have been interfered by the emergence of resistant subpopulations, which were indeed observed in a higher proportion in PAO1 compared to HUB8 and using lower ceftazidime concentrations in both strains. Ceftazidime resistance is extensively related to β-lactamase overproduction (261, 262). Tam *et al*. examined the ability of BL dosages to suppress resistance emergence by using a hollow-fibre PK/PD model of infection by wild-type and clinical drug-resistant isolates of *Klebsiella pneumoniae* and *P. aeruginosa* (harbouring extended spectrum beta-lactamase and AmpC-overexpression, respectively) (263). They evaluated standard clinical doses of ceftazidime, cefepime and

meropenem administered every 8 h, and showed that beta-lactam resistance was prevented by ensuring C_{min}/MIC≥3.8. Although we only examined two *P. aeruginosa* strains, we observed minimal resistance with higher ratios of ceftazidime concentration (in CI) to MBIC, which were achieved only against HUB8 strain using 10, 20 and 40mg/L ceftazidime. Although more studies are needed, these results may have clear implications for ceftazidime dosing in clinical practice, supporting the use of high doses in CI.

In line with the PK/PD activity of ceftazidime monotherapy, we also noted that the greatest bactericidal efficacy against both strains was achieved with the higher concentrations of ceftazidime in CI plus colistin. Confocal microscopy imaging results also support the synergy of the combination of beta-lactams and colistin against *P. aeruginosa* biofilms. Ceftazidime plus colistin combinations were associated with a greater red fluorescence (damaged cells) across all biofilm layers, in contrast to monotherapies, in which a mix of live and damaged bacteria were generally found. Again, the importance of colistin and BL combinations for minimizing the emergence of resistance to colistin is highlighted, which may impact on the efficacy of the combination. In contrast, colistin monotherapy modified the initial heteroresistance profile of both strains, with higher proportion of colonies able to grow at concentrations of ≥2 mg/L at 54 h compared to baseline, a finding that was prevented with both combinations of ceftazidime plus colistin. Previous PK/PD *in vitro* studies, including *P. aeruginosa* biofilms or infections with planktonic bacteria, have also shown the benefits of combination therapy for preventing the emergence of colistin-resistant strains (151, 152, 159).

We hypothesize that higher ceftazidime concentrations may result in greater ceftazidime diffusion through the heterogeneous structure of biofilms, where bacterial subpopulations of different metabolic status and antibiotic tolerance may be present (52). The addition of colistin would make the combination more effective by disrupting the biofilm and facilitating the access of beta-lactams to subpopulations within biofilm layers (156, 158).

CONCLUSIONS

A. Impact and prognosis of bacteraemic osteoarticular infections

Aim 1 – To describe epidemiological and microbiological trends of bacteraemic osteoarticular infections across time.

1.1. Bacteraemic osteoarticular infections have increased across time, in the setting of an ageing population with more chronic diseases. This increase is parallel to a higher incidence of bacteraemia, but also associated with a greater osteoarticular involvement.

1.2. Vertebral osteomyelitis and device-associated infections have presented a major relative increase, as well as nosocomial and healthcare-acquired cases.

1.3. *Staphylococcus aureus* remains the most frequent causative microorganism, but we have observed the emergence of infections by methicillin-resistant isolates and the increase of streptococcal and enterococcal episodes.

Aim 2 – To analyse the characteristics of bacteraemic osteoarticular infections associated with the presence of infective endocarditis.

2.1. Bacteraemic osteoarticular infections affecting the axial skeleton (vertebral osteomyelitis and septic arthritis) seem to be associated with concomitant infective endocarditis.

2.2. Infections by *Staphylococcus aureus* may be associated with septic arthritis of the axial skeleton, whereas those by the group of less virulent microorganisms (*Streptococcus viridans*, *Streptococcus bovis*, enterococci and coagulase-negative staphylococci) with vertebral osteomyelitis.

2.3. The performance of a transoesophageal echocardiography should be accurately considered in these patients.

Aim 3 – To compare the characteristics of bacteraemic septic arthritis, according to the site of acquisition.

3.1. Healthcare-related cases of bacteraemic septic arthritis present with similar clinical characteristics as community-acquired cases, whereas their aetiology overlap with nosocomial-acquired cases.

3.2. The site of acquisition should be considered when planning diagnostic and therapeutic approaches in patients with bacteraemic septic arthritis.

Aim 4 – To analyse the mortality and associated risk factors in patients with bacteraemic osteoarticular infections.

4.1. Mortality rates associated with bacteraemic osteoarticular infections are significant, especially in the elderly, nosocomial and healthcare-related cases, those with rheumatoid arthritis or liver cirrhosis, and cases caused by *Staphylococcus aureus*.

4.2. Peripheral septic arthritis is associated with higher mortality compared to other types of bacteraemic osteoarticular infections.

4.3. Surgical debridement in peripheral septic arthritis is associated with lower mortality, which should encourage clinicians to definitely incorporate this procedure in its early global management.

B. Antimicrobial treatment of osteoarticular infections by *Pseudomonas aeruginosa*

Aim 5 – To analyse the efficacy and therapeutic drug monitoring of continuous beta-lactam infusion for osteoarticular infections by fluoroquinolone-resistant *Pseudomonas aeruginosa.*

5.1. The use of continuous beta-lactam infusion, properly guided by therapeutic drug monitoring, is a safe and a feasible therapeutic option, ensuring desirable target concentrations and avoiding toxicity.

5.2. The optimized use of continuous beta-lactam infusion, with or without colistin, may be a promising therapeutic strategy for the management of

osteoarticular infections by fluoroquinolone-resistant *Pseudomonas aeruginosa*.

Aim 6 – To evaluate the activity of ceftolozane-tazobactam, with and without colistin, against a biofilm infection by multidrug-resistant *Pseudomonas aeruginosa* in a dynamic *in vitro* model.

6.1. Ceftolozane-tazobactam in monotherapy shows low efficacy against a biofilm infection by multidrug-resistant *Pseudomonas aeruginosa* in a dynamic *in vitro* model, but its combination with colistin seems to be a good alternative for the treatment of ceftolozane-tazobactam susceptible, meropenem-resistant strains.

6.2. Meropenem, with or without colistin, shows higher efficacy than ceftolozane-tazobactam in this model against strains susceptible to both antibiotics.

6.3. The use of beta-lactams combined with colistin may have high efficacy and prevent the emergence of resistance against foreign-body infections by MDR/XDR *Pseudomonas aeruginosa*.

Aim 7 – To evaluate the pharmacokinetics and pharmacodynamics of ceftazidime in continuous infusion, with or without colistin, against a biofilm infection by *Pseudomonas aeruginosa* in a dynamic *in vitro* model.

7.1 Higher concentrations of ceftazidime in combination with colistin provide higher anti-biofilm activity and may be considered to protect the emergence of resistance in foreign-body infections caused by susceptible *Pseudomonas aeruginosa*.

7.2 Beta-lactams may have a concentration-dependent activity against biofilms by susceptible *Pseudomonas aeruginosa*. This phenomenon may have relevance and should be taken into account in clinical practice, by the use of high beta-lactam doses in extended infusions.

REFERENCES

1. Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. N Engl J Med. 2009;361(8):787-94.

2. Singh JA, Yu S. Septic Arthritis in Emergency Departments in the US: A National Study of Health Care Utilization and Time Trends. Arthritis Care Res (Hoboken). 2018;70(2):320-6.

3. Ariza J, Euba G, Murillo O. [Orthopedic device-related infections]. Enferm Infecc Microbiol Clin. 2008;26(6):380-90.

4. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. J Arthroplasty. 2012;27(8 Suppl):61-5.e1.

5. Peel TN, Dowsey MM, Daffy JR, Stanley PA, Choong PF, Buising KL. Risk factors for prosthetic hip and knee infections according to arthroplasty site. J Hosp Infect. 2011;79(2):129-33.

6. Pulido L, Ghanem E, Joshi A, Purtill JJ, Parvizi J. Periprosthetic joint infection: the incidence, timing, and predisposing factors. Clin Orthop Relat Res. 2008;466(7):1710-5.

7. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351(16):1645-54.

8. Kurtz SM, Ong KL, Lau E, Bozic KJ, Berry D, Parvizi J. Prosthetic joint infection risk after TKA in the Medicare population. Clin Orthop Relat Res. 2010;468(1):52-6.

9. Shirtliff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev. 2002;15(4):527-44.

10. Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS, et al. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis. 1998;27(5):1247-54.

11. Dowsey MM, Choong PF. Obese diabetic patients are at substantial risk for deep infection after primary TKA. Clin Orthop Relat Res. 2009;467(6):1577-81.

12. Tande AJ, Patel R. Prosthetic joint infection. Clin Microbiol Rev. 2014;27(2):302-45.

13. Murdoch DR, Roberts SA, Fowler VG, Jr., Shah MA, Taylor SL, Morris AJ, et al. Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. Clin Infect Dis. 2001;32(4):647-9.

14. Sendi P, Banderet F, Graber P, Zimmerli W. Periprosthetic joint infection following Staphylococcus aureus bacteremia. J Infect. 2011;63(1):17-22.

15. Donaldson AD, Jalaludin BB, Chan RC. Patient perceptions of osteomyelitis, septic arthritis and prosthetic joint infection: the psychological influence of methicillin-resistant *Staphylococcus aureus*. Intern Med J. 2007;37(8):536-42.

16. Peel TN, Dowsey MM, Buising KL, Liew D, Choong PF. Cost analysis of debridement and retention for management of prosthetic joint infection. Clin Microbiol Infect. 2013;19(2):181-6.

17. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med. 2002;137(10):791-7.

18. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988;16(3):128-40.

19. Chong BSW, Brereton CJ, Gordon A, Davis JS. Epidemiology, Microbiological Diagnosis, and Clinical Outcomes in Pyogenic Vertebral Osteomyelitis: A 10-year Retrospective Cohort Study. Open Forum Infect Dis. 2018;5(3):ofy037.

20. Lora-Tamayo J, Euba G, Narvaez JA, Murillo O, Verdaguer R, Sobrino B, et al. Changing trends in the epidemiology of pyogenic vertebral osteomyelitis: the impact of cases with no microbiologic diagnosis. Semin Arthritis Rheum. 2011;41(2):247-55.

21. Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. Lancet. 2010;375(9717):846-55.

22. Nolla JM, Ariza J, Gomez-Vaquero C, Fiter J, Bermejo J, Valverde J, et al. Spontaneous pyogenic vertebral osteomyelitis in nondrug users. Semin Arthritis Rheum. 2002;31(4):271-8.

23. Nolla JM, Lora-Tamayo J, Gomez Vaquero C, Narvaez J, Murillo O, Pedrero S, et al. Pyogenic arthritis of native joints in non-intravenous drug users: A detailed analysis of 268 cases attended in a tertiary hospital over a 22-year period. Semin Arthritis Rheum. 2015;45(1):94-102.

24. Pigrau C, Almirante B, Flores X, Falco V, Rodriguez D, Gasser I, et al. Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med. 2005;118(11):1287.

25. Burns TC, Stinner DJ, Mack AW, Potter BK, Beer R, Eckel TT, et al. Microbiology and injury characteristics in severe open tibia fractures from combat. J Trauma Acute Care Surg. 2012;72(4):1062-7.

26. Benito N, Franco M, Ribera A, Soriano A, Rodriguez-Pardo D, Sorli L, et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect. 2016;22(8):732.e1-8.

27. Benito N, Mur I, Ribera A, Soriano A, Rodríguez-Pardo D, Sorlí L, et al. The Different Microbial Etiology of Prosthetic Joint Infections according to Route of Acquisition and Time after Prosthesis Implantation, Including the Role of Multidrug-Resistant Organisms. J Clin Med. 2019;8(5).

28. Zappe B, Graf S, Ochsner PE, Zimmerli W, Sendi P. *Propionibacterium* spp. in prosthetic joint infections: a diagnostic challenge. Arch Orthop Trauma Surg. 2008;128(10):1039-46.

29. Zeller V, Ghorbani A, Strady C, Leonard P, Mamoudy P, Desplaces N. *Propionibacterium acnes*: an agent of prosthetic joint infection and colonization. J Infect. 2007;55(2):119-24.

30. Lora-Tamayo J, Euba G, Ribera A, Murillo O, Pedrero S, Garcia-Somoza D, et al. Infected hip hemiarthroplasties and total hip arthroplasties: Differential findings and prognosis. J Infect. 2013;67(6):536-44.

31. Trampuz A, Zimmerli W. Diagnosis and treatment of implant-associated septic arthritis and osteomyelitis. Curr Infect Dis Rep. 2008;10(5):394-403.

32. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318-22.

33. Pamp SJ, Tolker-Nielsen T. Multiple roles of biosurfactants in structural biofilm development by Pseudomonas aeruginosa. J Bacteriol. 2007;189(6):2531-9.

34. Pasmore M, Costerton JW. Biofilms, bacterial signaling, and their ties to marine biology. J Ind Microbiol Biotechnol. 2003;30(7):407-13.

35. Kragh KN, Hutchison JB, Melaugh G, Rodesney C, Roberts AE, Irie Y, et al. Role of Multicellular Aggregates in Biofilm Formation. MBio. 2016;7(2):e00237.

36. Melaugh G, Hutchison J, Kragh KN, Irie Y, Roberts A, Bjarnsholt T, et al. Shaping the Growth Behaviour of Biofilms Initiated from Bacterial Aggregates. PLoS One. 2016;11(3):e0149683.

37. Vasudevan R. Biofilms: microbial cities of scientific significance. J Microbiol Experiment. 2014;1(3):84-98.

38. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14(9):576-88.

39. Bassler BL, Losick R. Bacterially speaking. Cell. 2006;125(2):237-46.

40. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001;358(9276):135-8.

41. Boudarel H, Mathias JD, Blaysat B, Grédiac M. Towards standardized mechanical characterization of microbial biofilms: analysis and critical review. NPJ Biofilms Microbiomes. 2018;4:17.

42. Stewart PS, Zhang T, Xu R, Pitts B, Walters MC, Roe F, et al. Reaction-diffusion theory explains hypoxia and heterogeneous growth within microbial biofilms associated with chronic infections. NPJ Biofilms Microbiomes. 2016;2:16012.

43. Souli M, Giamarellou H. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. Antimicrob Agents Chemother. 1998;42(4):939-41.

44. Bagge N, Ciofu O, Hentzer M, Campbell JI, Givskov M, Høiby N. Constitutive high expression of chromosomal beta-lactamase in *Pseudomonas aeruginosa* caused by a new insertion sequence (IS1669) located in ampD. Antimicrob Agents Chemother. 2002;46(11):3406-11.

45. Bagge N, Hentzer M, Andersen JB, Ciofu O, Givskov M, Høiby N. Dynamics and spatial distribution of beta-lactamase expression in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2004;48(4):1168-74.

46. Bagge N, Schuster M, Hentzer M, Ciofu O, Givskov M, Greenberg EP, et al. *Pseudomonas aeruginosa* biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. Antimicrob Agents Chemother. 2004;48(4):1175-87.

47. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998;26(1):1-10; quiz 1-2.

48. Lewis K. Persister cells. Annu Rev Microbiol. 2010;64:357-72.

49. Moser C, Pedersen HT, Lerche CJ, Kolpen M, Line L, Thomsen K, et al. Biofilms and host response - helpful or harmful. Apmis. 2017;125(4):320-38.

50. Driffield K, Miller K, Bostock JM, O'Neill AJ, Chopra I. Increased mutability of *Pseudomonas aeruginosa* in biofilms. J Antimicrob Chemother. 2008;61(5):1053-6.

51. García-Castillo M, del Campo R, Baquero F, Morosini MI, Turrientes MC, Zamora J, et al. Stationary biofilm growth normalizes mutation frequencies and mutant prevention concentrations in *Pseudomonas aeruginosa* from cystic fibrosis patients. Clin Microbiol Infect. 2011;17(5):704-11.

52. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents. 2010;35(4):322-32.

53. Maurin M, Raoult D. Intracellular organisms. Int J Antimicrob Agents. 1997;9(1):61-70.

54. Murillo O, Doménech A, Garcia A, Tubau F, Cabellos C, Gudiol F, et al. Efficacy of high doses of levofloxacin in experimental foreign-body infection by methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother. 2006;50(12):4011-7.

55. Hanke ML, Kielian T. Deciphering mechanisms of staphylococcal biofilm evasion of host immunity. Front Cell Infect Microbiol. 2012;2:62.

56. Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, et al. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation *in vivo*. J Immunol. 2011;186(11):6585-96.

57. Uribe-Querol E, Rosales C. Control of Phagocytosis by Microbial Pathogens. Front Immunol. 2017;8:1368.

58. Wright JA, Nair SP. Interaction of staphylococci with bone. International journal of medical microbiology : IJMM. 2010;300(2-3):193-204.

59. Kavanagh N, Ryan EJ, Widaa A, Sexton G, Fennell J, O'Rourke S, et al. Staphylococcal Osteomyelitis: Disease Progression, Treatment Challenges, and Future Directions. Clin Microbiol Rev. 2018;31(2).

60. Lister JL, Horswill AR. *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. Front Cell Infect Microbiol. 2014;4:178.

61. Kavanaugh JS, Horswill AR. Impact of Environmental Cues on Staphylococcal Quorum Sensing and Biofilm Development. J Biol Chem. 2016;291(24):12556-64.

62. Thoendel M, Kavanaugh JS, Flack CE, Horswill AR. Peptide signaling in the staphylococci. Chem Rev. 2011;111(1):117-51.

63. Kahl BC, Becker K, Löffler B. Clinical Significance and Pathogenesis of Staphylococcal Small Colony Variants in Persistent Infections. Clin Microbiol Rev. 2016;29(2):401-27.

64. Sendi P, Rohrbach M, Graber P, Frei R, Ochsner PE, Zimmerli W. *Staphylococcus aureus* small colony variants in prosthetic joint infection. Clin Infect Dis. 2006;43(8):961-7.

65. Sendi P, Proctor RA. *Staphylococcus aureus* as an intracellular pathogen: the role of small colony variants. Trends Microbiol. 2009;17(2):54-8.

66. Hoiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. Future Microbiol. 2010;5(11):1663-74.

67. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. Science. 2000;288(5469):1251-4.

68. Zimmerli W. Clinical practice. Vertebral osteomyelitis. N Engl J Med. 2010;362(11):1022-9.

69. Mylona E, Samarkos M, Kakalou E, Fanourgiakis P, Skoutelis A. Pyogenic vertebral osteomyelitis: a systematic review of clinical characteristics. Semin Arthritis Rheum. 2009;39(1):10-7.

70. Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004;364(9431):369-79.

71. Uçkay I, Jugun K, Gamulin A, Wagener J, Hoffmeyer P, Lew D. Chronic osteomyelitis. Curr Infect Dis Rep. 2012;14(5):566-75.

72. Tsukayama DT, Estrada R, Gustilo RB. Infection after total hip arthroplasty. A study of the treatment of one hundred and six infections. J Bone Joint Surg Am. 1996;78(4):512-23.

73. Zimmerli W. Clinical presentation and treatment of orthopaedic implant-associated infection. J Intern Med. 2014;276(2):111-9.

74. Amanatullah D, Dennis D, Oltra EG, Marcelino Gomes LS, Goodman SB, Hamlin B, et al. Hip and Knee Section, Diagnosis, Definitions: Proceedings of International Consensus on Orthopedic Infections. J Arthroplasty. 2019;34(2s):S329-s37.

75. Tande AJ, Palraj BR, Osmon DR, Berbari EF, Baddour LM, Lohse CM, et al. Clinical Presentation, Risk Factors, and Outcomes of Hematogenous Prosthetic Joint Infection in Patients with *Staphylococcus aureus* Bacteremia. Am J Med. 2016;129(2):221.e11-20.

76. Della Valle CJ, Sporer SM, Jacobs JJ, Berger RA, Rosenberg AG, Paprosky WG. Preoperative testing for sepsis before revision total knee arthroplasty. J Arthroplasty. 2007;22(6 Suppl 2):90-3.

77. Spangehl MJ, Masri BA, O'Connell JX, Duncan CP. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of two hundred and two revision total hip arthroplasties. J Bone Joint Surg Am. 1999;81(5):672-83.

78. Khan MH, Smith PN, Rao N, Donaldson WF. Serum C-reactive protein levels correlate with clinical response in patients treated with antibiotics for wound infections after spinal surgery. Spine J. 2006;6(3):311-5.

79. Tigges S, Stiles RG, Roberson JR. Complications of hip arthroplasty causing periprosthetic radiolucency on plain radiographs. AJR Am J Roentgenol. 1994;162(6):1387-91.

80. Palestro CJ, Love C, Miller TT. Infection and musculoskeletal conditions: Imaging of musculoskeletal infections. Best Pract Res Clin Rheumatol. 2006;20(6):1197-218.

81. Euba G, Narváez JA, Nolla JM, Murillo O, Narváez J, Gómez-Vaquero C, et al. Long-term clinical and radiological magnetic resonance imaging outcome of abscess-associated spontaneous pyogenic vertebral osteomyelitis under conservative management. Semin Arthritis Rheum. 2008;38(1):28-40.

82. Hain SF, O'Doherty MJ, Smith MA. Functional imaging and the orthopaedic surgeon. J Bone Joint Surg Br. 2002;84(3):315-21.

83. Smith SL, Wastie ML, Forster I. Radionuclide bone scintigraphy in the detection of significant complications after total knee joint replacement. Clin Radiol. 2001;56(3):221-4.

84. De Winter F, Vogelaers D, Gemmel F, Dierckx RA. Promising role of 18-F-fluoro-D-deoxyglucose positron emission tomography in clinical infectious diseases. Eur J Clin Microbiol Infect Dis. 2002;21(4):247-57.

85. Kälicke T, Schmitz A, Risse JH, Arens S, Keller E, Hansis M, et al. Fluorine-18 fluorodeoxyglucose PET in infectious bone diseases: results of histologically confirmed cases. Eur J Nucl Med. 2000;27(5):524-8.

86. Trampuz A, Hanssen AD, Osmon DR, Mandrekar J, Steckelberg JM, Patel R. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. Am J Med. 2004;117(8):556-62.

87. Freed JF, Nies KM, Boyer RS, Louie JS. Acute monoarticular arthritis. A diagnostic approach. Jama. 1980;243(22):2314-6.

88. Weston VC, Jones AC, Bradbury N, Fawthrop F, Doherty M. Clinical features and outcome of septic arthritis in a single UK Health District 1982-1991. Ann Rheum Dis. 1999;58(4):214-9.

89. Atkins BL, Athanasou N, Deeks JJ, Crook DW, Simpson H, Peto TE, et al. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol. 1998;36(10):2932-9.

90. Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med. 2007;357(7):654-63.

91. Schäfer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis. 2008;47(11):1403-9.

92. Athanasou NA, Pandey R, de Steiger R, McLardy Smith P. The role of intraoperative frozen sections in revision total joint arthroplasty. J Bone Joint Surg Am. 1997;79(9):1433-4.

93. Byren I, Bejon P, Atkins BL, Angus B, Masters S, McLardy-Smith P, et al. One hundred and twelve infected arthroplasties treated with 'DAIR' (debridement, antibiotics and implant retention): antibiotic duration and outcome. J Antimicrob Chemother. 2009;63(6):1264-71.

94. Vilchez F, Martinez-Pastor JC, Garcia-Ramiro S, Bori G, Macule F, Sierra J, et al. Outcome and predictors of treatment failure in early post-surgical prosthetic joint infections due to *Staphylococcus aureus* treated with debridement. Clin Microbiol Infect. 2011;17(3):439-44.

95. Lora-Tamayo J, Murillo O, Iribarren JA, Soriano A, Sanchez-Somolinos M, Baraia-Etxaburu JM, et al. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. Clin Infect Dis. 2013;56(2):182-94.

96. Cobo J, Del Pozo JL. Prosthetic joint infection: diagnosis and management. Expert Rev Anti Infect Ther. 2011;9(9):787-802.

97. El Helou OC, Berbari EF, Lahr BD, Eckel-Passow JE, Razonable RR, Sia IG, et al. Efficacy and safety of rifampin containing regimen for staphylococcal prosthetic joint infections treated with debridement and retention. Eur J Clin Microbiol Infect Dis. 2010;29(8):961-7.

98. Fisman DN, Reilly DT, Karchmer AW, Goldie SJ. Clinical effectiveness and cost-effectiveness of 2 management strategies for infected total hip arthroplasty in the elderly. Clin Infect Dis. 2001;32(3):419-30.

99. Sierra RJ, Trousdale RT, Pagnano MW. Above-the-knee amputation after a total knee replacement: prevalence, etiology, and functional outcome. J Bone Joint Surg Am. 2003;85(6):1000-4.

100. Biring GS, Kostamo T, Garbuz DS, Masri BA, Duncan CP. Two-stage revision arthroplasty of the hip for infection using an interim articulated Prostalac hip spacer: a 10- to 15-year follow-up study. J Bone Joint Surg Br. 2009;91(11):1431-7.

101. Hart WJ, Jones RS. Two-stage revision of infected total knee replacements using articulating cement spacers and short-term antibiotic therapy. J Bone Joint Surg Br. 2006;88(8):1011-5.

102. Lange J, Troelsen A, Søballe K. Chronic Periprosthetic Hip Joint Infection. A Retrospective, Observational Study on the Treatment Strategy and Prognosis in 130 Non-Selected Patients. PLoS One. 2016;11(9):e0163457.

103. Cabo J, Euba G, Saborido A, González-Panisello M, Domínguez MA, Agulló JL, et al. Clinical outcome and microbiological findings using antibiotic-loaded spacers in two-stage revision of prosthetic joint infections. J Infect. 2011;63(1):23-31.

104. Della Valle CJ, Scher DM, Kim YH, Oxley CM, Desai P, Zuckerman JD, et al. The role of intraoperative Gram stain in revision total joint arthroplasty. J Arthroplasty. 1999;14(4):500-4.

105. Murillo O, Euba G, Calatayud L, Domínguez MA, Verdaguer R, Pérez A, et al. The role of intraoperative cultures at the time of reimplantation in the management of infected total joint arthroplasty. Eur J Clin Microbiol Infect Dis. 2008;27(9):805-11.

106. Leone S, Borrè S, Monforte A, Mordente G, Petrosillo N, Signore A, et al. Consensus document on controversial issues in the diagnosis and treatment of prosthetic joint infections. Int J Infect Dis. 2010;14 Suppl 4:S67-77.

107. Whiteside LA, Peppers M, Nayfeh TA, Roy ME. Methicillin-resistant *Staphylococcus aureus* in TKA treated with revision and direct intra-articular antibiotic infusion. Clin Orthop Relat Res. 2011;469(1):26-33.

108. Castellanos J, Flores X, Llusà M, Chiriboga C, Navarro A. The Girdlestone pseudarthrosis in the treatment of infected hip replacements. Int Orthop. 1998;22(3):178-81.

109. Johns BP, Loewenthal MR, Dewar DC. Open Compared with Arthroscopic Treatment of Acute Septic Arthritis of the Native Knee. J Bone Joint Surg Am. 2017;99(6):499-505.

110. Kerbel YE, Lieber AM, Kirchner GJ, Stump NN, Prodromo JP, Petrucelli PM, et al. In-Hospital Complications following Arthrotomy versus Arthroscopy for Septic Knee Arthritis: A Cohort-Matched Comparison. J Knee Surg. 2019.

111. Peres LR, Marchitto RO, Pereira GS, Yoshino FS, de Castro Fernandes M, Matsumoto MH. Arthrotomy versus arthroscopy in the treatment of septic arthritis of the knee in adults: a randomized clinical trial. Knee Surg Sports Traumatol Arthrosc. 2016;24(10):3155-62.

112. Macià MD, Rojo-Molinero E, Oliver A. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin Microbiol Infect. 2014;20(10):981-90.

113. Coenye T, Goeres D, Van Bambeke F, Bjarnsholt T. Should standardized susceptibility testing for microbial biofilms be introduced in clinical practice? Clin Microbiol Infect. 2018;24(6):570-2.

114. Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis. 2013;56(1):e1-e25.

115. Zimmerli W, Sendi P. Role of Rifampin against Staphylococcal Biofilm Infections In Vitro, in Animal Models, and in Orthopedic-Device-Related Infections. Antimicrob Agents Chemother. 2019;63(2).

116. Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. Jama. 1998;279(19):1537-41.

117. Landersdorfer CB, Bulitta JB, Kinzig M, Holzgrabe U, Sorgel F. Penetration of antibacterials into bone: pharmacokinetic, pharmacodynamic and bioanalytical considerations. Clin Pharmacokinet. 2009;48(2):89-124.

118. Zeller V, Durand F, Kitzis MD, Lhotellier L, Ziza JM, Mamoudy P, et al. Continuous cefazolin infusion to treat bone and joint infections: clinical efficacy, feasibility, safety, and serum and bone concentrations. Antimicrob Agents Chemother. 2009;53(3):883-7.

119. Roblot F, Besnier JM, Juhel L, Vidal C, Ragot S, Bastides F, et al. Optimal duration of antibiotic therapy in vertebral osteomyelitis. Semin Arthritis Rheum. 2007;36(5):269-77.

120. Bernard L, Dinh A, Ghout I, Simo D, Zeller V, Issartel B, et al. Antibiotic treatment for 6 weeks versus 12 weeks in patients with pyogenic vertebral osteomyelitis: an open-label, non-inferiority, randomised, controlled trial. Lancet. 2015;385(9971):875-82.

121. Bernard L, Legout L, Zürcher-Pfund L, Stern R, Rohner P, Peter R, et al. Six weeks of antibiotic treatment is sufficient following surgery for septic arthroplasty. J Infect. 2010;61(2):125-32.

122. Cobo J, Miguel LG, Euba G, Rodríguez D, García-Lechuz JM, Riera M, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. Clin Microbiol Infect. 2011;17(11):1632-7.

123. Lora-Tamayo J, Euba G, Cobo J, Horcajada JP, Soriano A, Sandoval E, et al. Short- versus long-duration levofloxacin plus rifampicin for acute staphylococcal prosthetic joint infection managed with implant retention: a randomised clinical trial. Int J Antimicrob Agents. 2016;48(3):310-6.

124. Li HK, Agweyu A, English M, Bejon P. An unsupported preference for intravenous antibiotics. PLoS Med. 2015;12(5):e1001825.

125. Li HK, Rombach I, Zambellas R, Walker AS, McNally MA, Atkins BL, et al. Oral versus Intravenous Antibiotics for Bone and Joint Infection. N Engl J Med. 2019;380(5):425-36.

126. Vergidis P, Rouse MS, Euba G, Karau MJ, Schmidt SM, Mandrekar JN, et al. Treatment with linezolid or vancomycin in combination with rifampin is effective in an animal model of methicillin-resistant *Staphylococcus aureus* foreign body osteomyelitis. Antimicrob Agents Chemother. 2011;55(3):1182-6.

127. Saleh-Mghir A, Muller-Serieys C, Dinh A, Massias L, Crémieux AC. Adjunctive rifampin is crucial to optimizing daptomycin efficacy against rabbit prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2011;55(10):4589-93.

128. Tanaka G, Shigeta M, Komatsuzawa H, Sugai M, Suginaka H, Usui T. Effect of the growth rate of *Pseudomonas aeruginosa* biofilms on the susceptibility to antimicrobial agents: beta-lactams and fluoroquinolones. Chemotherapy. 1999;45(1):28-36.

129. Aboltins CA, Dowsey MM, Buising KL, Peel TN, Daffy JR, Choong PF, et al. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. Clin Microbiol Infect. 2011;17(6):862-7.

130. Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis. 2009;49(7):1036-43.

131. Martinez-Pastor JC, Munoz-Mahamud E, Vilchez F, Garcia-Ramiro S, Bori G, Sierra J, et al. Outcome of acute prosthetic joint infections due to gram-negative bacilli treated with open debridement and retention of the prosthesis. Antimicrob Agents Chemother. 2009;53(11):4772-7.

132. Rodriguez-Pardo D, Pigrau C, Lora-Tamayo J, Soriano A, del Toro MD, Cobo J, et al. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect. 2014;20(11):O911-9.

133. Gilbert P, Brown MR. Biofilms and beta-lactam activity. J Antimicrob Chemother. 1998;41(5):571-2.

134. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

135. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(1):1-12.

136. Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis. 2006;6(9):589-601.

137. Li J, Milne RW, Nation RL, Turnidge JD, Smeaton TC, Coulthard K. Pharmacokinetics of colistin methanesulphonate and colistin in rats following an intravenous dose of colistin methanesulphonate. J Antimicrob Chemother. 2004;53(5):837-40.

138. Nation RL, Garonzik SM, Thamlikitkul V, Giamarellos-Bourboulis EJ, Forrest A, Paterson DL, et al. Dosing guidance for intravenous colistin in critically-ill patients. Clin Infect Dis. 2017;64(5):565-71.

139. Plachouras D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother. 2009;53(8):3430-6.

140. Cheah SE, Wang J, Nguyen VT, Turnidge JD, Li J, Nation RL. New pharmacokinetic/pharmacodynamic studies of systemically administered colistin against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in mouse thigh and lung infection models: smaller response in lung infection. J Antimicrob Chemother. 2015;70(12):3291-7.

141. Tsuji BT, Pogue JM, Zavascki AP, Paul M, Daikos GL, Forrest A, et al. International Consensus Guidelines for the Optimal Use of the Polymyxins: Endorsed by the American College of Clinical Pharmacy (ACCP), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Infectious Diseases Society of America (IDSA), International Society for Anti-infective Pharmacology (ISAP), Society of Critical Care Medicine (SCCM), and Society of Infectious Diseases Pharmacists (SIDP). Pharmacotherapy. 2019;39(1):10-39.

142. Bergen PJ, Bulitta JB, Forrest A, Tsuji BT, Li J, Nation RL. Pharmacokinetic/pharmacodynamic investigation of colistin against *Pseudomonas aeruginosa* using an *in vitro* model. Antimicrob Agents Chemother. 2010;54(9):3783-9.

143. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2006;50(9):2946-50.

144. Poudyal A, Howden BP, Bell JM, Gao W, Owen RJ, Turnidge JD, et al. *In vitro* pharmacodynamics of colistin against multidrug-resistant *Klebsiella pneumoniae*. J Antimicrob Chemother. 2008;62(6):1311-8.

145. Horcajada JP, Sorlí L, Luque S, Benito N, Segura C, Campillo N, et al. Validation of a colistin plasma concentration breakpoint as a predictor of nephrotoxicity in patients treated with colistin methanesulfonate. Int J Antimicrob Agents. 2016;48(6):725-7.

146. Sorlí L, Luque S, Grau S, Berenguer N, Segura C, Montero MM, et al. Trough colistin plasma level is an independent risk factor for nephrotoxicity: a prospective observational cohort study. BMC Infect Dis. 2013;13:380.

147. Azad MA, Finnin BA, Poudyal A, Davis K, Li J, Hill PA, et al. Polymyxin B Induces Apoptosis in Kidney Proximal Tubular Cells. Antimicrob Agents Chemother. 2013;57(9):4329-35.

148. Nation RL, Li J. Colistin in the 21st century. Curr Opin Infect Dis. 2009;22(6):535-43.

149. Tran TB, Velkov T, Nation RL, Forrest A, Tsuji BT, Bergen PJ, et al. Pharmacokinetics/pharmacodynamics of colistin and polymyxin B: are we there yet? Int J Antimicrob Agents. 2016;48(6):592-7.

150. Hancock RE, Wong PG. Compounds which increase the permeability of the *Pseudomonas aeruginosa* outer membrane. Antimicrob Agents Chemother. 1984;26(1):48-52.

151. Bergen PJ, Forrest A, Bulitta JB, Tsuji BT, Sidjabat HE, Paterson DL, et al. Clinically relevant plasma concentrations of colistin in combination with imipenem enhance pharmacodynamic activity against multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula. Antimicrob Agents Chemother. 2011;55(11):5134-42.

152. Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, et al. Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an *in vitro* pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother. 2011;55(12):5685-95.

153. Phee LM, Kloprogge F, Morris R, Barrett J, Wareham DW, Standing JF. Pharmacokinetic-pharmacodynamic modelling to investigate *in vitro* synergy between colistin and fusidic acid against MDR *Acinetobacter baumannii*. J Antimicrob Chemother. 2019;74(4):961-9.

154. Khawcharoenporn T, Pruetpongpun N, Tiamsak P, Rutchanawech S, Mundy LM, Apisarnthanarak A. Colistin-based treatment for extensively drug-resistant *Acinetobacter baumannii* pneumonia. Int J Antimicrob Agents. 2014;43(4):378-82.

155. Zusman O, Altunin S, Koppel F, Dishon Benattar Y, Gedik H, Paul M. Polymyxin monotherapy or in combination against carbapenem-resistant bacteria: systematic review and meta-analysis. J Antimicrob Chemother. 2017;72(1):29-39.

156. Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol. 2008;68(1):223-40.

157. Brochmann RP, Toft A, Ciofu O, Briales A, Kolpen M, Hempel C, et al. Bactericidal effect of colistin on planktonic *Pseudomonas aeruginosa* is independent of hydroxyl radical formation. Int J Antimicrob Agents. 2014;43(2):140-7.

158. Kolpen M, Appeldorff CF, Brandt S, Mousavi N, Kragh KN, Aydogan S, et al. Increased bactericidal activity of colistin on *Pseudomonas aeruginosa* biofilms in anaerobic conditions. Pathog Dis. 2016;74(1):ftv086.

159. Lora-Tamayo J, Murillo O, Bergen PJ, Nation RL, Poudyal A, Luo X, et al. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant *Pseudomonas aeruginosa* in an *in vitro* dynamic biofilm model. J Antimicrob Chemother. 2014;69(9):2434-42.

160. Ribera A, Benavent E, Lora-Tamayo J, Tubau F, Pedrero S, Cabo X, et al. Osteoarticular infection caused by MDR *Pseudomonas aeruginosa*: the benefits of combination therapy with colistin plus beta-lactams. J Antimicrob Chemother. 2015;70(12):3357-65.

161. van Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation beta-Lactam/beta-Lactamase Inhibitor Combinations. Clin Infect Dis. 2016;63(2):234-41.

162. Goodlet KJ, Nicolau DP, Nailor MD. In Vitro Comparison of Ceftolozane-Tazobactam to Traditional Beta-Lactams and Ceftolozane-Tazobactam as an Alternative to Combination Antimicrobial Therapy for *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2017;61(12).

163. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant *Pseudomonas aeruginosa* Infections. Clin Microbiol Rev. 2019;32(4).

164. Solomkin J, Hershberger E, Miller B, Popejoy M, Friedland I, Steenbergen J, et al. Ceftolozane/Tazobactam Plus Metronidazole for Complicated Intra-abdominal Infections in an Era of Multidrug Resistance: Results From a Randomized, Double-Blind, Phase 3 Trial (ASPECT-cIAI). Clin Infect Dis. 2015;60(10):1462-71.

165. Wagenlehner FM, Umeh O, Steenbergen J, Yuan G, Darouiche RO. Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract

infections, including pyelonephritis: a randomised, double-blind, phase 3 trial (ASPECT-cUTI). Lancet. 2015;385(9981):1949-56.

166. Haidar G, Philips NJ, Shields RK, Snyder D, Cheng S, Potoski BA, et al. Ceftolozane-Tazobactam for the Treatment of Multidrug-Resistant *Pseudomonas aeruginosa* Infections: Clinical Effectiveness and Evolution of Resistance. Clin Infect Dis. 2017;65(1):110-20.

167. Munita JM, Aitken SL, Miller WR, Perez F, Rosa R, Shimose LA, et al. Multicenter Evaluation of Ceftolozane/Tazobactam for Serious Infections Caused by Carbapenem-Resistant *Pseudomonas aeruginosa*. Clin Infect Dis. 2017;65(1):158-61.

168. Dietl B, Sanchez I, Arcenillas P, Cuchi E, Gomez L, Gonzalez de Molina FJ, et al. Ceftolozane/tazobactam in the treatment of osteomyelitis and skin and soft-tissue infections due to extensively drug-resistant *Pseudomonas aeruginosa*: clinical and microbiological outcomes. Int J Antimicrob Agents. 2018;51(3):498-502.

169. McKinnon PS, Paladino JA, Schentag JJ. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents. 2008;31(4):345-51.

170. Van Herendael B, Jeurissen A, Tulkens PM, Vlieghe E, Verbrugghe W, Jorens PG, et al. Continuous infusion of antibiotics in the critically ill: The new holy grail for beta-lactams and vancomycin? Ann Intensive Care. 2012;2(1):22.

171. Alou L, Aguilar L, Sevillano D, Gimenez MJ, Echeverria O, Gomez-Lus ML, et al. Is there a pharmacodynamic need for the use of continuous versus intermittent infusion with ceftazidime against *Pseudomonas aeruginosa*? An *in vitro* pharmacodynamic model. J Antimicrob Chemother. 2005;55(2):209-13.

172. Cappelletty DM, Kang SL, Palmer SM, Rybak MJ. Pharmacodynamics of ceftazidime administered as continuous infusion or intermittent bolus alone and in combination with single daily-dose amikacin against *Pseudomonas aeruginosa* in an *in vitro* infection model. Antimicrob Agents Chemother. 1995;39(8):1797-801.

173. Mouton JW, Vinks AA. Continuous infusion of beta-lactams. Curr Opin Crit Care. 2007;13(5):598-606.

174. Dulhunty JM, Roberts JA, Davis JS, Webb SA, Bellomo R, Gomersall C, et al. Continuous infusion of beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. Clin Infect Dis. 2013;56(2):236-44.

175. Roberts JA, Abdul-Aziz MH, Davis JS, Dulhunty JM, Cotta MO, Myburgh J, et al. Continuous versus Intermittent beta-Lactam Infusion in Severe Sepsis. A Meta-analysis of Individual Patient Data from Randomized Trials. Am J Respir Crit Care Med. 2016;194(6):681-91.

176. Vardakas KZ, Voulgaris GL, Maliaros A, Samonis G, Falagas ME. Prolonged versus short-term intravenous infusion of antipseudomonal beta- lactams for patients with sepsis: a systematic review and meta-analysis of randomised trials. Lancet Infect Dis. 2018;18(1):108-20.

177. Rigo-Bonnin R, Cobo-Sacristan S, Padulles A, Ribera A, Arbiol-Roca A, Murillo O, et al. Measurement of ceftazidime concentration in human plasma by ultra-performance liquid chromatography-tandem mass spectrometry. Application to critically ill patients and patients with osteoarticular infections. Biomed Chromatogr. 2016;30(3):410-8.

178. Rigo-Bonnin R, Ribera A, Arbiol-Roca A, Cobo-Sacristan S, Padulles A, Murillo O, et al. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of beta-lactam antibiotic concentration in human plasma. Clin Chim Acta. 2017;468:215-24.

179. Baddour LM, Wilson WR, Bayer AS, Fowler VG, Jr., Tleyjeh IM, Rybak MJ, et al. Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications: A Scientific Statement for Healthcare Professionals From the American Heart Association. Circulation. 2015;132(15):1435-86.

180. Ordooei Javan A, Shokouhi S, Sahraei Z. A review on colistin nephrotoxicity. Eur J Clin Pharmacol. 2015;71(7):801-10.

181. Grayson M, Crowe S, McCarthy J, Mills J, Mouton J, Norrby S, et al. Kucers' The Use of Antibiotics. Sixth Edition ed: CRC Press; 2010.

182. Ribera A, Soldevila L, Rigo-Bonnin R, Tubau F, Padulles A, Gomez-Junyent J, et al. Beta-lactams in continuous infusion for Gram-negative bacilli osteoarticular infections: an easy method for clinical use. Infection. 2018;46(2):239-44.

183. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

184. Hayashi Y, Roberts JA, Paterson DL, Lipman J. Pharmacokinetic evaluation of piperacillin-tazobactam. Expert Opin Drug Metab Toxicol. 2010;6(8):1017-31.

185. Xu H, Zhou W, Zhou D, Li J, Al-Huniti N. Evaluation of Aztreonam Dosing Regimens in Patients With Normal and Impaired Renal Function: A Population Pharmacokinetic Modeling and Monte Carlo Simulation Analysis. J Clin Pharmacol. 2017;57(3):336-44.

186. Moriyama B, Henning SA, Childs R, Holland SM, Anderson VL, Morris JC, et al. High-dose continuous infusion beta-lactam antibiotics for the treatment of resistant Pseudomonas aeruginosa infections in immunocompromised patients. Ann Pharmacother. 2010;44(5):929-35.

187. Drusano GL, Standiford HC, Fitzpatrick B, Leslie J, Tangtatsawasdi P, Ryan P, et al. Comparison of the pharmacokinetics of ceftazidime and moxalactam and their microbiological correlates in volunteers. Antimicrob Agents Chemother. 1984;26(3):388-93.

188. Miller B, Hershberger E, Benziger D, Trinh M, Friedland I. Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. Antimicrob Agents Chemother. 2012;56(6):3086-91.

189. Van der Auwera P, Santella PJ. Pharmacokinetics of cefepime: a review. J Antimicrob Chemother. 1993;32 Suppl B:103-15.

190. Vinks AA, van Rossem RN, Mathot RA, Heijerman HG, Mouton JW. Pharmacokinetics of aztreonam in healthy subjects and patients with cystic fibrosis and evaluation of dose-exposure relationships using monte carlo simulation. Antimicrob Agents Chemother. 2007;51(9):3049-55.

191. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA, USA: CLSI; 2015.

192. European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoints. EUCAST; 2020.

193. El Haj C, Murillo O, Ribera A, Vivas M, Garcia-Somoza D, Tubau F, et al. Daptomycin combinations as alternative therapies in experimental foreign-body infection caused by meticillin-susceptible *Staphylococcus aureus*. Int J Antimicrob Agents. 2015;46(2):189-95.

194. Garrigós C, Murillo O, Euba G, Verdaguer R, Tubau F, Cabellos C, et al. Efficacy of usual and high doses of daptomycin in combination with rifampin versus alternative therapies in experimental foreign-body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2010;54(12):5251-6.

195. Garrigós C, Murillo O, Euba G, Verdaguer R, Tubau F, Cabellos C, et al. Efficacy of tigecycline alone and with rifampin in foreign-body infection by methicillin-resistant *Staphylococcus aureus*. J Infect. 2011;63(3):229-35.

196. Murillo O, Domenech A, Euba G, Verdaguer R, Tubau F, Cabo J, et al. Efficacy of linezolid alone and in combination with rifampin in staphylococcal experimental foreign-body infection. J Infect. 2008;57(3):229-35.

197. Ribera A, Benavent E, El-Haj C, Gomez-Junyent J, Tubau F, Rigo-Bonnin R, et al. Comparative Antibiofilm Efficacy of Meropenem Alone and in Combination with Colistin in an *In Vitro* Pharmacodynamic Model by Extended-Spectrum-β-Lactamase-Producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2019;63(11).

198. Oliver A, Mulet X, López-Causapé C, Juan C. The increasing threat of *Pseudomonas aeruginosa* high-risk clones. Drug Resist Updat. 2015;21-22:41-59.

199. Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol. 1999;37(6):1771-6.

200. Moskowitz SM, Foster JM, Emerson J, Burns JL. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. J Clin Microbiol. 2004;42(5):1915-22.

201. Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis. 2007;115(8):891-9.

202. Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. Statistical assessment of a laboratory method for growing biofilms. Microbiology. 2005;151(Pt 3):757-62.

203. El Haj C, Murillo O, Ribera A, Lloberas N, Gomez-Junyent J, Tubau F, et al. Evaluation of linezolid or trimethoprim/sulfamethoxazole in combination with rifampicin as alternative oral treatments based on an *in vitro* pharmacodynamic model of staphylococcal biofilm. Int J Antimicrob Agents. 2018;51(6):854-61.

204. Bui KQ, Ambrose PG, Nicolau DP, Lapin CD, Nightingale CH, Quintiliani R. Pharmacokinetics of high-dose meropenem in adult cystic fibrosis patients. Chemotherapy. 2001;47(3):153-6.

205. Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother. 2011;55(7):3284-94.

206. Li J, Milne RW, Nation RL, Turnidge JD, Coulthard K. Stability of colistin and colistin methanesulfonate in aqueous media and plasma as determined by high-performance liquid chromatography. Antimicrob Agents Chemother. 2003;47(4):1364-70.

207. Institut d'Estadística de Catalunya Website. [Available from: http://www.idescat.net/.

208. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95-108.

209. Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev. 2014;78(3):510-43.

210. Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int J Antimicrob Agents. 2010;36 Suppl 2:S50-4.

211. Mader JT, Shirtliff ME, Bergquist S, Calhoun JH. Bone and joint infections in the elderly: practical treatment guidelines. Drugs Aging. 2000;16(1):67-80.

212. Kak V, Chandrasekar PH. Bone and joint infections in injection drug users. Infect Dis Clin North Am. 2002;16(3):681-95.

213. Ross JJ, Shamsuddin H. Sternoclavicular septic arthritis: review of 180 cases. Medicine (Baltimore). 2004;83(3):139-48.

214. Sapico FL, Liquete JA, Sarma RJ. Bone and joint infections in patients with infective endocarditis: review of a 4-year experience. Clin Infect Dis. 1996;22(5):783-7.

215. Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med. 2004;350(14):1422-9.

216. Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. J Bone Joint Surg Am. 2005;87(7):1487-97.

217. Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am. 2007;89(4):780-5.

218. Dubost JJ, Soubrier M, De Champs C, Ristori JM, Sauvezie B. Streptococcal septic arthritis in adults. A study of 55 cases with a literature review. Joint Bone Spine. 2004;71(4):303-11.

219. Nolla JM, Gómez-Vaquero C, Corbella X, Ordóñez S, García-Gómez C, Pérez A, et al. Group B streptococcus (*Streptococcus agalactiae*) pyogenic arthritis in nonpregnant adults. Medicine (Baltimore). 2003;82(2):119-28.

220. Ross JJ, Saltzman CL, Carling P, Shapiro DS. Pneumococcal septic arthritis: review of 190 cases. Clin Infect Dis. 2003;36(3):319-27.

221. Ho J, Tambyah PA, Paterson DL. Multiresistant Gram-negative infections: a global perspective. Curr Opin Infect Dis. 2010;23(6):546-53.

222. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med. 2010;362(19):1804-13.

223. Rodríguez-Baño J, López-Prieto MD, Portillo MM, Retamar P, Natera C, Nuño E, et al. Epidemiology and clinical features of community-acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals. Clin Microbiol Infect. 2010;16(9):1408-13.

224. Mulleman D, Philippe P, Senneville E, Costes C, Fages L, Deprez X, et al. Streptococcal and enterococcal spondylodiscitis (vertebral osteomyelitis). High incidence of infective endocarditis in 50 cases. J Rheumatol. 2006;33(1):91-7.

225. Murillo O, Roset A, Sobrino B, Lora-Tamayo J, Verdaguer R, Jiménez-Mejias E, et al. Streptococcal vertebral osteomyelitis: multiple faces of the same disease. Clin Microbiol Infect. 2014;20(1):O33-8.

226. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-61.

227. Watkins RR, David MZ, Salata RA. Current concepts on the virulence mechanisms of meticillin-resistant *Staphylococcus aureus*. J Med Microbiol. 2012;61(Pt 9):1179-93.

228. Bossert M, Prati C, Bertolini E, Toussirot E, Wendling D. Septic arthritis of the acromioclavicular joint. Joint Bone Spine. 2010;77(5):466-9.

229. Ross JJ, Hu LT. Septic arthritis of the pubic symphysis: review of 100 cases. Medicine (Baltimore). 2003;82(5):340-5.

230. Pigrau C, Rodríguez-Pardo D, Fernández-Hidalgo N, Moretó L, Pellise F, Larrosa MN, et al. Health care associated hematogenous pyogenic vertebral osteomyelitis: a severe and potentially preventable infectious disease. Medicine (Baltimore). 2015;94(3):e365.

231. Kaandorp CJ, Dinant HJ, van de Laar MA, Moens HJ, Prins AP, Dijkmans BA. Incidence and sources of native and prosthetic joint infection: a community based prospective survey. Ann Rheum Dis. 1997;56(8):470-5.

232. Fangtham M, Baer AN. Methicillin-resistant *Staphylococcus aureus* arthritis in adults: case report and review of the literature. Semin Arthritis Rheum. 2012;41(4):604-10.

233. Gasch O, Ayats J, Angeles Dominguez M, Tubau F, Liñares J, Peña C, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection: secular trends over 19 years at a university hospital. Medicine (Baltimore). 2011;90(5):319-27.

234. Lessa FC, Mu Y, Davies J, Murray M, Lillie M, Pearson A, et al. Comparison of incidence of bloodstream infection with methicillin-resistant *Staphylococcus aureus* between England and United States, 2006-2007. Clin Infect Dis. 2010;51(8):925-8.

235. Sendi P, Banderet F, Graber P, Zimmerli W. Clinical comparison between exogenous and haematogenous periprosthetic joint infections caused by *Staphylococcus aureus*. Clin Microbiol Infect. 2011;17(7):1098-100.

236. Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum. 2002;46(9):2287-93.

237. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet. 2010;376(9746):1094-108.

238. Singh JA, Cameron C, Noorbaloochi S, Cullis T, Tucker M, Christensen R, et al. Risk of serious infection in biological treatment of patients with rheumatoid arthritis: a systematic review and meta-analysis. Lancet. 2015;386(9990):258-65.

239. van Dartel SA, Fransen J, Kievit W, Dutmer EA, Brus HL, Houtman NM, et al. Predictors for the 5-year risk of serious infections in patients with rheumatoid arthritis treated with anti-tumour necrosis factor therapy: a cohort study in the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry. Rheumatology (Oxford). 2013;52(6):1052-7.

240. Fowler VG, Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med. 2003;163(17):2066-72.

241. Tom S, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schonheyder HC, et al. Case fatality ratio and mortality rate trends of community-onset *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect. 2014;20(10):O630-2.

242. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis. 2003;36(1):53-9.

243. Gasch O, Camoez M, Dominguez MA, Padilla B, Pintado V, Almirante B, et al. Predictive factors for mortality in patients with methicillin-resistant *Staphylococcus aureus* bloodstream

infection: impact on outcome of host, microorganism and therapy. Clin Microbiol Infect. 2013;19(11):1049-57.

244. Gupta MN, Sturrock RD, Field M. A prospective 2-year study of 75 patients with adult-onset septic arthritis. Rheumatology (Oxford). 2001;40(1):24-30.

245. Kaandorp CJ, Krijnen P, Moens HJ, Habbema JD, van Schaardenburg D. The outcome of bacterial arthritis: a prospective community-based study. Arthritis Rheum. 1997;40(5):884-92.

246. Grossi O, Asseray N, Bourigault C, Corvec S, Valette M, Navas D, et al. Gram-negative prosthetic joint infections managed according to a multidisciplinary standardized approach: risk factors for failure and outcome with and without fluoroquinolones. J Antimicrob Chemother. 2016;71(9):2593-7.

247. Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2011;55(9):4469-74.

248. Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. *In vivo* pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. Antimicrob Agents Chemother. 2012;56(5):2683-90.

249. Mouton JW, den Hollander JG. Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an *in vitro* pharmacokinetic model. Antimicrob Agents Chemother. 1994;38(5):931-6.

250. Huttner A, Harbarth S, Hope WW, Lipman J, Roberts JA. Therapeutic drug monitoring of the beta-lactam antibiotics: what is the evidence and which patients should we be using it for? J Antimicrob Chemother. 2015;70(12):3178-83.

251. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. Clin Pharmacokinet. 2011;50(2):99-110.

252. Wong G, Briscoe S, Adnan S, McWhinney B, Ungerer J, Lipman J, et al. Protein binding of beta-lactam antibiotics in critically ill patients: can we successfully predict unbound concentrations? Antimicrob Agents Chemother. 2013;57(12):6165-70.

253. Hassan S, Kahn MD, Saraiya N, Nori P. Treatment of a complex orthopaedic infection due to extensively drug-resistant *Pseudomonas aeruginosa*. BMJ Case Rep. 2018;2018.

254. Velez Perez AL, Schmidt-Malan SM, Kohner PC, Karau MJ, Greenwood-Quaintance KE, Patel R. *In vitro* activity of ceftolozane/tazobactam against clinical isolates of *Pseudomonas aeruginosa* in the planktonic and biofilm states. Diagn Microbiol Infect Dis. 2016;85(3):356-9.

255. Riera E, Macia MD, Mena A, Mulet X, Perez JL, Ge Y, et al. Anti-biofilm and resistance suppression activities of CXA-101 against chronic respiratory infection phenotypes of *Pseudomonas aeruginosa* strain PAO1. J Antimicrob Chemother. 2010;65(7):1399-404.

256. Haagensen J, Verotta D, Huang L, Engel J, Spormann AM, Yang K. Spatiotemporal pharmacodynamics of meropenem- and tobramycin-treated *Pseudomonas aeruginosa* biofilms. J Antimicrob Chemother. 2017;72(12):3357-65.

257. Haagensen JA, Verotta D, Huang L, Spormann A, Yang K. New *in vitro* model to study the effect of human simulated antibiotic concentrations on bacterial biofilms. Antimicrob Agents Chemother. 2015;59(7):4074-81.

258. Bergen PJ, Bulman ZP, Landersdorfer CB, Smith N, Lenhard JR, Bulitta JB, et al. Optimizing Polymyxin Combinations Against Resistant Gram-Negative Bacteria. Infect Dis Ther. 2015;4(4):391-415.

259. Gunderson BW, Ibrahim KH, Hovde LB, Fromm TL, Reed MD, Rotschafer JC. Synergistic activity of colistin and ceftazidime against multiantibiotic-resistant *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic model. Antimicrob Agents Chemother. 2003;47(3):905-9.

260. Hengzhuang W, Ciofu O, Yang L, Wu H, Song Z, Oliver A, et al. High beta-lactamase levels change the pharmacodynamics of beta-lactam antibiotics in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2013;57(1):196-204.

261. Cabot G, Zamorano L, Moya B, Juan C, Navas A, Blazquez J, et al. Evolution of *Pseudomonas aeruginosa* Antimicrobial Resistance and Fitness under Low and High Mutation Rates. Antimicrob Agents Chemother. 2016;60(3):1767-78.

262. Feng Y, Jonker MJ, Moustakas I, Brul S, Ter Kuile BH. Dynamics of Mutations during Development of Resistance by *Pseudomonas aeruginosa* against Five Antibiotics. Antimicrob Agents Chemother. 2016;60(7):4229-36.

263. Tam VH, Chang KT, Zhou J, Ledesma KR, Phe K, Gao S, et al. Determining beta-lactam exposure threshold to suppress resistance development in Gram-negative bacteria. J Antimicrob Chemother. 2017;72(5):1421-8.

ANNEXES

Annex I – Written informed consent for the study on continuous beta-lactam infusion

HOJA DE INFORMACIÓN AL PACIENTE

TÍTULO DEL ESTUDIO: Uso optimizado de antibióticos beta-lactámicos: administración en infusión continua y monitorización farmacocinética / farmacodinámica (Proyecto βELIC).

PROMOTOR: Oscar Murillo Rubio. Servicio de Enfermedades Infecciosas.

CENTRO: Hospital Universitari de Bellvitge. C/ Feixa Llarga, s/n; 08907 Hospitalet de Llobregat (Barcelona).

TELÉFONO DE CONTACTO: 932607625.

INTRODUCCION

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por el Comité Ético de Investigación Clínica correspondiente y la Agencia Española del Medicamento y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 223/2004, de 6 de febrero, por el que se regulan los ensayos clínicos con medicamentos. Nuestra intención es tan solo que usted reciba la información correcta y suficiente para que pueda evaluar y juzgar si quiere o no participar en este estudio. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir después de la explicación. Además, puede consultar con las personas que considere oportuno.

PARTICIPACIÓN VOLUNTARIA

Debe saber que su participación en este estudio es voluntaria y que puede decidir no participar o cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su tratamiento.

DESCRIPCIÓN GENERAL DEL ESTUDIO

Las infecciones osteoarticulares son aquellas que afectan a los huesos y a las articulaciones e incluyen también las que asientan sobre prótesis articulares o material de osteosíntesis utilizado para fijar fracturas. El tratamiento antibiótico de las infecciones osteoarticulares se realiza habitualmente durante varias semanas. Un grupo de antimicrobianos frecuentemente utilizados en estas infecciones son los antibióticos beta-lactámicos (que incluyen a la penicilina y derivados). Éstos son administrados por vía endovenosa varias veces al día de forma más frecuente, aunque también pueden administrarse en perfusión contínua (a lo largo de 24 horas). Esta administración presenta un conjunto de ventajas teóricas en cuanto a sus propiedades farmacológicas, efectividad frente a microorganismos y protección frente al desarrollo de resistencias a los antibióticos.

La Unidad de Traumatología-Sépticos, en la que usted se encuentra ingresado/a, ha implantado en los últimos años en su práctica clínica habitual la administración de los antibióticos beta-lactámicos en perfusión contínua. Esto significa que durante el tiempo de hospitalización, los pacientes con infecciones osteoarticulares que requieren tratamiento con antibióticos beta-lactámicos por vía endovenosa, éstos son administrados en perfusión contínua. De forma paralela, el Servicio de

Bioquímica ha puesto en marcha la técnica necesaria para la determinación de la concentración de estos antibióticos, ya que esta es una condición indispensable para su monitorización y optimización de la posología. La determinación de la concentración de estos antibióticos se hace habitualmente una vez por semana junto a una analítica rutinaria de control de diversos parámetros bioquímicos, aunque en última instancia puede variar esta frecuencia, según el juicio clínico del equipo médico, en función de las condiciones concretas del paciente.

Con el presente estudio, se pretende describir la experiencia clínica del tratamiento de infecciones osteoarticulares con antibióticos beta-lactámicos en perfusión contínua o extendida. Para ello, le pedimos su consentimiento para recoger de forma totalmente anónima datos de su historia clínica. También le pedimos su consentimiento para poder almacenar de forma anónima las muestras de sangre que se recojan de forma rutinaria durante el tratamiento para determinar la concentración de los antibióticos. Estas muestras se almacenarán en una colección creada para este motivo, que se encuentra en el Servicio de Bioquímica del Hospital Universitari de Bellvitge y está registrada en el Instituto de Salud Carlos III.

Las muestras de sangre toda la información que nos proporcione serán almacenadas con un código de identificación por lo que su identidad en el estudio se mantendrá de manera confidencial. Dichas muestras podrán ser utilizadas en un futuro con fines de investigación. De igual modo, guardaremos toda la información del estudio bajo llave en nuestras oficinas y solamente el personal del estudio tendrá acceso a esta información. Su identidad en el estudio será confidencial.

Su participación en el estudio no va a conllevar ningún tipo de asistencia clínica extraordinaria, esto es, ningún elemento adicional a la práctica clínica habitual tanto en el número de visitas (hospitalización y ambulatoria), como en el número y tipo de pruebas complementarias realizadas.

BENEFICIOS Y RIESGOS DERIVADOS DE SU PARTICIPACIÓN EN EL ESTUDIO

Es posible que su participación en el estudio no implique beneficios directos sobre su salud. Sin embargo, la información derivada del estudio puede suponer beneficios en el tratamiento de los pacientes con infecciones osteoarticulares. De acuerdo a la experiencia previa con el uso de los antibióticos beta-lactámicos en infusión continua, la participación en el estudio no debe suponer riesgos añadidos a los ya existentes por la práctica clínica habitual.

Su participación en el estudio no le supondrá ningún gasto. Usted no tendrá que pagar por los medicamentos del estudio.

CONFIDENCIALIDAD

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los sujetos participantes se ajustará a lo dispuesto en la Ley Orgánica 15/1999, de 13 de diciembre de protección de datos de carácter personal. De acuerdo a lo que establece la legislación mencionada, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse a su médico del estudio. Los datos recogidos para el estudio estarán identificados mediante un código y solo su médico del estudio/colaboradores podrán relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a persona alguna.

Sólo se transmitirán a terceros y a otros países los datos recogidos para el estudio que en ningún caso contendrán información que le pueda identificar directamente, como nombre y apellidos, iniciales, dirección, nº de la seguridad social, etc. En el caso de que se produzca esta cesión, será para los mismos fines del estudio descrito y garantizando la confidencialidad como mínimo con el nivel de protección de la legislación vigente en nuestro país. El acceso a su información personal quedará

restringido al médico del estudio/colaboradores, autoridades sanitarias (Agencia Española del Medicamento y Productos Sanitarios), al Comité Ético de Investigación Clínica y personal autorizado por el promotor, cuando lo precisen para comprobar los datos y procedimientos del estudio, pero siempre manteniendo la confidencialidad de los mismos de acuerdo a la legislación vigente.

OTRA INFORMACIÓN RELEVANTE

Cualquier nueva información referente a los fármacos utilizados en el estudio y que pueda afectar a su disposición para participar en el estudio, que se descubra durante su participación, le será comunicada por su médico lo antes posible.

Si usted decide retirar el consentimiento para participar en este estudio, ningún dato nuevo será añadido a la base de datos y, puede exigir la destrucción de todas las muestras identificables previamente retenidas para evitar la realización de nuevos análisis.

También debe saber que puede ser excluido del estudio si el promotor los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la medicación en estudio o porque consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

Al firmar la hoja de consentimiento adjunta, se compromete a cumplir con los procedimientos del estudio que se le han expuesto. Cuando acabe su participación recibirá el mejor tratamiento disponible y que su médico considere el más adecuado para su enfermedad.

CONSENTIMIENTO INFORMADO

Datos del estudio para el que se otorga el consentimiento:
Título del proyecto: Uso optimizado de antibióticos beta-lactámicos: administración en infusión continua y monitorización farmacocinética / farmacodinámica (Proyecto βELIC).

Yo (nombre y apellidos)

……

He leído la hoja de información que se me ha entregado.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio.

He hablado con:

……

(nombre del investigador)

Comprendo que mi participación es voluntaria.

Comprendo que puedo retirarme del estudio:

1º Cuando quiera

2º Sin tener que dar explicaciones.

3º Sin que esto repercuta en mis cuidados médicos.

- Presto libremente mi conformidad para participar en el estudio y doy mi consentimiento para el acceso y utilización de mis datos en las condiciones detalladas en la hoja de información.

SÍ ☐ NO ☐

- Accedo a que las muestras de sangre obtenidas para el estudio puedan ser utilizadas en el futuro para nuevos análisis relacionados con la enfermedad o fármacos del estudio no previstos en el protocolo actual (quedando excluidos los análisis genéticos):

SÍ ☐ NO ☐

Fecha:
Firma del participante/paciente:

Fecha:
Firma del Investigador que proporciona la información y la hoja de consentimiento:

Versión 1. 31 de agosto de 2016.

CONSENTIMIENTO INFORMADO

Datos del estudio para el que se otorga el consentimiento:
Título del proyecto: Uso optimizado de antibióticos beta-lactámicos: administración en infusión continua y monitorización farmacocinética / farmacodinámica (Proyecto βELIC).

Yo (nombre y apellidos) ……en calidad de ……. (relación con el participante) de …… (nombre y apellidos del participante)

He leído la hoja de información que se me ha entregado.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio.

He hablado con:

……

(nombre del investigador)

Comprendo que la participación del paciente es voluntaria.

Comprendo que puede retirarse del estudio:

1º Cuando quiera

2º Sin tener que dar explicaciones.

3º Sin que esto repercuta en mis cuidados médicos.

- En mi presencia se ha dado a……(nombre del participante) toda la información pertinente adaptada a su nivel de entendimiento y está de acuerdo en participar. Presto mi conformidad para que ……(nombre del participante) participe en este estudio y doy mi consentimiento para el acceso y utilización de los datos en las condiciones detalladas en la hoja de información.

SÍ □ NO □

- Accedo a que las muestras de sangre obtenidas para el estudio puedan ser utilizadas en el futuro para nuevos análisis relacionados con la enfermedad o fármacos del estudio no previstos en el protocolo actual (quedando excluidos los análisis genéticos):

SÍ □ NO □

Fecha:
Nombre:
Firma del representante:

Fecha:
Nombre:
Firma del Investigador que proporciona la información y la hoja de consentimiento:

Versión 1. 31 de agosto de 2016.

Annex II – Articles

The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century

O. Murillo[1], I. Grau[1], J. Lora-Tamayo[1], J. Gomez-Junyent[1], A. Ribera[1], F. Tubau[2], J. Ariza[1] and R. Pallares[1]

1) *Infectious Disease Service and* 2) *Microbiology Department, Hospital Universitari de Bellvitge, Barcelona, Spain*

Abstract

Osteoarticular infections (OAI), which are often associated with bacteraemia, seem to be increasing. We studied all patients with bacteraemia and concomitant OAI: septic arthritis (SA), vertebral osteomyelitis (VOM) or peripheral osteomyelitis (POM), which were seen at our institution (1985–2011). Data were extracted from a prospective protocol of bacteraemia cases recorded. Trends in main findings were considered in five periods. Major antibiotic resistance patterns were studied. A total of 601 cases of bacteraemic OAI, accounting for 1.8% of total bactaeremias, were studied: SA (48%), VOM (40%) and POM (17%). When comparing the 1985–91 and 2007–11 periods, the incidence of bacteraemic OAI increased from 2.34 to 5.78 episodes/100 000 inhabitants per year ($p < 0.001$); and nosocomial and healthcare-related cases increased from 18% to 30% ($p < 0.001$) and from 10% to 25% ($p < 0.001$), respectively. Also, there was an increase of age (median, from 49 to 65 years, $p < 0.001$), patients with comorbidities (23% to 59%, $p < 0.001$), and device-related OAI (7% to 28%, $p < 0.001$). Patterns of OAI were changing over time. Compared with younger patients, older adults ($\geq$ 65 years) had more VOM, prosthetic-joint infections and enterococcal OAI. The percentage of OAI caused by methicillin-susceptible *Staphylococcus aureus* decreased, while those caused by methicillin-resistant *S. aureus*, streptococci, enterococci, and Gram-negative bacilli increased. There was a link between certain microorganisms with specific OAI and age of patients. Over the past three decades, bacteraemic OAI increased in association with aging and use of orthopaedic devices. Nosocomial and healthcare-related OAI increased, with a rise in multidrug-resistant bacteria. These trends should be considered when planning diagnostic and therapeutic guidelines for OAI.

Keywords: Bacteraemia, epidemiology, osteoarticular infections, osteomyelitis, septic arthritis, spondylodiscitis
Original Submission: 29 April 2014; **Revised Submission:** 1 September 2014; **Accepted:** 11 September 2014
Editor: D. Raoult
Article published online: 12 October 2014

Corresponding author: O. Murillo, Infectious Disease Service, Hospital Universitari de Bellvitge, Feixa Llarga s/n, 08907 L'Hospitalet, Barcelona, Spain
E-mail: omurillo@bellvitgehospital.cat

Introduction

Osteoarticular infections (OAI) are often associated with bacteraemia with a variable risk depending on the type of infection, this being higher in native joint and vertebral osteomyelitis than in prosthetic joint or peripheral osteomyelitis. In any case, bacteraemia may occur as a complication of the OAI itself or secondary to a distant infection [1–4].

Little is known about changes in epidemiological and clinical findings of OAI over long periods of time. Conceivably, there may have been major changes in the microbiology, types of OAI and the characteristics of patients at risk. For example, the widespread use of medical devices in orthopaedic surgery and increased life expectancy of the population are all factors related to the increased rates of some OAI [5–8]. Also, the emergence of multidrug-resistant microorganisms (e.g. methicillin-resistant *Staphylococcus aureus*, MRSA) in hospitals and other healthcare institutions has been associated with increased rates of bacteraemic infections caused by difficult-to-treat pathogens [9–11]. Lastly, an increasing incidence of

http://dx.doi.org/10.1016/j.cmi.2014.09.007

infections has been related to the improvement of identification methods of microorganism [12,13].

In the present study we analysed a large cohort of patients with bacteraemic OAI who attended at our institution over the past three decades. The main objectives were to determine changes over time in the types and characteristics of OAI, the microorganisms causing these infections, associated comorbidities and other patient characteristics.

Patients and methods

This study was carried out in Bellvitge hospital, a 700-bed teaching institution in Barcelona, Spain. The hospital does not attend paediatric, obstetric or burns patients. It has a Bone and Joint Infection Unit, run by a multidisciplinary medical team.

Over the past three decades, information of all patients with bloodstream infections has been collected in a prospective database, including data on patients' baseline characteristics, clinical presentation and source of bacteraemia, and microbiological records.

Patient's characteristics and definitions

All patients with OAI and bacteraemia who attended at our institution from 1985 to 2011 were analysed. This included both patients with an osteoarticular focus as the source of bacteraemia ('primary' bacteraemic OAI) and patients with bacteraemia from a distant focus, in whom the osteoarticular infection was the result of a septic metastasis ('secondary' bacteraemic OAI).

Blood samples were cultured following standard recommendations by the automated BacTEC method with both aerobic and anaerobic media (systems used were as follows: in the 1990s Bactec NR-860, in 2000s Bactec 9240, and in 2010s Bactec FX; Becton-Dickinson Microbiology Systems, Franklin Lakes, NJ, USA). During the study period, identification of microorganisms and their antibiotic susceptibility were performed by using standard biochemical reactions, disc diffusion or microdilution method, and the MicroScan system (Dade Behring, West Sacramento, CA, USA). Antimicrobial susceptibility was defined according to CLSI criteria [14]. We applied the definitions for multidrug-resistant and extensively drug-resistant microorganisms described by Magiorakos *et al.* [15].

Bacteraemic OAI were divided into three categories: (i) septic arthritis, including both episodes of native septic arthritis and prosthetic joint infections (PJI); (ii) vertebral osteomyelitis, including cases of infection with or without the presence of spine arthrodesis; and (iii) non-vertebral or peripheral osteomyelitis, which included episodes of peripheral osteomyelitis (with or without osteosynthesis). OAI were defined by the presence of bacteraemia, and a concomitant compatible clinical picture of septic arthritis (inflammatory local signs, macroscopic pus in joint fluid or the presence of sinus tract) [8,16], vertebral osteomyelitis (back pain, motion limitation, and/or macroscopic pus through the spine surgery wound) [4], or peripheral osteomyelitis (inflammatory signs and/or prolonged sinus drainage) [1]. Computed tomography or magnetic resonance imaging was performed if deemed appropriate by clinicians. Microbiology of OAI was always identified by blood samples, and in most cases by additional local samples obtained from the affected joint or bone. In line with the Lancefield and Sherman classifications, *Streptococcus* species were divided into two groups: pyogenic species (*Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*) and *viridans* streptococci (*Streptococcus bovis* and *Streptococcus milleri*, along with the remaining species) [17]. Endocarditis was diagnosed using Duke criteria [18].

Cases were considered to be nosocomial, healthcare-acquired or community-acquired according to the definitions provided by Friedman *et al.* [19].

Statistical analysis

To assess the epidemiological changes over time, the bacteraemic episodes were grouped into five periods: 1985–91 (P1), 1992–96 (P2), 1997–2001 (P3), 2002–06 (P4) and 2007–11 (P5). According to the total duration of the study (27 years), we considered 5-year intervals for all periods except for the first one, in which there was the lower number of cases. Incidence rates (the number of episodes per 100 000 inhabitants per year) were calculated after estimating the population that attends our hospital; the distribution of the population for each hospital in the region of Barcelona is pre-determined according to the area where they live. Data on this population were obtained from the public website page of the *Official Statistics in Catalonia* [20]: 2 991 146 inhabitants (P1), 2 468 378 (P2), 2 740 127 (P3), 3 021 176 (P4) and 3 185 079 (P5). Changes of several characteristics of bacteraemic OAI episodes were also analysed regarding the age of patients. The Maentel–Haenszel chi-squared test was used to assess the changing trends in the absolute number of incidence and in the characteristics of the episodes of osteoarticular bacteraemia. The changing trends of continuous parameters over time were studied with the Jonckheere–Terpstra test. Comparative analyses were performed with chi-squared or Fisher's test for categorical variables, and the Mann–Whitney *U*-test for continuous variables.

Data were analysed with SPSS (version 20.0). A p value <0.05 was considered as statistically significant.

Results

A total of 32 727 episodes of clinically significant bacteraemia were registered during the period of study. Of these, 601 episodes (1.8%) had a concomitant OAI. There was an increasing incidence of bacteraemic OAI in five periods (P1 to P5), rising from 2.34 to 5.78 episodes/100 000 inhabitants per year (p <0.001); this was also the case for the incidence of total bacteraemia in these periods, rising from 162 to 238 episodes/100 000 inhabitants per year (p <0.001). The proportion of bacteraemic OAI in relation to all bloodstream infections increased, from 1.45% in P1 to 2.43% in P5 (p <0.001; Fig. 1).

The bacteraemic OAI were considered to be 'primary' (456 cases, 76%) or 'secondary' (145, 24%). Among the latter, the most frequent distant foci of infection were vascular catheter-related infections (*n* = 49, 8%) and infective endocarditis (*n* = 42, 7%). The prevalence of nosocomial episodes of bacteraemic OAI significantly increased during the study period, whereas community-acquired infections diminished (P1 to P5, 18% and 72% to 30% and 45%, respectively; p <0.001). Concurrently, we observed the progressive appearance of healthcare-associated episodes (from 10% to 25%; p <0.001).

Table 1 shows the most important characteristics of the patients with bacteraemic OAI, and the changes observed over time. Median age increased significantly throughout the period studied; this was also the case when intravenous drug users were excluded (data not shown) taking into account that the rate of this population decreased over time (p <0.001). Concurrently, we observed an increasing percentage of patients suffering from comorbid conditions (p <0.001).

Out of 601 cases of bacteraemic OAI, 36 (6%) had multiple OAI. Overall, septic arthritis was the most frequent (*n* = 291, 46%), followed by vertebral osteomyelitis (*n* = 241, 38%) and peripheral osteomyelitis (*n* = 105, 16%). Changes over time for different types of OAI are presented in Table 1. Although the number of episodes of septic arthritis increased over the period, its frequency with respect to all OAI significantly decreased (57% in P1 and 38% in P5; p <0.001). The type of septic arthritis changed between the first (P1) and the last (P5) period of the study, with a lower incidence of native arthritis and an increase in the number of PJI (p <0.001). Although the incidence of peripheral osteomyelitis remained stable throughout the period studied, the number of vertebral osteomyelitis increased significantly. In fact, the number of all foreign body-related infections increased from 7% in P1 to 28% in P5 (p <0.001**).**

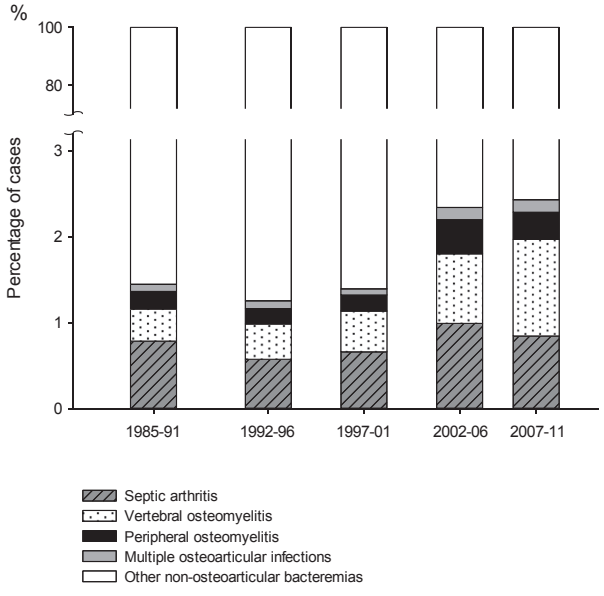


FIG. 1. Proportion of total bacteraemic cases compared with the total cases and different types of bacteraemic osteoarticular infections.

Microbiology of bacteraemic OAI

Gram-positive cocci caused 80% of OAI. The proportion of polymicrobial and anaerobic OAI was very low (2.4% and 1%, respectively) (Table 2). *Staphylococcus aureus* was by far the most frequent cause of episodes of all types of OAI, whereas other microorganisms showed more affinity for a particular infection (Table 3). In comparison with *S. aureus*, the *viridans* streptococci and *Enterococcus faecalis* groups produced more vertebral osteomyelitis (56% and 57%, respectively), whereas pyogenic streptococci species caused septic arthritis (67%). Among cases of bacteraemic OAI caused by *S. aureus*, MRSA strains were more commonly involved in cases of PJI than methicillin-susceptible *S. aureus* (MSSA) strains (p <0.05).

Fig. 2 illustrates the increasing incidence of the more frequent aetiologies in the study period. The number of episodes of OAI caused by *S. aureus* increased throughout the period of study, but its global proportion with respect to other microorganisms fell from 71% in P1 to 57% in P5 (p 0.1). Significant changes were observed in the proportion of episodes caused by MSSA (a decrease from 71% in P1 to 45% in P5; p <0.001) and those by MRSA (an increase from 0% to 12%, respectively; p <0.001). The episodes of OAI caused by *Streptococcus* sp. and *Enterococcus* sp. showed a trend towards an increase (from 10% to 19%; p 0.141). The frequency of *Enterobacteriaceae* and *Pseudomonas aeruginosa* bacteraemia remained more stable (17% in P1 and 21% in P5). The number

TABLE 1. Characteristics of patients and episodes of bacteraemic osteoarticular infections (OAI) and their changes over time

		Periods of the study					
	All OAI *n* = 601	**1985–91** *n* = 70	**1992–96** *n* = 70	**1997–2001** *n* = 97	**2002–06** *n* = 181	**2007–11** *n* = 183	**p value**[a]
Age (median, IQR)	63 (50–74)	49 (24–64)	58 (32–68)	64 (50–74)	64 (53–74)	65 (53–77)	<0.001
Male	366 (61)	39 (56)	42 (60)	68 (70)	114 (63)	103 (56)	0.2
Underlying diseases							
One or more	307 (51)	16 (23)	22 (31)	51 (53)	111 (61)	107 (59)	**<0.001**
Intravenous drug users	57 (10)	17 (24)	9 (13)	13 (13)	11 (6)	7 (4)	**<0.001**
Diabetes mellitus	153 (26)	11 (16)	13 (19)	24 (25)	55 (30)	50 (27)	**0.013**
Neoplasm	71 (12)	1 (1)	3 (4)	15 (15)	30 (16)	22 (12)	**0.006**
Cardiopathy	88 (15)	1 (1)	2 (3)	14 (14)	29 (16)	42 (23)	**<0.001**
Chronic renal insufficiency	44 (7)	0	2 (3)	5 (5)	17 (9)	20 (11)	**<0.001**
Immunosuppressive therapy	88 (15)	6 (9)	4 (6)	14 (14)	34 (19)	30 (16)	**0.01**
Reumathoid arthritis	30 (5)	5 (7)	0	7 (7)	10 (6)	8 (4)	0.2
Systemic autoimmune disease	16 (3)	1 (1)	2 (3)	3 (3)	3 (2)	7 (1)	0.4
Type of OAI							
Septic arthritis[b]	291 (46)	41 (57)	37 (49)	50 (49)	88 (45)	75 (38)	**0.006**
Native[c]	228 (78)	37 (90)	32 (86)	40 (80)	70 (79)	49 (65)	
Prosthetic[c]	63 (22)	4 (10)	5 (14)	10 (20)	18 (21)	26 (35)	**0.001**
Vertebral osteomyelitis[b]	241 (38)	18 (25)	26 (35)	37 (36)	71 (37)	89 (46)	**0.001**
Native[c]	206 (86)	18 (100)	25 (96)	37 (100)	60 (85)	66 (74)	
Spine instrumentation[c]	35 (14)	0	1 (4)	0	11 (15)	23 (26)	**<0.001**
Peripheral osteomyelitis[b]	105 (16)	13 (18)	12 (16)	15 (15)	34 (18)	31 (16)	0.9
With osteosynthesis[c]	16 (15)	1 (7)	3 (25)	1 (7)	8 (23)	3 (10)	0.3
Device-related infections[d]	114 (19)	5 (7)	9 (13)	11 (11)	37 (20)	52 (28)	**<0.001**

Data presented as numbers of cases (percentage).
[a]p value represents the changing trends over periods of the study (Jonckheere–Terpstra test).
[b]Total: 637 OAI types in 601 cases of bacteraemic OAI, with 36 cases that presented more than one concurrent infection (6%).
[c]Percentages of episodes are calculated with respect to total episodes of septic arthritis, vertebral osteomyelitis or peripheral osteomyelitis, respectively.
[d]Device-related infections include prosthetic joint infections and osteosynthesis hardware.

of extended-spectrum β-lactamase-producing *Enterobacteriaceae* and multidrug-resistant *P. aeruginosa* was very low (two and three episodes, respectively), but they increased in the last two periods (from none in the three first periods to two cases in P4 and three cases in P5; p 0.082).

Characteristics of bacteraemic OAI regarding the age of patients

Table 4 shows the main characteristics of episodes of OAI and the differences between age groups (group 1, patients aged 49 or less, group 2, patients aged 50 to 64, and group 3, patients aged 65 or over).

In comparison with younger patients (group 1), patients in groups 2 and 3 had more underlying diseases, and OAI were more frequently hospital-acquired or healthcare-related. Peripheral osteomyelitis was more frequent in the younger patients, whereas there were more episodes of vertebral osteomyelitis in groups 2 and 3 ($p < 0.05$). There were no differences in the total number of episodes of arthritis, but older patients (groups 2 and 3) had significantly more PJI.

Regarding microbiology, *S. aureus* was involved in a greater number of episodes of OAI in younger patients than in the older ones ($p \leq 0.002$); when these staphylococcal OAI occurred, MSSA strains were responsible for almost all episodes in group 1, whereas MRSA strains were significantly more frequent in patients in groups 2 and 3. Streptococcal infections (either pyogenic or *viridans* species) increased in older patients, and enterococcal OAI were significantly related with the oldest patients (group 3). OAI caused by Gram-negative bacilli were less common among younger patients (group 1).

The main differences between groups 2 and 3 were that the oldest group presented significantly less native arthritis and more PJI, and a higher frequency of enterococcal infections.

Additionally, we compared the median age of patients with bacteraemic OAI during the study period by types of infection and microbiology (see Supporting information, Table S1). Of note, median age of all septic arthritis significantly increased from 48 years in P1 to 68 years in P5 (p 0.001); this was mainly due to the rise in the number of PJI cases, which occurred in an older population that did not significantly change over time. In a similar way, a significant increase in the age of patients was observed for staphylococcal OAI, this being related to the rise in the number of episodes caused by MRSA strains in older patients. All of these differences were maintained when young population of intravenous drug users were excluded. In contrast, no significant changes in the median age of patients over time were observed for episodes of vertebral osteomyelitis and for episodes of OAI caused by other relevant microorganisms.

Discussion

The present study shows the progressive rise in the number of patients with OAI and bacteraemia, this being the cause or the

TABLE 2. Microbiology of the 601 episodes of bacteraemic osteoarticular infections

Gram-positive microorganisms		**492 (80%)**
Staphylococcus spp.		386 (62%)
S. aureus[a]	368 (59%)	
Coagulase-negative staphylococci	18 (3%)	
Streptococcus spp.		86 (17%)
S. pneumoniae	14 (2%)	
S. pyogenes	12 (2%)	
S. agalactiae	23 (4%)	
S. group anginosus	7 (1%)	
S. bovis[b]	7 (1%)	
Other group viridans streptococci [c]	15 (2%)	
Other *Streptococcus* spp. [d]	8 (1%)	
Enterococcus spp.		15 (2%)
E. faecalis	14 (2%)	
E. faecium	1 (0.2%)	
Other Gram-positive microorganisms [e]	5 (0.8%)	5 (0.8%)
Gram-negative microorganisms		**126 (20%)**
Enterobacteriaceae		87 (14%)
Escherichia coli	58 (9%)	
Klebsiella pneumoniae	8 (1%)	
Proteus mirabilis	8 (1%)	
Salmonella enteritidis	5 (0.8%)	
Morganella morgani	3 (0.4%)	
Enterobacter cloacae	2 (0.3%)	
Citrobacter spp	2 (0.3%)	
Serratia marcescens	1 (0.2%)	
Non-fermenting Gram-negative bacilli		13 (2%)
Pseudomonas aeruginosa	11 (2%)	
Aeromonas hydrophila	1 (0.2%)	
Acinetobacter baumanii	1 (0.2%)	
Other Gram-negative microorganisms		18 (3%)
Neisseria meningitidis	8 (1%)	
Haemophilus spp	4 (0.6%)	
Veillonella spp	2 (0.3%)	
Eikenella corrodens	2 (0.3%)	
Kingella kingae	1 (0.2%)	
Campylobacter sp.	1 (0.2%)	
Anaerobic Gram-negative microorganisms		8 (1%)
Bacteroides spp.	7 (1%)	
Porphyromonas sp.	1 (0.2%)	

Total: 618 isolates in 601 episodes of bacteraemic osteoarticular infections, with 15 episodes (2.4%) of polymicrobial bacteraemia. Microbiology of polymicrobial infections: *S. aureus* + *S. agalactiae* (2); *S. aureus* + *Streptococcus* group G, *S. aureus* + *P. mirabilis* (2); *S. aureus* + *E. coli*; *E. faecalis* + coagulase-negative staphylococci (CoNS); *E. faecalis* + *E. coli*; *E. faecalis* + *A. baumanii*; *K. pneumoniae* + *CoNS*; *K. pneumoniae* + *P. aeruginosa*; *E. coli* + *P. aeruginosa*; *E. corrodens* + CoNS; *P. mirabilis* + *S. intermedius*; *C. glabrata* + CoNS.
[a]This included 49 isolates (13%) of methicillin-resistant *S. aureus*.
[b]*S. bovis* type I (5 isolates) and *S. bovis* type II (2 isolates).
[c]*S. sanguis* (6 isolates), *S. mitis* (6 isolates), *S. mutans* (2 isolates) and *S. salivarius* (1 isolates).
[d]*S. equi* group C (3 isolates), β-haemolyticus *Streptococcus* of group G (4 isolates) and β-haemolyticus *Streptococcus* of group F (1 isolate).
[e]*Abiotrofia* sp (1 isolate), *Aerococcus viridans* (1 isolate), *Corynebacterium* sp (1 isolate), *Eubacterium lentum* (1 isolate) and *Arcanobacterium* sp (1 isolate).

consequence of osteoarticular infection itself, and describes the trends observed in our area during the last three decades. Our study shows the significant rise in the number of all types of OAI over a long period (three decades), as has previously been reported for the particular case of vertebral osteomyelitis [1,6].

These increased rates of OAI have been associated with longer life expectancy and the development of chronic diseases [1,6,21]. In agreement with previous reports, we observed a significant increase in the age of patients with OAI. Previous studies highlighted an association between OAI and patients with intravenous drug abuse, who are usually younger [3,22,23]. Our results suggest that this harmful social habit has been progressively abandoned, and that it no longer has an effect on the current pattern of bacteraemic OAI.

Given the increase in life expectancy, the higher prevalence of chronic diseases observed in our patients may be aging-related. In addition, modern medical advances have generated new technologies that can be offered to a high number of older patients [24]. We observed a progressive increase in the number of episodes of OAI related with orthopaedic devices. It is known that the implantation and revision of joint prostheses have risen significantly [7,25], which implies an increase in the absolute number of episodes of infection [8,24]. Our results agree with these reports, and included not just joint prostheses but osteosynthesis hardware as well. In fact, the current pattern of bacteraemic OAI has been modified by the impact of these foreign material-associated infections, which represented 28% of all infections in the most recent period. Although the incidence of PJI and osteosynthesis-related infections all rose, among 'native' OAI vertebral osteomyelitis was the only one to record an increase. Interestingly, due to the increase of older patients, the pattern of non-device-related OAI has also changed. While the presence of septic arthritis did not differ between patients of different ages, peripheral osteomyelitis was predominant among patients under 50 years of age and vertebral osteomyelitis was more frequent in older patients. Overall, the pattern of OAI in younger patients reflected the predominance of native arthritis and peripheral osteomyelitis, whereas older patients had more PJI and vertebral osteomyelitis. Accordingly, the increase in the median age of patients observed in some OAI (i.e. septic arthritis) was mainly related to the rise in the number of episodes that occurred in older patients (i.e. PJI).

Finally, regarding these epidemiological changes, the source and acquisition of OAI has also changed. We observed a progressive increase in nosocomial episodes, the emergence of healthcare-related infections, and a decrease in the number of strictly community-acquired OAI. These results seem to agree with recent works in other fields which reflect the improvements obtained with modern medical and social assistance [11,19,26].

Staphylococcus aureus is recognized as the most important aetiology of OAI [1,2,5], as was the case in the present study, but we noted a progressive decline in its proportion with respect to other microorganisms. Moreover, the pattern of these infections has clearly changed with a significant emergence of MRSA, mostly among older patients, and a decrease in MSSA infections. The presence of *Streptococcus* and *Enterococcus* species is also notable and, together with staphylococcal episodes, highlights the major role of gram-positive cocci in this setting. Our results emphasize the greater presence of *S. aureus* in young patients (75% of OAI, MSSA strains in almost all cases) than in older patients (56–58%, with the emergence of MRSA strains), this being related with the increased age of patients

TABLE 3. Differences between more frequent microorganisms and their type of osteoarticular infections caused

	Arthritis			Vertebral osteomyelitis			Peripheral osteomyelitis (POM)			
	All	Native	Prosthetic	All	Native	Spine	All	DM Foot	POM-no OS	OS
Staphylococcus aureus (*n* = 384)	182 (47)	141 (36)	41 (11)	131 (35)	109 (29)	22 (6)	71 (18)	16 (4)	42 (11)	13 (3)
MSSA (*n* = 333)[a]	156 (47)	126 (38)	30 (9)	120 (36)	100 (30)	20 (6)	57 (17)	11 (3)	35 (11)	11 (3)
MRSA (*n* = 51)	26 (51)	15 (29)	11 (22)	11 (22)	9 (19)	2 (3)	14 (27)	5 (10)	7 (14)	2 (3)
pyogenic streptococci (*n* = 57)[b]	38 (67)	32 (56)	6 (11)	15 (26)	15 (26)	0	4 (7)	3 (5)	1 (2)	0
viridans streptococci (*n* = 41)[c]	12 (29)	10 (24)	2 (5)	23 (56)	22 (54)	1 (2)	6 (15)	0	6 (15)	0
Enterococcus (*n* = 14)[d]	5 (36)	3 (22)	2 (14)	8 (57)	7 (50)	1 (7)	1 (7)	0	0	1 (7)
GNB (*n* = 118)[e]	44 (37)	34 (28)	10 (9)	51 (44)	40 (35)	11 (9)	23 (19)	11 (9)	10 (8)	2 (2)

Note: Data presented are number of cases (percentage); cases with multiple OAI (*n* = 36) are included.
Abbreviations: POM, peripheral osteomyelitis; DM foot, diabetic foot infections; POM-no OS, peripheral osteomyelitis without osteosynthesis; OS, osteosynthesis; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; pyogenic streptococci, *Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus pneumoniae*; GNB, Gram-negative bacilli.
[a]Differences between MSSA and MRSA, p 0.03 (chi-square)
[b]Differences between *S. aureus* (All) and pyogenic streptococci (All), p 0.02 (chi-square)
[c]Differences between *S. aureus* (All) and *viridans* streptococci (All), p 0.02 (chi-square)
[d]No significant differences between *S. aureus* (All) and *Enterococcus* sp. (All)
[e]No significant differences between *S. aureus* (All) and GNB (All).

with staphylococcal OAI observed over time; and also, our results show the predominance of streptococcal and enterococcal OAI in the group of older patients. Finally, these changes in the Gram-positive microbiology of OAI may also be associated with the differences in the types of OAI observed over time. Hence, our results support the higher affinity of *viridans* streptococci and *Enterococcus* species for causing vertebral osteomyelitis, or that of pyogenic streptococcus strains for causing arthritis. These results seem to corroborate those of previous authors [27–29], but further studies addressing this specific field are required to confirm them.

Regarding the world-wide emergence of multidrug-resistant Gram-negative bacilli [30,31], we have witnessed only anecdotal episodes in the last 10 years. These data are surprising because drug-resistant Gram-negative bacterial infections, including bacteraemia, have been described in our country and elsewhere [30,32]. It is beyond the scope of our work to identify why these microorganisms did not cause OAI, but we may hypothesize that they will emerge in the future. In addition, we note that the low rate of polymicrobial and anaerobic OAI of our work should be interpreted taken into account that we included only bacteraemic infections and so, both entities can be underestimated.

The present study has some limitations that are inherent to observational studies, but in any case it offers a global perspective of a great number of OAI over a long period of

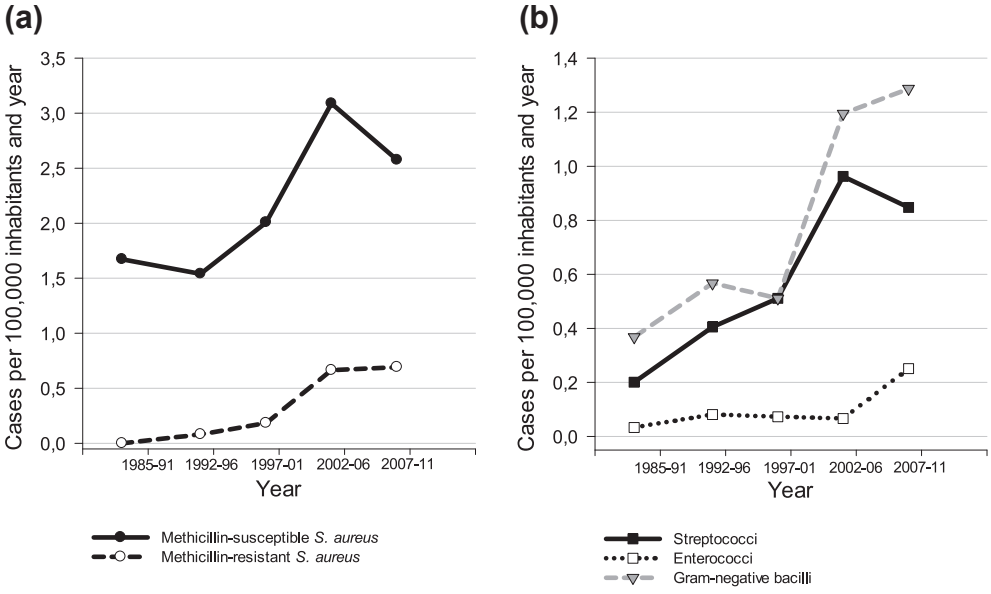


FIG. 2. Incidence of the more frequent microorganisms responsible for bacteraemic osteoarticular infections in the study period. (a) Incidence of cases caused by methicillin-susceptible and -resistant *Staphylococcus aureus*. (b) Incidence of cases caused by *Streptococcus* sp., *Enterococcus* sp. and Gram-negative bacilli.

TABLE 4. Clinical and microbiological characteristics of cases of osteoarticular infections according to patients' age: Group 1 (age ≤ 49 years), Group 2 (age 50–64 years), and Group 3 (age ≥ 65 years)

	Group 1 *n* = 149	Group 2 *n* = 178	p value (1 vs 2)	Group 3 *n* = 274	p value (2 vs 3)	p value (1 vs 3)
One or more underlying disease	24 (16)	106 (60)	**<0.001**	177 (65)	0.3	**<0.001**
Intravenous drug users	56 (38)	1 (1)	**<0.001**	0	0.2	**<0.001**
Place of acquisition						
Community	108 (72)	92 (52)		134 (49)		
Health-care	10 (7)	35 (20)		54 (20)		
Nosocomial	31 (21)	51 (29)	**<0.001**	85 (31)	0.8	**<0.001**
Device-related OAI[a]	19 (13)	26 (15)	0.6	68 (25)	**0.009**	**0.003**
Arthritis	76 (51)	82 (46)	0.3	133 (48)	0.6	0.6
Native	74 (50)	72 (40)	0.09	82 (30)	**0.02**	**<0.001**
Prosthetic	2 (1)	10 (6)	**0.04**	51 (18)	**<0.001**	**<0.001**
Vertebral osteomyelitis	37 (25)	66 (37)	**0.02**	102 (37)	0.9	**0.01**
Spine instrumentation	9 (6)	16 (9)	0.3	10 (4)	**0.02**	0.2
Peripheral osteomyelitis	37 (25)	27 (15)	**0.03**	40 (15)	0.9	**0.009**
Staphylococcus aureus	111 (75)	104 (58)	**0.002**	153 (56)	0.6	**<0.001**
methicillin-susceptible *S. aureus*	108 (73)	90 (50)	**<0.001**	120 (44)	0.2	**<0.001**
methicillin-resistant *S. aureus*	3 (2)	14 (8)	**0.02**	32 (12)	0.2	**0.001**
Pyogenic streptococci	8 (5)	13 (7)	0.4	28 (10)	0.3	0.09
Viridans streptococci	3 (2)	16 (9)	**0.007**	20 (7)	0.5	**0.02**
Enterococcus sp.	2 (1)	1 (1)	0.4	12 (4)	**0.02**	0.09
Gram-negative bacilli	20 (13)	39 (22)	**0.047**	53 (19)	0.5	0.1

[a]Device-related OAI include infections of prosthetic joint, spine instrumentation and osteomyelitis with osteo-synthesis hardware.

time. It should be noted that our study refers only to the incidence of bacteraemic OAI and we do not know the overall incidence of OAI, including bacteraemic and non-bacteraemic cases; therefore in the present report the incidence of overall OAI has been underestimated. Indeed, the increased incidence of bacteraemic OAI in our study could be due to either a greater number of patients with OAI or to a higher prevalence of bacteraemia among patients with OAI.

In conclusion, we have observed an increased number of bacteraemic OAI over the last three decades in the setting of an aging population with more chronic diseases. Cases associated with orthopaedic devices have increased, and the rates of nosocomially acquired and healthcare-related cases have also risen. *Staphylococcus aureus* remains the most frequent cause of OAI, but we stress the emergence of infections by MRSA and the increase of streptococcal and enterococcal episodes. Overall, these significant epidemiological changes reflect the pattern of development of OAI for the early twenty-first century, which is likely to involve older patients with several comorbidities, particular types of OAI, and specific microorganisms with a predominance of multidrug-resistant bacteria.

Transparency declaration

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III—co-financed by European Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015).

It was presented in part at the 23rd European Congress of Clinical Microbiology and Infectious Diseases, Berlin 27–30 April 2013 (oral session O-286).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2014.09.007.

References

[1] Lew DP, Waldvogel FA. Osteomyelitis. Lancet 2004;364:369–79.
[2] Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. Lancet 2010;375:846–55.
[3] Sapico FL, Liquete JA, Sarma RJ. Bone and joint infections in patients with infective endocarditis: review of a 4-year experience. Clin Infect Dis 1996;22:783–7.
[4] Zimmerli W. Clinical practice. Vertebral osteomyelitis. N Engl J Med 2010;362:1022–9.
[5] Carragee EJ. Pyogenic vertebral osteomyelitis. J Bone Joint Surg Am 1997;79:874–80.
[6] Lora-Tamayo J, Euba G, Narvaez JA, Murillo O, Verdaguer R, Sobrino B, et al. Changing trends in the epidemiology of pyogenic vertebral osteomyelitis: the impact of cases with no microbiologic diagnosis. Semin Arthritis Rheum 2011;41:247–55.
[7] Kurtz S, Mowat F, Ong K, Chan N, Lau E, Halpern M. Prevalence of primary and revision total hip and knee arthroplasty in the United States from 1990 through 2002. J Bone Joint Surg Am 2005;87:1487–97.
[8] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645–54.

[9] Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 2003;36:53–9.

[10] Gasch O, Ayats J, Angeles Dominguez M, Tubau F, Liñares J, Peña C, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection: secular trends over 19 years at a university hospital. Medicine (Baltimore) 2011;90:319–27.

[11] Lye DC, Earnest A, Ling ML, Lee TE, Yong HC, Fisher DA, et al. The impact of multidrug resistance in healthcare-associated and nosocomial Gram-negative bacteraemia on mortality and length of stay: cohort study. Clin Microbiol Infect 2012;18:502–8.

[12] Seng P, Abat C, Rolain JM, Colson P, Lagier JC, Gouriet F, et al. Identification of rare pathogenic bacteria in a clinical microbiology laboratory: impact of matrix-assisted laser desorption ionization-time of flight mass spectometry. J Clin Microbiol 2013;51:2182–94.

[13] Walter G, Vernier N, Pinelli PO, Million M, Coulange M, Seng P, et al. Bone and joint infections due to anaerobic bacteria: an analysis of 61 cases and review of the literature. Eur J Clin Microbiol Infect Dis 2014;33:1355–64.

[14] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard. 8th ed. Wayne, PA: CLSI; 2009M7–A8.

[15] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.

[16] Shirtliff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev 2002;15:527–44.

[17] Sherman JM. The Streptococci. Bacteriol Rev 1937;1:3–97.

[18] Li JS, Sexton DJ, Mick N, Nettles R, Fowler Jr VG, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30:633–8.

[19] Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 2002;137:791–7.

[20] Institut d'Estadística de Catalunya Webside. Available online: http://www.idescat.net. [accessed 07.03.12].

[21] Mader JT, Shirtliff ME, Bergquist S, Calhoun JH. Bone and joint infections in the elderly: practical treatment guidelines. Drugs Aging 2000;16:67–80.

[22] Kak V, Chandrasekar PH. Bone and joint infections in injection drug users. Infect Dis Clin North Am 2002;16:681–95.

[23] Ross JJ, Shamsuddin H. Sternoclavicular septic arthritis: review of 180 cases. Medicine (Baltimore) 2004;83:139–48.

[24] Darouiche RO. Treatment of infections associated with surgical implants. N Engl J Med 2004;350:1422–9.

[25] Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007;89:780–5.

[26] Micek ST, Kollef KE, Reichley RM, Roubinian N, Kollef MH. Health care-associated pneumonia and community-acquired pneumonia: a single-center experience. Antimicrob Agents Chemother 2007;51:3568–73.

[27] Murillo O, Roset A, Sobrino B, Lora-Tamayo J, Verdaguer R, Jimenez-Mejias E, et al. Streptococcal vertebral osteomyelitis: multiple faces of the same disease. Clin Microbiol Infect 2013;20:O33–8.

[28] Pigrau C, Almirante B, Flores X, Falco V, Rodriguez D, Gasser I, et al. Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med 2005;118:1287.

[29] Mulleman D, Philippe P, Senneville E, Costes C, Fages L, Deprez X, et al. Streptococcal and enterococcal spondylodiscitis (vertebral osteomyelitis). High incidence of infective endocarditis in 50 cases. J Rheumatol 2006;33:91–7.

[30] Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med 2010;362:1804–13.

[31] Ho J, Tambyah PA, Paterson DL. Multiresistant Gram-negative infections: a global perspective. Curr Opin Infect Dis 2010;23:546–53.

[32] Rodriguez-Baño J, Lopez-Prieto MD, Portillo MM, Retamar P, Natera C, Nuño E, et al. Epidemiology and clinical features of community-acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals. Clin Microbiol Infect 2010;16:1408–13.

Infection (2018) 46:245–251
https://doi.org/10.1007/s15010-018-1121-9

ORIGINAL PAPER

Endocarditis associated with vertebral osteomyelitis and septic arthritis of the axial skeleton

Oscar Murillo[1] · Imma Grau[1] · Joan Gomez-Junyent[1] · Celina Cabrera[1] · Alba Ribera[1] · Fe Tubau[2,3] · Carmen Peña[1] · Javier Ariza[1] · Roman Pallares[1]

Received: 28 July 2017 / Accepted: 27 January 2018 / Published online: 2 February 2018

Abstract

Purpose The relationship between infective endocarditis (IE) and osteoarticular infections (OAIs) are not well known. We aimed to study the characteristics of patients with IE and OAIs, and the interactions between these two infections.

Methods An observational study (1993–2014) which includes two cohorts: (1) patients with IE ($n = 607$) and (2) patients with bacteremic OAIs ($n = 458$; septic arthritis of peripheral and axial skeleton, and vertebral and peripheral osteomyelitis). These two cohorts were prospectively collected, and we retrospectively reviewed the clinical and microbiological variables.

Results There were 70 cases of IE with concomitant OAIs, representing 11.5% of IE cases and 15% of bacteremic OAI cases. Among cases with IE, the associated OAIs mainly involved the axial skeleton ($n = 54$, 77%): 43 were vertebral osteomyelitis (61%), mainly caused by "less virulent" bacteria (*viridans* and *bovis* streptococci, enterococci, and coagulase-negative staphylococci), and 15 were septic arthritis of the axial skeleton (21%), which were mainly caused by *Staphylococcus aureus*. OAIs with involvement of the axial skeleton were associated with IE (adjusted OR = 2.2; 95% CI 1.1–4.3) independently of age, sex, and microorganisms.

Conclusions Among patients with IE, the associated OAIs mainly involve the axial skeleton. Transesophageal echocardiography should be carefully considered in patients presenting with these bacteremic OAIs.

Keywords Endocarditis · Osteoarticular infections · Bacteremia · Septic arthritis · Axial skeleton · Vertebral osteomyelitis

Introduction

Infective endocarditis (IE) produces continuous bacteremia, and may secondarily cause metastatic bone and joint infections [1, 2]. However, some studies have described osteoarticular symptoms such as arthralgia related to immunological disorders associated with IE [3–5], while others have reported truly pyogenic osteoarticular infections (OAIs) and have focused on the particular association between IE and vertebral osteomyelitis [6, 7].

As the prevalence of metastatic OAIs in patients with IE and the prevalence of IE in patients with OAIs are considered low, the particularities of this relationship are not well known. Some previous reports emphasized the role of *S. aureus* etiology in cases of IE with metastatic OAI among intravenous drug users (IDUs) [1, 8, 9], and more recent studies have highlighted the association between streptococcal OAIs with concomitant IE in non-drug users [10, 11]. However, with regard to the notable epidemiologic changes in OAIs in recent years, their impact on the overall prevalence of OAIs and IE is not well explored [12].

The aim of this study, which includes a large number of patients (without IDUs), was to analyze the clinical, epidemiological and microbiological characteristics of bacteremic OAI (septic arthritis and vertebral and peripheral osteomyelitis) associated with the presence of IE.

✉ Oscar Murillo
omurillo@bellvitgehospital.cat

[1] Infectious Diseases Department, IDIBELL-Hospital Universitari de Bellvitge, University of Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

[2] Microbiology Department, IDIBELL-Hospital Universitari de Bellvitge, Feixa Llarga s/n, 08907 Hospitalet de Llobregat, Barcelona, Spain

[3] Spain Ciber de Enfermedades Respiratorias ISCIII, Madrid, Spain

Methods

Setting and study design

This observational study was carried out at Hospital Bellvitge, a teaching hospital in Barcelona, Spain. The hospital has a Bone and Joint Infection Unit, run by a multidisciplinary medical team comprising orthopedic surgeons, infectious disease specialists, rheumatologists and microbiologists. Our institution does not attend pediatric, obstetric or burn patients.

At our institution, all cases with IE (group 1) were prospectively studied and followed-up at our hospital between 1993 and 2014. The presence of metastatic osteoarticular infection was specifically recorded and these cases were retrospectively analyzed.

In addition, all patients with bloodstream infection were recorded in a prospective database including patients' baseline characteristics, clinical presentation and source of bacteremia, microbiological data, and outcome. For the present study, all patients with bacteremic OAIs (group 2) attending at our institution during the study period (1993–2014) were extracted from the above-mentioned protocol and retrospectively analyzed.

Patients' characteristics and definitions

Cases of IE included in the study were diagnosed using the initial Duke criteria and its modified criteria [13]. Among them, cases with negative blood cultures, unknown or fungal etiology, or those with etiologic diagnosis obtained by serologic test (e.g., *Coxiella* sp., *Bartonella* sp.) were not included in the present study. The concomitant diagnosis of IE and OAI was considered as metastatic OAI; in fact, this bacteremic OAI was assumed to be due to IE. Transthoracic echocardiography was performed in all cases, and the transesophageal echocardiography was performed if deemed appropriate by clinicians to confirm diagnosis of IE (especially from the year 2000 and onwards).

The study also included patients with bacteremia and concomitant OAI, in whom the OAI was assumed to have been acquired hematogenously. Thus, postsurgical cases of bacteremic OAI and diabetic foot infections with concomitant bacteremia, which present a different pathogenic way of acquisition, were excluded. Cases of bacteremia caused by *Neisseria sp*. were not considered to avoid cases with reactive arthritis. Also, bacteremic OAIs and IE occurring in IDUs were excluded due to the particularities of this association (almost all cases are caused by *S. aureus* and mainly involve certain locations such as the sacroiliac), and the decline of this population in recent years. [8, 9]. [12].

Blood samples were processed according to standard recommendations by the automated BacTEC method with both aerobic and anaerobic media (current Bactec FX system, Becton–Dickinson Microbiology Systems, USA). Identification of microorganisms and their antibiotic susceptibility were performed by using standard biochemical reactions, disk-diffusion or microdilution methods, and the MicroScan system (Dade Behring, West Sacramento, CA, USA). Antimicrobial susceptibility was defined according to previous NCCLS and current CLSI criteria [14]. In accordance with the Lancefield and Sherman classifications, *Streptococcus* species were divided into pyogenic (*S. pyogenes*, *S. agalactiae*, and *S. pneumoniae*), *S. bovis*, and *viridans* streptococci (*S. milleri*, along with the remaining species) [15].

OAIs were classified in three different categories: (i) septic arthritis (SA), including native SA and prosthetic joint infections; (ii) vertebral osteomyelitis; and (iii) peripheral osteomyelitis (non-vertebral osteomyelitis). Joint involvement was classified as "peripheral" (joints of the appendicular skeleton) and "axial" (those forming part of the axis of the skeleton: acromioclavicular, sternoclavicular, sternocostal, pubic symphysis, interapophyseal, and sacroiliac). For the particular case of interapophyseal location, it was diagnosed and differentiated from vertebral osteomyelitis with the wide use of MRI (so, these cases were diagnosed especially from the year 2000 and onwards).

All OAI cases included had bacteremia and met the main diagnostic criteria for each type of OAI [16–19]. According to our protocol, we consider patients with a short history of a warm, swollen and tender joint to have SA until proven otherwise, an arthrocentesis is routinely performed to obtain synovial fluid samples for microbiological analyses and a pair of blood cultures is taken; in cases in which the process affects joints with difficult access (i.e., the axial skeleton) only blood cultures are initially taken. In addition, prosthetic joint infection was defined by the isolation of a pathogenic microorganism from two or more surgical, joint-aspirated or blood cultures, or by one such positive culture plus the presence of typical signs and clinical symptoms (inflammatory signs, the presence of a sinus tract or purulence around the prosthesis during surgery). Vertebral and peripheral osteomyelitis was diagnosed by the presence of a compatible clinical picture (back pain and motion limitation for the former and inflammatory signs and/or prolonged sinus drainage for the latter) and characteristic imaging findings (computed tomography or magnetic resonance imaging). The microbiology of OAIs was always identified by blood samples, and in most cases by additional local samples obtained from the affected joint or bone. For the particular case of coagulase-negative *staphylococci* (CoNS), and in order to exclude potential contaminated cultures, all cases had at least four blood cultures showing the same microorganism (in accordance with the susceptibility pattern) and

accomplished the respective diagnostic criteria. Computed tomography or magnetic resonance imaging was carried out in all cases of SA of the axial skeleton and osteomyelitis (vertebral and peripheral).

Statistical analysis

Data were analyzed using SPSS (version 20.0). Continuous variables are expressed as mean ± SD or median and range, according to normality tests; categorical variables are expressed as counts and valid percentages. Comparative analyses were performed with X^2 or Fisher's test for categorical variables, and the Mann–Whitney U-test for continuous variables. Univariate and multivariate analyses of parameters predicting IE were performed by logistic regression, and adjusted OR and 95% CI are shown. All tests were two-tailed, and a p value < 0.05 was considered statistically significant.

Results

During the period of study (1993–2014), there were 607 cases of IE. The most frequent etiologies were: *S. viridans* group ($n = 181$, 30%), *S. aureus* ($n = 123$, 20%; 20 cases caused by methicillin-resistant strains), CoNS ($n = 96$, 16%), enterococci ($n = 94$, 15%), *S. bovis group* ($n = 64$, 10%), and others ($n = 49$, 9%). The proportion of metastatic OAIs (70 cases) among patients with IE was 11.5% (70/607 cases); the microorganisms responsible were *S. aureus* (27/123, 22%), enterococci (10/94, 11%), *S. viridans* (16/181, 9%), *S. bovis* group (6/64, 9%), and CoNS (6/96, 6%). The type of OAI observed in these patients (see below) had involvement of the axial skeleton in 77% of cases (vertebral osteomyelitis, septic arthritis or both). Patients with IE and associated OAIs, compared with those without OAIs, were older, had less frequently cardiac predisposing factors, aortic valve was less commonly affected, had fewer prosthetic valves, and were caused more often by *S. aureus* (Table 1).

During the same period, there were 458 cases of bacteremic OAI (36 had multiple OAI): vertebral osteomyelitis ($n = 202$), peripheral SA ($n = 175$), SA of the axial skeleton ($n = 67$), and peripheral osteomyelitis ($n = 50$). Seventy out of 458 OAIs (15%) were associated with IE. Table 2 shows the microorganisms causing OAIs and the proportion with concomitant IE. This proportion differed according to the type of OAI: 22% (15/67) for SA of the axial skeleton, 21% (43/202) for vertebral osteomyelitis, 11% (19/175) for peripheral SA, and 4% (2/50) for peripheral osteomyelitis. Moreover, Fig. 1 shows the proportion of different OAIs for

Table 1 Characteristics of patients with infective endocarditis (IE) comparing those with and without osteoarticular infections (OAIs)

	IE with OAI, $n = 70$	IE without OAI, $n = 537$	*P* value
Age (median, IQR)	68 (59–74)	65 (54–74)	0.1
Sex (male)	50 (71%)	347 (65%)	0.9
Cardiac predisposing factor for IE[a]	30 (43%)	333 (62%)	**0.001**
Additional emboli (other than OAI)[b]	14 (20%)	134 (25%)	0.3
Valvular location			
Mitral valve	42 (60%)	270 (50%)	0.1
Aortic valve	29 (41%)	301 (56%)	**0.02**
Tricuspid valve	5 (7%)	17 (3%)	0.1
Prosthetic valve	11 (16%)	167 (31%)	**0.002**
Presence of vegetation	46 (66%)	331 (62%)	0.4
Positive blood cultures (≥ 4)	41 (59%)	305 (57%)	0.2
Microorganisms			
S. aureus	27 (39%)	96 (18%)	**<0.001**
Coagulase-negative *staphylococci*	6 (9%)	90 (17%)	0.1
S. viridans	16 (23%)	165 (31%)	0.5
S. bovis	6 (9%)	58 (11%)	0.8
Enterococci	10 (14%)	84 (16%)	0.7
Pyogenic *streptococci* #	3 (4%)	20 (4%)	0.9
Others	2 (3%)	24 (4%)	0.9

[a]Includes degenerative or rheumatic valvulopathy, mitral prolapse, bivalve aorta, congenital valvulopathy, previous IE, or prosthetic valve

[b]Includes emboli to brain, spleen, vascular peripheral artery or kidneys, or presence of Roth spots

Pyogenic *streptococci*: *S. pneumoniae*, *S. pyogenes* and *S. agalactiae*

$P < 0.05$ (bold values) was considered statistically significant

Table 2 Comparison between the presence or not of infective endocarditis (IE) among cases of bacteremic osteoarticular infections (OAIs) according to causative microorganisms

Microorganisms (no. of bacteremic OAIs)	Presence of IE		*P* value
	Yes	No	
S. aureus (*n* = *254*)	27 (11)	227 (89)	**0.002**
Coagulase-negative Staphylococcus (*n* = *17*)	6 (35)	11 (65)	**0.02**
S. viridans group (*n* = *37*)	16 (43)	21 (56)	**0.001**
S. bovis (*n* = *9*)	6 (67)	3 (33)	**< 0.001**
Enterococcus (*n* = *11*)	10 (91)	1 (9)	**< 0.001**
Pyogenic streptococci (*n* = *56*)	3 (5)	52 (95)	**0.03**
Gram-negative bacilli (*n* = *74*)	1 (1)	73 (99)	**< 0.001**

Data presented are number of cases (percentage)

$P < 0.05$ (bold values) was considered statistically significant

each microorganism. The ratio of vertebral osteomyelitis vs peripheral SA for each microorganism was as follows: 0.6 for pyogenic *streptococci*, 0.99 for *S. aureus*, 1.2 for Gram-negative bacilli, 2 *S. bovis*, 2.4 viridans *streptococci,* 4.3 coagulase-negative *staphylococci*, and 8 *enterococci*.

Analyzing the types of OAIs and their relationship with the presence of IE (Table 3), we observed that *S. aureus* was the main agent responsible for the association with SA of the axial skeleton, whereas the *viridans streptococci*, *S. bovis, enterococci* and CoNS were mainly responsible for the association with vertebral osteomyelitis. Particularly, there were 17 cases of bacteremic OAI caused by CoNS (13 vertebral osteomyelitis, 76%; and 4 SA, one prosthetic joint infection); 6 of them had IE (5 native and 1 prosthetic valve) and 9 cases had peripheral or central venous catheter. Pyogenic streptococci also caused a large number of SA of the axial skeleton (15/67, 22%), but only a few cases of IE (3/607, 0.5%, all these cases had involvement of the axial skeleton).

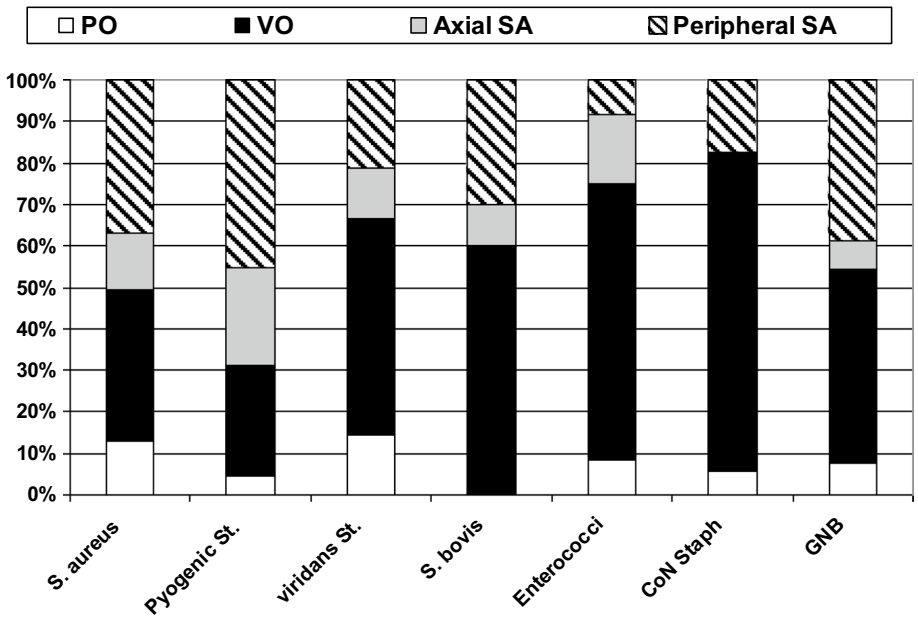


Fig. 1 Proportion of different types of bacteremic osteoarticular infection according to causative microorganism. *PO* peripheral osteomyelitis, *VO* vertebral osteomyelitis, *SA* septic arthritis, *CoN Staph* coagulase-negative staphylococci, *GNB* Gram-negative bacilli

Table 3 Types of bacteremic osteoarticular infections (OAIs) caused by *S. aureus* compared with *S. viridans* group, *S. bovis*, *enterococci* and coagulase-negative *staphylococci* according to the presence or absence of infective endocarditis (IE)

Bacteremic OAI	*S. aureus* (*n* = 254)			*S. viridans*, *S. bovis*, *Enterococcus* and coagulase-negative *staphylococci* (*n* = 73)		
	With IE (*n* = 27)	Without IE (*n* = 227)	*P* value	With IE (*n* = 38)	Without IE (*n* = 35)	*P* value
Septic arthritis (SA; all)	16 (59)	120 (53)	0.5	11 (29)	11 (31)	0.8
SA of the axial skeleton	8 (30)	29 (13)	**0.02**	5 (13)	3 (9)	0.5
Peripheral SA	11 (41)	89 (39)	0.9	7 (18)	9 (26)	0.4
Vertebral osteomyelitis	10 (37)	89 (39)	0.8	30 (79)	19 (54)	**0.02**
Peripheral osteomyelitis	2 (7)	33 (14)	0.3	0	8 (23)	**0.002**

$P < 0.05$ (bold values) was considered statistically significant

Table 4 Comparison between bacteremic osteoarticular infection (OAI) cases with or without concomitant infective endocarditis (IE)

	OAI with IE, $n = 70$	OAI without IE, $n = 388$	*P* value	Unadjusted OR (95% CI)
Age (median, IQR)	68 (59–74)	67 (55–77)	0.8	
Sex (male)	50 (71)	236 (61)	0.09	
Type and location of OAI[a]				
All OAIs of the axial skeleton	54 (77)	203 (52)	**< 0.001**	3.1 (1.7–5.6)
Vertebral osteomyelitis	43 (61)	159 (41)	**0.002**	2.3 (1.4–3.9)
Septic arthritis of the axial skeleton	15 (21)	52 (13)	0.08	1.8 (0.9–3.3)
Peripheral septic arthritis	19 (27)	156 (40)	**0.04**	0.6 (0.3–1)
Peripheral osteomyelitis	2 (3)	48 (12)	**0.02**	0.2 (0.05–0.9)
Microbiology				
S. aureus	27 (39)	227 (58)	**0.002**	0.4 (0.3–0.7)
CoNS	6 (9)	11 (3)	**0.02**	3.2 (1.1–9)
S. viridans	16 (23)	20 (5)	**< 0.001**	5.4 (2.7–11.2)
S. bovis and enterococci	16 (23)	4 (1)	**< 0.001**	28.4 (9.2–88.2)
Pyogenic streptococci	3 (4)	52 (13)	**0.03**	0.3 (0.1–0.9)
Gram-negative bacilli	1 (1)	72 (19)	**< 0.001**	0.06 (0.01–0.5)
30-day mortality	9 (13)	44 (11)	0.7	

[a]Among OAI cases with IE, five presented with concomitant vertebral osteomyelitis and septic arthritis (two septic arthritis of the axial skeleton + peripheral septic arthritis; two septic arthritis of the axial skeleton; one peripheral septic arthritis); three cases of septic arthritis presented concomitant involvement of the peripheral and axial skeletons

$P < 0.05$ (bold values) was considered statistically significant

A comparative analysis of bacteremic OAI cases with or without IE (univariate analysis) is presented in Table 4. The main parameters associated with IE were: OAIs with involvement of the axial skeleton (OR 3.1), vertebral osteomyelitis (OR 2.3), and several etiologies such as CoNS (OR 3.2), *S. viridans* group (OR 5.4), and *enterococci* and *S. bovis* (OR 28.4). The OAI with involvement of the axial skeleton was associated with IE (adjusted OR = 2.2; 95% CI 1.1–4.3) after adjusting for age, sex and microorganisms.

Discussion

The association of IE with OAI has been reported previously, but several questions remain to be clarified. In this study, we analyze in a large series of cases the association of IE and bacteremic OAI. Previous reports emphasized the role of young IDUs, who mainly presented staphylococcal infection, and a predominance of septic arthritis at certain sites such as sacroiliac joint [8, 9]. Due to the decline in IDUs in recent years, they represent only a small percentage of recent OAI cases [12]. Taking these particularities into account, we decided to analyze the association of IE and bacteremic OAIs among the non-IDU population in more detail.

In accordance with previous studies, our results show that Gram-positive cocci, that is, *S. aureus* and the group of "less virulent" microorganisms such as *viridans streptococci*, *S. bovis*, *enterococci*, and CoNS, have a particular affinity for causing IE (above 90% in our series) [20, 21]. Although continuous bacteremia occurs in almost all cases of IE, we observed differences between microorganisms in their capacity to produce metastatic OAI, with *S. aureus* being the most prevalent. Of note, the aortic valve was less commonly affected in patients with IE and associated OAIs, this fact being in the line with previous studies analyzing the occurrence of other embolic events.

We also found that the microorganisms presented different capacities for causing a particular type of bacteremic OAI. Thus, *S. aureus*, pyogenic streptococci and Gram-negative bacilli were able to produce either peripheral SA or vertebral osteomyelitis in a similar proportion, but the group of "less virulent" microorganisms (*S. viridans*, *S. bovis, enterococci* and CoNS strains) were between two and eight times more likely to cause vertebral osteomyelitis. Taking all these data together, the association of IE and bacteremic OAI presented some particular features that deserve further discussion. The prevalence of IE as a source of bacteremic OAI has been reported to range from ratios as low as < 5–10% to as high as 25–30% when analyzing a particular OAI such as vertebral osteomyelitis [6, 10, 11, 22]. In a large series of all types of bacteremic OAI reported here, the presence of a concomitant diagnosis of IE was 15%, but it clearly differed between types of OAI. The highest incidence of IE was among OAIs with involvement of the axial skeleton (SA of the axial skeleton in 22% of cases and vertebral osteomyelitis in 20%). In fact, we observed that most OAIs associated with IE had involvement of the axial skeleton (77%).

Regarding microbiology, *S. aureus* was the most frequent etiology of metastatic OAI associated with IE, but the whole group of "less virulent" microorganisms that included *S. viridans*, *S. bovis, enterococci* and coagulase-negative staphylococci strains caused a greater percentage of these cases. Previous studies have reported the association between vertebral osteomyelitis and IE, especially in the case of streptococcal etiology [11, 12, 23]. In the present study, we confirmed that this group of less virulent microorganisms was responsible for this association. In fact, we showed that when these microorganisms caused a bacteremic OAI, they almost exclusively produced vertebral osteomyelitis, and this was mainly caused by the existence of IE that may facilitate continuous bacteremia. In fact, we consider that these streptococcal, enterococcal and coagulase-negative staphylococcal microorganisms, all of which have affinity for causing IE, also have a great ability for causing vertebral osteomyelitis for reasons that are not well understood.

S. aureus can cause all types of bacteremic OAIs. It is known that this microorganism has a mixture of virulence factors that facilitate either the adherence or the invasion of several tissues [24, 25]. Therefore, *S aureus* is able to produce IE, like the group of less virulent microorganisms mentioned, but it also caused all types of OAIs regardless of the presence or absence of IE. In fact, bacteremic OAI caused by *S. aureus* was significantly more frequent in the absence of concomitant IE. Overall, the contrast between OAIs caused by *S. aureus* and less virulent microorganisms illustrates that particular types of OAIs are less likely to be produced by some bacteria. Interestingly, our results support the notion that the main relationship between staphylococcal OAIs and IE was established with the presence of SA of axial skeleton. In our experience, *S. aureus*, together with pyogenic streptococcus, is responsible for SA involving the axial skeleton [12, 26]. While *S. aureus* caused a large number of both OAI and IE, pyogenic streptococci only rarely produced IE, but in all these cases the OAI involved the axial skeleton. There is little previous information about SA of the axial skeleton, and the few studies available have reported its higher predominance among IDUs [27–29]. The results of our study (which did not include the IDU population) suggest that, in the presence of staphylococcal bacteremia affecting the axial skeleton (especially in the case of SA of the axial skeleton) the concomitant presence of IE should be ruled out.

The association of IE and OAI with involvement of the axial skeleton was observed regardless of the microorganisms responsible or the sex and age of patients. It seems that some particular features of bone and joints that change throughout life, the characteristics of the continuous low-grade bacteremia of IE, and the differences observed in the osteoarticular tropism of microorganisms may explain this association.

The present study has some limitations that are inherent to long-term observational studies, some of them may be in relation with the availability of more sophisticated technologies in recent years (such as MRI or transesophageal echocardiography). The misdiagnosis of IE among bacteremic OAI cases or of OAIs among IE cases should be kept to minimum, since cases included in both groups were prospectively followed by infectious diseases specialists in our hospital. Finally, it should be noted that our study refers only to bacteremic OAIs and thus, the non-bacteremic cases that can be associated with IE were not included. In any case, we think it may offer some new insights regarding the interaction of IE and OAIs.

In conclusion, bacteremic OAIs of the axial skeleton (vertebral osteomyelitis and septic arthritis) were associated with the presence of concomitant IE. While *S. aureus* was the most frequent etiology of all types of metastatic OAIs in association with IE, it was the main agent responsible for the SA of the axial skeleton. In contrast, the group of "less virulent" microorganisms (*S. viridans*, *S. bovis, enterococci* and CoNS) was more prevalent in cases of vertebral osteomyelitis associated with IE. From a practical point of view, the performance of transesophageal echocardiography should be strongly considered in patients presenting with bacteremic vertebral osteomyelitis and SA of the axial skeleton.

Acknowledgements We thank Michael Maudsley for helping with the English in this manuscript.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The institutional review board approved this study and publication of the results. The need for informed consent was waived as the study analysed data retrospectively and the data were anonymized.

References

1. Sapico FL, Liquete JA, Sarma RJ. Bone and joint infections in patients with infective endocarditis: review of a 4-year experience. Clin Infect Dis. 1996;22:783–7.
2. Mansur AJ, Grinberg M, da Luz PL, Bellotti G. The complications of infective endocarditis. A reappraisal in the 1980s. Arch Intern Med. 1992;152:2428–32.

3. Sapico FL, Montgomerie JZ. Pyogenic vertebral osteomyelitis: report of nine cases and review of the literature. Rev Infect Dis. 1979;1:754–76.
4. Churchill MA, Geraci JE, Hunder GG. Musculoskeletal manifestations of bacterial endocarditis. Ann Intern Med. 1977;87:754–9.
5. González-Juanatey C, González-Gay MA, Llorca J, et al. Rheumatic manifestations of infective endocarditis in non-addicts. A 12-year study. Medicine (Baltimore). 2001;80:9–19.
6. Pigrau C, Almirante B, Flores X, et al. Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med. 2005;118:1287.
7. Le Moal G, Roblot F, Paccalin M, et al. Clinical and laboratory characteristics of infective endocarditis when associated with spondylodiscitis. Eur J Clin Microbiol Infect Dis. 2002;21:671–5.
8. Sapico FL, Montgomerie JZ. Vertebral osteomyelitis in intravenous drug abusers: report of three cases and review of the literature. Rev Infect Dis. 1980;2:196–206.
9. Scheidegger C, Zimmerli W. Infectious complications in drug addicts: seven-year review of 269 hospitalized narcotics abusers in Switzerland. Rev Infect Dis. 1989;11:486–93.
10. Murillo O, Roset A, Sobrino B, et al. Streptococcal vertebral osteomyelitis: multiple faces of the same disease. Clin Microbiol Infect. 2014;20:O33–8.
11. Mulleman D, Philippe P, Senneville E, et al. Streptococcal and enterococcal spondylodiscitis (vertebral osteomyelitis). High incidence of infective endocarditis in 50 cases. J Rheumatol. 2006;33:91–7.
12. Murillo O, Grau I, Lora-Tamayo J, et al. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. Clin Microbiol Infect. 2015;21:254.e1–8.
13. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633–8.
14. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard, 8th ed, M7-A8 CLSI, Wayne; 2009.
15. Sherman JM. The streptococci. Bacteriol Rev. 1937;1:3–97.
16. Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004;364:369–79.
17. Shirtliff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev. 2002;15:527–44.
18. Zimmerli W. Clinical practice. Vertebral osteomyelitis. N Engl J Med. 2010;362:1022–9.
19. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351:1645–54.
20. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation. 2015;132:1435–86.
21. Habib G, Lancellotti P, Antunes MJ, et al. 2015 ESC guidelines for the management of infective endocarditis. Eur Heart J. 2015;36:3075–128.
22. Lamas C, Bóia M, Eykyn SJ. Osteoarticular infections complicating infective endocarditis: a study of 30 cases between 1969 and 2002 in a tertiary referral centre. Scand J Infect Dis. 2006;38:433–40.
23. Weber M, Gubler J, Fahrer H, et al. Spondylodiscitis caused by viridans streptococci: three cases and a review of the literature. Clin Rheumatol. 1999;18:417–21.
24. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28:603–61.
25. Watkins RR, David MZ, Salata RA. Current concepts on the virulence mechanisms of meticillin-resistant Staphylococcus aureus. J Med Microbiol. 2012;61:1179–93.
26. Nolla JM, Lora-Tamayo J, Gómez Vaquero C, et al. Pyogenic arthritis of native joints in non-intravenous drug users: a detailed analysis of 268 cases attended in a tertiary hospital over a 22-year period. Semin Arthritis Rheum. 2015;45:94–102.
27. Bossert M, Prati C, Bertolini E, Toussirot E, Wendling D. Septic arthritis of the acromioclavicular joint. Joint Bone Spine. 2010;77:466–9.
28. Ross JJ, Hu LT. Septic arthritis of the pubic symphysis: review of 100 cases. Medicine (Baltimore). 2003;82:340–5.
29. Ross JJ, Shamsuddin H. Sternoclavicular septic arthritis: review of 180 cases. Medicine (Baltimore). 2004;83:139–48.

European Journal of Internal Medicine 28 (2016) 38–42

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original Article

Clinical findings of bacteremic septic arthritis according to the site of acquisition: The overlap between health care-related and community- and nosocomial-acquired cases

Oscar Murillo [a,*], Joan Gomez-Junyent [a], Imma Grau [a], Alba Ribera [a], Celina Cabrera [a], Salvador Pedrero [b], Fe Tubau [c], Joan M. Nolla [d], Javier Ariza [a], Roman Pallares [a]

[a] Infectious Disease Department, Hospital Bellvitge, Feixa Llarga s/n, 08907 L'Hospitalet, Barcelona, Spain
[b] Orthopaedic Surgery Department, Hospital Bellvitge, Barcelona, Spain
[c] Microbiology Department, Hospital Bellvitge, Barcelona, Spain
[d] Rheumatology Department, Hospital Bellvitge, Barcelona, Spain

ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 7 November 2015
Accepted 11 November 2015
Available online 27 November 2015

Keywords:
Septic arthritis
Bacteremia
Healthcare-related
Nosocomial
Community

ABSTRACT

Background: The site of acquisition of infection may have a major impact on outcome. The health care-related (HCR) environment has recently come under scrutiny. In a group of patients with bacteremic septic arthritis (SA), we compared their characteristics, type of SA, microbiology and prognosis according to the site of acquisition: community-acquired (CA), nosocomial-acquired (NA), and HCR.

Methods: We studied all patients with bacteremic SA seen at our institution between 1985 and 2013. Data were obtained from a protocol of prospectively recorded bacteremia cases.

Results: There were 273 cases of bacteremic SA (CA: 51%; NA: 31%; and HCR: 18%). NA and HCR sites were more frequent in older and fragile patients. SA of peripheral joints was the most common presentation; infections of the axial skeleton predominated in CA and HCR (24%), and prosthetic joint infection in NA (44%). MRSA and *Pseudomonas aeruginosa* were mainly found in NA (21% and 6% respectively) and HCR (14% and 8% respectively), whereas *Streptococcus* spp. was more frequent in CA (30%) and HCR (28%). The 30-day mortality rates were: CA 7%, HCR 18%, and NA 26%.

Conclusion: The characteristics of HCR-SA overlapped with those of the CA or NA-SA cases. The HCR and NA cases presented more advanced age, greater fragility, and the predominance of difficult-to-treat microorganisms, while the HCR and CA cases presented an involvement of the axial skeleton, streptococcal etiology, and a lower number of prosthetic joint infections. Our data show that the site of acquisition should be considered when planning diagnostic and therapeutic management for SA.

1. Introduction

Septic arthritis (SA) remains a significant health concern and presents high rates of morbidity and mortality. Its treatment requires emergency medical and surgical care, including antibiotic therapy and debridement to avoid joint destruction and loss of functionality. The presence of bacteremia is common and may be either the cause or the consequence of SA [1–3].

Several medical conditions have been identified as the risk factors for SA, such as the presence of rheumatoid arthritis, diabetes mellitus, corticosteroid therapy, intravenous drug abuse or joint prosthesis [3,4]. In addition, recent changes in patients' characteristics and social habits and the use of more aggressive therapies have increased the numbers of elderly individuals with more chronic debilitating conditions. This may predispose them to a greater number of joint infections, either native or prosthetic (PJI) [5–8]. Indeed, although native SA and PJI present notable differences, several epidemiological studies have stressed the value of addressing both entities together [5–8].

The site of acquisition of infection may have a strong influence on microbiology or on patients' characteristics. While the distinction between community and nosocomial acquisition has been well established, health care-related infections have only recently been described as a specific entity [9–11]. To our knowledge, differences in the current pattern of SA in relation to the site of acquisition have not been previously reported. Moreover, mortality due to SA may also be influenced by the site of acquisition, but this has not been specifically evaluated to date.

In the present study we analyzed a large cohort of patients suffering from bacteremic SA (native or prosthetic) over the last three decades. Our main objective was to compare patients' characteristics, microbiology and prognosis according to the site of acquisition.

* Corresponding author. Tel.: +34 93 260 76 25; fax: +34 93 260 76 37.
E-mail address: omurillo@bellvitgehospital.cat (O. Murillo).

http://dx.doi.org/10.1016/j.ejim.2015.11.013
0953-6205/

2. Patients and methods

2.1. Setting and study design

This study was performed at the Hospital Bellvitge, a 700-bed tertiary care teaching institution in Barcelona, Spain. The hospital does not have pediatric, obstetric or burn wards.

Over the past three decades, information on all patients with bloodstream infection has been collected in a prospective database which contains data on patients' baseline characteristics, clinical presentation and source of bacteremia, microbiologic data, and outcome.

3. Patients' characteristics, microbiological studies and definitions

All patients with SA and bacteremia who were attended at our institution from 1985 to 2013 were analyzed. The study included both patients with an SA focus as the source of bacteremia (defined as "primary" bacteremic SA) and patients with bacteremia from a distant focus, in whom the SA was the result of a septic metastasis (defined as "secondary" bacteremic SA). Specifically, the location of SA, the number of joints involved, and the presence of joint prosthesis were recorded. In view of the recent epidemiology of bacteremic osteoarticular infection [5] and in order to analyze a representative sample of SA cases according to the site of acquisition, we decided to exclude intravenous drug users from the study because this young population presents its own particular pattern of osteoarticular infections which are almost exclusively community-acquired [12–14].

Blood samples were cultured following the standard recommendations by the automated Bactec method with both aerobic and anaerobic media (current Bactec FX system, Becton-Dickinson Microbiology Systems, USA). Microorganisms were identified and their antibiotic susceptibility was assessed using standard biochemical reactions, disk-diffusion, and the MicroScan system (Dade Behring, West Sacramento, CA, USA). Antimicrobial susceptibility was defined according to CLSI criteria [15]. We applied the definitions for multidrug-resistant and extensively drug-resistant microorganisms described by Magiorakos et al [16].

SA cases were defined according to the modified criteria used by Newman [3]. All cases included met at least one of these two criteria: i) isolation of a pathogenic microorganism from an affected joint; or ii) isolation of a pathogenic organism from another source (blood) in the context of compatible clinical picture of SA (inflammatory local signs). In addition, PJI was defined by the isolation of a pathogenic microorganism from two or more surgical, joint-aspirated or blood cultures, or by one such positive culture plus the presence of typical signs and clinical symptoms (inflammatory signs, the presence of a sinus tract or purulence around the prosthesis during surgery) [1,3]. According to our protocol, arthrocentesis is routinely performed to obtain synovial fluid samples and a pair of blood cultures is obtained for microbiological analyses; in cases in which the process affects joints with difficult access (i.e., the axial skeleton) only blood cultures are initially taken.

Joint involvement was divided into "peripheral" (joints of the appendicular skeleton) and "axial". Specifically, axial SA cases were those that involved joints forming part of the axis of the skeleton: acromioclavicular, sternoclavicular, sternocostal, pubic symphysis and sacroiliac (obviously, cases of spondylodiscitis were not considered).

Cases of bacteremia by *Neisseria* sp. (*Neisseria meningitidis* and *Neisseria gonorrhoeae*) were not considered, so as to avoid the inclusion of patients with reactive arthritis. Cases of interapophyseal arthritis were excluded because their diagnosis is mainly confirmed by the use of magnetic resonance imaging, which was only available for part of the study period [17]. Microbiology was always identified by blood samples, and in most cases by additional local samples obtained from the affected joint. In accordance with the Lancefield and Sherman classifications, the *Streptococcus* species were divided into two groups: *Pyogenic* (*Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*) and *Other* streptococci (*Streptococcus viridans*, *Streptococcus bovis* and *Streptococcus milleri*, along with the remaining species) [18]. Cases were considered to be nosocomial-acquired (NA), health care-related (HCR) or community-acquired (CA) in accordance with the definitions provided by Friedman et al. [9]; thus, the SA cases recorded prior to that publication were classified retrospectively. Briefly, health care-related SA was defined by a diagnosis obtained from a patient at the time of admission or within 48 h of admission if he/she: i) had received intravenous therapy or specialized nursing care at home in the 30 days before the infection; ii) had attended a hospital or hemodialysis clinic or received intravenous chemotherapy in the 30 days before the infection; iii) had been hospitalized in an acute care hospital for 2 or more days in the 90 days before the infection; or iv) resided in a nursing home or a long-term care facility. Nosocomial-acquired SA was defined by a diagnosis obtained from patients who had been hospitalized for 48 h or longer, and community-acquired SA by a diagnosis obtained at the time of hospital admission or within 48 h of hospital admission in patients who did not meet the criteria for a health care-related SA.

Mortality associated with bacteremic SA (30-day mortality) was considered when the patient died within 30 days of diagnosis of bacteremia with concomitant SA.

4. Statistical analysis

Data were analyzed with SPSS (version 20.0). Continuous variables are expressed as mean ± SD or median and range, according to normality tests; categorical variables are expressed as counts and valid percentages. Comparative analyses were performed with X^2 or Fisher's test for categorical variables, and the Mann–Whitney *U*-test for continuous variables. All tests were two-tailed, and a P value < 0.05 was considered as statistically significant.

5. Results

A total of 35,250 episodes of clinically significant bacteremia were recorded during the period of study. Among these, 273 cases (0.8%) had a concomitant SA; the source of bacteremia was considered "primary" in 200 cases (73%), and "secondary" in the remaining 73 cases (27%). Among the latter, the most frequent initial origins of bacteremia were vascular-catheter infection ($n = 27$, 10%), infectious endocarditis ($n = 20$, 7%), and soft tissue infections ($n = 13$, 5%).

The site of acquisition of SA was classified as: community-acquired ($n = 139$, 51%), nosocomial-acquired ($n = 84$, 31%), and health-care related ($n = 50$, 18%). Differences in the source of bacteremia regarding the site of acquisition were observed between primary and vascular-catheter foci (which represented 81% and 0% of community-acquired cases, 69% and 21% of nosocomial-acquired cases, and 62% and 18% of health care-related cases respectively; $P < 0.001$ and $P = 0.05$).

SA occurred more frequently in male patients (56%), and the median age was 67 years (IQR 55–77). The most frequent baseline conditions are presented in Table 1. Older and more fragile patients with SA were more likely to have nosocomial or health care-related sites of acquisition. Nosocomial-acquired and health care-related cases were more likely than community-acquired cases to present relevant risk factors for SA such as immunosuppressive therapy, chronic renal insufficiency or prosthesis infection.

The location of SA also differed depending on the site of acquisition: while in community-acquired and health care-related cases the location was similar (peripheral joints in 76% and the axial skeleton in 24%), in nosocomial-acquired cases it was mainly the peripheral joints (92% vs 8% for the axial skeleton; $P = 0.003$ and $P = 0.01$, respectively) (Table 1). The higher number of PJIs acquired in the hospital environment was responsible for the differences in the overall percentage of peripheral joint SA between nosocomial-acquired and community-acquired or health care-related cases ($P < 0.001$ and $P = 0.005$

Table 1
Patients' characteristics, location, microbiology and mortality of SA cases according to the site of acquisition.

	All ($n = 273$)	Community ($n = 139$)	Nosocomial ($n = 84$)	*P* value C vs N	Healthcare ($n = 50$)	*P* value N vs H	*P* value C vs H
Age (median, IQR)	67 (55–77)	65 (52–76)	73 (58–79)	0.03	66 (60–76)	0.3	0.2
Male	153 (56)	79 (57)	41 (49)	0.2	32 (64)	0.09	0.4
Underlying disease							
One or more	167 (61)	74 (54)	53 (63)	0.2	40 (80)	0.04	0.001
Diabetes mellitus	74 (27)	39 (28)	21 (25)	0.6	14 (28)	0.7	0.9
Neoplasm	37 (14)	9 (6)	14 (17)	0.02	14 (28)	0.1	<0.001
Immunosuppressive therapy	67 (25)	28 (20)	17 (20)	0.9	22 (44)	0.003	0.001
Cardiopathy	51 (19)	21 (15)	19 (23)	0.2	11 (22)	0.9	0.3
Chronic renal insufficiency	20 (7)	0	3 (4)	0.02	17 (34)	<0.001	<0.001
Rheumatoid arthritis	27 (10)	13 (9)	7 (8)	0.8	7 (14)	0.3	0.4
Location of SA							
Axial	53 (19)	33 (24)	7 (8)	0.003	12 (24)	0.01	0.9
Peripheral	220 (81)	105 (76)	77 (92)	0.003	38 (76)	0.01	0.9
Native	150 (55)	81 (59)	40 (48)	0.1	28 (56)	0.3	0.7
Prosthesis	70 (26)	24 (17)	37 (44)	<0.001	10 (20)	0.005	0.6
Microbiology							
MSSA	129 (47)	75 (54)	37 (44)	0.1	17 (34)	0.2	0.01
MRSA	27 (10)	2 (1)	18 (21)	<0.001	7 (14)	0.3	<0.001
Streptococcus sp.	59 (22)	41 (30)	4 (5)	<0.001	14 (28)	<0.001	0.8
Pyogenic[a]	43 (16)	32 (23)	2 (2)	<0.001	9 (18)	0.001	0.5
Other	16 (6)	9 (6)	2 (2)	0.2	5 (10)	0.05	0.4
Enterococcus sp.	5 (2)	1 (1)	2 (2)	0.3	2 (4)	0.6	0.1
GNB	49 (18)	19 (14)	21 (25)	0.03	9 (18)	0.3	0.5
Escherichia coli	19 (7)	13 (9)	4 (5)	0.2	2 (4)	0.8	0.2
Pseudomonas aeruginosa	9 (3)	0	5 (6)	0.004	4 (8)	0.6	0.001
30-day mortality	41 (15)	10 (7)	22 (26)	<0.001	9 (18)	0.3	0.03

Data presented as numbers of cases (percentage).
Abbreviations: C, community-acquired; N, nosocomial-acquired; H, health-care related; SA, septic arthritis; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; GNB, Gram-negative bacilli.
[a] Pyogenic streptococci: *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*.

respectively; Table 1). There were 70 episodes (26%) of PJI, all of them monoarticular and mostly affecting the hip ($n = 41$) and the knee ($n = 26$). The main differences between PJI and native SA cases were that PJIs occurred more frequently in older patients (median age, IQR: 76, 71–81 vs 64, 52–74; $P < 0.001$), had more infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterobacteriaceae* strains (17% vs 8%, and 20% vs 10%; $P = 0.03$ and $P = 0.04$, respectively), and had a higher mortality rate (24% vs 12%; $P = 0.01$). With regard to the site of acquisition, native SA and PJI were broken down as follows: nosocomial-acquired, 23% and 53% ($P < 0.001$); health care-related, 20% and 14% ($P = 0.3$); and community-acquired, 57% and 33% ($P = 0.001$).

Cases of SA with involvement of the axial skeleton (in comparison with involvement of peripheral joints) tended to affect younger people (median 59 vs 69 years; $P = 0.001$), were more frequently associated with concomitant infectious endocarditis (19% vs 4%, $P < 0.001$) and were more likely to be caused by the *Streptococcus* species (34% vs 19%; $P = 0.01$).

Microorganisms responsible for bacteremic SA are presented in Table 2. These episodes were mainly caused by Gram-positive bacteria (81%), and only five cases (2%) were polymicrobial. *S. aureus* was by far the most frequent microorganism ($n = 157$, 57%), the strains being methicillin-susceptible (MSSA) in 82% of the cases and MRSA in 18%. *Streptococcus* sp. caused 21% ($n = 59$) of SA cases, with *S. agalactiae* predominating ($n = 21$). Among Gram-negative bacilli (GNB), *Escherichia coli* ($n = 19$) and *Pseudomonas aeruginosa* ($n = 9$) were the most frequent isolates.

Significant differences in the etiology of SA with regard to the site of acquisition are shown in Table 1. *S. aureus* was the most frequently involved microorganism in all cases; MSSA strains were less common than other etiologies in health care-related cases than in the others. In addition, the presence of MRSA strains was significantly higher in nosocomial-acquired and health care-related cases (33% and 29% of all *S. aureus* respectively) than in community-acquired cases (1%; $P < 0.001$). The proportion of streptococcal SA was significantly higher among community-acquired and health care-related cases than among nosocomial-acquired cases, especially in those caused by the pyogenic *Streptococcus* species. Infections caused by GNB were more frequent in nosocomial than in community acquisition ($P = 0.03$); all episodes of SA caused by *P. aeruginosa* were nosocomial-acquired or health care-related. Microorganisms that were more typically related to PJI than to native SA were MRSA and *Enterobacteriaceae* strains ($P = 0.03$ and $P = 0.04$ respectively).

Forty-one patients died within 30 days of SA diagnosis, a mortality rate of 15%. In 19 out of 41 cases (46%) death occurred within a week of diagnosis, being closely related with the acute phase of sepsis. Differences in mortality rate according to the site of acquisition are presented in Table 1; health care-related cases presented higher mortality than community-acquired but lower than nosocomial-acquired. In addition, prosthetic joint infections were associated with higher mortality (24%) than native joint SA (12%) ($P = 0.01$).

6. Discussion

The present study identifies the main differences in patients' characteristics, type of SA, and microbiology according to the site of acquisition of bacteremic SA.

The site of acquisition of various infections has changed in the last few years [9]. Recently, we reported a significant increase in nosocomial-acquired and health care-related bacteremic osteoarticular infections, and a decrease in the number of strictly community-acquired cases [5]. In the SA setting, the results of our current study confirmed that only half of the cases were community-acquired. While the distinction between nosocomial- and community-acquired infections is well known, several recent articles have reported the particularities of infections in the health care environment [9–11]. To our knowledge, no previous studies have focused on the site of infection in the setting of SA; the results of the present study show that health care-related cases share some features

Table 2
Microorganisms responsible for all cases of septic arthritis.

Gram-positive microorganisms			226 (81%)
Staphylococcus sp.			160 (58%)
	S. aureus	157 (57%)	
	MSSA	129 (47%)	
	MRSA	28 (10%)	
	Coagulase-negative	3 (1%)	
Streptococcus sp.		59 (21%)	
	S. agalactiae	21 (7%)	
	S. pneumoniae	13 (5%)	
	S. pyogenes	9 (3%)	
	Other [a]	16 (5%)	
Enterococcus sp.			5 (2%)
	Enterococcus faecalis	5 (2%)	
Other Gram-positive microorganisms			2 (0.7%)
	Corynebacterium sp.	1 (0.4%)	
	Gemella	1 (0.4%)	
Gram-negative microorganisms			52 (19%)
Enterobacteriaceae			35 (12%)
	E. coli	19 (7%)	
	Klebsiella pneumoniae	6 (2%)	
	Salmonella enteritidis	3 (1%)	
	Proteus mirabilis	2 (0.8%)	
	Enterobacter cloacae	2 (0.8%)	
	Morganella morgagnii	2 (0.8%)	
	Serratia marcescens	1 (0.4%)	
Non-fermenting Gram-negative bacilli		9 (4%)	
	P. aeruginosa	9 (3%)	
Other Gram-negative microorganisms			8 (3%)
	Bacteroides fragilis	4 (1.5%)	
	Haemophilus sp.	2 (0.8%)	
	Eubacterium sp.	1 (0.4%)	
	Aeromonas hydrophila	1 (0.4%)	

Total: 278 isolates in 273 cases of bacteremic SA, with 5 cases of polymicrobial bacteremia.
[a] Other *Streptococcus* groups included: *Streptococcus equi* ($n = 3$), *Streptococcus* group G (3), *Streptococcus bovis* (3), *Streptococcus intermedius* (2), *Streptococcus mitis* (2), *Streptococcus sanguis* (1), *Streptococcus salivarius* (1), and *Streptococcus* group F (1).

with community-acquired cases and others with nosocomial-acquired cases.

We found the characteristics of patients with nosocomial-acquired and health care-related SA to be quite similar. These patients tended to be older and had more underlying diseases than patients with community-acquired infection. In contrast, the clinical pattern of SA with regard to the joints involved showed a clear predominance of peripheral joints (92%) among nosocomial-acquired cases, whereas the axial skeleton was involved in almost 25% of health care-related and community-acquired cases. Our results confirmed that the peripheral joints (i.e., knee, hip, shoulder) are the most affected in all sites of acquisition; however, while the percentage of native peripheral SA was similar for all three sites, PJI presented a significant association with nosocomial acquisition. The notable differences between native SA and PJI have been mentioned in the previous studies [6–8]; once again, we confirmed these previous results but our study also highlighted some interesting differences from the epidemiological point of view. As is well known, PJIs are usually divided into acute and late chronic according to the time elapsed after the implantation of prosthesis (1–3 months vs longer) [8]. The clinical presentation of these two kinds of PJI reveals clear differences: while acute infections usually present with bacteremia, this is extremely uncommon in late chronic infections. Thus, the high rates of PJI in nosocomial cases of SA may be due to the predominance of postsurgical acute infections. Less frequently, hematogenous seeding of microorganisms to the prosthesis may occur over time, and this may be reflected in the community and health care-related cases of PJI.

As for SA involving the axial skeleton, classic studies have reported higher predominance in young people, especially among intravenous-drug users [1,13,14,19]. Our results support these findings (even in the absence of the latter population in our series), and showed the association with the presence of endocarditis and streptococcal etiology.

S. aureus was the main cause of SA at all sites of acquisition, but other interesting microbiological features were also observed. The presence of MRSA was clearly associated with nosocomial-acquired and health care-related sites, and was practically non-existent among community-acquired sites (1%). These results seem to be in agreement with others from elsewhere in Europe which have reported a lower frequency of community-acquired MRSA infections than in the USA [20–23]. As noted above, the association between nosocomial acquisition and PJI cases may partially explain the higher presence of MRSA infections at this site. The *Streptococcus* species were the second most frequent etiology of SA, with *S. agalactiae* being the most common. This seems to be in agreement with recent reports of the increasing incidence of *Streptococcus* sp. as a cause of osteoarticular infections [24–26]. In this case, our results showed that almost all these infections were acquired in the community and the health care environment, and were extremely rare among nosocomial-acquired cases. The great variety of *Streptococcus* species makes it hard to draw firm conclusions, but the *viridans* group (as a common cause of endocarditis) and the pyogenic species seem to be more related to community-acquired infections. In this regard, our results for streptococcal SA underline the similarities between the health care-related and community-acquired populations. Finally, GNB was a more common etiology of SA in the hospital environment, and was frequent in the health care-related setting as well. *P. aeruginosa* was observed only at these sites, and *Enterobacteriaceae* strains were significantly more frequent as causes of PJI.

Overall, identification of health care-related SA appears to be important in clinical practice. The particular features of its etiology, as well as the greater fragility of patients (who tend to be older and present more comorbid conditions), make an early distinction between health care-related and community-acquired SA mandatory in order to plan the emergency indications of empirical antibiotic therapy and surgical procedures.

The mortality observed in our study (15%) is close to the highest rates reported in previous studies of SA [1,4,27]. We stress that our study included only bacteremic PJI and SA, which have both been associated with greater mortality in other SA series [4,8,27,28]. Our results support the notion that mortality is mainly due to sepsis, since almost half of the deaths occurred in the first week after bacteremia. With regard to the site of acquisition, our results showed that health care-related SA presented a higher risk for mortality than community-acquired cases but a lower risk than nosocomial-acquired cases. Our results also showed a higher mortality rate for PJIs and nosocomial-acquired cases. Previous reports have noted the greater risk of mortality for PJI [8,28]; therefore, the association between these cases and nosocomial acquisition could partially explain the higher mortality associated with this site.

7. Conclusions

The pattern of bacteremic SA presents notable differences regarding the site of acquisition and has changed over time; half of the cases are now health care-related or nosocomial-acquired. The health care-related cases presented clinical findings, etiology and prognosis that overlapped with those for community- or nosocomial-acquired SA. An older, more fragile population and the predominance of MRSA and *P. aeruginosa* infections were characteristics shared by health care-related and nosocomial-acquired SA. In contrast, health care-related cases seemed to be more similar to community-acquired SA in terms of the involvement of the axial skeleton, the streptococcal etiology, and the lower number of PJI cases. Overall, bacteremic SA had a high mortality rate; specifically, mortality was higher in health care-related SA cases than in community-acquired cases, but not as high as in nosocomial-acquired SA. Our data show that the site of acquisition should be considered when planning diagnostic and therapeutic approaches in patients with septic arthritis.

Conflict of interest

The authors state that they have no conflicts of interest.

Acknowledgments

This work was supported by Ministerio de Economía y Competitividad, Instituto de Salud Carlos III — co-financed by European Development Regional Fund "A way to achieve Europe" ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015). We thank Michael Maudsley for revising the English manuscript.

References

[1] Shirtliff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev 2002;15:527–44.
[2] Smith JW, Chalupa P, Shabaz Hasan M. Infectious arthritis: clinical features, laboratory findings and treatment. Clin Microbiol Infect 2006;12:309–14.
[3] Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. Lancet 2010;375:846–55.
[4] Kaandorp CJ, Van Schaardenburg D, Krijnen P, Habbema JD, van de Laar MA. Risk factors for septic arthritis in patients with joint disease. A prospective study. Arthritis Rheum 1995;38:1819–25.
[5] Murillo O, Grau I, Lora-Tamayo J, Gomez-Junyent J, Ribera A, Tubau F, et al. The changing epidemiology of bacteremic osteoarticular infections in the early 21st century. Clin Microbiol Infect 2015;21:254e1–8. http://dx.doi.org/10.1016/j.cmi.2014.09.007.
[6] Kaandorp CJ, Dinant HJ, van de Laar MA, Moens HJ, Prins AP, Dijkmans BA. Incidence and sources of native and prosthetic joint infection: a community based prospective survey. Ann Rheum Dis 1997;56:470–5.
[7] Geirsson AJ, Statkevicius S, Vikingsson A. Septic arthritis in Iceland 1990–2002: increasing incidence due to iatrogenic infections. Ann Rheum Dis 2008;67:638–43.
[8] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645–54.
[9] Friedman ND, Kaye KS, Stout JE, et al. Health care—associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 2002;137:791–7.
[10] Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988;16:128–40.
[11] Pigrau C, Rodriguez-Pardo D, Fernandez-Hidalgo N, Moreto L, Pellise F, Larrosa MN, et al. Health care associated hematogenous pyogenic vertebral osteomyelitis: a severe and potentially preventable infectious disease. Medicine (Baltimore) 2015;94:e365 [doi:10.1097].
[12] Peterson TC, Pearson C, Zekaj M, Hudson I, Fakhouri G, Vaidya R. Septic arthritis in intravenous drug abusers: a historical comparison of habits and pathogens. J Emerg Med 2014;47:723–8.
[13] Ross JJ, Hu LT. Septic arthritis of the pubic symphysis: review of 100 cases. Medicine (Baltimore) 2003;82:340–5.
[14] Ross JJ, Shamsuddin H. Sternoclavicular septic arthritis: review of 180 cases. Medicine (Baltimore) 2004;83:139–48.
[15] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard. M7-A8 CLSI, Wayne, PA8th ed. ; 2009.
[16] Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[17] Narvaez J, Nolla JM, Narvaez JA, et al. Spontaneous pyogenic facet joint infection. Semin Arthritis Rheum 2006;35:272–83.
[18] Sherman JM. The streptococci. Bacteriol Rev 1937;1:3–97.
[19] Bossert M, Prati C, Bertolini E, Toussirot E, Wendling D. Septic arthritis of the acromioclavicular joint. Joint Bone Spine 2010;77:466–9.
[20] Lessa FC, Mu Y, Davies J, Murray M, Lille M, Pearson A, et al. Comparison of incidence of bloodstream infection with methicillin-resistant *Staphylococcus aureus* between England and United States, 2006–2007. Clin Infect Dis 2010;51:925–8.
[21] Gasch O, Ayats J, Angeles Dominguez M, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection: secular trends over 19 years at a university hospital. Medicine (Baltimore) 2011;90:319–27.
[22] Fangtham M, Baer AN. Methicillin-resistant *Staphylococcus aureus* arthritis in adults: case report and review of the literature. Semin Arthritis Rheum 2012;41:604–10.
[23] Frazee BW, Fee C, Lambert L. How common is MRSA in adult septic arthritis? Ann Emerg Med 2009;54:695–700.
[24] Nolla JM, Gomez-Vaquero C, Corbella X, et al. Group b *Streptococcus* (*Streptococcus agalactiae*) pyogenic arthritis in nonpregnant adults. Medicine (Baltimore) 2003;82:119–28.
[25] Ross JJ, Saltzman CL, Carling P, Shapiro DS. Pneumococcal septic arthritis: review of 190 cases. Clin Infect Dis 2003;36:319–27.
[26] Dubost JJ, Soubrier M, De Champs C, Ristori JM, Sauvezie B. Streptococcal septic arthritis in adults. A study of 55 cases with a literature review. Joint Bone Spine 2004;71:303–11.
[27] Kaandorp CJ, Krijnen P, Moens HJ, Habbema JD, van Schaardenburg D. The outcome of bacterial arthritis: a prospective community-based study. Arthritis Rheum 1997;40:884–92.
[28] Lora-Tamayo J, Euba G, Ribera A, et al. Infected hip hemiarthroplasties and total hip arthroplasties: differential findings and prognosis. J Infect 2013;67:536–44.

Seminars in Arthritis and Rheumatism 48 (2018) 327–333

Contents lists available at ScienceDirect

Seminars in Arthritis and Rheumatism

journal homepage: www.elsevier.com/locate/semarthrit

Analysis of mortality in a cohort of 650 cases of bacteremic osteoarticular infections

Joan Gomez-Junyent, MD[a], Oscar Murillo, PhD[a,*], Imma Grau, PhD[a], Eva Benavent, MD[a], Alba Ribera, PhD[a], Xavier Cabo, PhD[b], Fe Tubau, MD[c], Javier Ariza, PhD[a], Roman Pallares, PhD[a]

[a] Department of Infectious Diseases, Hospital Universitari de Bellvitge, IDIBELL, Universidad de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain
[b] Department of Orthopaedics, Hospital Universitari de Bellvitge, IDIBELL, Universidad de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain
[c] Department of Microbiology, Hospital Universitari de Bellvitge, IDIBELL, Universidad de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

ARTICLE INFO

Keywords:
Bacteremia
Osteoarticular infections
Mortality
Septic arthritis
Surgical debridement

ABSTRACT

Objectives: The mortality of patients with bacteremic osteoarticular infections (B-OAIs) is poorly understood. Whether certain types of OAIs carry higher mortality or interventions like surgical debridement can improve prognosis, are unclarified questions.
Methods: Retrospective analysis of a prospective cohort of patients with B-OAIs treated at a teaching hospital in Barcelona (1985–2014), analyzing mortality (30-day case-fatality rate). B-OAIs were categorized as peripheral septic arthritis or other OAIs. Factors influencing mortality were analyzed using logistic regression models. The association of surgical debridement with mortality in patients with peripheral septic arthritis was evaluated with a multivariate logistic regression model and a propensity score matching analysis.
Results: Among 650 cases of B-OAIs, mortality was 12.2% (41.8% of deaths within 7 days). Compared with other B-OAI, cases of peripheral septic arthritis were associated with higher mortality (18.6% vs 8.3%, $p < 0.001$). In a multiple logistic regression model, peripheral septic arthritis was an independent predictor of mortality (adjusted odds ratio [OR] 2.12; 95% CI: 1.22–3.69; $p = 0.008$). Cases with peripheral septic arthritis managed with surgical debridement had lower mortality than those managed without surgery (14.7% vs 33.3%; $p = 0.003$). Surgical debridement was associated with reduced mortality after adjusting for covariates (adjusted OR 0.23; 95% CI: 0.09–0.57; $p = 0.002$) and in the propensity score matching analysis (OR 0.81; 95% CI: 0.68–0.96; $p = 0.014$).
Conclusions: Among patients with B-OAIs, mortality was greater in those with peripheral septic arthritis. Surgical debridement was associated with decreased mortality in cases of peripheral septic arthritis.

Introduction

Osteoarticular infections (OAIs) are often associated with bacteremia and can either be metastatic OAIs, when they are the consequence of a bacteremia from another origin, or primary OAIs, if they cause the bacteremia and no other distant infection is present. The prevalence of bacteremia among patients with OAIs may differ substantially, but it is usually higher among cases of septic arthritis (SA) [1,2] and vertebral osteomyelitis (VO) [3,4].

In recent years, the incidence of bacteremic OAIs (B-OAIs) has increased [5], and there have been changes in the characteristics of patients with OAIs. For example, there has been a marked decrease in the rate of OAIs in intravenous drug users (IVDU), but an increase in OAIs among older adults and in patients with chronic medical conditions. The microbiology of B-OAIs has also changed, with increased rates of Gram-negative bacilli (GNB) and *Streptococcus* spp., though *Staphylococcus aureus* remains the main etiological agent.

The presence of bacteremia in patients with OAIs is a major concern. It is considered a major risk factor for poor outcomes [6–8], but no detailed analysis of factors influencing mortality in

Abbreviations: B-OAI, Bacteremic Osteoarticular Infection; OR, Odds ratio; SA, septic arthritis; VO, vertebral osteomyelitis; IVDU, intravenous drug users; GNB, Gram-negative bacilli; PJI, prosthesis joint infection; IQR, interquartile range; 95% CI, 95% confidence interval.

*Corresponding author.
E-mail address: omurillo@bellvitgehospital.cat (O. Murillo).

https://doi.org/10.1016/j.semarthrit.2018.01.007
0049-0172/

large cohorts of patients with B-OAIs has been published to date. Moreover, little information is available on the type of OAIs, the presence of prior host factors, or the causative microorganisms as determinants of mortality in patients with B-OAIs. For example, SA is reported as a major cause of morbidity and mortality [1,9], but its impact on the overall mortality in B-OAIs is unclear. Therefore, it is essential that we understand the medical and surgical interventions, together with other factors, that influence mortality in patients with B-OAI.

In this study, our aim was to analyze mortality (30-day case-fatality rate) among a large cohort of patients with B-OAIs, and to investigate the host, microbiological, and interventional factors that may influence prognosis.

Materials and methods

Study design and setting

This study was performed at the Hospital Universitari de Bellvitge, a large teaching hospital in Barcelona, Spain. The hospital does not attend pediatric, obstetric or burn patients, and has a dedicated multidisciplinary Bone and Joint Infection Unit comprising orthopedic surgeons, infectious diseases specialists and rheumatologists. Patients with OAIs are admitted to this Unit for antibiotic therapy and/or surgical interventions, based on the assessments of the attending physicians and surgeons. Usually, SA of native joints is routinely managed by open arthrotomy.

Inclusion and exclusion criteria

Over the last three decades, a team of microbiologists and infectious disease specialists have prospectively studied all cases of bacteremia at our hospital. As a result, they have produced a database that contains information on patients' characteristics, clinical presentation, source of bacteremia, microbiological records, follow-up, and outcomes. The institutional review board approved this study and publication of the results. The need for informed consent was waived as the study analyzed data retrospectively and the data were anonymized.

All episodes of bacteremia (some patients had more than one episode) and associated OAIs managed between 1985 and 2014 were included. Patients were included if OAI was the primary source of bacteremia, or if they had metastatic OAI secondary to bacteremia from a distant focus. Patients were excluded if they were younger than 18 years old or IVDU. Cases caused by *Neisseria* spp. were also excluded so as to avoid including cases with reactive rather than septic arthritis.

Definitions

All cases included in the study fulfilled the main diagnostic criteria for each type of OAI; they presented with bacteremia and a compatible clinical picture. According to our protocol, we consider patients with a short history of a warm, swollen and tender joint to have SA until proven otherwise [2,10]. An arthrocentesis is routinely performed to obtain synovial fluid samples for microbiological analyses and a pair of blood cultures is taken; in cases in which the process affects joints with difficult access (i.e., the axial skeleton) only blood cultures are initially taken. Non-infectious etiologies of acute arthritis are also ruled out prior to establishing the diagnosis of SA. In addition, prosthetic joint infection (PJI) was defined by the isolation of a pathogenic microorganism from two or more surgical, joint-aspirated or blood cultures, or by one such positive culture plus the presence of typical signs and clinical symptoms (inflammatory signs, the presence of a sinus tract or purulence around the prosthesis during surgery). Joint involvement was classified as 'peripheral' (i.e., joints of the appendicular skeleton, which were either native or PJI) and 'axial' (i.e., the axial skeleton, including the acromioclavicular, sternoclavicular, sternocostal, pubic symphysis, inter-apophyseal, and sacroiliac joints). VO, with or without spine arthrodesis, was defined by the presence of back pain, motion limitation, spinal tenderness, and/or macroscopic pus through the surgical wound, together with characteristic imaging findings (computed tomography or magnetic resonance imaging) [4]. Peripheral osteomyelitis was defined by the presence of typical signs and symptoms such as a draining fistula, bone tenderness, and/or local swelling, and characteristic imaging findings (bone radiograph, computed tomography or magnetic resonance imaging) [3] and included cases with or without an orthopedic device. Microbiology of OAI was always identified by blood samples and in most cases by additional local samples obtained from the affected joint or bone.

The severity of the underlying diseases was estimated by the McCabe Jackson score (I = nonfatal disease [survival $>$ 5 years]; II = ultimately fatal disease [survival 1–5 years], III = rapidly fatal disease [survival $<$ 1 year]) [11]. Mortality was recorded if death occurred within 30 days from the diagnosis, and early mortality if death occurred within 7 days.

Microbiological studies

Blood samples were cultured following standard criteria by the automated BACTEC™ method, using both aerobic and anaerobic media. We used the following BACTEC™ systems (Becton-Dickinson Microbiology Systems, Franklin Lakes, NJ, USA): the BACTEC™ NR-860 in the 1990s, the BACTEC™ 9240 in the 2000s, and the BACTEC™ FX in the 2010s. During the study period, identification of microorganisms and their antibiotic susceptibility were performed by standard biochemical reaction, disc diffusion or microdilution, and the MicroScan system (Dade Behring, West Sacramento, CA, USA). Antimicrobial susceptibility was defined according to Clinical and Laboratory Standards Institute [12]. Streptococci were divided into two groups according to the Lancefield and Sherman classifications: pyogenic species *(Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*) and viridans species (*Streptococcus bovis* and *Streptococcus milleri*, along with the remaining species) [13].

Statistical analysis

Data were analyzed by Stata 13.1 (Stata Corporation, TX, USA). Categorical variables were described by counts and percentages, while mean and standard deviation or median and interquartile range (IQR) were used to summarize continuous variables. Comparisons between groups were performed with either the chi-square test or Fisher exact test for categorical variables, and the *t*-test or Mann–Whitney *U* test was used for continuous variables. A non-parametric test for trend was performed to analyze the changes in mortality across the study periods: 1985–1994 (P1), 1995–2004 (P2), and 2005–2014 (P3).

For the mortality analysis, OAIs were divided into two categories depending on the presence or absence of peripheral SA. Those without peripheral SA included patients with osteomyelitis (vertebral and peripheral osteomyelitis) and SA of the axial skeleton. To determine the predictors of mortality, logistic regression models were built to estimate unadjusted and adjusted odds ratios (ORs) with their 95% confidence intervals (95% CIs). Clinically relevant variables associated with the outcome in the univariate analysis were entered in the multivariate model. We did not include variables related to infection severity (e.g., shock), which

may be considered intermediate variables rather than risk factors for mortality. The likelihood ratio test was used to obtain *p*-values.

Since most cases of peripheral SA are managed with surgical debridement, in contrast with other OAIs such as VO or axial SA, we also aimed to estimate the association of surgical debridement with mortality among those with bacteremic SA. We used two different approaches. First, a multivariate logistic regression model was built, adjusting by clinically relevant variables. In a second analysis, we performed a propensity score matching analysis including factors that could potentially affect the decision of receiving surgical debridement. Clinically relevant variables were introduced in the propensity model, together with baseline characteristics found to have a univariate association with surgical debridement ($p < 0.1$). Variables finally included in the propensity score matching model were age, McCabe and Jackson score, shock upon admission, PJI and *S. aureus* infection. 1:1 nearest neighbor matching with replacement was performed with a caliper of 0.1. The association between surgical debridement and mortality among those with surgical debridement after matching was expressed with OR and 95% CI.

Results

Between 1985 and 2014, a total of 36,507 episodes of bacteremia were recorded in our institution, of which 749 (2.1%) had a concomitant OAI. Of these, we excluded 18 that occurred in patients < 18 years, 70 that occurred in IVDU, 5 that were caused by *Neisseria* spp., and 6 that lacked data on mortality. Finally, 650 episodes were therefore analyzed (Fig. 1).

The median age of the 650 included patients was 66 years (IQR 54–75), of which 59.7% were males. The most frequent baseline medical conditions were diabetes mellitus (31.3%), immunosuppressive therapy (16%) and cancer (13.4%). Of note, there were 36 patients with rheumatoid arthritis, representing 25% of cases in the whole cohort of bacteremia (36/144 patients), in contrast with those without rheumatoid arthritis (614/36363; 1.7%) ($p < 0.001$).

These 650 patients presented with 691 B-OAIs, which were 286 SA (41.4%), 278 VO (40.2%) and 127 peripheral osteomyelitis (18.4%). Among the 286 cases of SA, axial arthritis was present in 44 (15.4%), native peripheral arthritis in 168 (58.7%) and PJI in 74 (25.9%). Thus, 242 patients (84.6%) presented with peripheral SA. The main microorganism involved in B-OAIs was *S. aureus* (56.8%), of which 16.3% of strains were methicillin-resistant. Cases in those with rheumatoid arthritis were mainly caused by *S. aureus* (80.6%). Other groups of microorganisms were: GNB (22.2%), with *E. coli* accounting for more than 10% of all cases, pyogenic streptococci (9.1%) and viridans-group streptococci (6.5%).

Analysis of mortality

Among 650 patients with B-OAIs, mortality (30-day case-fatality rate) was observed in 79 cases (12.2%), without significant differences across the study periods: 7/71 (9.9%) in P1, 34/226 (15%) in P2 and 38/353 (10.8%) in P3 ($p = 0.564$). Early mortality (≤7 days) was 5.1% (33 cases), representing 41.8% of patients who died (Fig. 2).

Mortality was greater in cases with PJI (19/74; 25.7%) and native SA (26/168; 15.5%), compared with axial SA (2/44; 4.6%), VO (22/278; 7.9%), and peripheral osteomyelitis (11/127; 8.7%). Overall mortality was greater in those with peripheral SA, including native SA and PJI, (45/242; 18.6%) than in those with other OAIs (34/408; 8.3%) ($p < 0.001$). Early mortality was also greater in cases with peripheral SA (22/242; 9.1%) than in cases with other OAIs (11/408; 2.7%) ($p < 0.001$). Figure 3 shows the cumulative mortality in patients with peripheral SA and other B-OAIs. No significant differences in overall mortality were found between primary and metastatic B-OAIs (11.2% vs 15.09%; $p = 0.192$) and primary and metastatic peripheral SA (17.6% vs 21.7%; $p = 0.481$).

The 30-day mortality for each covariate, together with the unadjusted ORs, are summarized in Table 1. Patients older than 65 years were more likely to die than younger patients (17.2% vs 7.1%; $p < 0.001$). In fact, mortality increased clearly with age, though

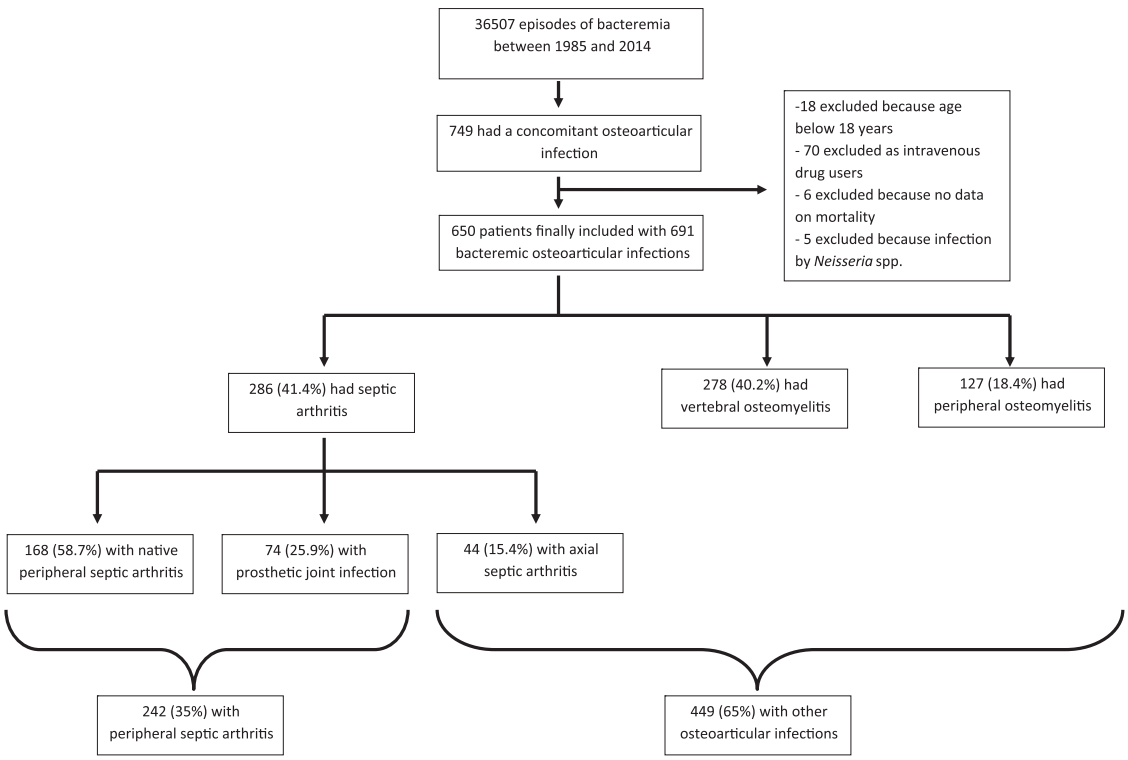


Fig. 1. Flowchart of participants into the study.

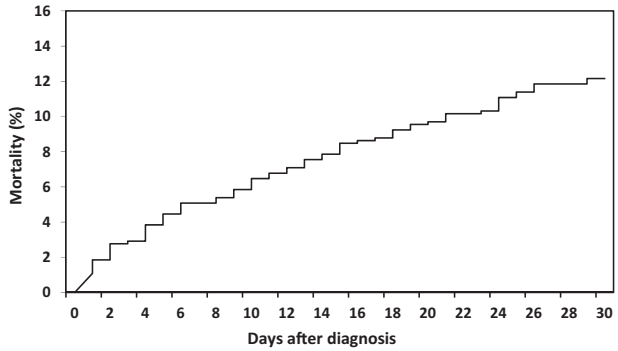


Fig. 2. Cumulative mortality among all 650 cases with bacteraemic osteoarticular infections.

patients with peripheral SA had higher mortality in all age groups (Fig. 4).

Liver cirrhosis and rheumatoid arthritis were associated with greater mortality (deaths/total): 13/38 [34.2%] in patients with liver cirrhosis vs 66/612 [10.8%] in those without ($p < 0.001$); 10/36 [27.8%] in patients with rheumatoid arthritis vs 69/614 [11.2%] in those without ($p = 0.003$). These higher mortality rates were also observed in patients from the whole cohort of bacteremia cases: 872/2965 [29.4%] in patients with liver cirrhosis vs 6005/33542 [17.9%] in those without ($p < 0.001$); 43/144 [29.9%] in patients with rheumatoid arthritis vs 8145/36,363 [22.4%] in those without ($p = 0.032$). Thus, no significant differences in mortality rates were found in patients with these comorbidities when they had B-OAIs or bacteremia from other sources. It should be noted that patients with rheumatoid arthritis had more often peripheral SA (77.8% vs 34.9%; $p < 0.001$) and, among the 10 patients who died, 9 had peripheral SA.

B-OAIs caused by *S. aureus* was associated with a mortality rate of 14.9% (55/361), which was greater in cases of methicillin-resistant than in methicillin-sensitive *S. aureus* (26.7% vs 12.6%; $p = 0.005$). *S. aureus* was the causative agent in 69.6% of patients who died. Although mortality associated with *S. aureus* was higher in all B-OAI, those associated with peripheral SA had the greatest mortality (Fig. 5). Mortality was 5.1% (3/59) for patients with pyogenic *streptococci*, whereas it was 11.9% (5/42) for those with viridans *streptococci*, and 7.6% (11/144) for those with GNB.

Adjustment in a multivariate model (Table 1) which included all statistically significant variables from the univariate analysis, indicated that peripheral SA was associated with a two-fold increased odds of mortality (adjusted OR 2.12; 95% CI: 1.22–3.69; $p = 0.008$). Other variables with adjusted ORs significantly associated with mortality were age older than 65 years, liver cirrhosis, rheumatoid arthritis, the McCabe and Jackson score, and *S. aureus* infection.

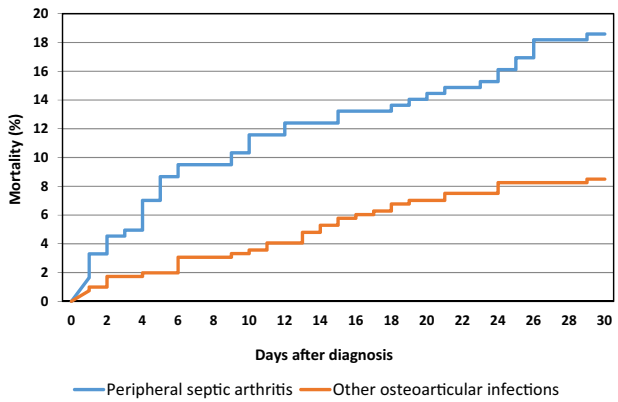


Fig. 3. Cumulative mortality in patients with peripheral septic arthritis compared with other bacteraemic osteoarticular infections.

Peripheral septic arthritis

We evaluated the impact of surgical debridement on mortality in 239 patients with peripheral SA (3 patients had no information available). There were not significant differences between those treated with surgical debridement (191; 79.9%) compared to those who were not (48; 20.1%), according to age, comorbidities, type of OAI and McCabe and Jackson score (Table 2). *S. aureus* infection was more frequent among those treated with surgical debridement (62.8% vs 45.8%; $p = 0.032$).

Importantly, mortality was lower among patients treated with surgical debridement compared with those who were not (28/191 [14.7%] vs 16/48 [33.3%]; $p = 0.003$); in particular, early mortality was significantly lower among patients who underwent surgical debridement compared with those who did not (9/191 [4.7%] vs 12/48 [25%]; $p < 0.001$). After adjusting for potential confounders (age, liver cirrhosis, rheumatoid arthritis, McCabe and Jackson score, PJI, and *S. aureus* infection) surgical debridement remained significantly associated with reduced mortality (adjusted OR 0.23; 95% CI: 0.09–0.57; $p = 0.002$). After propensity score matching, surgical debridement was still associated with decreased mortality in patients with bacteremic SA (OR 0.81; 95% CI: 0.68–0.96; $p = 0.014$).

Discussion

The main findings of this study are: first, mortality in patients with B-OAIs was greater if the patient was elderly or had peripheral SA. Second, early mortality (≤7 days) accounted for more than 40% of all deaths. Third, underlying conditions like rheumatoid arthritis and liver cirrhosis were important risk factors for mortality. Fourth, *S. aureus* was the main causative microorganism of B-OAIs, and was associated with high mortality. Fifth, surgical debridement was associated with decreased mortality in patients with peripheral SA.

Although bacteremia is frequent among some OAIs and it has been associated with a poor prognosis, to our knowledge, no previous studies had addressed mortality in a large cohort of patients with B-OAIs [6–9,14]. However, restricting our study to patients with bacteremia alone makes it difficult to compare our mortality figures with those of other studies. Comparison is further confounded by the lack of definitions of mortality, the use of different time cut-offs for defining mortality [7,9,14], or because some studies were limited to particular microorganisms [6,8,15].

Patients with rheumatoid arthritis, in comparison with the general population, are considered to have an increased risk of mortality and greater susceptibility to infection, including bacteremia, SA and osteomyelitis [16,17]. Our data reinforce this concept, showing that rheumatoid arthritis was a major risk factor for increased mortality. Most patients with rheumatoid arthritis also presented with peripheral SA, with this being the most frequent OAI among those who died. In current clinical practice, the increasing use and availability of biological therapies and steroids for patients with rheumatoid arthritis may be associated with this increased likelihood of severe infections [18,19]. Liver cirrhosis was also associated with increased mortality in our patients with B-OAIs.

The impact of *S. aureus* bacteremia has been extensively evaluated, with research showing that OAIs are one of its major complications [20,21]. In our study, *S. aureus* was the most frequent etiology among those who died, and it was a predictor

Table 1
Risk factors for 30-day mortality in patients with bacteraemic osteoarticular infections (n = 650)

Variable	Number of individuals	Dead within 30 days (%)	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age						
≤65 years	325	23 (7.1)	1		1	
> 65 years	325	56 (17.2)	2.73 (1.64–4.56)	< 0.001	2.51 (1.41–4.49)	0.001
Sex						
Female	262	33 (12.6)	1			
Male	388	46 (11.9)	0.93 (0.58–1.50)	0.777		
Cancer						
No	562	66 (11.7)	1			
Yes	87	13 (14.9)	1.32 (0.69–2.51)	0.408		
Diabetes mellitus						
No	446	54 (12.1)	1			
Yes	203	25 (12.3)	1.02 (0.61–1.69)	0.940		
Liver cirrhosis						
No	612	66 (10.8)	1		1	
Yes	38	13 (34.2)	4.30 (2.10–8.81)	< 0.001	3.10 (1.29–7.46)	0.014
Chronic kidney disease						
No	587	69 (11.8)	1			
Yes	63	10 (15.9)	1.42 (0.69–2.91)	0.359		
Rheumatoid arthritis						
No	614	69 (11.2)	1		1	
Yes	36	10 (27.8)	3.04 (1.41–6.57)	0.009	3.02 (1.20–7.55)	0.024
McCabe & Jackson score						
I	528	34 (6.4)	1		1	
II–III	122	45 (36.9)	8.49 (5.12–14.08)	< 0.001	8.28 (4.71–14.56)	< 0.001
Peripheral septic arthritis						
No	408	34 (8.3)	1		1	
Yes	242	45 (18.6)	2.51 (1.56–4.05)	< 0.001	2.12 (1.22–3.69)	0.008
S. aureus infection						
No	281	24 (8.5)	1		1	
Yes	369	55 (14.9)	1.88 (1.13–3.11)	0.012	2.19 (1.23–3.90)	0.006

OR: Odds ratio. CI: confidence interval. McCabe & Jackson score (I = nonfatal disease; II = ultimately fatal disease, and III = rapidly fatal disease).

of 30-day mortality in the whole cohort. What is clear is that *S. aureus* bacteremia is associated with significant mortality rates [22] that tend to be higher with methicillin-resistant *S. aureus* bacteremia [23,24]. Our results are consistent with such research findings.

Cases of peripheral SA presented the highest mortality among all types of B-OAIs (18.6%), with the lower mortality observed in patients with axial SA. In accordance with previous reports [25–27], the latter pattern of SA has been highlighted in young people and IVDU, but has also been shown in other populations. In the present study, we confirmed that mortality was clearly different when associated with SA of the peripheral joints or of the axial skeleton, supporting the argument that they should be considered two separate entities of SA.

Previous reports that included bacteremic and non-bacteremic cases of SA had shown mortality rates around 10% [28,29], possibly representing a midpoint between our rates for peripheral and axial SA. The higher mortality of peripheral SA may partly be explained

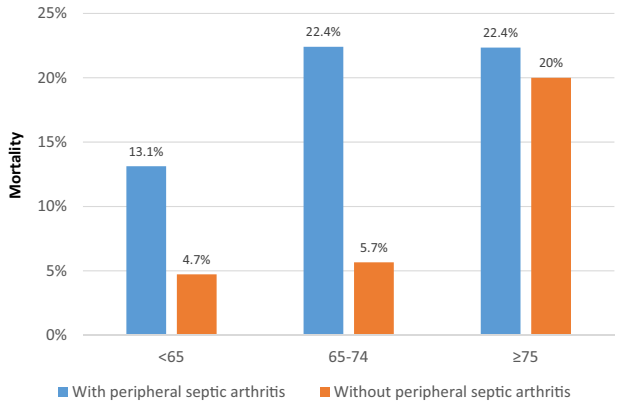


Fig. 4. Mortality by age group and presence or absence of peripheral septic arthritis.

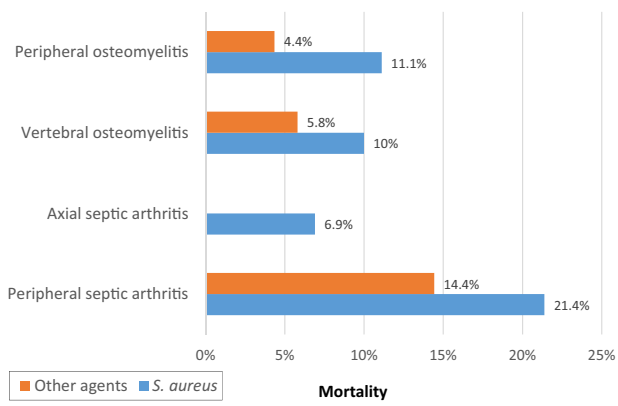


Fig. 5. Mortality rates by the type of osteoarticular infection and whether *Staphylococcus aureus* was the causative agent.

Table 2
Baseline characteristics of 239 patients with bacteremic peripheral septic arthritis, according to receiving or not surgical debridement

	No surgical debridement (n = 48)	Surgical debridement (n = 191)	p value
Age (median, IQR)	67 (54–77)	70 (58–78)	0.262
Age > 65 years	25 (52.1)	114 (59.7)	0.340
Male	22 (45.8)	103 (53.9)	0.316
One or more underlying diseases	37 (77.1)	141 (73.8)	0.643
Diabetes mellitus	9 (18.8)	56 (29.3)	0.141
Neoplasm	9 (18.8)	25 (13.1)	0.316
Cardiopathy	7 (14.6)	25 (13.1)	0.786
Liver cirrhosis	5 (10.4)	9 (4.7)	0.132
Chronic kidney disease	3 (6.3)	14 (7.3)	0.795
Immunosuppressive therapy	10 (20.8)	47 (24.6)	0.583
Rheumatoid arthritis	6 (12.5)	21 (11.0)	0.768
McCabe-Jackson score I-II	12 (25.0)	37 (19.4)	0.388
Prosthesis joint infection	10 (20.8)	63 (33.0)	0.102
Post-surgical infections	5 (10.4)	28 (14.7)	0.446
S. aureus infection	22 (45.8)	120 (62.8)	0.032

Data expressed as No. (%), if not stated otherwise. McCabe & Jackson score (I = nonfatal disease; II = ultimately fatal disease, and III = rapidly fatal disease). IQR: interquartile range.

by the inclusion only of bacteremic cases and partly by the patients' characteristics, because these individuals were older and more frequently had chronic medical conditions. However, the features of peripheral SA itself may play a major role, especially regarding the accumulation of purulent collections in enclosed spaces, the high inoculum associated with the infection, or the enhanced inflammatory response. Thus, bacteremic peripheral SA should be considered an emergency, and physicians should manage it accordingly.

We recommend that surgical debridement be considered for any patient presenting with peripheral SA, especially if large joints are affected. This approach has also been recommended for native SA [1] and PJI [10]. We showed that surgical debridement reduced the risk of mortality, especially early mortality. In this context, we believe that surgery should ensure the removal of the purulent content in the joint, and that this may improve the effectiveness of antibiotics and reduce the inflammatory response. In turn, these factors affect the mortality observed in these patients. In those who may not be fit enough for surgery or transfer to the operation theatre, physicians may consider joint drainage by arthrocentesis, even repeated if needed, and defer the debridement until the patient has been stabilized.

Our study has several limitations. It was an observational study performed in a single center, thereby limiting the generalizability of the results. In addition, since it was performed in a tertiary teaching centre with a specialized Bone and Joint Infection Unit, referral bias may have occurred. Despite the inclusion of a large cohort, the sample size may have been too small for the analysis of some patient subsets. We also included patients treated in our hospital for a long period of time and treatment strategies might have changed, which may have affected our results. Although we evaluated the role of surgical debridement in those with bacteremic SA using a multivariate regression model and a propensity-score matching analysis, it is still possible that other variables may have affected our results. Factors affecting the physician's decision-making process of performing surgical debridement, such as the patient's sickness, could have caused selection bias. In this line, adjusting by the McCabe-Jackson score or introducing shock in the propensity-score matching analysis try to reduce the impact in the association between surgical debridement and mortality, but the possibility of residual bias cannot be excluded.

Conclusions

In conclusion, we believe that the findings of this study can help clinicians identify patients with B-OAIs at higher risk of mortality. Clinicians should be aware that mortality rates associated with B-OAIs are significant, especially in the elderly, in those with rheumatoid arthritis or liver cirrhosis, and in cases caused by *S. aureus*. Our results also indicated that mortality was higher from peripheral SA than from other types of OAIs. In this context, we observed a protective role of surgical debridement that should encourage clinicians to definitely incorporate this procedure in the early global management of patients with peripheral SA.

Funding information

J. G.-J. was supported by a grant from the Spanish Ministry of Education (FPU 14/03124).

Conflict of interest

None.

Acknowledgments

We thank Michael Maudsley for revising the English manuscript. The preliminary results of this study were reported in part at the XX Congress of the Spanish Society of Infectious Diseases and Clinical Microbiology (Barcelona, Spain, 2016).

References

[1] Mathews CJ, Weston VC, Jones A, Field M, Coakley G. Bacterial septic arthritis in adults. Lancet 2010;375:846–55, http://dx.doi.org/10.1016/s0140-6736(09)61595-6.
[2] Shirtliff ME, Mader JT. Acute septic arthritis. Clin Microbiol Rev 2002;15:527–44.
[3] Lew DP, Waldvogel FA. Osteomyelitis. Lancet 2004;364:369–79, http://dx.doi.org/10.1016/s0140-6736(04)16727-5.
[4] Zimmerli W. Clinical practice. Vertebral osteomyelitis. N Engl J Med 2010;362:1022–9, http://dx.doi.org/10.1056/NEJMcp0910753.
[5] Murillo O, Grau I, Lora-Tamayo J, Gomez-Junyent J, Ribera A, Tubau F, et al. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. Clin Microbiol Infect 2015;21:254.e1–8, http://dx.doi.org/10.1016/j.cmi.2014.09.007.
[6] Lora-Tamayo J, Murillo O, Iribarren JA, Soriano A, Sanchez-Somolinos M, Baraia-Etxaburu JM, et al. A large multicenter study of methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* prosthetic joint infections managed with implant retention. Clin Infect Dis 2013;56:182–94, http://dx.doi.org/10.1093/cid/cis746.
[7] Pigrau C, Almirante B, Flores X, Falco V, Rodriguez D, Gasser I, et al. Spontaneous pyogenic vertebral osteomyelitis and endocarditis: incidence, risk factors, and outcome. Am J Med 2005;118:1287, http://dx.doi.org/10.1016/j.amjmed.2005.02.027.
[8] Sendi P, Banderet F, Graber P, Zimmerli W. Clinical comparison between exogenous and haematogenous periprosthetic joint infections caused by *Staphylococcus aureus*. Clin Microbiol Infect 2011;17:1098–100, http://dx.doi.org/10.1111/j.1469-0691.2011.03510.x.
[9] Nolla JM, Lora-Tamayo J, Gomez Vaquero C, Narvaez J, Murillo O, Pedrero S, et al. Pyogenic arthritis of native joints in non-intravenous drug users: a detailed analysis of 268 cases attended in a tertiary hospital over a 22-year period. Semin Arthritis Rheum 2015;45:94–102, http://dx.doi.org/10.1016/j.semarthrit.2015.01.009.
[10] Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645–54, http://dx.doi.org/10.1056/NEJMra040181.
[11] Mc CW, Jackson G. Gram-negative bacteremia: I. etiology and ecology. Arch Intern Med 1962;110:847–55, http://dx.doi.org/10.1001/archinte.1962.03620240029006.
[12] Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition 2012.
[13] Sherman JM. The Streptococci. Bacteriol Rev 1937;1:3–97.

[14] Maneiro JR, Souto A, Cervantes EC, Mera A, Carmona L, Gomez-Reino JJ. Predictors of treatment failure and mortality in native septic arthritis. Clin Rheumatol 2015;34:1961–7, http://dx.doi.org/10.1007/s10067-014-2844-3.
[15] Park KH, Cho OH, Jung M, Suk KS, Lee JH, Park JS, et al. Clinical characteristics and outcomes of hematogenous vertebral osteomyelitis caused by gram-negative bacteria. J Infect 2014;69:42–50, http://dx.doi.org/10.1016/j.jinf.2014.02.009.
[16] Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum 2002;46:2287–93, http://dx.doi.org/10.1002/art.10524.
[17] Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094–108, http://dx.doi.org/10.1016/s0140-6736(10)60826-4.
[18] Singh JA, Cameron C, Noorbaloochi S, Cullis T, Tucker M, Christensen R, et al. Risk of serious infection in biological treatment of patients with rheumatoid arthritis: a systematic review and meta-analysis. Lancet 2015;386:258–65, http://dx.doi.org/10.1016/s0140-6736(14)61704-9.
[19] van Dartel SA, Fransen J, Kievit W, Dutmer EA, Brus HL, Houtman NM, et al. Predictors for the 5-year risk of serious infections in patients with rheumatoid arthritis treated with anti-tumour necrosis factor therapy: a cohort study in the Dutch Rheumatoid Arthritis Monitoring (DREAM) registry. Rheumatology (Oxford) 2013;52:1052–7, http://dx.doi.org/10.1093/rheumatology/kes413.
[20] Fowler VG Jr., Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated Staphylococcus aureus bacteremia. Arch Intern Med 2003;163:2066–72, http://dx.doi.org/10.1001/archinte.163.17.2066.
[21] Murdoch DR, Roberts SA, Fowler VG Jr., Shah MA, Taylor SL, Morris AJ, et al. Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. Clin Infect Dis 2001;32:647–9, http://dx.doi.org/10.1086/318704.
[22] Tom S, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schonheyder HC, et al. Case fatality ratio and mortality rate trends of community-onset *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 2014;20:O630–2, http://dx.doi.org/10.1111/1469-0691.12564.
[23] Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 2003;36:53–9, http://dx.doi.org/10.1086/345476.
[24] Gasch O, Camoez M, Dominguez MA, Padilla B, Pintado V, Almirante B, et al. Predictive factors for mortality in patients with methicillin-resistant *Staphylococcus aureus* bloodstream infection: impact on outcome of host, microorganism and therapy. Clin Microbiol Infect 2013;19:1049–57, http://dx.doi.org/10.1111/1469-0691.12108.
[25] Bossert M, Prati C, Bertolini E, Toussirot E, Wendling D. Septic arthritis of the acromioclavicular joint. Joint Bone Spine 2010;77:466–9, http://dx.doi.org/10.1016/j.jbspin.2010.03.010.
[26] Ross JJ, Hu LT. Septic arthritis of the pubic symphysis: review of 100 cases. Medicine (Baltimore) 2003;82:340–5, http://dx.doi.org/10.1097/01.md.0000091180.93122.1c.
[27] Ross JJ, Shamsuddin H. Sternoclavicular septic arthritis: review of 180 cases. Medicine (Baltimore) 2004;83:139–48.
[28] Gupta MN, Sturrock RD, Field M. A prospective 2-year study of 75 patients with adult-onset septic arthritis. Rheumatology (Oxford) 2001;40:24–30.
[29] Kaandorp CJ, Krijnen P, Moens HJ, Habbema JD, van Schaardenburg D. The outcome of bacterial arthritis: a prospective community-based study. Arthritis Rheum 1997;40:884–92, http://dx.doi.org/10.1002/1529-0131(199705)40:5<884::AID-ART15>3.0.CO;2-6.

European Journal of Drug Metabolism and Pharmacokinetics
https://doi.org/10.1007/s13318-020-00625-5

ORIGINAL RESEARCH ARTICLE

Efficacy and Therapeutic Drug Monitoring of Continuous Beta-Lactam Infusion for Osteoarticular Infections Caused by Fluoroquinolone-Resistant *Pseudomonas aeruginosa*: A Prospective Cohort Study

Joan Gómez-Junyent[1] · Raul Rigo-Bonnin[2] · Eva Benavent[1] · Laura Soldevila[1] · Ariadna Padullés[3] · Xavier Cabo[4] · Fe Tubau[5] · Javier Ariza[1] · Oscar Murillo[1]

Abstract

Background and Objectives Osteoarticular infections (OIs) caused by fluoroquinolone-resistant *Pseudomonas aeruginosa*, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, have poor outcomes. We evaluated the outcomes of an optimized strategy of continuous beta-lactam infusion (BL-CI) guided by therapeutic drug monitoring (TDM) for OIs caused by fluoroquinolone-resistant *P. aeruginosa*.

Methods A prospective observational study of patients with *P. aeruginosa* OIs in a hospital-based BL-CI program (2016–2018) was carried out. TDM targeting free BL concentrations in plasma (*f*Css) of at least 3–4 × MIC was performed. We compared failure rates between patients with OIs caused by fluoroquinolone-resistant strains who were treated with BL-CI, with or without colistin, and patients with OIs caused by fluoroquinolone-susceptible strains who were treated with ciprofloxacin.

Results Fifty-two patients were included in the study, 19 (36.5%) of whom had OIs caused by fluoroquinolone-resistant *P. aeruginosa* (13 (68.4%) MDR/XDR strains; 11 (57.9%) device-related infections). The median duration of BL-CI was 36 days; ten patients (52.6%) received BL–colistin combinations. Eighty-two samples were utilized in the TDM, and most patients were found to have a median *f*Css of 3–10 × MIC; 17 dose adjustments were performed and eight patients needed dose decreases, five of which were due to chronic kidney disease or acute kidney injury (AKI). BL-CI was well tolerated, with the most frequent adverse event being AKI. Failure occurred to 4 patients (21.1%), which was similar to the failure rate of patients with OIs caused by fluoroquinolone-susceptible *P. aeruginosa* treated with ciprofloxacin (5/30 [16.7%]) ($p = 0.699$). TDM was also used in the initial BL treatment of patients with OIs caused by susceptible strains before those patients were switched to treatment with ciprofloxacin alone (33 patients, 110 samples, 19 dose adjustments).

Conclusions BL-CI used with/without colistin and supported by TDM may be an alternative and effective treatment option for OIs caused by fluoroquinolone-resistant *P. aeruginosa*, where limited available therapeutic options exist, especially in the setting of multidrug resistance. Future research should elucidate whether this strategy can produce outcomes similar to those of patients treated for OIs caused by fluoroquinolone-susceptible strains.

1 Introduction

Osteoarticular infections (OIs) represent a challenge for clinicians due to the presence of bacterial biofilms, which impair the activities of most antibiotics [1–3]. While OIs are mainly caused by gram-positive microorganisms, the incidence of cases caused by gram-negative bacteria (GNB) is increasing [4, 5]. Among these GNB, *Pseudomonas aeruginosa* is a frequent causative agent of OIs.

Fluoroquinolones are the mainstay of the antimicrobial therapy of patients with GNB OIs, and have yielded good results [6, 7] even in the most demanding cases involving prosthetic joint infection (PJI) managed with debridement, antibiotics, and implant retention (DAIR). In contrast, beta-lactams (BL) are usually associated with worse outcomes [7, 8].

The emergence of increasing resistance of GNB to fluoroquinolones is concerning because it is associated with the appearance of multidrug-resistant (MDR) and extensively

✉ Oscar Murillo
omurillo@bellvitgehospital.cat

Extended author information available on the last page of the article

Published online: 21 May 2020

Key Points

Osteoarticular infections caused by quinolone-resistant *P. aeruginosa* have poor cure rates, but continuous beta-lactam infusion supported by therapeutic drug monitoring may improve outcomes.

This strategy, with or without colistin, yielded desirable target concentrations, was well tolerated, and resulted in high cure rates.

This strategy could be a promising therapeutic option for osteoarticular infections caused by quinolone- and multidrug-resistant *P. aeruginosa*.

drug-resistant (XDR) strains, which are frequently resistant to most antibiotics in vitro. Colistin, which is often the only available option and has shown good anti-biofilm activity [9, 10], has been recommended for the treatment of MDR/XDR *P. aeruginosa* OIs in combination with BL [11].

Given the limited treatment options available for MDR/XDR *P. aeruginosa* OIs, new therapeutic alternatives are clearly needed. The use of continuous BL infusion (BL-CI) could optimize the pharmacodynamics of BL [12, 13] and improve the outcomes of patients with OIs caused by *P. aeruginosa*. Moreover, the use of BL-CI may represent an opportunity to recover the activity of BLs against MDR/XDR *P. aeruginosa*, which are resistant in vitro to these drugs when administered via conventional intermittent infusion. We recently reported our initial experience using BL-CI in patients with GNB OIs after the development of an institutional program (2012–2015) [14]. We described an easy-to-use method of calculating suitable initial antibiotic dosages, and noted the convenience of implementing therapeutic drug monitoring (TDM) to ensure that the target concentration is achieved and support this clinical practice.

Thus, in this study, we present our prospective experience with the TDM of BL-CI within this program (2016–2018) and compare the outcomes of patients with fluoroquinolone-resistant *P. aeruginosa* OIs managed with an optimized therapeutic strategy to the outcomes of patients with *P. aeruginosa* OIs who could receive fluoroquinolones.

2 Patients and Methods

2.1 Study Design, Setting, and Inclusion/Exclusion Criteria

A prospective observational study was performed at Hospital Universitari de Bellvitge (January 2016 to December 2018), a large teaching hospital in Spain. Patients with OIs caused by *P. aeruginosa* who were admitted to our Bone and Joint Infection Unit, irrespective of their in vitro antibiotic susceptibility testing, were systematically given BL-CI over 24 h and managed with TDM to maintain a drug concentration that optimized the pharmacokinetics/pharmacodynamics ($T > \text{MIC} = 100\%$, where $T >$ MIC is the time spent above the minimum inhibitory concentration) during the treatment course (see below). In particular, carbapenems were administered as an extended infusion due to stability issues [15] and to allow for a potential postantibiotic effect; thus, even though carbapenem administration can also be guided by TDM, patients who received carbapenems were not included in the present study. We aimed to compare the outcomes of patients with fluoroquinolone-resistant *P. aeruginosa* OIs treated using a BL-CI strategy, which may allow recovering antipseudomonal activity in most cases, to the outcomes of patients with OIs caused by fluoroquinolone-susceptible strains who received first-line treatment with fluoroquinolones. Considering that previous experience of the treatment of fluoroquinolone-resistant *P. aeruginosa* (including MDR/XDR strains) is scarce, a specific sample size could not be estimated.

In order to evaluate the feasibility of TDM in this setting and how it performed throughout the treatment course, including dose adjustments, only patients who had at least two serial blood samples extracted to determine BL concentrations were included. Polymicrobial infections that included *P. aeruginosa* and cases where *P. aeruginosa* was involved after a different primary infection (superinfection) were also included. Diabetic foot infections were excluded.

This study was approved by the hospital's ethics committee (reference EPA045/16) and authorized by the Spanish Agency of Drugs and Sanitary Products (AEMPS) (reference 16744/RG 29045). All patients signed an informed consent before they were included in the study.

2.2 Definitions

All cases included in the study fulfilled the main diagnostic criteria for each type of OI [11]. The term "OI" included PJIs and osteoarthritis (with or without an orthopedic device).

OIs by *P. aeruginosa* were identified by positive cultures from two or more surgical samples or by one positive surgical culture, joint aspirate, or blood culture, together with classical symptoms and signs of infection. Microbiological studies were performed as previously reported [11]. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains were defined according to the criteria proposed by Magiorakos et al. [16].

Chronic kidney disease (CKD) and acute kidney injury (AKI) were defined according to standardized criteria [17, 18].

2.3 Clinical/Surgical Management

Patients with an acute postsurgical PJI or device-associated osteoarthritis were commonly managed with DAIR, according to current recommendations [1, 2]. Implant removal was performed otherwise.

Patients were usually treated with the selected antibiotic plan for 4–6 weeks. We aimed to use combination therapy with BL and another antipseudomonal agent, when possible, as follows. For fluoroquinolone-resistant *P. aeruginosa*, we combined BL with colistin, which was started at 2 million IU (MIU) every 8 h (without a loading dose) when renal function was normal, but was adjusted in those with CKD according to available recommendations [19]. In order to prevent potential toxicity, colistin was generally not considered for fragile patients (those aged ≥ 75 years and those with comorbidities), especially if they had CKD [20] and/or were managed with implant removal or bone resection. The antipseudomonal beta-lactam used was generally ceftazidime, except in cases with MDR/XDR strains, when the BL with the lowest MIC was usually chosen. For fluoroquinolone-susceptible *P. aeruginosa*, oral ciprofloxacin (750–1000 mg twice daily but adjusted according to renal function) was combined with BL for at least 7–14 days, after which the patient was switched to ciprofloxacin alone. Doses of BL and combination agents were also adjusted if AKI occurred during therapy, as per available recommendations [21].

2.4 Continuous Infusion of Beta-Lactams and Therapeutic Drug Monitoring

Our protocol recommends that patients should be commenced on empiric antibiotic treatment, mainly consisting of intermittent BL infusion plus glycopeptides, while awaiting microbiological results from surgical procedures or tissue samples. When OI caused by *P. aeruginosa* strains was confirmed, the BL therapy was switched to CI. The daily dosage of BL-CI was based on a previously described formula and our own experience [12, 14]. A loading dose was commonly administered to rapidly attain the target steady-state concentration (*f*Css). Loading doses of BL were 2 g (ceftazidime, cefepime, aztreonam), 2/1 g (ceftolozane-tazobactam), and 4/0.5 g (piperacillin-tazobactam).

The first blood samples that were used to check the *f*Css were extracted from each patient at least 24 h after BL-CI initiation (corresponding to a period of 4–5 half-lives of the BL). Samples were then extracted at the clinicians' discretion (weekly if possible) to check that the *f*Css was being attained. Clinicians were encouraged to obtain a new sample at least 24–48 h after modifying the daily BL dose. Once extracted, the samples were immediately centrifuged and frozen at − 80 °C until analysis to avoid disturbing their stability. In our hospital, BL measurements are performed twice weekly, so samples were analyzed a maximum of 72 h after they were collected. The stability of the samples as well as the UPLC-MS/MS methodology used to measure plasma concentrations have been validated, as reported elsewhere [22].

In general, we aimed to optimize the most relevant PK/PD target for BL by ensuring that $T > \text{MIC} = 100\%$ and that *f*Css was at least 3 or 4 times the MIC (3–4 × MIC), since this concentration has been correlated with the maximum killing effect of BL [12]. However, as higher concentrations can also be desirable in OIs due to poor bone penetration or the potential concentration-dependent effect of BL in these infections, we used *f*Css values of between 3–4 × MIC and 10 × MIC. The daily dosage was adjusted based on the measured BL concentrations in plasma; our protocol suggests that dose adjustments (increases or decreases) should be 50% of the initial dose. Dose reductions were usually performed when concentrations were 10–15 × MIC, higher than 100 mg/L [23], or when toxicity occurred. However, the criteria for adjusting the dosage was ultimately decided by the clinicians, and the decision to adjust the dose was taken by consensus after a multidisciplinary discussion. For isolates with high MICs, 3–4 × MIC was often difficult to achieve, so lower levels above the MIC (≥ 1.5–2 × MIC) were considered acceptable. Free BL fractions were estimated from reported protein binding in healthy subjects [24–28].

2.5 Outcome

After the treatment, the patients were clinically assessed in the outpatient clinic at months 3, 6, and 12; the patients were then reviewed at the clinicians' discretion. Failure was defined as (a) death related to the infection, (b) implant removal due to infection persistence/relapse in patients with an orthopedic device, (c) new debridements > 30 days after the first, due to persistence/relapse, and (d) relapsing symptoms during follow-up. Superinfection or orthopedic problems were not considered failures.

We also monitored adverse events throughout the treatment course by performing either clinical assessments or routine blood tests. Adverse events attributable to BL were defined according to the common side effects associated with their use [21] and/or an absence of a plausible alternative explanation.

2.6 Statistical Analysis

Data were analyzed with Stata 13.1 (Stata Corporation, USA). Categorical variables were described by counts and

percentages, while the median and interquartile range (IQR) were used to summarize continuous variables. Comparisons between groups were performed with the chi-square test or Fisher's exact test for categorical variables and the Mann–Whitney test for continuous variables.

The failure rate of patients with OIs caused by fluoroquinolone-resistant *P. aeruginosa* was compared to the failure rate of patients with fluoroquinolone-susceptible *P. aeruginosa*. To achieve this, we excluded patients in the susceptible group who were not treated with fluoroquinolones and we avoided survivor's bias by restricting the analysis to those who did not fail in the first 21 days following treatment initiation. Antibiotic therapy for osteoarticular infections has a relatively long duration (6–8 weeks), which could be shortened in cases failing prematurely. Therefore, the antimicrobial therapy parameters were only analyzed when the comparison groups had the same possibility of receiving antibiotics. Kaplan–Meier curves and the log-rank test were used to compare the cumulative likelihood of failure between groups. A p value of ≤ 0.05 was considered to be statistically significant.

3 Results

During the study period, 61 patients with OIs caused by *P. aeruginosa* were identified, of whom nine were excluded (one had a diabetic foot infection, three were treated with meropenem, and five patients each provided only one sample for TDM). Therefore, 52 patients were ultimately included in the analysis, 19 (36.5%) of whom had OIs caused by fluoroquinolone-resistant *P. aeruginosa*.

3.1 Patients with OIs Caused by Fluoroquinolone-Resistant *P. aeruginosa*

3.1.1 Characteristics of the Patients and Infections

The main characteristics of the cases included in this study are summarized in Table 1. The majority were female (11, 57.9%) and the median age was 67 years (IQR 55–76). Most patients had at least one comorbidity. Three patients (15.8%) had CKD, but none were on hemodialysis.

Device-related infections accounted for more than half of all OIs (osteoarthritis, $n=6$, 31.6%; PJI, $n=5$, 26.3%). Most infections were caused by MDR or XDR strains (13, 68.4%), and 11 (57.9%) were carbapenem resistant.

Almost one-third of all OIs were polymicrobial. More than half of the patients had postsurgical OIs, and the OI was cataloged as a superinfection in two patients (10.0%).

3.1.2 Management of Infections

All but two patients were treated with surgery. Most (7, 63.6%) of those who had device-associated infections managed with surgery were treated by implant removal.

The median duration of BL-CI was 36 days (IQR 28–39). Only one patient was initiated on BL-CI immediately; the rest received intermittent infusion for a median of 6 days (IQR 4–7) prior to CI. The median global duration of antibiotic therapy was 42 days (IQR 30–46). Ten patients (52.6%) received combinations of BL with colistin.

MIC values for each BL and doses used in BL-CI are summarized in Table 2. In general, patients were treated with median BL doses that were lower than the doses they may have received with intermittent infusion, according to product data sheets. BLs were chosen according to susceptibility, but two cases were treated with ceftazidime with a MIC of 16 mg/L. This was the lowest BL MIC available upon testing; alternatives such as ceftolozane-tazobactam or ceftazidime-avibactam were not yet available in our hospital.

3.1.3 Therapeutic Drug Monitoring

Overall, 82 plasma samples were taken from the patients in order to monitor BL concentrations (a median of five per patient, IQR 3–6) during CI (Table 2). The median *f*Css expressed in mg/L and as multiples of the respective *P. aeruginosa* MIC in each patient is shown in Table 3. Most patients had a median *f*Css of between 3 and $10\times$MIC. Median *f*Css values of less than $3\times$MIC were mainly found in patients with OIs caused by *P. aeruginosa* strains with MICs of 8–16 mg/L (cases 4, 14, 15, and 16), whereas four of the five patients with a *f*Css above $10\times$MIC had CKD or AKI (cases 2, 3, 7, and 8).

A total of 17 dose adjustments were performed in 12 patients (63.2%) during BL-CI (nine patients had one adjustment, one had two adjustments, and two had three adjustments). The median time from sample collection to dose adjustment was 2 days (IQR 1–3). Three patients did not initially achieve a *f*Css of at least $3\times$MIC, two of whom had OIs by *P. aeruginosa* with MIC of 8–16 mg/L to the BL used. The other patient, despite dose adjustment, did not meet that PK/PD target on subsequent samples. TDM values prompted ten dose decreases in eight patients; three of those patients presented AKI (cases 8, 9, and 17), two had CKD (cases 2 and 3), and two also received colistin (cases 5 and 10). Seven dose increases were performed in six patients, three of whom (cases 16, 17, and 19) had OIs caused by strains with a BL MIC of 4–8 mg/L.

Table 1 Baseline and clinical characteristics of 52 patients with osteoarticular infection caused by *Pseudomonas aeruginosa* and treated with continuous beta-lactam infusion

Characteristic	All patients ($n=52$)	Patients with fluoroquinolone-resistant strains ($n=19$)	Patients with fluoroquinolone-susceptible strains ($n=33$)	p value
Demographics				
Age (years) (median, IQR)	68 (55–75)	67 (55–76)	69 (55–74)	0.985
Male sex	27 (51.9)	8 (42.1)	19 (57.6)	0.282
Any comorbidity	31 (59.6)	13 (68.4)	18 (54.6)	0.326
Diabetes mellitus	12 (23.1)	6 (31.6)	6 (18.2)	0.270
Chronic heart disease	14 (26.9)	7 (36.8)	7 (21.2)	0.221
Chronic lung disease	10 (19.2)	5 (26.3)	5 (15.2)	0.325
Malignancy	1 (1.9)	0	1 (3.0)	0.444
Chronic kidney disease	8 (15.4)	3 (15.8)	5 (15.2)	0.951
Rheumatologic autoimmune disease	7 (13.5)	3 (15.8)	4 (12.1)	0.324
Immunosuppressive therapy	6 (11.5)	3 (15.8)	3 (9.1)	0.467
Chronic steroid therapy	6 (11.5)	4 (21.1)	2 (6.1)	0.103
Clinical data				
Type of infection				
Prosthetic joint infection	13 (25.0)	5 (26.3)	8 (24.2)	
Osteoarthritis (without device)	23 (44.2)	8 (42.1)	15 (45.5)	
Osteoarthritis (with device)	16 (30.8)	6 (31.6)	10 (30.3)	0.972
Device-related infections	29 (55.8)	11 (57.9)	18 (54.6)	0.815
Postsurgical infections	26 (50.0)	10 (52.6)	16 (48.5)	0.773
Microbiological data				
Bacteremia	4 (7.7)	1 (5.3)	3 (9.1)	0.618
Polymicrobial infection	19 (36.5)	6 (31.6)	13 (39.4)	0.573
Superinfection	6 (11.5)	2 (10.5)	4 (12.1)	0.862
MDR or XDR strains	13 (25.0)	13 (68.4)	0	<0.001
Carbapenem-resistant strains	11 (21.2)	11 (57.9)	0	<0.001
Antibiotic therapy				
Duration of therapy (days) (median, IQR)	45 (42–56)	42 (30–46)	55 (42–59)	0.002
Duration of antibiotic therapy in CI (days) (median, IQR)	21 (14–36)	36 (28–39)	18 (13–23)	<0.001
Combination therapy[a]	39 (75.0)	10 (52.6)	29 (87.9)	0.004
Duration of combination therapy (days)[b] (median, IQR)	14 (10–21)	35 (20–36)	13 (10–15)	0.006
Surgical therapy				
None	3 (5.8)	2 (10.5)	1 (3.0)	
Debridement[c]	20 (38.5)	6 (31.6)	14 (42.4)	
DAIR	13 (25.0)	4 (21.1)	9 (27.3)	
Implant removal	16 (30.8)	7 (36.8)	9 (27.3)	0.315
Outcome				
Failure[d]	9 (18.4)	4 (21.1)	5 (16.7)	0.699
Adverse events	11 (21.2)	5 (26.3)	6 (18.2)	0.489

All data are expressed as the number (percentage) unless stated otherwise

IQR interquartile range, *MDR* multidrug resistant, *XDR* extensively drug resistant, *DAIR* debridement, antibiotics, and implant retention

[a]Combination therapy included colistin for those with fluoroquinolone-resistant strains or ciprofloxacin for those with fluoroquinolone-susceptible strains

[b]Among those who received antibiotics in combination

[c]Individuals without an associated device

[d]49 individuals were analyzed (three individuals were excluded from the fluoroquinolone-susceptible group: two were not treated with ciprofloxacin and one received ciprofloxacin for <21 days)

Table 2 The median MIC, median dose, and estimated free concentration in plasma of each continuously infused beta-lactam used to treat fluoroquinolone-resistant *Pseudomonas aeruginosa* OIs in the study

Beta-lactam (number of plasma samples)[a]	MIC (range), mg/L	Dose (IQR), g/day	Estimated free beta-lactam concentration (IQR), mg/L[b]	Estimated free beta-lactam concentration (IQR), × MIC
Aztreonam ($n=19$)	4 (2–8)	3 (1.5–4)	12.1 (9.1–18.2)	2.3 (2.0–3.9)
Ceftazidime ($n=49$)	2 (1–16)	2 (2–4)	18.2 (11.9–31.8)	9.1 (7.0–18.9)
Cefepime ($n=10$)	6 (4–8)	2 (2–2.5)	21.6 (14.2–30.4)	3.7 (3.5–4.5)
Ceftolozane-tazobactam ($n=4$)	4	5 (4–6)	16.7 (15.4–21.9)[c]	4.2 (3.9–5.5)[c]

MIC minimum inhibitory concentration, *IQR* interquartile range, *OIs* osteoarticular infections

[a]Plasma samples were collected from four patients treated with aztreonam, 12 treated with ceftazidime, two treated with cefepime, and one treated with ceftolozane-tazobactam

[b]Median free beta-lactam concentrations based on patient samples obtained on various occasions; these concentrations reflect a mix of inter- and intraindividual variability

[c]Only ceftolozane concentrations are considered

3.2 Patients with OIs Caused by Fluoroquinolone-Susceptible *P. aeruginosa*

Thirty-three patients had OIs caused by fluoroquinolone-susceptible *P. aeruginosa* that were treated with BL-CI; those patients had similar characteristics to the patients infected with fluoroquinolone-resistant strains (Table 1).

The median duration of BL-CI was significantly shorter (18 days, IQR 13–23), and the most frequently used BL was ceftazidime (Table 4). Again, patients received lower median BL doses than those they may have received with intermittent infusion, according to product data sheets. The majority received combination therapy with ciprofloxacin for a median of 13 days (IQR 10–15). Ciprofloxacin was used after BL discontinuation in 31 patients (median 31 days, IQR 22–37); two patients with a fluoroquinolone allergy were treated with BL-CI only.

Regarding the TDM, a total of 110 plasma samples were taken for BL concentration analysis (a median of three per patient, IQR 2–4) (Table 4). A total of 19 dose adjustments were performed in 15 patients (45.5%) during therapy (12 had one adjustment, 2 had two adjustments, and 1 had three adjustments). The median time from sample collection to dose adjustment was 1 day (IQR 0–3). Only four patients did not initially achieve a *f*Css of at least 3 × MIC; those patients had OIs by *P. aeruginosa* strains with a BL MIC of 4–8 mg/L. Three of those patients achieved the PK/PD target after dose adjustment, and the remaining patient had an OI by *P. aeruginosa* with MIC of 8 mg/L to the BL used (piperacillin-tazobactam). Dose decreases were needed after receiving the results for ten of the samples during TDM. Five of those decreases were performed in patients with CKD/AKI.

3.3 Comparison of Outcomes and Adverse Events

We evaluated treatment failure rates for 49 patients; three of the patients with fluoroquinolone-susceptible strains were excluded from this analysis because they were not treated with fluoroquinolones (two patients) or they had received ciprofloxacin for < 21 days before failure (one patient).

After a median follow-up of 444 days (IQR 338–617), the treatment had failed in nine patients (18.4%). There was no significant difference in failure rate between the patients with fluoroquinolone-resistant strains and those with susceptible strains (21.1% [4/19] vs 16.7% [5/30], respectively; $p=0.699$) (Fig. 1). There was also no significant difference in baseline characteristics or in the achievement of the pharmacokinetics/pharmacodynamics target (a median *f*Css of at least 3 × MIC) between the patients in whom the treatment failed and those in whom it did not fail (Table 5). Among the patients in whom treatment failed, one had a PJI and eight had osteoarthritis (four of whom had a device and four did not). In the patients with device-related OIs, 4/5 (80%) patients in whom treatment failed were managed with implant retention, compared to 8/22 (36.4%) patients in whom treatment succeeded ($p=0.049$).

Adverse events occurred in five (26.3%) and six (18.2%) patients with fluoroquinolone-resistant and -susceptible strains, respectively ($p=0.489$), at a median of 17 days (IQR 8–20) following BL-CI initiation. In patients with OIs caused by fluoroquinolone-resistant *P. aeruginosa*, the adverse event was AKI in four (three of whom also received colistin) and diarrhea in one (not associated with *Clostridium difficile*). Among those with susceptible strains, the adverse events were AKI ($n=3$), encephalopathy (1), BL-related fever (1), and candidemia (1). All of the patients recovered aside from one (with AKI) who died due to a worsening of their baseline medical condition.

Table 3 Baseline characteristics, treatment details, and outcomes of patients with osteoarticular infections caused by fluoroquinolone-resistant *Pseudomonas aeruginosa* who were treated with continuous beta-lactam infusion

Case number	Age/sex	CKD/AKI while on therapy?	Type of infection	Surgical management	MDR or XDR?	BL	MIC of BL used[a]	Median dose of BL[b] (range[c])	Median BL *f*conc[c] (range)	Median BL *f*conc as multiple of the MIC (range)	Colistin?	Cured?
1	62/M	No/no	PJI	Implant retention	No	Ceftazidime	1	2 (1–3)	8.8 (3.9–15.9)	8.8 (3.9–15.9)	Yes	Yes
2	75/F	Yes/no	PJI	Implant retention	No	Ceftazidime	1	1.5 (1–2)	33.9 (17.8–42.6)	33.9 (17.8–42.6)	No	No
3	76/F	Yes/no	PJI	Implant removal	No	Ceftazidime	1	1.5 (1–2)	13.0 (8.1–42.7)	13.0 (8.1–42.7)	No	Yes
4	63/M	No/no	Osteoarthritis with device	Implant removal	MDR	Ceftazidime	16	6	25.8 (24.3–27.4)	1.6 (1.5–1.7)	Yes	Yes
5	75/F	No/no	Osteoarthritis with device	Implant retention	XDR	Ceftazidime	8	5.5 (5–6)	36.4 (32.2–40.7)	4.6 (4.0–5.1)	Yes	No
6	55/F	No/no	Osteoarthritis with device	Implant removal	MDR	Ceftazidime	2	2	12.5 (11.7–13.3)	6.2 (5.8–6.6)	No	Yes
7	69/M	No/yes	Osteoarthritis without device	Debridement	No	Ceftazidime	1	2	24.0 (18.9–29.5)	24.0 (18.9–29.5)	Yes	Yes
8	80/M	No/yes	Osteoarthritis without device	Debridement	MDR	Ceftazidime	2	2 (1–4)	29.7 (15.4–49.8)	14.8 (7.7–24.9)	Yes	No
9	68/M	No/yes	Osteoarthritis without device	Debridement	XDR	Ceftazidime	16	4.5 (2–7)	132.3 (124.3–169.1)	8.3 (7.8–10.6)	Yes	Yes
10	49/M	No/no	Osteoarthritis without device	Debridement-bone resection	MDR	Ceftazidime	2	5.5 (4–7)	44.5 (22.3–56.3)	22.2 (11.2–28.2)	Yes	Yes
11	63/F	No/no	Osteoarthritis without device	Debridement	No	Ceftazidime	2	3	15.0 (11.9 – 16.6)	7.5 (6–8.3)	Yes	Yes
12	40/M	No/no	Osteoarthritis without device	Debridement-bone resection	MDR	Ceftazidime	1.5	2 (1.5–2)	8.8 (4.2–12.7)	5.8 (2.8–8.5)	No	Yes
13	67/M	No/no	PJI	Implant removal	XDR	Aztreonam	2	2	9.7 (7.7–10.9)	4.3 (3.9–5.4)	Yes	Yes
14	81/F	Yes/no	PJI	Implant removal	XDR	Aztreonam	2	1.25 (1–1.5)	4.1 (3.9–6.7)	2.0 (1.9–3.3)	No	Yes

Table 3 (continued)

Case number	Age/sex	CKD/AKI while on therapy?	Type of infection	Surgical management	MDR or XDR?	BL	MIC of BL used[a]	Median dose of BL[b] (range[c])	Median BL *f*conc[c] (range)	Median BL *f*conc as multiple of the MIC (range)	Colistin?	Cured?
15	90/F	No/no	Osteoarthritis with device	None	MDR	Aztreonam	8	4	18.2 (11.6–25)	2.3 (1.4–3.1)	No	Yes
16	80/F	No/no	Osteoarthritis without device	None	XDR	Aztreonam	8	4.5 (4–5)	17.6 (12.1–20.6)	2.2 (1.5–2.6)	No	Yes
17	46/F	No/yes	Osteoarthritis with device	Implant removal	MDR	Cefepime	8	2 (1–3)	29.2 (14.2–49.8)	3.7 (1.8–6.2)	No	Yes
18	68/F	No/no	Osteoarthritis without device	Debridement-bone resection	No	Cefepime	4	2.5	16.0 (14.1–17.9)	4.0 (3.5–4.5)	No	Yes
19	40/F	No/no	Osteoarthritis with device	Implant retention	XDR	Ceftolozane-tazobactam	4	5 (4–6)	16.7 (14.8–26.3)	4.2 (3.7–6.6)	Yes	No

CKD chronic kidney disease, *AKI* acute kidney failure, *MDR* multidrug resistant, *XDR* extensively drug resistant, *BL* beta-lactam, *fconc* free concentration, *M* male, *F* female, *PJI* prosthetic joint infection

[a]Expressed in mg/L

[b]Expressed in g

[c]Only includes patients who changed dose during treatment

Table 4 The median MIC, median dose, and estimated free concentration in plasma of each continuously infused beta-lactam used to treat fluoroquinolone-susceptible *Pseudomonas aeruginosa* OIs in the study

Beta-lactam (number of plasma samples)[a]	MIC (range), mg/L	Dose (IQR), g/day	Estimated free beta-lactam concentration (IQR), mg/L[b]	Estimated free beta-lactam concentration (IQR), × MIC
Ceftazidime ($n=84$)	1 (0.25–8)	2 (1–4)	12.1 (8.1–19.0)	9.2 (5.8–13.8)
Cefepime ($n=17$)	1 (1–2)	2 (1–2.5)	19.2 (11.4–26.0)	11.0 (8.9–13)
Piperacillin-tazobactam ($n=9$)	2 (1–4)	12 (10–12)	13.2 (11.5–17.5)[c]	2.3 (2.0–3.3)[c]

MIC minimum inhibitory concentration, *IQR* interquartile range, *OIs* osteoarticular infections

[a]Plasma samples were collected from 25 patients treated with ceftazidime, six treated with cefepime, and two treated with piperacillin-tazobactam

[b]Median free beta-lactam concentrations based on patient samples obtained on various occasions; these concentrations reflect a mix of inter- and intraindividual variability

[c]Only piperacillin concentrations are considered

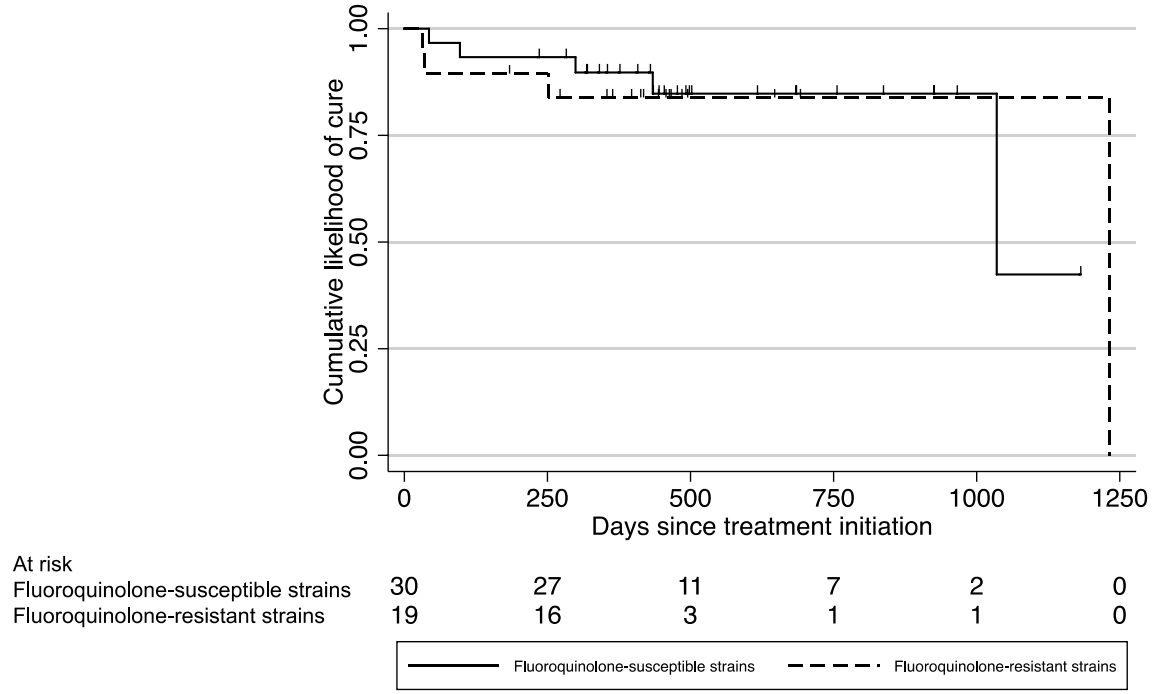


Fig. 1 Kaplan–Meier curves of cure likelihood among patients with osteoarticular infections caused by fluoroquinolone-susceptible (*continuous line*) and fluoroquinolone-resistant (*dashed line*) strains of *Pseudomonas aeruginosa*

4 Discussion

Treatment with fluoroquinolones is considered the first-line antimicrobial therapy for OIs caused by *P. aeruginosa* [1, 2]. Some fluoroquinolone-resistant *P. aeruginosa* strains are MDR/XDR, which limits the probability of success even further and thus demands new therapeutic strategies. In this study, we found that an optimized treatment strategy of TDM-supported BL-CI, often used in combination with colistin, led to similar patient outcomes to those attained when OIs caused by fluoroquinolone-susceptible *P. aeruginosa* were managed with ciprofloxacin. Importantly, our study did not aim to provide evidence for TDM, but it did show that it was feasible to use TDM in the management of difficult-to-treat OIs caused by *P. aeruginosa*.

CI has been suggested as a method that could optimize the pharmacodynamics of BL by ensuring *f*Css for $T>\text{MIC} \approx 100\%$. Extending the $T>\text{MIC}$ may be appropriate in challenging scenarios (such as for critically ill patients or biofilm-related infections), as it has been linked to better outcomes [29–31]. BL-CI may also recover the activities of BL against MDR/XDR strains (which usually exhibit

Table 5 Comparative analysis of patients in whom treatment of an osteoarticular infection caused by *Pseudomonas aeruginosa* failed or did not fail

Characteristics	Patients in whom treatment failed ($n=9$)	Patients in whom treament did not fail ($n=40$)	p value
Demographics			
Age (median, IQR)	73 (65–75)	67 (55–75)	0.416
Female sex	5 (55.6)	19 (47.5)	0.662
Any comorbidity	4 (44.4)	26 (65.0)	0.253
Diabetes mellitus	2 (22.2)	10 (25.0)	0.861
Chronic heart disease	1 (11.1)	12 (30.0)	0.246
Chronic lung disease	1 (11.1)	9 (22.5)	0.444
Malignancy	0	1 (2.5)	0.632
Chronic kidney disease	2 (22.2)	5 (12.5)	0.451
Rheumatologic autoimmune disease	2 (22.2)	5 (12.5)	0.226
Immunosuppressive therapy	1 (11.1)	5 (12.5)	0.909
Chronic steroid therapy	1 (11.1)	5 (12.5)	0.909
Clinical data			
Type of infection			
Prosthetic joint infection	1 (11.1)	10 (25.0)	
Osteoarthritis (without device)	4 (44.4)	18 (45.0)	
Osteoarthritis (with device)	4 (44.4)	12 (30.0)	0.577
Device-related infections	5 (55.6)	22 (55.0)	0.976
Postsurgical infections	5 (55.6)	19 (47.5)	0.662
Microbiological data			
Bacteremia	1 (11.1)	3 (7.5)	0.721
Polymicrobial infection	2 (22.2)	15 (37.5)	0.384
Superinfection	1 (11.1)	5 (12.5)	0.909
MDR or XDR strains	3 (33.3)	10 (25.0)	0.593
Carbapenem-resistant strains	2 (22.2)	9 (22.5)	0.763
Treatment data			
Achievement of PK/PD target[a]	8 (88.9)	36 (90.0)	0.921
Implant retention[b]	4 (80%)	8 (36.4%)	0.049

IQR interquartile range, *MDR* multidrug resistant, *XDR* extensively drug resistant, *PK/PD* pharmacokinetics/pharmacodynamics

[a]Number (%) of patients who had an estimated median free BL concentration of at least 3 × MIC

[b]27 patients with device-related infections were considered (five failed, 22 did not fail; two patients were not eligible for failure analysis)

high BL MICs otherwise), and may optimize PK/PD indices ($T > \text{MIC} = 100\%$) [23].

Colistin has shown remarkable antibiofilm activity against *P. aeruginosa* in previous experimental models and a few clinical studies, mainly when used in combination with BL against MDR/XDR *P. aeruginosa* [9–11]. However, when treating OIs caused by fluoroquinolone-resistant *P. aeruginosa*, we avoided using colistin in older fragile patients, especially if they were managed by removing the implant or performing extensive bone resection, in order to prevent potential toxicity. Even in this situation, most of the patients were cured, further supporting the efficacy of BL-CI and suggesting that it is an effective therapeutic option for improving the outcomes of patients with OIs caused by *P. aeruginosa* when fluoroquinolones cannot be used.

Previous studies in this area have shown that significantly poorer outcomes result when OIs caused by GNB (including *P. aeruginosa*) are treated with regimens that do not include a quinolone—usually BL administered via intermittent infusion or orally [7, 8]. Interestingly, Grossi et al. emphasized the importance of optimizing the intravenous administration of BL to improve the outcomes of orthopedic device-related infections caused by fluoroquinolone-resistant GNB [32]. Although we also included other osteoarticular infections aside from prosthetic joint infections, the results we obtained with BL-CI (with or without colistin) support the role of this therapeutic strategy in such a setting.

When optimizing BL pharmacodynamics through the use of CI ($T > \text{MIC} = 100\%$), as was done in our study, the importance of achieving BL concentrations exceeding

four times the MIC in cases of biofilm-related infection is unclear. However, it may be important to achieve this target due to the antibiotic tolerance of biofilms. In contrast to previous assumptions inferred from studies involving planktonic bacteria [12], results from a limited number of studies using high bacterial inoculums or biofilm experimental models have suggested that BL may exhibit concentration-dependent activity against *P. aeruginosa* [33–36].

In our study, TDM was found to be useful for guiding an optimized BL-CI therapy for difficult-to-treat infections. We employed an easy-to-use method to calculate the appropriate initial BL-CI dosing regimen for each clinical case, based on the MIC and antibiotic clearance. This method, despite being a practical approximation for bedside use, shows poor correlation between observed and predicted BL concentractions in patients with CKD or weight > 75 kg, and for BL that are not exclusively cleared renally [14]. In our experience, most patients had a *f*Css of 3–10 × MIC. Patients with a *f*Css exceeding 10 × MIC generally had CKD/AKI, whereas patients with a *f*Css of less than 3 × MIC had OIs by *P. aeruginosa* with high MIC. This suggests that TDM may be especially relevant in cases where the *f*Css of the beta-lactam may be less predictable or highly variable (CKD/AKI) and in cases where it may be difficult to achieve a relevant *f*Css, such as cases with a high MIC [37]. Although our approach is feasible and practical for clinical use, truly individualized treatments may require population pharmacokinetics models for an OI setting, as they should provide more reliable dose adjustments.

The target *f*Css of the beta-lactam was generally obtained with lower doses compared to standard intermittent infusion, without affecting the outcome. It is unclear whether BL concentrations in plasma correlate with BL concentrations at the infection site [38], but CI probably maintains free (and active) local concentrations of BL, which may partly explain the good clinical courses observed in this study. However, despite their limitations, most pharmacokinetics studies that have evaluated BL bone concentrations have found bone-to-serum BL concentration ratios of less than one [39]. Therefore, the local BL concentrations in our study may have been significantly lower than those obtained in plasma samples.

Overall, BL-CI was well tolerated and all but one of the patients with adverse events recovered. The most frequent adverse event was AKI, which was difficult to associate solely with BL-CI, since most patients had underlying conditions, had undergone surgery, or had received other nephrotoxic drugs such as colistin. Our experience indicates that BL-CI is a safe therapeutic option, as has also been found in previous studies [29].

The observational design of our single-center study may limit the generalizability of the results. Patients included in this study were heterogeneous in terms of their clinical presentations, *P. aeruginosa* susceptibility profiles, and, consequently, the use of several beta-lactams. Unfortunately, our sample size was too small to allow subgroup analyses or other comparative studies such as that between cases receiving BL-CI with TDM and cases receiving BL-CI without TDM. Due to variability in protein binding and the potential effect of hypoalbuminemia [40, 41], the real free BL concentrations may have differed from those estimated here, especially for highly protein-bound BL (i.e., aztreonam). As mentioned before, the use of colistin in some patients may partly explain the good outcomes observed. Despite all of these considerations, we believe that our results support the use of BL-CI for the management of OIs caused by fluoroquinolone-resistant *P. aeruginosa*.

5 Conclusions

The optimized use of BL-CI, with or without colistin, for OIs caused by fluoroquinolone-resistant *P. aeruginosa* resulted in an alternative therapeutic option with favorable outcomes. BL-CI guided by TDM was found to be a safe and feasible therapeutic option which ensured that desirable target concentrations were maintained and avoided toxicity. While further robust studies are awaited, our data should encourage physicians to incorporate BL-CI and TDM into an optimized strategy for the management of OIs caused by fluoroquinolone-resistant *P. aeruginosa*.

Acknowledgements The preliminary results were presented in part at the 21st Spanish Congress of Infectious Diseases and Clinical Microbiology, Málaga, Spain, 11–13 May 2017.

Compliance with Ethical Standards

Funding J. G.-J. was supported by a grant from the Spanish Ministry of Education (FPU 14/03124). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest All authors declare that they did not have any conflict of interest concerning this article.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was obtained from the Ethics Committee of Hospital Universitari de Bellvitge (reference EPA045/16).

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med. 2004;351(16):1645–54.
2. Trampuz A, Zimmerli W. Diagnosis and treatment of implant-associated septic arthritis and osteomyelitis. Curr Infect Dis Rep. 2008;10(5):394–403.
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318–22.
4. Benito N, Franco M, Ribera A, Soriano A, Rodriguez-Pardo D, Sorli L, et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect. 2016;22(8):732.e1–8.
5. Murillo O, Grau I, Lora-Tamayo J, Gomez-Junyent J, Ribera A, Tubau F, et al. The changing epidemiology of bacteraemic osteoarticular infections in the early 21st century. Clin Microbiol Infect. 2015;21(3):254.e1–8.
6. Aboltins CA, Dowsey MM, Buising KL, Peel TN, Daffy JR, Choong PF, et al. Gram-negative prosthetic joint infection treated with debridement, prosthesis retention and antibiotic regimens including a fluoroquinolone. Clin Microbiol Infect. 2011;17(6):862–7.
7. Rodriguez-Pardo D, Pigrau C, Lora-Tamayo J, Soriano A, del Toro MD, Cobo J, et al. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. Clin Microbiol Infect. 2014;20(11):O911–9666666666.
8. Hsieh PH, Lee MS, Hsu KY, Chang YH, Shih HN, Ueng SW. Gram-negative prosthetic joint infections: risk factors and outcome of treatment. Clin Infect Dis. 2009;49(7):1036–43.
9. Lora-Tamayo J, Murillo O, Bergen PJ, Nation RL, Poudyal A, Luo X, et al. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant *Pseudomonas aeruginosa* in an in vitro dynamic biofilm model. J Antimicrob Chemother. 2014;69(9):2434–42.
10. Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol. 2008;68(1):223–40.
11. Ribera A, Benavent E, Lora-Tamayo J, Tubau F, Pedrero S, Cabo X, et al. Osteoarticular infection caused by MDR *Pseudomonas aeruginosa*: the benefits of combination therapy with colistin plus beta-lactams. J Antimicrob Chemother. 2015;70(12):3357–65.
12. Mouton JW, Vinks AA. Continuous infusion of beta-lactams. Curr Opin Crit Care. 2007;13(5):598–606.
13. Roberts JA, Paratz J, Paratz E, Krueger WA, Lipman J. Continuous infusion of beta-lactam antibiotics in severe infections: a review of its role. Int J Antimicrob Agents. 2007;30(1):11–8.
14. Ribera A, Soldevila L, Rigo-Bonnin R, Tubau F, Padulles A, Gomez-Junyent J, et al. Beta-lactams in continuous infusion for Gram-negative bacilli osteoarticular infections: an easy method for clinical use. Infection. 2018;46(2):239–44.
15. Fawaz S, Barton S, Whitney L, Swinden J, Nabhani-Gebara S. Stability of meropenem after reconstitution for administration by prolonged infusion. Hosp Pharm. 2019;54(3):190–6.
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
17. Anon. Decreased GFR. In: Chapter 1: Definition and classification of CKD. Kidney Int Suppl. 2013;3(1):19–62.
18. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure-definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care. 2004;8(4):R204–R212212.
19. Nation RL, Garonzik SM, Thamlikitkul V, Giamarellos-Bourboulis EJ, Forrest A, Paterson DL, et al. Dosing guidance for intravenous colistin in critically-ill patients. Clin Infect Dis. 2017;64(5):565–71.
20. Ordooei Javan A, Shokouhi S, Sahraei Z. A review on colistin nephrotoxicity. Eur J Clin Pharmacol. 2015;71(7):801–10.
21. Grayson ML, Crowe SM, McCarthy JS, Mills J, Mouton JW, Norrby SR, Paterson DL, Pfaller MA. Kucers' the use of antibiotics. 6th edn. Boca Raton: CRC Press; 2010.
22. Rigo-Bonnin R, Ribera A, Arbiol-Roca A, Cobo-Sacristan S, Padulles A, Murillo O, et al. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of beta-lactam antibiotic concentration in human plasma. Clin Chim Acta. 2017;468:215–24.
23. Moriyama B, Henning SA, Childs R, Holland SM, Anderson VL, Morris JC, et al. High-dose continuous infusion beta-lactam antibiotics for the treatment of resistant *Pseudomonas aeruginosa* infections in immunocompromised patients. Ann Pharmacother. 2010;44(5):929–35.
24. Drusano GL, Standiford HC, Fitzpatrick B, Leslie J, Tangtatsawasdi P, Ryan P, et al. Comparison of the pharmacokinetics of ceftazidime and moxalactam and their microbiological correlates in volunteers. Antimicrob Agents Chemother. 1984;26(3):388–93.
25. Hayashi Y, Roberts JA, Paterson DL, Lipman J. Pharmacokinetic evaluation of piperacillin-tazobactam. Expert Opin Drug Metab Toxicol. 2010;6(8):1017–31.
26. Miller B, Hershberger E, Benziger D, Trinh M, Friedland I. Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. Antimicrob Agents Chemother. 2012;56(6):3086–91.
27. Van der Auwera P, Santella PJ. Pharmacokinetics of cefepime: a review. J Antimicrob Chemother. 1993;32(Suppl B):103–15.
28. Vinks AA, van Rossem RN, Mathot RA, Heijerman HG, Mouton JW. Pharmacokinetics of aztreonam in healthy subjects and patients with cystic fibrosis and evaluation of dose-exposure relationships using Monte Carlo simulation. Antimicrob Agents Chemother. 2007;51(9):3049–55.
29. Dulhunty JM, Roberts JA, Davis JS, Webb SA, Bellomo R, Gomersall C, et al. Continuous infusion of beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. Clin Infect Dis. 2013;56(2):236–44.
30. Roberts JA, Abdul-Aziz MH, Davis JS, Dulhunty JM, Cotta MO, Myburgh J, et al. Continuous versus intermittent beta-lactam infusion in severe sepsis. A meta-analysis of individual patient data from randomized trials. Am J Respir Crit Care Med. 2016;194(6):681–91.
31. Vardakas KZ, Voulgaris GL, Maliaros A, Samonis G, Falagas ME. Prolonged versus short-term intravenous infusion of antipseudomonal beta-lactams for patients with sepsis: a systematic review and meta-analysis of randomised trials. Lancet Infect Dis. 2018;18(1):108–20.
32. Grossi O, Asseray N, Bourigault C, Corvec S, Valette M, Navas D, et al. Gram-negative prosthetic joint infections managed according to a multidisciplinary standardized approach: risk factors for failure and outcome with and without fluoroquinolones. J Antimicrob Chemother. 2016;71(9):2593–7.
33. Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and nonmucoid *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother. 2011;55(9):4469–74.

34. Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. Antimicrob Agents Chemother. 2012;56(5):2683–90.
35. McKinnon PS, Paladino JA, Schentag JJ. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T > MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents. 2008;31(4):345–51.
36. Mouton JW, den Hollander JG. Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. Antimicrob Agents Chemother. 1994;38(5):931–6.
37. Huttner A, Harbarth S, Hope WW, Lipman J, Roberts JA. Therapeutic drug monitoring of the beta-lactam antibiotics: what is the evidence and which patients should we be using it for? J Antimicrob Chemother. 2015;70(12):3178–83.
38. Zeller V, Durand F, Kitzis MD, Lhotellier L, Ziza JM, Mamoudy P, et al. Continuous cefazolin infusion to treat bone and joint infections: clinical efficacy, feasibility, safety, and serum and bone concentrations. Antimicrob Agents Chemother. 2009;53(3):883–7.
39. Landersdorfer CB, Bulitta JB, Kinzig M, Holzgrabe U, Sorgel F. Penetration of antibacterials into bone: pharmacokinetic, pharmacodynamic and bioanalytical considerations. Clin Pharmacokinet. 2009;48(2):89–124.
40. Wong G, Briscoe S, Adnan S, McWhinney B, Ungerer J, Lipman J, et al. Protein binding of beta-lactam antibiotics in critically ill patients: can we successfully predict unbound concentrations? Antimicrob Agents Chemother. 2013;57(12):6165–70.
41. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. Clin Pharmacokinet. 2011;50(2):99–110.

Affiliations

Joan Gómez-Junyent[1] · Raul Rigo-Bonnin[2] · Eva Benavent[1] · Laura Soldevila[1] · Ariadna Padullés[3] · Xavier Cabo[4] · Fe Tubau[5] · Javier Ariza[1] · Oscar Murillo[1]

[1] Department of Infectious Diseases, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

[2] Department of Clinical Laboratory, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

[3] Department of Pharmacy, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

[4] Department of Orthopedics, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

[5] Department of Microbiology, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

Clinica Chimica Acta 488 (2019) 50–60

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Measurement of ceftolozane and tazobactam concentrations in plasma by UHPLC-MS/MS. Clinical application in the management of difficult-to-treat osteoarticular infections

Raúl Rigo-Bonnin[a,*], Joan Gomez-Junyent[b], Laura García-Tejada[a], Eva Benavent[b], Laura Soldevila[b], Fe Tubau[c,d], Oscar Murillo[b,e]

[a] Laboratori Clínic Department, IDIBELL, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[b] Infectious Diseases Department, IDIBELL, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[c] Microbiology Department, IDIBELL, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[d] Spanish Network for Biomedical Research in Respiratory Diseases (CIBERES CB06/060037), Instituto de Salud Carlos III, Madrid, Spain
[e] Spanish Network for Research in Infectious Diseases (REIPI RD12/0012), Instituto de Salud Carlos III, Madrid, Spain

ARTICLE INFO

Keywords:
Ceftolozane
Osteoarticular infections
Therapeutic drug monitoring
UHPLC-MS/MS
Tazobactam

ABSTRACT

Background: Ceftolozane, in combination with the β-lactamase inhibitor tazobactam, is a new option in the pipeline against multidrug-resistant Gram-negative bacilli. As for other β-lactam antibiotics, optimizing the use of ceftolozane-tazobactam is advisable, especially in difficult-to-treat infections. In this regard, therapeutic drug monitoring would be required to guide the treatment of ceftolozane-tazobactam. Thus, we aimed to develop and validate procedures based on UHPLC-MS/MS for measurement of ceftolozane and tazobactam plasma concentrations in clinical practice.

Material and methods: Analyses were conducted using an Acquity® UPLC® integrated system coupled to an Acquity® TQD® tandem-quadrupole mass spectrometer. Ceftolozane, tazobactam and their internal standards (ceftazidime-D_5 and sulbactam) were detected by electrospray ionization mass spectrometry in positive and negative ion multiple reaction monitoring modes, using transitions of 667.2 → 199.3/139.0 and 551.9 → 467.9 for ceftolozane and ceftazidime-D_5, and 299.0 → 138/254.9 and 232.0 → 140.0 for tazobactam and sulbactam. Measurement procedures developed were used for guiding the treatment and adjusting daily dose of ceftolozane-tazobactam in patients with osteoarticular infections.

Results: Coefficients of variation and absolute relative biases were < 7.9% and 6.5% in all cases. The lower limit of quantification, linearity, normalized-recoveries, normalized-matrix effects and measurement uncertainties for ceftolozane were: 0.97 mg/L, (0.97–125) mg/L, ≤113.6%, ≤108.7%, and ≤ 18.7%, respectively; and for tazobactam: 1.04 mg/L, (1.04–125) mg/L, ≤103.6%, ≤101.9%, and ≤ 20.0%. No interferences and carry-over were observed. Patients plasma concentrations were higher than the recommended 3–4 times the minimal inhibitory concentrations.

Conclusions: Our measurement procedures are suitable for therapeutic drug monitoring of ceftolozane-tazobactam in patients with osteoarticular infections.

Abbreviations: β-LA, β-lactam antibiotic; CLSI, Clinical and Laboratory Standards Institute; CO, Carry-over; *CV*, coefficient of variation; δ_r, Relative bias associated to calibration procedure; Δ_{max}, Maximum permissible root mean square of the relative error of measurement (measurement uncertainty metrological requirement); DMSO, Dimethyl sulfoxide; EMA, European Medicines Agency; ESI, Electrospray ionization; EUROLAB, European Federation of National Associations of Measurement, Testing and Analytical Laboratories; GNB, Gram-negative bacilli; HPLC, High-performance liquid chromatography; IFCC, International Federation of Clinical Chemistry; IUPAC, International Union of Pure and Applied Chemistry; IS, Internal standard; LC, Liquid chromatography; LLOQ, Lower limit of quantification; ME, Matrix effect; MDR, Multidrug-resistant; MIC, Minimal inhibitory concentration; MRM, Multiple reaction monitoring; MS, Mass spectrometer; MS/MS, Tandem mass spectrometry; *m/z*, Mass-to-charge; QC, Quality control; REC, Recovery of extracted samples; SEL, Selectivity; TDM, Therapeutic drug monitoring; UHPLC, Ultra-high performance liquid chromatography; ULOQ, Upper limit of quantification; UV, Ultraviolet; %D, Percent deviation; T > MIC, Time the drug concentration remains above the MIC

* Corresponding author at: Laboratori Clínic Department, Hospital Universitari de Bellvitge, Feixa Llarga s/n, L'Hospitalet de Llobregat, Barcelona 08907, Spain.
E-mail address: raulr@bellvitgehospital.cat (R. Rigo-Bonnin).

https://doi.org/10.1016/j.cca.2018.10.034
Received 1 September 2018; Received in revised form 11 October 2018; Accepted 28 October 2018
Available online 30 October 2018

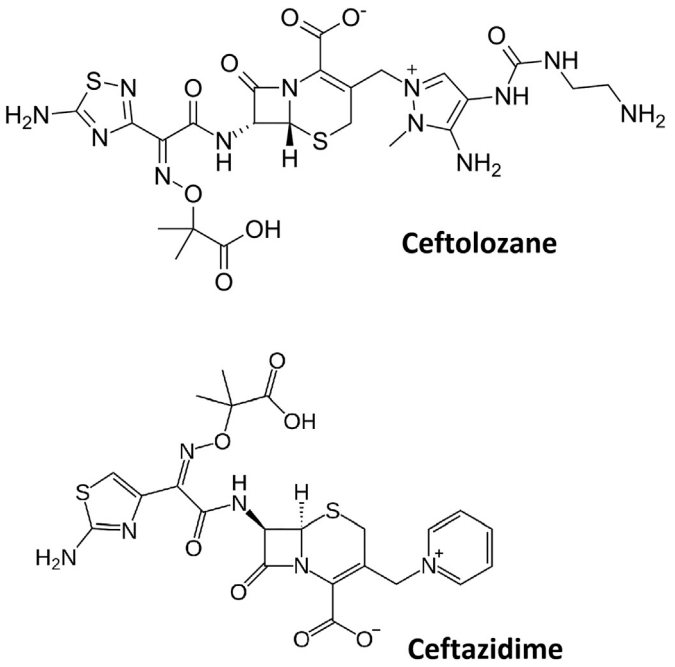


Fig. 1. Chemical structures of ceftolozane and ceftazidime.

1. Introduction

Ceftolozane is a fifth-generation cephalosporin structurally similar to the previous β-lactam antibiotic (β-LA) ceftazidime, but with a modified side chain (Fig. 1). It is administered with the β-lactamase inhibitor tazobactam and this combination is active against several multidrug-resistant (MDR) Gram-negative bacilli (GNB) [1–3]. In the current worldwide concern of infections caused by MDR microorganisms and particularly, when these microorganisms are responsible of difficult-to-treat infections (i.e., biofilm-related osteoarticular infections), an optimized use of antibiotics is advisable [4,5]. In this line, the administration of β-LA in continuous infusion has been used to optimize their pharmacokinetic/pharmacodynamic indices and then to expect an improvement in the efficacy and protection against the emergence of resistance [6–8]. In these cases, therapeutic drug monitoring (TDM) of β-LA to guide this therapy should be considered and thus, this could be also the case for ceftolozane-tazobactam [9].

For the particular measurement of mass concentrations of ceftolozane and tazobactam in plasma, a limited number of studies have applied high-performance liquid chromatography (HPLC) with UV detection procedures [10–12], and only few cases included HPLC coupled to the tandem mass spectrometry (MS/MS) [13–15]. Among these HPLC-MS/MS procedures reported, several methodological limitations can be identified in regard with the lacked information about the selectivity, carry over, matrix effect, dilution integrity, and measurement of uncertainty.

The aim of this work was to develop ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) procedures for the measurement of ceftolozane and tazobactam mass concentrations in plasma, and to validate widely their performance characteristics. Additionally, we aimed to apply these procedures for guiding the treatment of cases of osteoarticular infections.

2. Material and methods

2.1. Chemical, materials and reagents

Reference material of ceftolozane sulfate (pure ceftolozane content of 76.9%) was donated by Merck Sharp & Dohme S.A. (Barcelona, Spain). Certified reference material of tazobactam (purity of 99.6%) was purchased from United States Pharmacopeia (Rockville, MD, USA). Ceftazidime-D_5 (purity of 90.0%, isotopic purity of 98.6%), used as ceftolozane-internal standard (IS), was supplied by Toronto Research Chemicals (Ontario, Canada). Certified reference material of sulbactam (purity of 99.9%; IS for tazobactam) was obtained from European Pharmacopeia (European Directorate for the Quality of Medicines-Council of Europe, Strasburg, France)

LC-MS-grade acetonitrile, dimethyl sulfoxide (DMSO), formic acid, methanol and water were purchased from Sigma-Aldrich Química S.L. (Madrid, Spain).

Ceftolozane/tazobactam-free human plasma (blank plasma) was obtained from blood of patients arrived at Emergency Laboratory of Hospital de Bellvitge. Blood was collected in BD Vacutainer® lithium-heparin tube (Becton Dickinson, Franklin Lakes, NJ, USA) and centrifuged at 2000*g* for 10 min at room temperature. Subsequently, the obtained plasma was pooled and stored at (−75 ± 3) °C until use. An aliquot was separated to confirm the absence of ceftolozane and tazobactam using the current UHPLC-MS/MS procedures.

2.2. Preparation of calibration, control and internal standard materials

For ceftolozane and tazobactam, two stock solutions of each one from independent weighing were prepared at a concentration of 2.00 g/L. The stock solutions for ceftolozane were prepared by weighing 5.20 mg of ceftolozane sulfate and dissolving this one in 2 mL water:metanol:DMSO (50:25:25, *v/v/v*); and for tazobactam, weighing 4.02 mg in 2 mL of water:metanol:DMSO (50:25:25, *v/v/v*). For each drug, eight working standards (10.0, 50.0, 100, 250, 450, 750, 1000 and 1250 mg/L) with a volume of 1-mL each one were prepared pipetting, respectively, 5.0, 25, 50, 125, 225, 375, 500, and 625 µL of stock solution and 995, 975, 950, 875, 775, 625, 500, and 375 µL of water. These solutions were stored light-protected at (−75 ± 3) °C. On the day of analysis, 100 µL of calibration materials at 1.00, 5.00, 10.0, 25.0, 45.0, 75.0, 100 and 125 mg/L were prepared pipetting 10 µL of working standard and 90 µL of drug-free plasma. Four plasma quality control (QC) materials were prepared in the same manner as the calibration materials at 3.00, 7.50, 30.0 and 80.0 mg/L.

Stock solution of ceftazidime-D_5 was prepared by diluting 1 mg of ceftazidime-D_5 in 10.0 mL of metanol:DMSO (50:50, *v/v/v*). Sulbactam stock solution was made weighing 2.5 mg in 25 mL of methanol. These solutions were stored at (−75 ± 3) °C. A working solution of IS was prepared freshly for 20 samples analysis by adding 150 µL of each stock solution to 5.70 mL of acetonitrile.

2.3. Instrumentation, measurement procedures and equipment

Analyses were conducted using an Acquity® UPLC® chromatographic system coupled to an Acquity® TQD® tandem-quadrupole mass spectrometer (Waters, Milford, MA, USA).

Given that, at the present time, we use a previously developed and validated UHPLC-MS/MS procedure to measure different β-LA concentrations in plasma [16] in routine practice, for practical reasons, we used the same sample preparation (protein precipitation with acetonitrile and subsequent dilution with water containing 0.1% (*v/v*) formic acid), chromatographic separation conditions (analytical column, mobile phases, flow rate, elution conditions based on gradient mode, injected volume, and autosampler temperature), and generic mass spectrometer parameters (capillary voltage, extractor voltage, RF lens voltage, source temperature, desolvation temperature, desolvation gas flow rate, and collision gas flow).

Ceftolozane and its IS were detected by multiple reaction monitoring (MRM) operating in positive electrospray ionization (ESI+) mode, and using the following transitions of mass-to-charge (*m/z*): ceftolozane, 667.2 → 199.3 (quantifier ion) and 667.2 → 139.0 (qualifier ion); ceftazidime-D_5, 551.9 → 467.9. On the other hand, for

tazobactam and its IS, the mass spectrometer operated in MRM and ESI- modes, and their *m/z* transitions were 299.0 → 138.0 (quantifier ion) and 299.0 → 254.9 (qualifier ion) for tazobactam, and 232.0 → 140.0 for sulbactam. Cone voltages of 23 V for ceftolozane, 20 V for ceftazidime-D_5 and tazobactam, and 25 V for sulbactam were used. Collision energies were 16/25 eV for ceftolozane and 14/15 eV for tazobactam as quantifier/qualifier ions, as well as, 12 eV for ceftazidime-D_5 and sulbactam. The dwell time was set to 100 ms for every channel.

Also, the following equipment was used: ADA-120/L analytical balance (Adam Equipment, Bletchley, UK), Acura® 825 adjustable 100–1000 µL volume pipette from Socorex Isba (Ecublens, Switzerland) (pipette A), Nichipet® EX II adjustable 10–100 µL (pipette B) and 0.5–10 µL (pipette C) volume pipettes from Nichiryo Co Ltd. (Koshigaya-shi, Saitama, Japan), and 2-mL BLAUBRAND® class A, USP certified bulb pipette with two marks (BRAND GMBH + CO KG, Wertheim, Germany).

2.4. Validation

Validations were carried out according to the European Medicines Agency (EMA) guideline [17], Clinical and Laboratory Standards Institute (CLSI) [18–22] and EUROLAB guidelines [23,24].

2.4.1. Intermediate imprecision and bias associated to calibration procedure

According to CLSI EP05-A3 guideline [18], to estimate intermediate imprecision (within-laboratory coefficient of variation) and bias, plasma QC materials were processed in 20 non-consecutive days over 2 months. Relative bias (δ_r) were calculated using the following equation:

$$\delta_r = \left(\frac{\bar{x} - \mu}{\mu}\right)\cdot 100$$

where $\bar{x}$ is the mean value obtained in the imprecision study for each control material and μ, the conventional values assigned by weighing.

According to the EMA guideline [17], *CV* should be ≤15% and δ_r should be within the acceptance criteria of ± 15%.

2.4.2. Lower limit of quantification

For each drug, lower limit of quantification (LLOQ) was estimated in the same way than the intermediate imprecision and bias, but processing a plasma sample at concentration of 1.00 mg/L.

According to the EMA acceptance criteria [17], the LLOQ should have a signal-to-noise (*S/N*) ratio ≥ 5, and an acceptable imprecision (20%) and bias (± 20%).

2.4.3. Selectivity

Selectivity (SEL) studies were performed according to the EMA guideline [17]. Thus, a double blank plasma sample (containing a neither analyte nor IS), a blank plasma sample (spiked only with IS), a sample at LLOQ and 9 drug-free plasma samples from volunteers not treated with ceftolozane-tazobactam but receiving other antibiotics (amikacin, ampicillin, aztreonam, cefepime, ceftazidime, gentamycin, meropenem, tobramycin, and vancomycin) were analyzed for interferences with the analytes. Antibiotic concentrations of the drug-free plasma patient samples were within therapeutic intervals for the aminoglycosids or above 3–5 of the minimal inhibitory concentration (MIC) for β-LA.

Based on the EMA criteria, peak area response of all possible interfering peaks at the retention time of analyte (ceftolozane or tazobactam) should be ≤20% of the LLOQ for the analyte and ≤5% for the IS (ceftazidime-D_5 or sulbactam).

2.4.4. Carry-over

In accordance with the EMA guideline [17], carry-over (CO) studies were assessed by injecting a double blank plasma sample, a sample at LLOQ, and the highest calibration material (125 mg/L) in the following order:

Sample at LLOQ − Highest calibration material

− Double blank plasma sample

The peak are responses obtained in the double blank plasma sample were evaluated at the retention time of ceftolozane, tazobactam and their IS.

Following the EMA criteria [17], carry-over was acceptable if peak area response in the double blank sample was ≤20% of the analyte peak area response at the LLOQ, and ≤5% of the peak area response of their IS.

2.4.5. Calibration curve

Eight plasma calibration samples were prepared and daily calibration curves for each batch were assessed. Integration of smoothed peak areas, calibration curves and calculation of ceftolozane or tazobactam concentrations were performed using the TargetLynx™ v4.1 software (Waters, Milford, MA, USA). Plasma calibration level 1 (1.00 mg/L) served as the LLOQ and calibration level 8 (125 mg/L) as the upper limit of quantification (ULOQ).

The calibration curves were generated by linear fit of the analyte/IS area response ratio multiplied by IS concentration vs. analyte concentration (1/X weighting; excluding the option to force through the point of origin). According to the EMA guideline [17], calculated concentrations of the calibration standards should be all within ± 15% of the nominal value (± 20% for the LLOQ).

2.4.6. Linearity

According to the CLSI EP06-A guidelines [19], to verify statistically the linearity interval of the measurement procedure for concentrations of ceftolozane and tazobactam in plasma, the highest calibrator material (125 mg/L), was either diluted with the lowest calibrator material (LLOQ, 1.00 mg/L) at ratios of 0:5 (dilution factor 1), 1:4 (dilution factor 2), 2:3 (dilution factor 3), 3:2 (dilution factor 4), 4:1 (dilution factor 5), and 5:0 (dilution factor 6). Dilution samples were randomly processed in triplicate. The measured values obtained were plotted on the y-axis versus the dilution factor on the x-axis. Using the Analyse-it® v5.10 statistical software (Analyse-it Software, Ltd., Leeds, UK), the polynomial regression method was used in order to evaluate the non-linearity. Briefly, this method consists of two parts. The first part examines whether a nonlinear polynomial fits the data better than a linear one. The second part, performed in cases when a nonlinear polynomial fits the data better than a linear one, assesses whether the difference between the best-fitting nonlinear and linear polynomial is less than a previously predefined bias requirement of the measurement procedure (in our case 15%, which was established from EMA [17]). Also, taking into account that the random variability can lead to poor ability to detect nonlinearity, imprecision values obtained for each dilution should be lower than the imprecision requirement previously predefined of the measurement procedure (in our case 15%, which was established from EMA [17]).

2.4.7. Dilution integrity

According to the EMA guideline [17], for ceftolozane and tazobactam, the dilution integrity experiment was carried out analyzing six replicates of a plasma sample prepared at two times the ULOQ, and subsequently diluted 1/10 with drug-free plasma. According to the EMA guideline, imprecision should be ≤15% and bias should be within ± 15%.

2.4.8. Recovery of extracted samples and matrix effect

According to the EMA, the CLSI-IFCC C50-A and the C62-A guidelines [17,20,21] and Viswanathan et al. [25], to examine the recovery of extracted samples (REC) and the matrix effect (ME), 4 concentrations

of ceftolozane and tazobactam (3.00, 7.50, 30.0 and 80.0 mg/L) and 1 concentration of their IS (2.5 mg/L) were tested. Recovery and ME (based on estimation of matrix factor) were calculated according to the following equations:

$$\text{REC (\%)} = \frac{\text{Peak Area response of extracted sample (with analyte)}}{\text{Peak Area response of post} - \text{extracted spiked sample}} \cdot 100$$

$$\text{ME (\%)} = \left(\frac{\text{Peak Area response of post} - \text{extracted spiked sample}}{\text{Peak Area response of analyte in pure solution}} \right) \cdot 100$$

Six different batches of plasma matrix samples were analyzed.

Considering the REC and ME of the IS, IS-normalized REC and IS-normalized matrix factors were also calculated by dividing the REC or ME of each analyte by the REC or ME of its IS.

According to the EMA [17] or CLSI [20,21] criteria, the variation in recoveries and matrix effects among all concentrations should be ≤15%.

2.4.9. Stability

Stability studies included working standard and control solutions stabilities of ceftolozane and tazobactam, IS-stock solutions, extracted samples in-autosampler stability and short- and long-term stabilities for concentration of ceftolozane and tazobactam. These studies were performed following the EMA guideline recommendations [17].

For stability of working standard, and control material solutions, these one were freshly prepared, diluted 10-fold with water, and processed. Afterwards, these solutions were kept at (−75 ± 3) °C until their respective analyses either 6 months later.

For stability IS-stock solutions, solutions were freshly prepared, diluted 10-fold with metanol:DMSO (50:50, *v/v/v*) for ceftazidime-D_5 and with methanol for sulbactam, and processed at the day of preparation and 6 months later, after a storage at (−75 ± 3) °C.

Stability of extracted samples in the autosampler was tested by processing the extracted samples and reinjecting them after 6 h, 12 h and 24 h storage at (4 ± 1) °C into autosampler.

Stability studies of concentration of analytes in the studied matrix (plasma in our case) were performed using the four plasma QC materials (3.00, 7.50, 30.0 and 80.0 mg/L). Three different batches of plasma QC materials were freshly prepared and processed, and left on the bench-top at room temperature, (5 ± 3) °C and at (−75 ± 3) °C until their respective analyses at different times. For short-term stability, the batch plasma QC stored at room temperature were processed 2, 4, 8 and 12 h later, and the batch plasma QC stored at (5 ± 3) °C, were processed 1, 2, 3 and 5 days later. On the other hand, for the long-term stability, the batch plasma QC stored at (−75 ± 3) °C were processed 6 months later.

All solutions, extracted samples and plasma QC materials were analyzed against a freshly prepared calibration curve and using ten replicates of each one. In all cases, to estimate the stability, percent deviations (%D) from the nominal concentrations (areas in the case of IS), i.e. biases, were calculated as:

$$\text{\%D} = \left(\frac{\text{Mean value of the 10 replicates} - \text{Nominal concentration}}{\text{Nominal concentration}} \right) \cdot 100$$

According to the EMA criteria [15], the %D should be within ± 15%.

2.4.10. Measurement uncertainty

Measurement uncertainties of ceftolozane and tazobactam concentration in plasma values were estimated using the top-down approach called *single-validation approach*, according to different guidelines [22–24]. Fig. 2. shows a *cause and effect* diagram used to identify the different sources of uncertainty. The main uncertainty sources considered were related with calibrator's-assigned values, the intermediate precision and the bias of the measurement system. These standard uncertainties were estimated using information from certified reference materials manufacturers (certificates of analysis), mass and volumetric equipment calibration certificates, and the performance characteristic results obtained from the current measurement procedures. Once the standard uncertainties were quantified, they were combined to give a combined uncertainty for, finally, to estimate an expanded uncertainty using a coverage factor of 2 ($k = 2$).

Uncertainties associated with the assigned values of calibration materials (u_{cal}) were due to the ceftolozane sulfate and tazobactam certified reference materials mass weighted into the balance, the different pipetted volumes used to prepare calibration materials, and the stability of working standards.

The measuring mass uncertainty from analytical balance (u_{bal}) was indicated in the certificate of external calibration as (5.00 ± 0.17) mg ($k = 2$).

According to the manufacturer's data, the 2-mL bulb pipette accuracy is 0.006 mL. To estimate its uncertainty (u_{bulb}) a type-B estimation using a triangular distribution was used.

The uncertainty associated to the measuring volume from pipetting (u_{pip}) was: (1000.0 ± 2.6) µL ($k = 2$), (500.0 ± 1.8) µL ($k = 2$) and (100.0 ± 0.4) µL ($k = 2$) for pipette A; (10.00 ± 0.18) µL ($k = 2$), (50.00 ± 0.22) µL ($k = 2$) and (100.0 ± 0.4) µL ($k = 2$) for pipette B; and (0.500 ± 0.02) µL ($k = 2$), (5.00 ± 0.08) µL ($k = 2$) and (10.00 ± 0.16) µL ($k = 2$) for pipette C.

Additionally, u_{bulb} and u_{pip} include the uncertainty related to the temperature change (u_{temp}) associated to the volume. By assuming a rectangular distribution, the uncertainty for the temperature variation for 2 mL bulb pipette and pipettes were calculated using the following equation:

$$u_{temp} = \frac{V_x \cdot \alpha \cdot \Delta T}{\sqrt{3}}$$

where V_x is the volume of the bulb pipette or the volume pipetted; α, the volume expansion coefficient for water ($2.14 \cdot 10^{-4}$ °C^{-1}); and ΔT, the difference between the actual laboratory temperature and the calibration temperature indicated in the bulb pipette ($\Delta T = 5$ °C) or the temperature during the calibration of the pipettes ($\Delta T = 2$ °C).

Uncertainty related to the stability of working standards (u_{stab}) was estimated as [26]:

$$u_{stab} = \frac{L_s}{\sqrt{18}}$$

where L_s is the absolute %D value obtained in the stability study.

Taking into account the procedure to prepare each calibration material described above, the relative u_{cal} was obtained as:

$$u_{cal} = \sqrt{u_{bal}^2 + u_{bulb}^2 + u_{pip,stock}^2 + u_{pip,water}^2 + u_{pip,ws}^2 + u_{pip,plasma}^2 + u_{stab}^2}$$

where $u_{pip,\ stock}$ is the relative uncertainty related to the stock solution volume pipetted for the preparation of the working standards; $u_{pip,water}$, the relative uncertainty associated to the water volume pipetted for the preparation of the working standards; $u_{pip,ws}$, the relative uncertainty related to the working standard solution volume pipetted for the preparation of the calibration materials; and $u_{pip,plasma}$, the relative uncertainty associated to the blank plasma volume pipetted for the preparation of the calibration materials.

The relative uncertainties associated to intermediate imprecision (u_p) were the *CV* obtained in the present study.

The different possible sources of bias considered were: calibration procedure (δ_r), REC, ME, CO and SEL. The REC and the ME biases are corrected by using the IS, whereas the other sources remain uncorrected. Treatment of corrected and uncorrected biases was performed according to Magnusson et al. recommendations [27].

Since there are no high-order reference materials to be used, we used the control materials as reference to estimate the δ_r as described above. The relative uncertainties related to the δ_r (u_δ) were calculated as follows:

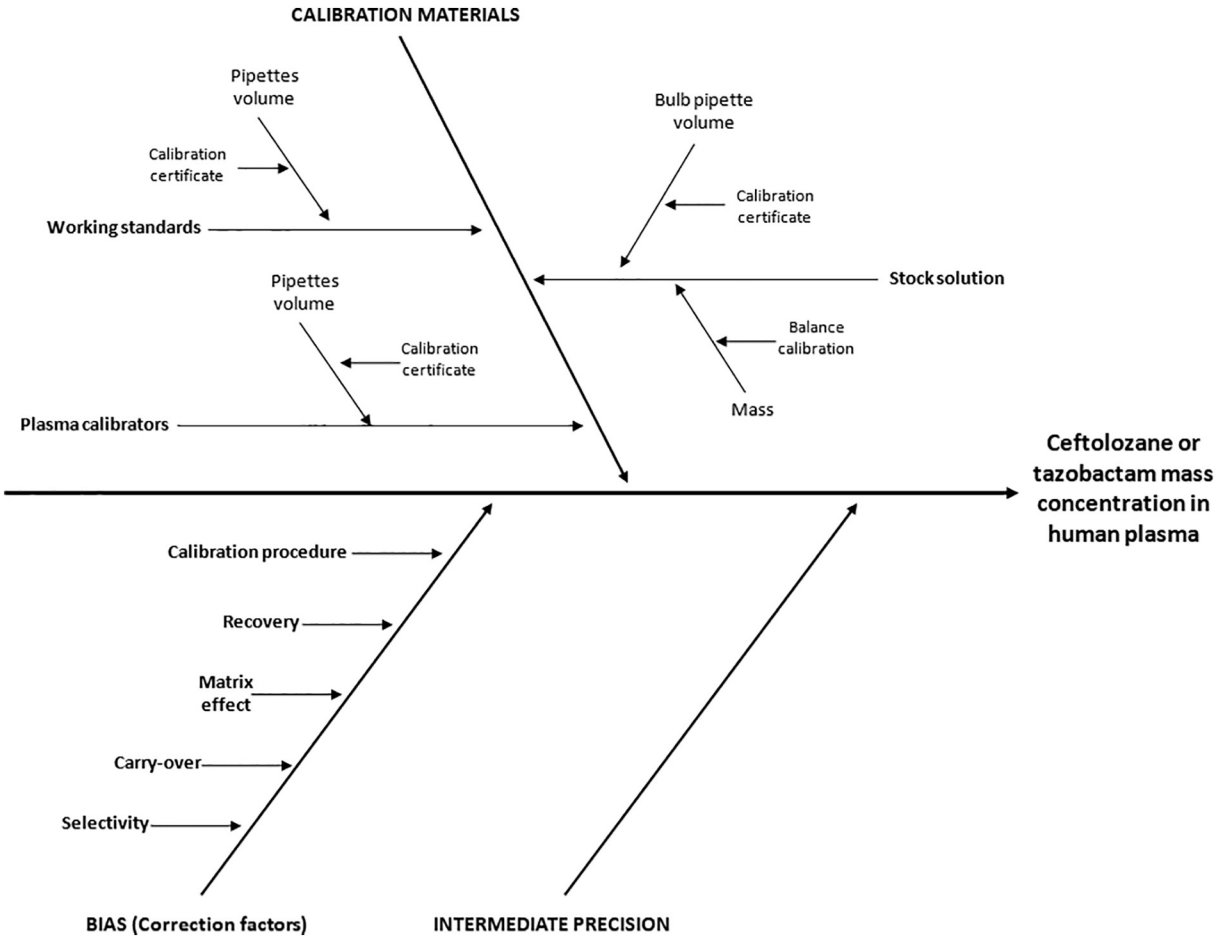


Fig. 2. Cause and effect diagram of the most relevant measurement uncertainty sources of ceftolozane or tazobactam mass concentration in human plasma using the single laboratory approach.

$$u_\delta = \sqrt{\delta_r^2 + \left(\frac{u_p}{n}\right)^2 + u_\mu^2}$$

where n, the number of reference materials processed ($n = 80$ in our case); u_μ, the relative uncertainty associated with the assigned value of the reference material estimated in the same manner than the u_{cal} described above but for the control materials.

Biases related to the REC, ME, CO and SEL were estimated using the following equations:

$$\delta_{REC} = \frac{1}{n} \cdot \sum_{i=1}^{n} (REC_i - \mu_{REC})$$

$$\delta_{ME} = \frac{1}{n} \cdot \sum_{i=1}^{n} (ME_i - \mu_{ME})$$

$$\delta_{CO} = CO - \mu_{CO}$$

$$\delta_{SEL} = \frac{1}{n} \cdot \sum_{i=1}^{n} (SEL_i - \mu_{SEL})$$

where n is the number of samples used to perform the REC, ME and SEL studies ($n = 12$ for REC and ME; and $n = 9$ for SEL); REC_i is the normalized REC in % (100·REC sample/REC internal standard) value obtained for the sample i; μ_{REC}, the REC reference value assigned as 100% (indicating 100% of REC); ME_i is the normalized ME in % (100·ME sample/ME internal standard) value obtained for the sample i; μ_{ME}, the ME reference value assigned as 100% (indicating that no ME exist); CO, the carry-over value in %; μ_{CO}, the CO reference value assigned as 0% (indicating that no CO exist); SEL_i, the selectivity value in % for the possible interference i; μ_{SEL}, the SEL reference value assigned as 0% (indicating that no interference exist).

The relative uncertainty associated to the REC (u_{REC}) and the ME (u_{ME}) were their respective coefficient of variations obtained divided by the root square of their number of samples used to perform the REC and ME studies. The relative uncertainties related to CO (u_{CO}) and the SEL (u_{SEL}) were calculated as:

$$u_{CO} = \sqrt{\delta_{CO}^2 + u_{s-CO}^2}$$

$$u_{SEL} = \sqrt{\delta_{SEL}^2 + \sum_{i=1}^{n} (u_{s-SEL})_i^2}$$

where u_{s-CO} and u_{s-SEL} were estimated using a right-angled triangle distribution (type-B approach) as:

$$u_{s-CO} \text{ or } u_{s-SEL} = \sqrt{\frac{(b-a)^2}{18}}$$

where a and b are, respectively, the lower and upper limits of the interval; being $a = 0\%$ in our case for CO and SEL; and b the CO value or the mean SEL value of all interferences considered.

All uncertainty bias sources were combined to obtain the uncertainty related to the bias (u_{bias}):

$$u_{bias} = \sqrt{u_\delta^2 + u_{REC}^2 + u_{ME}^2 + u_{CO}^2 + u_{SEL}^2}$$

Once the individual contribution of uncertainty sources was estimated, we combined them to give a relative combined standard uncertainty (u_c) according to the following equation:

Table 1
Characteristics of patients and the plasmatic concentrations of ceftolozane-tazobactam obtained in each case.

Patient	Age (years)	Weight (kg)	GFR (mL/min)	MIC (mg/L)	Ceftolozane-tazobactam dosage/frequency (g/h)*	Steady-state ceftolozane concentration (mg/L)	Steady-state tazobactam concentration (mg/L)
1	73	70	> 90	1.5	6–3/24	38.1	7.5
2	69	120	86	1.0	2–1/24	6.60	1.3
3	71	75	> 90	2.0	2–1/24	11.4	2.3
4**	39	77	> 90	4.0	4–2/24	18.5	2.7
					6–3/24	21.8	3.4
					6–3/24	32.9	4.2

In all cases, patients suffered for osteoarticular infections caused by multidrug-resistant *Pseudomonas aeruginosa*.
GFR, Glomerular filtration rate estimated using the CKD-EPI formula.
MIC, Minimum inhibitory concentration of ceftolozane-tazobactam for each *P. aeruginosa* strain.
* Ceftolozane-tazobactam was administered in continuous infusion in all cases.
** For the particular Case 4, three different samples are represented, which were obtained at different time during the treatment.

Table 2
Intermediate imprecision and bias values obtained in the UHPLC-MS/MS measurement system for ceftolozane and tazobactam mass concentrations in plasma.

Quantity	Material	n	$\bar{x}$ (mg/L)	CV (%)	μ (mg/L)	δ_r (%)
P—Ceftolozane; mass c.	LLOQ	80	0.97	12.5	1.00	−3.0
	QC1	80	3.05	7.2	3.00	1.7
	QC2	80	7.71	5.4	7.50	2.8
	QC3	80	31.5	3.6	30.0	5.0
	QC4	80	85.2	3.1	80.0	6.5
P—Tazobactam; mass c.	LLOQ	80	1.04	15.9	1.00	4.0
	QC1	80	2.99	7.9	3.00	−0.3
	QC2	80	7.28	6.6	7.50	−2.9
	QC3	80	28.6	4.9	30.0	−4.7
	QC4	80	75.4	3.8	80.0	−5.8

LLOQ, lower limit of quantification; QC1, control material 1; QC2, control material 2; QC3, control material 3; QC3, control material 4; n, number of materials processed; $\bar{x}$, mean value; CV, intermediate coefficient of variation; μ, reference value (conventional value); δ_r, intermediate relative bias.
Quantities are described according to the IFCC and IUPAC recommendations [30]. P, plasma; mass c., mass concentration.

$$u_c = \sqrt{u_{cal}^2 + u_p^2 + u_{bias}^2}$$

Relative expanded uncertainty (U) was obtained by multiplying u_c by a coverage factor of 2 [22].

Finally, the U values obtained were compared with the uncertainty requirement, whose was established based on the maximum permissible root mean square of the relative error of measurement (Δ_{max}) concept, in accordance to the guideline of the German Medical Association on quality assurance in medical laboratory testing [28]. The Δ_{max} value used was 21% and obtained as:

$$\Delta_{max} = \sqrt{\delta_{max}^2 + CV_{max}^2}$$

being δ_{max} and CV_{max}, the EMA relative bias and imprecision requirements (15%), respectively [17].

2.5. Application to biological samples

The current UHPLC-MS/MS procedures as well as another previously published by our group [16] have been developed to be introduced into an institutional antimicrobial stewardship program approved by our hospital. This program included the optimized use of antibiotics in difficult-to-treat infections, such as those occurring in critically-ill-patients and osteoarticular infections. Particularly, the administration of β-LA in continuous infusion or extended infusion against these difficult-to-treat infections is considered a routine clinical practice in our hospital.

2.5.1. Patients and sample collection

We evaluated the applicability of the UHPLC-MS/MS procedures by processing plasma samples from patients treated with ceftolozane-tazobactam admitted in Infectious Diseases Department. All these patients suffered osteoarticular infections.

Blood samples were obtained at least 72 h after the beginning of ceftolozane-tazobactam in order to assure that it represented

Table 3
Recoveries, internal standard-normalized recoveries, matrix factors, and internal standard-normalized matrix factors obtained in the UHPLC-MS/MS measurement system for ceftolozane and tazobactam mass concentrations in plasma.

Value	Ceftazidime-D_5 recovery (%)	Ceftolozane recovery (%)	IS-normalized recovery (%)	Ceftazidime-D_5 matrix factor (%)	Ceftolozane matrix factor (%)	IS-normalized matrix factor (%)
2.5 mg/L	75.4 (11.5)	–	–	112.4 (10.5)	–	–
3.0 mg/L	–	75.2 (5.9)	100.5 (9.4)	–	112.9 (6.9)	100.9 (7.9)
7.5 mg/L	–	78.4 (4.8)	104.7 (9.1)	–	113.9 (4.3)	102.3 (12.0)
30.0 mg/L	–	81.5 (2.6)	109.3 (11.9)	–	117.6 (2.5)	105.5 (10.3)
80.0 mg/L	–	85.1 (4.6)	113.6 (8.1)	–	121.1 (4.6)	108.7 (11.4)

Value	Sulbactam recovery (%)	Tazobactam recovery (%)	IS-normalized recovery (%)	Sulbactam matrix factor (%)	Tazobactam matrix factor (%)	IS-normalized matrix factor (%)
2.5 mg/L	80.9 (10.9)	–	–	105.9 (11.2)	–	–
3.0 mg/L	–	81.0 (13.5)	100.1 (12.4)	–	102.4 (9.9)	96.7 (10.2)
7.5 mg/L	–	81.7 (13.1)	101.0 (12.1)	–	104.0 (10.1)	98.2 (10.6)
30.0 mg/L	–	82.0 (13.9)	101.4 (12.8)	–	106.5 (7.3)	100.6 (9.7)
80.0 mg/L	–	83.4 (9.8)	103.6 (10.7)	–	107.9 (5.9)	101.9 (7.8)

Coefficients of variation (in %) between patients are indicated in brackets.
IS, internal standard.

Table 4
Measurement uncertainty budget for the measurement of ceftolozane and tazobactam mass concentrations in human plasma using the *single laboratory validation* approach.

Quantity	Material	Value (mg/L)	u_{cal} (%)	u_p (%)	u_δ (%)	u_{REC} (%)	u_{ME} (%)	u_{SEL} (%)	u_{CO} (%)	u_{bias} (%)	u_c (%)	U (%)	U (mg/L)
P—Ceftolozane; mass c.	QC1	3.05	2.65	7.20	3.29	2.71	2.28	2.06	3.39	5.32	9.34	18.67	0.57
	QC2	7.71	2.65	5.40	3.92	2.63	3.46	2.06	3.39	5.88	8.41	16.83	1.30
	QC3	31.5	2.65	3.60	5.67	3.43	2.97	2.06	3.39	6.03	7.51	15.0	4.7
	QC4	85.2	2.65	3.10	7.00	2.34	3.29	2.06	3.39	5.66	6.98	14.0	11.9
P—Tazobactam; mass c.	QC1	2.99	3.16	7.9	3.36	3.58	2.94	1.52	2.05	5.30	10.02	20.04	0.60
	QC2	7.28	3.16	6.6	4.39	3.49	3.06	1.52	2.05	5.30	9.04	18.07	1.32
	QC3	28.6	3.16	4.9	5.52	3.70	2.80	1.52	2.05	5.30	7.88	15.8	4.5
	QC4	75.4	3.16	3.8	6.59	3.09	2.25	1.52	2.05	4.60	6.75	13.5	10.2

QC1, control material 1; QC2, control material 2; QC3, control material 3; QC4, control material 4; u_{cal} (%), relative uncertainty associated with the assigned value of the calibrator materials; u_p (%), relative uncertainty related to the intermediate precision; u_δ (%), relative uncertainty related to the bias associated to the calibration procedure; u_{REC}, relative uncertainty associated to the bias related to the recovery of the extracted samples; u_{ME}, relative uncertainty related to the bias associated to the matrix effect; u_{SEL}, relative uncertainty associated to the bias related to the selectivity; u_{CO}, uncertainty related to the bias associated to carry-over; u_{bias} (%), relative uncertainty related to the bias; u_c (%), relative combined uncertainty; U (%), relative expanded uncertainty; U (mg/L), expanded uncertainty in mg/L units. Quantities are described according to the IFCC and IUPAC recommendations [30]. P, plasma; mass c., mass concentration.

concentrations at the steady-state condition. Samples were collected in BD Vacutainer® lithium-heparin tube (Becton Dickinson, Franklin Lakes, NJ, USA) and immediately refrigerated at (2–8) °C. Finally, they were then centrifuged at 2000*g* for 10 min at (4 ± 1) °C, aliquoted, and stored at (−75 ± 3) °C until analysis.

2.5.2. Microbiological studies

The isolation of the microorganisms was carried out by microbiological conventional procedures. Identification was performed with the MALDI-TOF Biotyper® system (Bruker, Billerica, MA, USA).

Antibiotic susceptibility was performed using the MicroScan® automated microdilution system (Beckman Coulter Inc., Brea, CA, USA). In addition, exact MIC values for ceftolozano-tazobactam was determined by *E*-test® method (bioMérieux, Marcy-l'Étoile, France). Minimal inhibitory concentration clinical breakpoints were defined according to the CLSI criteria [29].

3. Results

3.1. Intermediate imprecision and bias

Data for intermediate imprecision and relative bias are summarized in Table 2. The imprecision values ranged from 3.1% to 7.9% whereas relative bias values ranged between −5.8% and 6.5%. The imprecision and absolute relative bias values obtained were within the EMA criteria.

3.2. Lower limit of quantification

Lower limits of quantification were 0.97 mg/L (signal-to-noise ratio of 15.2) and 1.04 mg/L (signal-to-noise ratio of 8.9) for ceftolozane and tazobactam, respectively. Data for intermediate imprecision and relative bias at LLOQ are summarized in Table 2. All LLOQ obtained were in accordance to the EMA criteria.

3.3. Selectivity

Ceftolozane, tazobactam and their IS were clearly separated from endogenous peaks originating from the blank matrix.

No significant endogenous area response peaks were observed at the retention time of ceftolozane, tazobactam and their IS. For amikacin, ampicillin, aztreonam, cefepime, ceftazidime, gentamycin, meropenem, tobramycin, and vancomycin plasma batches, the peak area responses observed at ceftolozane retention times were 1.4%, 2.2%, 1.8%, 2.6%, 2.9%, 1.9%, 2.7%, 1.3%, and 1.6% of the LLOQ of ceftolozane, respectively, being 0.4%, 0.3%, 0.5%, 0.6%, 0.7%, 0.5%, 0.8%, 0.3%, and 0.6% at ceftazidime-D_5 retention time. Further, the peak area responses observed at tazbactam retention times were 1.7%, 1.1%, 2.7%, 1.4%, 1.3%, 2.1%, 1.8%, 0.9%, and 0.7% of the LLOQ of tazobactam, respectively; and being 0.3%, 0.4%, 1.0%, 0.5%, 0.4%, 0.8%, 0.5%, 0.4%, and 0.3% at sulbactam retention time. All peak area responses obtained were within the EMA criteria.

3.4. Carry-over

No significant area response peaks at the same retention time of ceftolozane, tazobactam and their IS were observed in the chromatogram of double blank plasma extract immediately after injection of highest calibration material. Peak areas responses observed in the double blank plasma sample after measurement of the highest calibration sample were 3.3% of the peak area response at LLOQ of ceftolozane, and 2.0% of the peak area response at LLOQ of tazobactam. On the other hand, peak area responses were 0.9% and 0.6% of the peak area responses of ceftazidime-D_5 and sulbactam, respectively. Peak area response obtained accomplished the EMA acceptance criteria.

3.5. Calibration curve

Typical calibration curve equations were $y = 0.7016 \cdot x - 1.061$ ($r^2 = 0.9957$) and $y = 0.3028 \cdot x + 0.1262$ ($r^2 = 0.9948$) for ceftolozane and tazobactam, respectively; where y is the analyte/IS standard area response ratio multiplied by the IS concentration, and x is the nominal concentration of analyte. The deviations of the calculated concentrations from their nominal values at LLOQ ranged from 5.6% to 12.2% for ceftolozane and − 10.4% to −3.5% for tazobactam. Calibration materials other than LLOQ were within (3.2–7.9)% and (1.9–9.3)% for ceftolozane and tazobactam, respectively. All these values accomplished the EMA criteria.

3.6. Linearity

For ceftolozane, mean values ± standard deviation obtained for each dilution were: (0.94 ± 0.08) mg/L, (25.2 ± 0.85) mg/L, (48.9 ± 1.27) mg/L, (74.6 ± 1.59) mg/L, (99.7 ± 2.08) mg/L, and (122 ± 1.53) mg/L. In the linearity analysis, no second or third order polynomial fit was statistically better than the linear fit at the 5% significant level, indicating that between the LLOQ and the ULOQ a linearity interval exists. Dilution integrities of all samples achieved the EMA acceptance criteria for imprecision and bias.

For tazobactam, mean values ± standard deviation obtained for each dilution were: (1.02 ± 0.09) mg/L, (25.2 ± 0.95) mg/L, (50.9 ± 1.53) mg/L, (74.3 ± 2.10) mg/L, (104 ± 2.08) mg/L, and (122 ± 2.08) mg/L. The linearity analysis showed a nonlinear

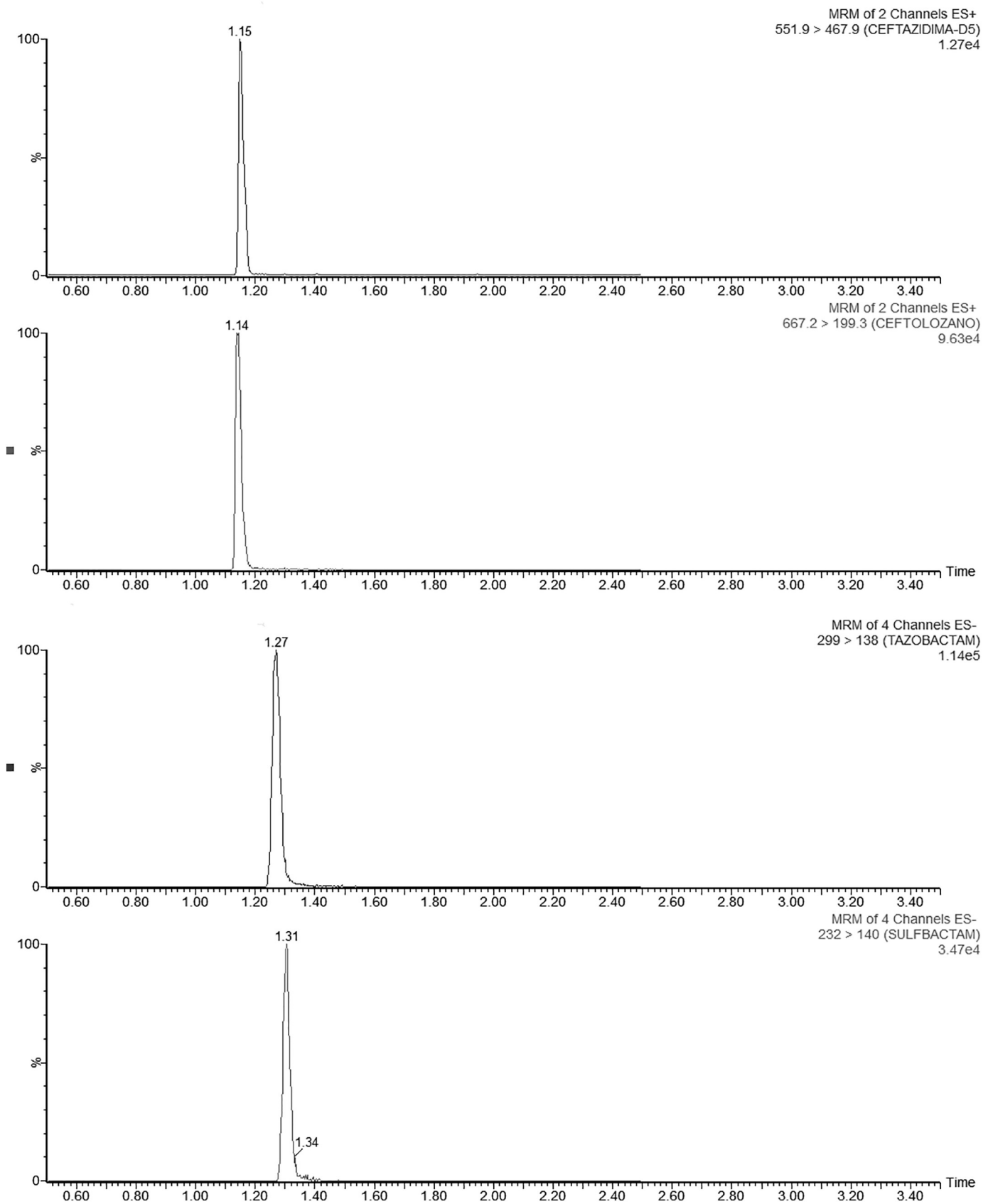


Fig. 3. Multiple reaction monitoring chromatograms for ceftolozane, tazobactam, and their internal standards (ceftazidime-D_5 and sulfactam) for a control sample at 3.00 mg/L.

response. The third-order term in the third-degree polynomial was significant ($p = 0.0399$) and the nonlinear terms in the second-order model were not significant ($p = 0.2363$). The standard errors also showed that the third-order model fitted better than the first- or second-order models. Percentage differences in predicted values between the first- and third-order models were within −1,7 and 9.4%. Taking into account that none of these differences exceeded the linearity (bias) criterion of 15%, as well as the imprecision requirement (15%), the measurement procedure was considered linear between the LLOQ and the ULOQ.

3.7. Dilution Integrity

Imprecision and bias values for dilution integrity at ten-fold dilution for ceftolozane were found to be 6.4 and −5.2%, respectively; and 7.8 and −7.3% for tazobactam, being lower than the EMA requirements.

3.8. Recovery of extracted samples and matrix effect

Values for REC, IS-normalized REC, matrix factor, IS-normalized matrix factor and variabilities of REC and ME of ceftolozane and tazobactam at different concentrations are showed in Table 3. Evaluation of the matrix effect showed ion enhancement for ceftolozane, tazobactam well compensated by their IS. The variation in recoveries and matrix effects among all concentrations accomplished with the EMA or CLSI criteria.

3.9. Stability

Working standard and control solutions (10.0, 50.0, 100, 250, 450, 750, 1000 and 1250 mg/L; and 30.0, 75.0, 300 and 800 mg/L) were stable during storage at (−75 ± 3) °C for at least 6 months with absolute %D values of 8.2%, 8.1%, 7.8%, 7.9%, 7.7%, 7.4%, 7.6%, 7.9%, 8.0%, 7.6%, 8.3%, and 7.2%, respectively for ceftolozane; and 9.7%, 9.3%, 8.9%, 7.5%, 10.1%, 9.4%, 9.3%, 8.2%, 9.8%, 9.1%, 8.8%, and 8.9% for tazobactam. Internal standard-stock solutions stored at (−75 ± 3) °C was stable for 6 months (absolute %D value of 9.2% for ceftolozane and 10.9% for tazobactam). On the other hand, ceftolozane and tazobactam concentrations in extracted samples were stable in the autosampler at (4 ± 1) °C for 12 h (absolute %D values ≤13.4% and ≤14.2% for ceftolozane and tazobactam, respectively). Furthermore, ceftolozane and tazobactam concentrations in plasma were stable during storage at (5 ± 3) °C for a period of 2 days (absolute %D ≤ 13.9% for ceftolozane and ≤14.5% for tazobactam), at room temperature for 8 h (absolute %D ≤ 12.9% and ≤13.3% for ceftolozane and tazobactam, respectively), and at (−75 ± 3) °C for at least 6 months (absolute %D ≤ 14.2% for ceftolozane and ≤14.7% for tazobactam).

All percent deviations were negative, indicating a decomposition or degradation of ceftolozane and tazobactam.

3.10. Measurement uncertainty

Table 4 shows the measurement uncertainty budget containing all main uncertainty sources, as well as the combined and expanded uncertainties. The *U* values obtained were lower than the maximum permissible root mean square of the relative error of measurement (21%).

3.11. Clinical application

Ceftolozane and tazobactam concentrations in plasma as well as clinical and microbiological information of selected cases are shown in Table 1. In all cases, ceftolozane-tazobactam was administered in continuous infusion. Plasma drug concentrations were above the MIC all the time (T > MIC 100%) and they were also higher than the recommended 3–4 times the MIC value for optimizing the pharmacodynamic parameters of these antibiotics [1–3,12–15].

4. Discusion

The UHPLC-MS/MS procedures were developed and validated for measurement of ceftolozane and tazobactam concentrations in plasma. These procedures were applied in the management of patients with osteoarticular infections caused by MDR *P. aeruginosa* to monitor the treatment with ceftolozane-tazobactam in continuous infusion.

Chromatographic separation and generic mass spectrometer conditions, and sample preparation were previously reported by our group as adequate for the analysis of different β-LA concentrations in plasma [16]. Taking into account that we use the reported measurement procedure for TDM of β-LA in routine practice, for practical reasons, we decided to use those conditions for measurement of ceftolozane and tazobactam concentrations in plasma. Under these chromatographic separation conditions, ceftolozane eluted at retention time of 1.14 min, ceftazidime-D_5 at 1.15, tazobactam at 1.27 min, and sulbactam at 1.31 min. Typical MRM chromatograms for the lowest control material (3.00 mg/L) are shown in Fig. 3. The UHPLC-MS/MS run times were 3.5 min, being shorter than other procedures previously reported [10–12]. On the other hand, setting the generic MS parameters described by our group [16], specific MS parameters as *m/z* precursor and product ions, cone voltage and collision energy were optimized injecting 10.0 mg/L of each drug and IS solution in a mixture of water:-acetonitrile 50:50 *v/v* containing 0.1% formic acid at a flow rate of 10 μL/min. The most abundant ions obtained were the $[M-H]^+$ adducts in ESI+ for ceftolozane, and ceftazidime-D_5, and the $[M-H]^-$ adducts in ESI- for tazobactam and sulbactam. Furthermore, because ceftolozane and ceftazidime-D_5 detected in ESI+ presented similar elution times than tazobactam and sulbactam in ESI-, we preferred not use the polarity switching mode option. Therefore, two injections were carried out, one to monitor ceftolozane and ceftazidime-D_5 and the second one to trace tazobactam and sulbactam. Furthermore, taking into account the REC and ME results obtained in this study, extraction procedure previously reported by our group [16] (based on protein precipitation with acetonitrile), besides simplifying the extraction procedures published by other groups [10–15], can also be applied for measurement of ceftazolane and tazobactam concentration.

Regarding to the measurement procedure performance characteristics, we evaluated intermediate imprecision, bias, selectivity, carry-over, calibration curve, linearity, dilution integrity, recovery of extracted samples, matrix effect, stability, and measurement of uncertainty. Of all of them, selectivity, carry-over, matrix effect and measurement uncertainty have not been evaluated by other groups [10–15], although the importance that these metrological characteristics have for obtaining accurate ceftolozane and tazobactam concentration results, and that these pharmacological quantities are measured for TDM or to perform pharmacokinetic/pharmacodynamic studies.

The imprecision, bias and LLOQ results obtained for the proposed UHPLC-MS/MS procedures were similar or better to those of previous publications [10–15]. These results indicate acceptable precisions and trueness. Also, we considered that the LLOQ were low enough given that ceftolozane-tazobactam MIC's for many multidrug-resistant Gram-negative bacilli are higher than 1.00 mg/L [28,31], and because patients receiving a continuous infusion administration rarely will have low concentrations of ceftolozane and tazobactam in plasma.

Selectivity data obtained showed that no endogenous interferences exist. Carry-over values obtained were negligible, indicating that there are no necessary include blank samples between patient samples to prevent the CO.

For each analyte, a calibration curve consisting of 8 plasma calibrators was prepared with a LLOQ and an ULOQ based on available literature and *in-house* experience. The calibration curves generated showed that linear regression with a weighting scheme of 1/X can

describe the data set generated in the intervals of (0.97–125) mg/L for ceftolozane, and (1.04–125) mg/L for tazobactam. Also, statistical linearity studies were performed to verify the linearities between the LLOQ and the ULOQ, indicating that the linear regression model used for the calibration curve were valid.

According to the results obtained in the dilution integrity study, for those samples with a concentration above the ULOQ, a 1/10 dilution can be applied. Higher dilution factors were not investigated because of the wide calibration range.

We showed steady REC and ME values, given that the use of the ceftazidime-D_5 and sulbactam as IS well compensated the lack of REC and the ME observed in the measurements of ceftolozane and tazobactam concentrations. For ceftolozane, we did not use a ceftolozane-labeled compound due to its high price, but we used a labeled-cephalosporin analogue structurally similar to the ceftolozane, the ceftazidime-D_5, which elutes simultaneously with ceftolozane. For tazobactam, we used a chemical structural analogue, sulbactam, as IS due to problems of availability at the moment of purchase of its stable labeled compound.

Ceftolozane and tazobactam working standard and control solutions, as well as the IS-stock solutions were stable during storage at (−75 ± 3) °C for at least 6 months. Extracted samples were stable in autosampler at (4 ± 1) °C for 12 h. The storage capabilities of plasma samples were investigated and deemed to be acceptable for a minimum of 8 h at room temperature, 2 days at (5 ± 3) °C and 6 months at (−75 ± 3) °C. Despite this, taking into account that the stability of concentration of β-LA in plasma at room temperature or refrigerated is low [32,33], mainly in sample extracts, we recommend process a maximum run of 30 samples and using a refrigerated autosampler temperature to prevent the gradual antibiotic decomposition.

Finally, in the validation process we included a detailed procedure to estimate a “new” performance characteristic: the measurement uncertainty, considering that it is essential to evaluate the reliability of β-LA concentration results facilitated by clinical laboratories and it is being required in accredited clinical laboratories [34].

5. Conclusions

In conclusion, we developed simple UHPLC-MS/MS procedures to measure concentrations of ceftolozane and tazobactam in plasma, and validated them following international guidelines. The mentioned procedures were useful for monitoring the treatment of difficult-to-treat osteoarticular infections caused by MDR *P. aeruginosa*. Overall, we believe our measurement procedures can be applied in the daily routine of the clinical laboratory, considering the performance characteristics obtained.

Funding

This study was supported by a research grant from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (PI14/00511) and funded in part by Merck Sharp & Dohme S.A (Merck Investigator Studies Program, IISP 54711).

Conflict of interest

The funders of the study did not play any role in the design, analysis or reporting of the results.

The authors declare that they have no potential conflict of interest.

References

[1] L. Nguyen, J. Garcia, K. Gruenberg, C. MacDougall, Multidrug-resistant pseudomonas infections: hard to treat, but hope on the horizon? Curr. Infect. Dis. Rep. 20 (2018) 23.

[2] M. Bassetti, A. Vena, A. Croxatto, E. Righi, B. Guery, How to manage *Pseudomonas aeruginosa* infections, Drugs Context 7 (2018) 212527.

[3] G.G. Zhanel, P. Chung, H. Adam, S. Zelenitsky, A. Denisuik, F. Schweizer, P.R. Lagacé-Wiens, E. Rubinstein, A.S. Gin, A. Walkty, D.J. Hoban, J.P. Lynch, J.A. Karlowsky, Ceftolozane/tazobactam: a novel cephalosporin/β-lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli, Drugs 74 (2014) 31–51.

[4] A. Ribera, E. Benavent, J. Lora-Tamayo, F. Tubau, S. Pedrero, X. Cabo, et al., Osteoarticular infection caused by MDR Pseudomonas aeruginosa: the benefits of combination therapy with colistin plus β-lactams, J. Antimicrob. Chemother. 70 (2015) 3357–3365.

[5] N. Hoiby, T. Bjarnsholt, C. Moser, G.L. Bassi, T. Coenye, G. Donelli, et al., ESCMID guideline for the diagnosis and treatment of biofilm infections 2014, Clin. Microbiol. Infect. 21 (2015) S1–S25.

[6] J.W. Mouton, A.A. Vinks, Is continuous infusion of beta-lactam antibiotics worthwhile? Efficacy and pharmacokinetic considerations, J. Antimicrob. Chemother. 38 (1996) 5–15.

[7] B. Moriyama, S.A. Henning, R. Childs, S.M. Holland, V.L. Anderson, J.C. Morris, et al., High-dose continuous infusion beta-lactam antibiotics for the treatment of resistant Pseudomonas aeruginosa infections in immunocompromised patients, Ann. Pharmacother. 44 (2010) 929–935.

[8] A. Ribera, L. Soldevila, R. Rigo-Bonnin, F. Tubau, A. Padulles, J. Gomez-Junyent, et al., Beta-lactams in continuous infusion for Gram-negative bacilli osteoarticular infections: an easy method for clinical use, Infection 46 (2018) 239–244.

[9] A. Huttner, S. Harbarth, W.W. Hope, J. Lipman, J.A. Roberts, Therapeutic drug monitoring of the β-lactam antibiotics: what is the evidence and which patients should we be using it for? J. Antimicrob. Chemother. 70 (2015) 3178–3183.

[10] C.E. Garin, R. Ferriols-Lisart, M. Alós-Almiñana, G. Aguilar-Aguilar, J.F. Belda-Nacher, J.A. Carbonell, Validated HPLC-UV detection method for the simultaneous determination of ceftolozane and tazobactam in human plasma, Bioanalysis 10 (2018) 461–473.

[11] C.A. Sutherland, D.P. Nicolau, Development of an HPLC method for the determination of ceftolozane/tazobactam in biological and aqueous matrixes, J. Chromatogr. Sci. 54 (2016) 1037–1040.

[12] A.P. MacGowan, A.R. Noel, S.G. Tomaselli, D. Nicholls, K.E. Bowker, Pharmacodynamics of ceftolozane plus tazobactam studied in an *in vitro* pharmacokinetic model of infection, Antimicrob. Agents Chemother. 60 (2015) 515–521.

[13] B. Yu, A. Adedoyin, E. Hershberger, L. Caro, A. Xiao, E.G. Rhee, J.A. Huntington, Safety, tolerability, and pharmacokinetics of 3 g of ceftolozane/tazobactam in healthy adults: a randomized, placebo-controlled, multiple-dose study, Clin. Pharm. Drug Dev. 7 (2018) 382–391.

[14] G. Chandorkar, A. Xiao, M.S. Mouksassi, E. Hershberger, G. Krishna, Population pharmacokinetics of ceftolozane/tazobactam in healthy volunteers, subjects with varying degrees of renal function and patients with bacterial infections, J. Clin. Pharmacol. 22 (2015) 230–239.

[15] B. Miller, E. Hershberger, D. Benziger, M. Trinh, I. Friedland, Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses, Antimicrob. Agents Chemother. 56 (2012) 3086–3091.

[16] R. Rigo-Bonnin, A. Ribera, A. Arbiol-Roca, S. Cobo-Sacristán, A. Padullés, Ò. Murillo, E. Shaw, R. Granada, X.L. Pérez-Fernández, F. Tubau, P. Alía, Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of β-lactam antibiotic concentration in human plasma, Clin. Chim. Acta 468 (2017) 215–224.

[17] European Medicines Agency, Guideline on Bioanalytical Method Validation, EMA, 2011, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf , Accessed date: 30 May 2018.

[18] Clinical and Laboratory Standards Institute, Evaluation of Precision of Quantitative Analysis Measurement Procedures; Approved Guideline, 3rd edition, CLSI, Wayne, PA, 2014 (CLSI EP05-A3).

[19] Clinical and Laboratory Standard Institute, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP06-A), CLSI, Wayne, PA, 2003.

[20] Clinical and Laboratory Standard Institute, Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline (CLSI C62-A), CLSI, Wayne, PA, 2014.

[21] Clinical and Laboratory Standards Institute, International Federation of Clinical Chemistry and Laboratory Medicine, Mass Spectrometry in the Clinical Laboratory: General Principles and Guidance; Approved Guideline (CLSI C50-A), CLSI, Wayne, PA, 2007.

[22] Clinical and Laboratory Standards Institute, Expression of Measurement Uncertainty in Laboratory Medicine; Approved Guideline (CLSI EP29-A), CLSI, Wayne, PA, 2012.

[23] European Federation of National Associations of Measurement, Testing and Analytical Laboratories, Measurement uncertainty revisited: alternative approaches to uncertainty evaluation, EUROLAB Technical Report No. 1/2007, http://www.eurolab.org/documents/1-2007.pdf, 2007 (accessed 30 May 2018).

[24] European Federation of National Associations of Measurement, Testing and Analytical Laboratories, Guide to the evaluation of measurement uncertainty for quantitative test results, EUROLAB Technical Report No. 1/2006, http://www.eurolab.org/documents/EL_11_01_06_387%20Technical%20report%20-%20Guide_Measurement_uncertainty.pdf, 2006 (accessed 30 May 2018).

[25] C.T. Viswanathan, S. Bansal, B. Booth, A.J. De Stefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays, Pharm. Res. 24 (2007) 1962–1973.

[26] X. Fuentes-Arderiu, Clinical sample stability and measurement uncertainty, Clin.

Chem. Lab. Med. 52 (2014) e37–e38.
[27] B. Magnusson, S.R.L. Ellison, Treatment of uncorrected measurement bias in uncertainty estimation for chemical measurements, Anal. Bioanal. Chem. 390 (2008) 201–213.
[28] Bundesärztekammer, Guidelines of the German Medical Association on quality assurance in medical laboratory testing, Doc03 (20150408), GMS Z Forder Qualitatssich Med. Lab. 6 (2015) 1–43 https://www.egms.de/static/pdf/journals/lab/2015-6/lab000018.pdf , Accessed date: 30 May 2018.
[29] Clinical and Laboratory Standard Institute, Performance Standards for Antimicrobial Susceptibility Testing, 28th edition, CLSI, Wayne, PA, 2018 (CLSI M100-S28).
[30] U. Magdal, R. Dybkær, H. Olesen, Properties and units in the clinical laboratory sciences, Part XXIII, the NPU terminology, principles, and implementation: a user's guide (IUPAC Technical Report), Pure Appl. Chem. 84 (2012) 137–165.
[31] European Committee on Antimicrobial Susceptibility Testing, European Society of Clinical Microbiology and Infectious Diseases, Antimicrobial Wild Type Distributions of Microorganisms, ESCMID, 2018, http://mic.eucast.org/Eucast2/SearchController/search.jsp?action=init , Accessed date: 30 May 2018.
[32] J. Zander, B. Maier, M. Zoller, G. Döbbeler, L. Frey, D. Teupser, M. Vogeser, Effects of biobanking conditions on six antibiotic substances in human serum assessed by a novel evaluation protocol, Clin. Chem. Lab. Med. 54 (2016) 265–274.
[33] M. Carlier, J.J. De Waele, A.G. Verstraete, V. Stove, Exploration of the preanalytical stability of β-lactam antibiotics in plasma and blood—Implications for therapeutic drug monitoring and pharmacokinetic studies, Clin. Chem. Lab. Med. 53 (2015) e227–e230.
[34] International Organization for Standardization, Medical Laboratories, Requirements for Quality and Competence, ISO 15189, ISO, Geneva, Switzerland, 2012.

International Journal of Antimicrobial Agents 53 (2019) 612–619

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Efficacy of ceftolozane/tazobactam, alone and in combination with colistin, against multidrug-resistant *Pseudomonas aeruginosa* in an *in vitro* biofilm pharmacodynamic model

Joan Gómez-Junyent[a], Eva Benavent[a], Yanik Sierra[b], Cristina El Haj[a], Laura Soldevila[a], Benjamín Torrejón[c], Raul Rigo-Bonnin[d], Fe Tubau[b], Javier Ariza[a,e], Oscar Murillo[a,e,*]

[a] *Infectious Diseases Service, Laboratory of Experimental Infection, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Barcelona, Spain*
[b] *Department of Microbiology, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Barcelona, Spain*
[c] *Centres Científics i Tecnològics, Universitat de Barcelona, Barcelona, Spain*
[d] *Department of Clinical Laboratory, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Barcelona, Spain*
[e] *Spanish Network for the Research in Infectious Diseases (REIPIRD12/0015), Instituto de Salud Carlos III, Madrid, Spain*

ARTICLE INFO

Article history:
Received 22 October 2018
Accepted 16 January 2019

Editor: Professor Paul Tulkins

Keywords:
Foreign-body infection
Pseudomonas aeruginosa
Multidrug resistance
Ceftolozane-tazobactam
Colistin
Biofilm

ABSTRACT

Objectives: Ceftolozane/tazobactam is a potential tool for infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa* (*P. aeruginosa*), but its efficacy against some difficult-to-treat infections has not been well defined.

Methods: Using an *in vitro* pharmacodynamic biofilm model, this study evaluated the comparative efficacy of ceftolozane/tazobactam against MDR/extensively drug-resistant (XDR) *P. aeruginosa* strains, alone and in combination with colistin. Simulated regimens of ceftolozane/tazobactam (2 g/1 g every 8 h), meropenem (2 g every 8 h) and ceftazidime (2 g every 8 h), alone and in combination with colistin (continuous infusion) were evaluated against three colistin-susceptible and ceftazidime-resistant strains: MDR-HUB1, ceftolozane/tazobactam-susceptible and meropenem-susceptible; XDR-HUB2, ceftolozane/tazobactam-susceptible and meropenem-resistant; MDR-HUB3, ceftolozane/tazobactam-resistant and meropenem-susceptible. Antibiotic efficacy was evaluated by decreases in bacterial counts (Δlog CFU/mL) from biofilm-embedded bacteria over 54 h. Resistance emergence was screened.

Results: Among monotherapies, ceftolozane/tazobactam had low killing but no resistance appeared, ceftazidime was ineffective, colistin was initially effective but regrowth and resistance occurred, and meropenem was bactericidal against carbapenem-susceptible strains. Ceftolozane/tazobactam plus colistin was the most effective combination against the meropenem-resistant XDR-HUB2 strain (Δlog CFU/mL 54–0 h = −4.42 vs. −3.54 for meropenem-colistin; $P = 0.002$), whereas this combination against MDR-HUB1 (−4.36) was less effective than meropenem-colistin (−6.25; $P < 0.001$). Ceftolozane/tazobactam plus colistin was ineffective against the ceftolozane/tazobactam-resistant strain; meropenem plus colistin was the most bactericidal therapy (−6.37; $P < 0.001$ vs. others). Combinations of active beta-lactams plus colistin prevented the emergence of colistin-resistant strains.

Conclusions: Combinations of colistin plus ceftolozane/tazobactam and meropenem were the most appropriate treatments for biofilm-related infections caused by XDR and MDR *P. aeruginosa* strains, respectively. These combinations could be considered as potential treatment options for these difficult to treat infections.

1. Introduction

Osteoarticular and orthopaedic device-related infections are among the most frequent concerns and therapeutic challenges of infectious diseases. While staphylococci are the most common microorganisms responsible for these infections, a significant rise in the proportion of device-related infections caused by

* Corresponding author. Oscar Murillo, Infectious Diseases Service, Hospital Universitari de Bellvitge. IDIBELL, Universitat de Barcelona, FeixaLlarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain. Tel.: 34 93 260 76 25; fax: 34 93 260 76 37.
E-mail address: omurillo@bellvitgehospital.cat (O. Murillo).

https://doi.org/10.1016/j.ijantimicag.2019.01.010
0924-8579/

Gram-negative bacilli (GNB) has been observed in recent years [1]. Moreover, the emergence of multidrug-resistant (MDR) GNB and, particularly, the global spread of MDR *Pseudomonas aeruginosa* (*P. aeruginosa*) is worrisome in the setting of device-related infections due to the presence of biofilms. Bacterial biofilms impair the activity of most antibiotics [2,3], and very limited options exist for the treatment of these MDR *P. aeruginosa* strains, which are commonly resistant to fluoroquinolones and have a decreased susceptibility to beta-lactams [4].

Colistin is often the only active drug that can be used against these MDR microorganisms [5,6]. Colistin may have notable activity against biofilm-embedded bacteria present in the inner layers of biofilms [7,8], but its clinical efficacy can be threatened by its toxicity and the ability to select for resistant subpopulations when given in monotherapy. Thus, its administration in combination with other antibiotics, such as beta-lactams, may provide a synergistic effect and protect against the emergence of resistant strains [9–11].

Recently, the appearance of ceftolozane/tazobactam, a novel cephalosporin in combination with a beta-lactamase inhibitor, has represented a promising opportunity for the treatment of serious infections by MDR *P. aeruginosa* [12]. It is approved for the treatment of intraabdominal and urinary tract infections, but in the current global era of multiresistance there is a need to improve the knowledge about its efficacy against other infections caused by MDR and XDR strains of *P. aeruginosa*, such as biofilm-related infections, in which scarce experience exists.

Thus, the objective of this study was to evaluate the activity of ceftolozane/tazobactam, in comparison with that of meropenem and ceftazidime, alone and in combination with colistin against MDR and XDR *P. aeruginosa* in an *in vitro* pharmacodynamic biofilm model. It also aimed to investigate the protection of resistance to colistin and ceftolozane/tazobactam after exposure to these antibiotics.

2. Materials and methods

2.1. Bacterial isolates

Three clinical isolates of *P. aeruginosa*, all colistin-susceptible but ceftazidime-resistant strains, were used: HUB1, a ceftolozane/tazobactam-susceptible and meropenem-susceptible MDR strain (ST308); HUB2, a ceftolozane/tazobactam-susceptible and meropenem-resistant XDR strain (ST175); and HUB3, a ceftolozane/tazobactam-resistant and meropenem-susceptible MDR strain (ST274). The three strains have spread worldwide and are considered to be high-risk clones [13]; mechanisms of resistance are AmpC hyperproduction for all strains (MDR-HUB3 having the AmpR mutation G154R), plus OprDporin deletion in XDR-HUB2. Multidrug resistant and XDR were defined in accordance with previous criteria [14]; antibiotic susceptibility was interpreted according to EUCAST criteria.

2.2. Antibiotics

Ceftolozane was provided by MSD (Merck Sharp & Dohme, Spain), whereas the remaining drugs were purchased from the manufacturers' laboratory (Sigma-Aldrich, Madrid, Spain). Stock solutions of antibiotics were re-suspended immediately prior to each experiment following the laboratories' recommendations.

2.3. Determination of MIC, MBC, and minimum biofilm inhibitory and eradication concentrations

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined by broth microdilution method using standard recommendations [15]. The minimum biofilm inhibitory (MBIC) and eradication (MBEC) concentrations were determined based on previously described methodology [16], using an MBEC™ device (Innovotech Inc., Canada). All tests were performed at least in triplicate.

2.4. In vitro pharmacokinetics/pharmacodynamics (PK/PD) biofilm model

A CDC Biofilm Reactor (CBR) (BioSurface Technologies, USA) was used, which consists of a glass vessel with an effluent spout giving place to an operational volume of 350 mL in continuous mixing by a magnetic baffled stir bar. Antibiotics and media can be added through the ports in the top lid of the reactor, from where eight rods descend, each housing three removable Teflon coupons (biofilm growth surfaces), for a total of 24 sampling opportunities throughout the experiment.

The protocol followed previously reported methods [17–19], and consisted of a biofilm conditioning phase, in which the biofilm was formed for 48 h, followed by a therapeutic phase. Briefly, the biofilm conditioning phase started with the bacteria inoculation into the reactor (initial inoculum of 7 log CFU/mL), followed by a 24-h batch culture at 37 °C in drug-free 20% TSB. Then, fresh sterile 20% TSB was infused into the model for 24 h using a peristaltic pump (Masterflex, Cole-Parmer, USA), to achieve a bacterial residence time within the reactor shorter than the generation time for the suspended bacteria. The generation time (g) was calculated according to the following equation:

$$g = \mathrm{Ln}\,2/\mu;$$

where μ is the growth rate,

$$\mu = \mathrm{Ln}\,N - \mathrm{Ln}\,N_0/t - t_0;$$

where N is the number of bacteria at time t, and N_0 is the number of bacteria at time t_0.

Thus, the generation times, infusion rates and estimated bacterial residence times within the reactor were as follows: 45 min, 8 mL/min and 43.75 min, respectively, for MDR-HUB3, and 60 min, 6 mL/min and 58.33 min, respectively, for MDR-HUB1 and XDR-HUB2.

Once the biofilm was formed, the therapeutic phase started (time zero, 0 h). For the three beta-lactam regimens, a bolus dose was injected into the model every 8 h to achieve the desired free-drug C_{max} (fC_{max}; in accordance with the protein binding for each drug). Then, fresh media (20% TSB) was pumped at a flow rate reproducing the respective beta-lactam $t_{1/2}$.

Evaluated regimens were as follows: ceftazidime, 2 g every 8 h (fC_{max} 134 mg/L, $t_{1/2}$ 2 h, flow rate 2 mL/min, protein binding considered 16%); meropenem, 2 g every 8 h (fC_{max} 90 mg/L, $t_{1/2}$ 1 h, flow rate 4 mL/min, protein binding considered 10%); ceftolozane/tazobactam, 2 g/1 g every 8 h (fC_{max} 111 mg/L, $t_{1/2}$ 2.5 h, flow rate 1.61 mL/min, protein binding considered 21%/fC_{max} 25 mg/L, $t_{1/2}$ 2.5 h, flow rate 1.61 mL/min, protein binding considered 30%, respectively) [20–22].

For the particular case of ceftolozane/tazobactam combination, with different $t_{1/2}$ (2.5 h and 1 h, respectively), the $t_{1/2}$ of ceftolozane was reproduced and it was assumed that tazobactam would be eliminated at the same $t_{1/2}$, thus providing tazobactam concentrations during the whole 8-h period always in adequate proportion with ceftolozane (at least 2:1). In all cases, flow rates were calibrated prior to each experiment and monitored throughout to ensure that the system was performing optimally.

Colistin was pumped into the CBR as a continuous infusion at 3.50 mg/L, which mimicked the plasma steady-state concentration observed in humans by 6–9 MU colistin every 24 h [23–24]. This

Table 1
Minimum inhibitory concentrations, minimum bactericidal concentrations, minimum biofilm inhibitory concentrations, and minimum eradication concentrations for the different antibiotics among all *Pseudomonas aeruginosa* strains.

Antibiotics	MDR-HUB1				XDR-HUB2				MDR-HUB3			
	MIC	MBC	MBIC	MBEC	MIC	MBC	MBIC	MBEC	MIC	MBC	MBIC	MBEC
CST	1	4	8	> 64	2	2	8	64	2	2	8	> 64
CAZ	64	128	> 256	> 256	32	32	> 256	> 256	64	> 256	> 256	> 256
MEM	2	4	2	> 256	16	16	16	> 256	2	4	2	> 256
*TOL/TZB	2	4	8	> 256	4	4	16	> 256	8	8	16	> 256

MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; MBIC = minimum biofilm inhibitory concentration; MBEC = minimum biofilm eradication concentration; CST = colistin; CAZ = ceftazidime; MEM = meropenem; TOL/TZB = ceftolozane/tazobactam.

* The MIC, MBC, MBIC and MBEC values refer to the concentration of ceftolozane in the presence of a fixed concentration of tazobactam at 4 mg/L.

was achieved by bolus administration at 0 h followed by infused medium with colistin at the appropriate concentration.

For all strains, the therapeutic regimens evaluated were ceftazidime, meropenem, ceftolozane/tazobactam and colistin, as monotherapies, the respective beta-lactams in combination with colistin, and controls (no antibiotic). All the experiments were performed at least in duplicate.

2.5. Pharmacodynamic analysis

One sample from medium (free-floating bacteria) and three coupons from a rod (biofilm-embedded bacteria) were collected at 0, 6, 24, 30, 48, and 54 h (two extra coupons were collected at the last time point). The removed coupons were processed following previously described methodology [17,19]; medium and coupon samples were serially diluted (10-fold), plated on agar plates (Beckton Dickinson, Spain), and incubated at 37 °C for 24–48 h.

Bacterial counts were expressed as log CFU/mL (means and standard deviations [SD]). Efficacy was evaluated against biofilm-embedded and free-floating bacteria using the log change method from 0 h to each *t* timepoint (Δlog CFU/mL *t*h-0h). Treatments were considered to be bactericidal (or to have bactericidal effect) when they led to a ≥ 3 log CFU/mL reduction, compared with the corresponding counts at zero time. Monotherapy or combination regimens causing a reduction of ≥ 1 log CFU/mL at a specified time were considered active. Synergy (or synergistic effect) was defined as ≥ 2 log CFU/mL killing for the combination relative to the most active monotherapy at a specified time; additivity was defined as 1–2 log CFU/mL greater killing for the combination.

2.6. Pharmacokinetic studies

For these studies, the CBR was filled with saline serum, antibiotic boluses were injected into the CBR, and peristaltic pumps were set up in accordance with simulated $t_{1/2}$ (described above). Samples were then collected into 1 mL polypropylene test tubes at different time points and stored at −20 °C until analysis. All antibiotics and tazobactam concentrations were analysed by Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC/MS-MS), following a methodology previously standardised by the current group [25]; a prior standardisation of this method was developed for ceftolozane and tazobactam.

2.7. Colistin population analysis profiles, and resistance studies

Baseline heteroresistance to colistin was studied by the screening of subpopulations (population analysis profiles, PAPs) able to grow in the presence of ≥ 2, 4, 8, and 16 mg/L of colistin, applying previously reported methods [9,17]. To evaluate the emergence of resistance to ceftolozane/tazobactam and colistin during the therapeutic experiments, samples from coupons at all time points were plated onto nutrient agar plates containing 4–4 mg/L of ceftolozane/tazobactam and 2 mg/L of colistin. Results were interpreted as positive if any macroscopic growth was observed. For the particular case of XDR-HUB2 strain, the PAPs of colistin from isolates recovered at the end of experiments were also analysed.

2.8. Confocal laser scanning microscopy

Coupons were evaluated by confocal laser scanning microscopy (CLSM) to confirm biofilm infection (0 h) and treatment activity (54 h). Images of the biofilms stained with LIVE/DEAD BacLight Bacterial Viability Kit (ThermoFisher Scientific, USA) were acquired using a Leica TCS-SL filter-free spectral confocal laser scanning microscope (Leica Microsystems, Germany) equipped with a 488 nm argon laser and 543 nm He/Ne laser (Centres CientíficsiTecnològics, Universitat Barcelona, Spain) using a 63x oil immersion objective (1.4 numerical aperture). Different image stacks were acquired with a 0.5 microns' distance between planes and the pinhole size was kept at 1 AU. The number of total planes was calculated according with the thickness of each biofilm. Three different stacks were obtained randomly of each coupon. Selected fields were acquired with zoom 4 and an image resolution of 1024 × 1024 pixels. The images obtained were processed with IMARIS software (Bitplane AG, Switzerland).

2.9. Statistical analysis

Data were analysed using Stata 13.1 (Stata Corporation, USA). An analysis of variance with Tukeyś post hoc test was performed for each treatment regimen to evaluate changes in the log CFU/mL for free-floating and biofilm-embedded bacteria. A *P*-value of ≤ 0.05 was considered statistically significant.

3. Results

Table 1 summarises the MIC, MBC, MBIC and MBEC for all strains. Targeted values of PK parameters for intermittent administration of beta-lactams were well reproduced; observed fC_{max} concentrations (mean ± SD) were within 15% of the targeted values: 115 mg/L ± 2.1 for ceftazidime ($t_{1/2}$ 2 h), 94 mg/L ± 1 for meropenem ($t_{1/2}$ 1 h), 100 mg/L ± 1.9 and 24 mg/L ± 0.4 for ceftolozane and tazobactam, respectively ($t_{1/2}$ 2.5 h).

3.1. Microbiological response

The bacterial growth of biofilm-embedded and free-floating cells in the absence of antibiotics for all strains is illustrated in Fig. 1. Mean inoculums for biofilm-embedded cells at 0 h were higher for MDR strains (HUB1 and HUB3) than for XDR-HUB2.

Bacterial counts (log changes) of biofilm-embedded in the presence of antibiotics throughout the experiments are shown in

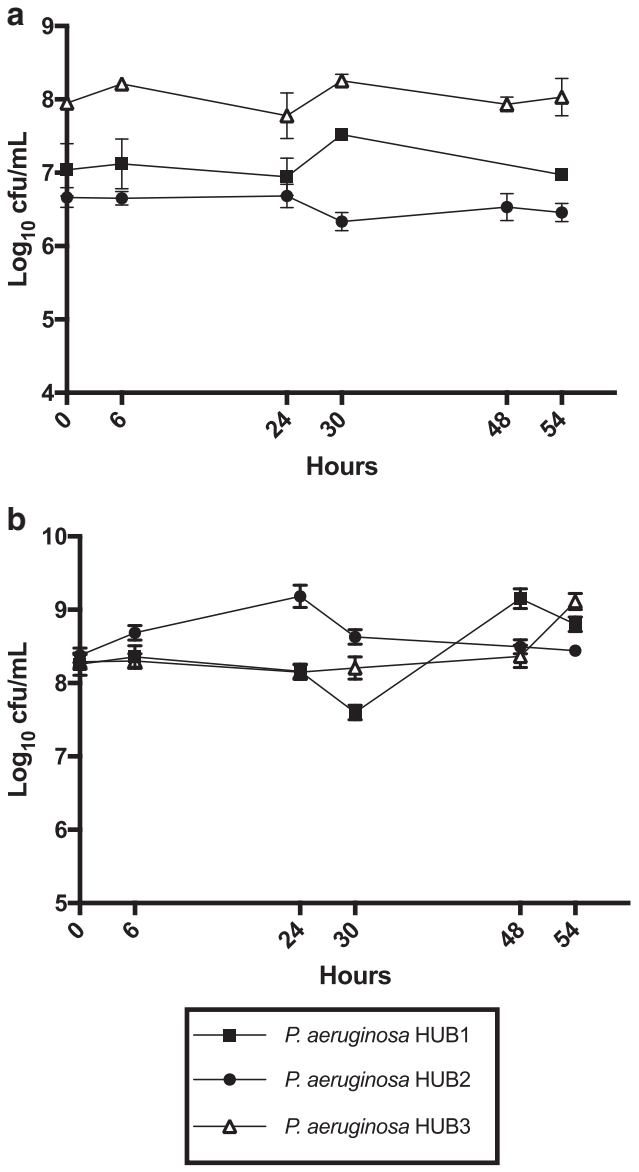


Fig. 1. Bacterial growth in the absence of antibiotics for biofilm-embedded (a) and free floating (b) cells for the three strains of *Pseudomonas aeruginosa*.
Time on the *x*-axis begins immediately after the 48-hour conditioning phase
Data presented as means ± SD
P. aeruginosa = *Pseudomonas aeruginosa*.

Fig. 2. Among monotherapies, at 54 h, ceftolozane/tazobactam achieved a low killing only against susceptible strains (MDR-HUB1 and XDR-HUB2), which was only greater than controls for MDR-HUB1 (Δlog CFU/mL = –0.91; $P = 0.002$), whereas ceftazidime was ineffective in all strains. Colistin therapy, overall, resulted in an initial killing against all strains, but regrowth appeared later in a different manner (Δlog CFU/mL at 54 h = –1.33 in MDR-HUB1, –1.85 in XDR-HUB2, and –2.07 in MDR-HUB3), this leading colistin to be the only effective monotherapy at 54 h against XDR-HUB2 ($P < 0.001$ vs. controls and other monotherapies). Of interest, meropenem alone was the most effective monotherapy and the only bactericidal regimen at 54 h against both carbapenem-susceptible strains (Δlog CFU/mL = –4.55 in MDR-HUB1 and –3.96 in MDR-HUB3; $P < 0.001$ vs. controls and other monotherapies).

Regarding drug combinations, the addition of colistin to ceftolozane/tazobactam significantly increased the activity of monotherapies against both ceftolozane/tazobactam-susceptible strains (MDR-HUB1 and XDR-HUB2) at 54 h ($P < 0.001$), this leading to a bactericidal and synergistic effect in both cases. Ceftolozane/tazobactam plus colistin was the most effective combination against the meropenem-resistant XDR-HUB2 strain (Δlog CFU/mL = –4.42 vs. –3.54 for meropenem-colistin; $P = 0.002$); whereas this combination against MDR-HUB1 (Δlog CFU/mL = –4.36) was less effective than meropenem-colistin (–6.25; $P < 0.001$) and showed similar efficacy as meropenem monotherapy ($P = 0.964$). In contrast, the combination ceftolozane/tazobactam-colistin was ineffective against the ceftolozane/tazobactam-resistant strain (MDR-HUB3), being meropenem plus colistin the most bactericidal therapy (Δlog CFU/mL = –6.37; $P < 0.001$ vs. other regimens). The combination ceftazidime-colistin was slightly effective against MDR-HUB1 and MDR-HUB3 (no synergism nor bactericidal effect), but it achieved a bactericidal effect against XDR-HUB2 (Δlog CFU/mL = –3.10).

Overall, low non-bactericidal activity was observed among free-floating cells of the three strains of *P. aeruginosa* (mean inoculums at 0 h around 8 log CFU/mL). Only meropenem and its combination with colistin showed activity at 54 h against MDR-HUB1 strain (Δlog CFU/mL = –2.67 and –2.23, respectively).

3.2. Resistance studies and colistin PAPs

Resistant strains to ceftolozane/tazobactam among biofilm-embedded cells were not detected with any treatment (monotherapy or combination) in ceftolozane/tazobactam-susceptible strains.

Colistin-heteroresistant subpopulations were detected at baseline in all strains (Fig. 3). The proportion of colonies able to grow at concentrations of colistin 2 mg/L, 4 mg/L and 8 mg/L was slightly higher for the XDR-HUB2 (from 1×10^{-4} to 1×10^{-6} CFU/mL) than for the MDR strains (from 1×10^{-5} to 1×10^{-8} CFU/mL). At the end of treatment (Fig. 4), colistin monotherapy led to the emergence of resistant subpopulations at 54 h among all strains. The combination of an active beta-lactam and colistin prevented the emergence of resistant subpopulations, in contrast with what occurred when the beta-lactam was non-active *in vitro*. For the XDR-HUB2 strain, the PAPs of cells recovered at the end of treatments with meropenem-colistin and ceftazidime-colistin showed the same proportion of colistin-resistant subpopulations than that obtained at baseline; in contrast, this proportion increased with colistin monotherapy (until 10^{-2} CFU/mL).

3.3. Confocal laser scanning microscopy images

Well-formed biofilms prior to the start of therapeutic experiments were observed in all strains. Treatment with beta-lactams altered the shape of isolates in both live and dead cells. Colistin in monotherapy mainly had activity within deeper layers of the biofilm structure, whereas beta-lactams plus colistin mainly resulted in activity against all the biofilm structure. Fig. 5 shows some CLSM images of the biofilm-embedded cells of *P. aeruginosa* HUB2, according to treatment regimens.

4. Discussion

The best treatment for osteoarticular and orthopaedic device-related infections caused by MDR *P. aeruginosa* is currently unknown. The presence of bacterial biofilms, where nutrient and oxygen penetration are limited, results in tolerance to antibiotics by expression of phenotypic changes and this impairs the activity of antibiotics, such as beta-lactams, which act against processes occurring in growing bacteria [2,3]. In this setting, the occurrence of MDR/XDR *P. aeruginosa* isolates dramatically limits the therapeutic

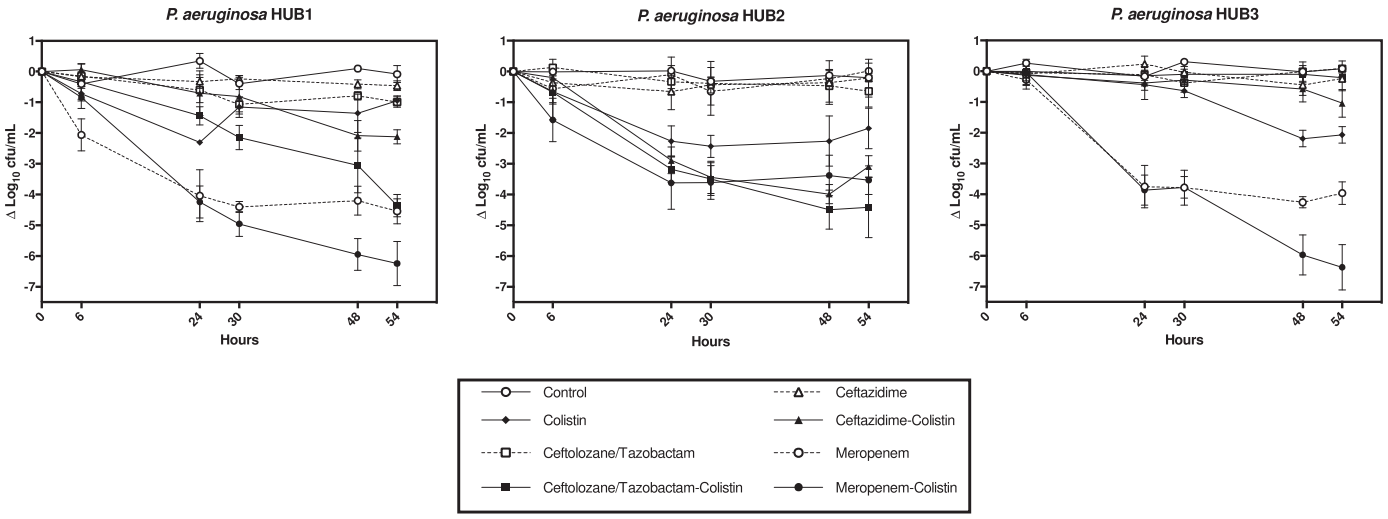


Fig. 2. Bacterial killing by monotherapies with colistin, ceftazidime, meropenem and ceftolozane-tazobactam, and the combination of colistin with beta-lactams against biofilm-embedded cells of three different *Pseudomonas aeruginosa* strains.
Results are expressed using the log change method
Data presented as means ± SD
P. aeruginosa = *Pseudomonas aeruginosa*.

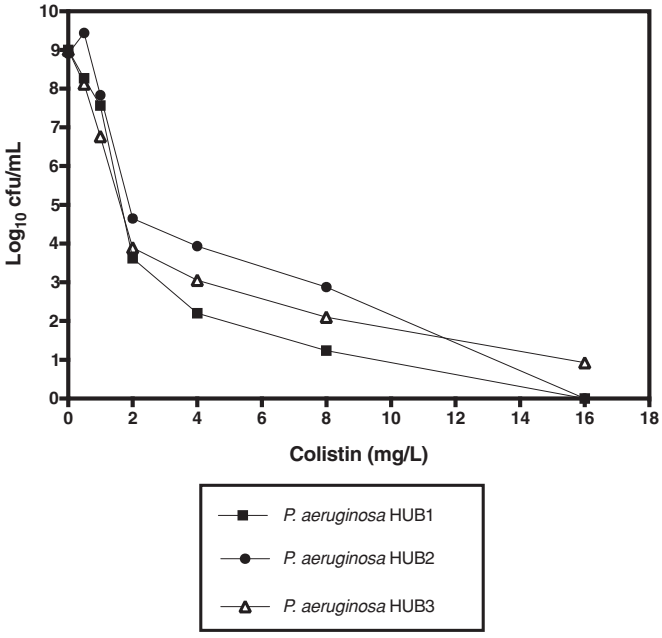


Fig. 3. Baseline Population Analysis Profiles of the three strains of *Pseudomonas aeruginosa* at an initial inoculum of 10^9 cfu/mL.
P. aeruginosa = *Pseudomonas aeruginosa*.

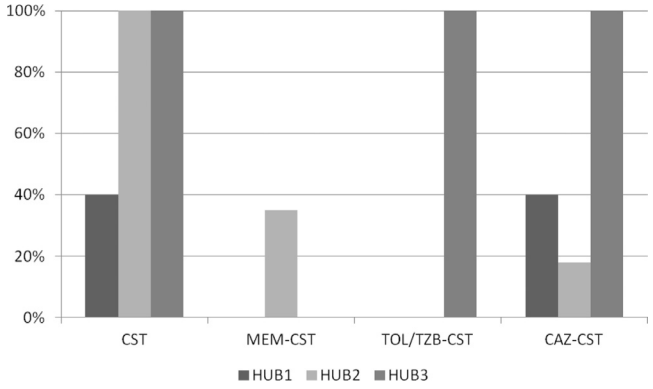


Fig. 4. Emergence of resistant colistin subpopulations among biofilm-embedded cells of the three *Pseudomonas aeruginosa* strains according to the treatment regimen at 54 hours.
Data expressed as proportion of samples with colonies growing at colistin concentration of 2 mg/L among all tested
CST = colistin; MEM = meropenem; TOL/TZB = ceftolozane-tazobactam; CAZ = ceftazidime.

alternatives, since these usually have a decreased susceptibility to beta-lactams and are often only susceptible to colistin.

The current study evaluated the comparative efficacy of ceftolozane/tazobactam, in monotherapy and in combination with colistin, using an *in vitro* pharmacodynamic biofilm model. This has previously been used to model *P. aeruginosa*, has been validated for evaluating the pharmacodynamic efficacy of antibiotics, and reasonably mimics foreign-body infections. Based on previous knowledge, antibiotic efficacy is evaluated by using free drug concentrations in order to reproduce the main PK/PD parameters achieved in human serum (i.e. AUC/MIC or $T_{>MIC}$), which are equivalent to those achieved in interstitial fluids [26]. However, it should be considered that antibiotic peak concentrations close to the biofilm infection within the reactor may be greater than those achieved locally in a human extravascular biofilm-related infection (i.e. prosthetic joint infection).

Ceftolozane/tazobactam is a novel cephalosporin in combination with a beta-lactamase inhibitor that is active among most resistant GNB, including MDR/XDR *P. aeruginosa*. It is approved for clinical use, but its efficacy against osteoarticular and biofilm-related infections is not well known. In the pre-clinical setting, previous experiences have suggested poor activity and the clinical efficacy is limited to few cases with contradictory success [27–29]. In the current model, ceftolozane/tazobactam in monotherapy showed low anti-biofilm efficacy against susceptible strains and it was ineffective against a ceftolozane/tazobactam-resistant strain (MDR-HUB3). Of note, the latter strain was considered resistant (MIC = 8 mg/L) in accordance with the current susceptibility breakpoints (EUCAST criteria) for the 1 g/0.5 g every 8 h regimen, but it could likely be considered susceptible according to the simulated PK/PD parameters for the purpose of 2 g/1 g dosage. Regarding the

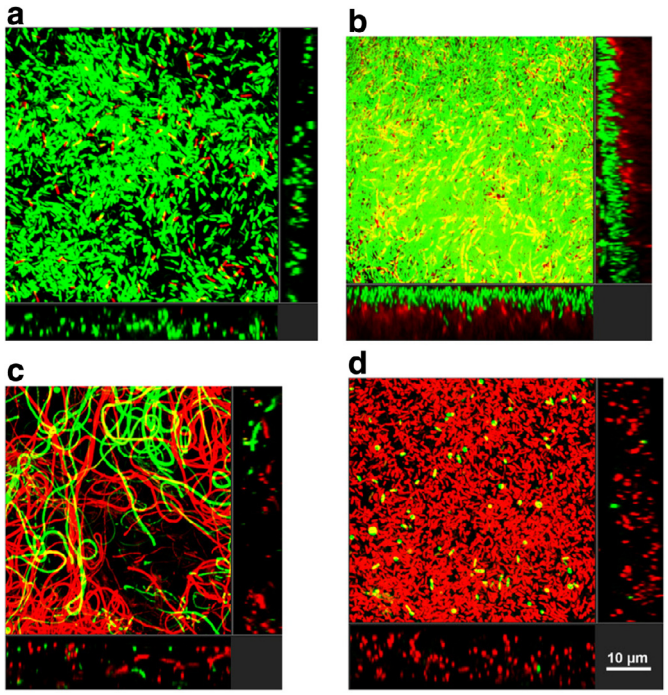


Fig. 5. Confocal laser scanning microscopy images of biofilm-embedded cells of *Pseudomonas aeruginosa* HUB2 at 0 hours (a), at 54 hours for the treatment of colistin in monotherapy (b), ceftolozane/tazobactam in monotherapy (c), and the combination of ceftolozane/tazobactam and colistin (d).
Live cells are green due to staining with Syto 9, whereas dead cells appear red due to staining with propidium iodide. Maximum intensity projection of confocal images of total biofilm thickness is represented as central image. Rectangle images below and to the right of the projection correspond to XZ and YZ planes, respectively.

low frequency of spontaneous resistant mutants previously reported [30], no ceftolozane/tazobactam-resistant strains among the biofilm-embedded population emerged. Overall, given conflicting data between this and other published *in vitro* studies and case reports, clinicians should be cautious when considering the use of ceftolozane/tazobactam in monotherapy against osteoarticular infections by MDR and XDR *P. aeruginosa*.

The current study also evaluated the comparative efficacy of other beta-lactams in monotherapy. All strains were resistant to ceftazidime and no significant activity was observed with this therapy. Interestingly, meropenem alone achieved bactericidal activity against the two meropenem-susceptible strains, suggesting a differential anti-biofilm activity by an unknown mechanism in comparison with other beta-lactams such as cephalosporins. Haagensen et al. used a dynamic biofilm model with flow cell technology and CLSM, and showed that meropenem initially targeted *P. aeruginosa* subpopulations present at the periphery of the biofilm structure but repeated doses resulted in progressive killing of cells in deeper layers [31,32].

Currently, colistin is often the only active drug for treating MDR-GNB and recent research suggests that it has a remarkable anti-biofilm effect mainly based on greater activity in anaerobic conditions and as a biofilm 'destabiliser' [7,8,33,34]. The current results with colistin in monotherapy showed initial killing against biofilm-embedded bacteria followed by regrowth and the progressive appearance of resistant strains; the final efficacy was variable but notable (almost 2 log CFU/mL killing). Interestingly, CLSM pictures from the current experiments showed how colistin has higher affinity for killing bacteria within inner layers of the biofilm population.

In agreement with previous reports [9,35,36], the current results have shown that combining beta-lactams with colistin substantially increases the activity of monotherapies against biofilm-related infections caused by MDR/XDR *P. aeruginosa* and also reinforce the opinion that combination therapy may prevent the emergence of colistin-resistant subpopulations. Moreover, the results also suggest that the anti-biofilm benefits of this combination extend to other subfamilies of beta-lactams, apart from carbapenems, but the efficacy of each beta-lactam plus colistin combination may significantly differ according to its prior activity and the strains' variability.

The combination of ceftolozane/tazobactam plus colistin achieved a bactericidal effect against susceptible strains, but it was ineffective against the ceftolozane/tazobactam-resistant strain. Overall, this combination was the most active treatment for the meropenem-resistant strain. It is believed that the combination ceftolozane/tazobactam-colistin has not been previously evaluated against biofilm-related infections by MDR *P. aeruginosa*, and few studies exist with time-kill analyses [37,38], which mainly reported a synergistic or additive effect even in the case of ceftolozane/tazobactam-resistant *P. aeruginosa* strains. This contrasts with the current results, which limited these beneficial effects against biofilm-embedded bacteria to the treatment of ceftolozane/tazobactam-susceptible strains. The combination of ceftazidime plus colistin was the least active in all strains, although bactericidal in the XDR strain. Finally, the combination of meropenem plus colistin was the most effective regimen for meropenem-susceptible MDR *P. aeruginosa* strains and, interestingly, this combination also achieved a synergistic and bactericidal effect against the meropenem-resistant XDR-HUB2 strain. It has previously been shown that doripenem plus colistin enhance the *in vitro* anti-biofilm killing of monotherapies against carbapenem-resistant MDR/XDR *P. aeruginosa* strains, which contained different mechanisms of resistance (VIM-2 metallo-beta-lactamase or PSE-1 beta-lactamase plus efflux pump) than the XDR-HUB2 strain used [17].

The synergy observed with the beta-lactam and colistin combination has been previously associated with mechanistic and subpopulation synergy effects [35], which may also be applied to biofilm-related infections by targeting different subpopulations. Whereas colistin may target subpopulations with low metabolic activity within inner layers of the biofilm [7,33,34], beta-lactams may act upon more metabolically active subpopulations present at the periphery of the biofilm structure [31,32]. In the particular setting of biofilm-related osteoarticular and orthopaedic device-related infections, clinical data have also emphasised the benefits of using colistin in combination, especially against *P. aeruginosa* isolates [39]. However, this combination has not been found to be superior to colistin alone among critically ill patients with other types of infection caused by MDR-GNB (not limited to *P. aeruginosa*) [40]. The different characteristics of biofilm-related infections and the particular activity of colistin in this field may explain these apparent contradictory results. The current study observed poor efficacy against free-floating bacteria for all monotherapies or combinations. Although it did not specifically study this bacteria population, it probably reflected a mix of microorganisms at high inoculums, forming small clusters or biofilm-like aggregates, either in a planktonic state or detached from biofilms. All these characteristics may impair the efficacy of treatments, which were clearly different from that obtained against biofilm-embedded cells, as also observed in a previous study [17].

Additionally, it was found that the combination of beta-lactams and colistin prevented the amplification of colistin-resistant subpopulations among heteroresistant strains in biofilm-embedded populations, depending on the strain's susceptibility to beta-lactams: protection was more likely if the strain was susceptible.

However, current analysis of colistin PAPs with XDR-HUB2 strain at the end of treatment showed a similar proportion of heteroresistant populations with the combined treatments compared to PAPs at baseline, thus suggesting a stochastic expression of resistance rather than the emergence of real mutants. In contrast, this proportion of heteroresistant strains did change at the end of treatment with colistin alone. Overall, this protective effect of combined therapies (independent of susceptibility or resistance to beta-lactam) should be evaluated for longer periods.

Although CBR can simulate the PK/PD profile of antibiotics similar to human dosage exposure, there is a clear limitation in mimicking the complex structures that biofilms constitute *in vivo*. Similarly, host-pathogen interactions were not considered and these may also affect the efficacy of treatments. Moreover, antibiotic concentrations near the biofilm infection site may differ depending on the biofilm location *in vivo*, which represents a limitation of the CBR. Even when ceftolozane/tazobactam was used in a 2:1 solution, the activity and dynamics of both compounds may have been different, as they were purchased from different companies. This study was also limited by the use of a small number of *P. aeruginosa* strains and, certainly, the use of more strains may provide a deeper understanding of the anti-biofilm activity of the treatments. However, these three strains are disseminated worldwide and very representative high-risk clones; specifically, ST274 clone is linked to chronic biofilm-related infections and ST175 is highly disseminated in Spanish hospitals. Finally, this model evaluated a 48-h-old biofilm, so different results may have been obtained with a more mature biofilm. For all these reasons, it is reasonable to be cautious when translating the results into clinical practice.

5. Conclusions

Monotherapies with beta-lactams mainly had little efficacy against biofilm-embedded MDR/XDR *P. aeruginosa*, with the exception of meropenem against susceptible strains. Colistin alone had notable efficacy, but it was influenced by the emergence of resistance. Based on the efficacy and protection against resistance, the results support the use of a beta-lactam plus colistin combination to treat foreign-body infections caused by MDR/XDR *P. aeruginosa*. Ceftolozane/tazobactam plus colistin was the most appropriate combination for meropenem-resistant (non-carbapenemase producer) *P. aeruginosa* strains, whereas the combination of meropenem-colistin was for the carbapenem-susceptible strains. More studies are needed to further evaluate the particular anti-biofilm activity of meropenem and its combination with colistin against carbapenem-resistant GNB and to provide more evidence for the use of these combinations in clinical practice.

Acknowledgments

The preliminary results were presented in part at the 28th European Congress of Clinical Microbiology and Infectious Diseases, Madrid, Spain, 21–24 April 2018 (Oral presentation, abstract number 2605).

Funding

This study was supported by a research grant from the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III, Spain (PI14/00511), and funded in part by Merck Sharp & Dohme S.A, USA (Merck Investigator Studies Program, IISP 54711). J. G-J was supported by a grant from the Spanish Ministry of Education (FPU 14/03124).

Competing Interests

All authors declare that they have no conflicts of interest to disclose. The funders of the study did not play any role in the design, analysis or reporting of the results.

Ethical Approval

Not required.

References

[1] Benito N, Franco M, Ribera A, Soriano A, Rodriguez-Pardo D, Sorli L, et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. Clin Microbiol Infect 2016;22 732.e1–8.
[2] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95–108.
[3] Lebeaux D, Ghigo JM, Beloin C. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol Mol Biol Rev 2014;78:510–43.
[4] Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int J Antimicrob Agents 2010;36:S50–4.
[5] Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner DR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589–601.
[6] Nation RL, Li J. Colistin in the 21st century. Curr Opin Infect Dis 2009;22:535–43.
[7] Pamp SJ, Gjermansen M, Johansen HK, Tolker-Nielsen T. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. Mol Microbiol 2008;68:223–40.
[8] Pletzer D, Hancock RE. Antibiofilm Peptides: Potential as Broad-Spectrum Agents. J Bacteriol 2016;198:2572–8.
[9] Bergen PJ, Tsuji BT, Bulitta JB, Forrest A, Jacob J, Sidjabat HE, et al. Synergistic killing of multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an *in vitro* pharmacokinetic/pharmacodynamic model. Antimicrob Agents Chemother 2011;55:5685–95.
[10] Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. Pharmacokinetics/pharmacodynamics of colistin and imipenem on mucoid and non-mucoid *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 2011;55:4469–74.
[11] Hengzhuang W, Wu H, Ciofu O, Song Z, Hoiby N. *In vivo* pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. Antimicrob Agents Chemother 2012;56:2683–90.
[12] van Duin D, Bonomo RA. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation beta-lactam/beta-lactamase inhibitor combinations. Clin Infect Dis 2016;63:234–41.
[13] Oliver A, Mulet X, Lopez-Causape C, Juan C. The increasing threat of Pseudomonas aeruginosa high-risk clones. Drug Resist Updat 2015;21-22:41–59.
[14] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[15] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA, USA: CLSI; 2015.
[16] Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J Clin Microbiol 1999;37:1771–6.
[17] Lora-Tamayo J, Murillo O, Bergen PJ, Nation RL, Poudyal A, Luo X, et al. Activity of colistin combined with doripenem at clinically relevant concentrations against multidrug-resistant *Pseudomonas aeruginosa* in an *in vitro* dynamic biofilm model. J Antimicrob Chemother 2014;69:2434–42.
[18] Goeres DM, Loetterle LR, Hamilton MA, Murga R, Kirby DW, Donlan RM. Statistical assessment of a laboratory method for growing biofilms. Microbiology 2005;151:757–62.
[19] El Haj C, Murillo O, Ribera A, Lloberas N, Gomez-Junyent J, Tubau F, et al. Evaluation of linezolid or trimethoprim/sulfamethoxazole in combination with rifampicin as alternative oral treatments based on an *in vitro* pharmacodynamic model of staphylococcal biofilm. Int J Antimicrob Agents 2018;51:854–61.
[20] Drusano GL, Standiford HC, Fitzpatrick B, Leslie J, Tangtatsawasdi P, Ryan P, et al. Comparison of the pharmacokinetics of ceftazidime and moxalactam and their microbiological correlates in volunteers. Antimicrob Agents Chemother 1984;26:388–93.
[21] Bui KQ, Ambrose PG, Nicolau DP, Lapin CD, Nightingale CH, Quintiliani R. Pharmacokinetics of high-dose meropenem in adult cystic fibrosis patients. Chemotherapy 2001;47:153–6.
[22] Miller B, Hershberger E, Benziger D, Trinh M, Friedland I. Pharmacokinetics and safety of intravenous ceftolozane-tazobactam in healthy adult subjects following single and multiple ascending doses. Antimicrob Agents Chemother 2012;56:3086–91.
[23] Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in

critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. Antimicrob Agents Chemother 2011;55:3284–94.
[24] Plachas D, Karvanen M, Friberg LE, Papadomichelakis E, Antoniadou A, Tsangaris I, et al. Population pharmacokinetic analysis of colistin methanesulfonate and colistin after intravenous administration in critically ill patients with infections caused by gram-negative bacteria. Antimicrob Agents Chemother 2009;53:3430–6.
[25] Rigo-Bonnin R, Ribera A, Arbiol-Roca A. Cobo-Sacristan S, Padulles A, Murillo O, et al. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of beta-lactam antibiotic concentration in human plasma. Clin Chim Acta 2017;468:215–24.
[26] Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998;26:1–10.
[27] Dietl B, Sanchez I, Arcenillas P, Cuchi E, Gomez L, Gonzalez de Molina FJ, et al. Ceftolozane/tazobactam in the treatment of osteomyelitis and skin and soft-tissue infections due to extensively drug-resistant *Pseudomonas aeruginosa*: clinical and microbiological outcomes. Int J Antimicrob Agents 2018;51:498–502.
[28] Hassan S, Kahn MD, Saraiya N, Nori P. Treatment of a complex orthopaedic infection due to extensively drug-resistant *Pseudomonas aeruginosa*. BMJ Case Rep 2018. https://doi.org/10.1136/bcr-2017-223202.
[29] Velez Perez AL, Schmidt-Malan SM, Kohner PC, Karau MJ, Greenwood-Quaintance KE, Patel R. *In vitro* activity of ceftolozane/tazobactam against clinical isolates of *Pseudomonas aeruginosa* in the planktonic and biofilm states. Diagn Microbiol Infect Dis 2016;85:356–9.
[30] Riera E, Macia MD, Mena A, Mulet X, Perez JL, Ge Y. Anti-biofilm and resistance suppression activities of CXA-101 against chronic respiratory infection phenotypes of *Pseudomonas aeruginosa* strain PAO1. J Antimicrob Chemother 2010;65:1399–404.
[31] Haagensen J, Verotta D, Huang L, Engel J, Spormann AM, Yang K. Spatiotemporal pharmacodynamics of meropenem- and tobramycin-treated *Pseudomonas aeruginosa* biofilms. J Antimicrob Chemother 2017;72:3357–65.
[32] Haagensen JA, Verotta D, Huang L, Spormann A, Yang K. New *in vitro* model to study the effect of human simulated antibiotic concentrations on bacterial biofilms. Antimicrob Agents Chemother 2015;59:4074–81.
[33] Brochmann RP, Toft A, Ciofu O, Briales A, Kolpen M, Hempel C, et al. Bactericidal effect of colistin on planktonic Pseudomonas aeruginosa is independent of hydroxyl radical formation. Int J Antimicrob Agents 2014;43:140–7.
[34] Kolpen M, Appeldorff CF, Brandt S, Mousavi N, Kragh KN, Aydogan S, et al. Increased bactericidal activity of colistin on *Pseudomonas aeruginosa* biofilms in anaerobic conditions. Pathog Dis 2016. https://doi.org/10.1093/femspd/ftv086.
[35] Bergen PJ, Bulman ZP, Landersdorfer CB, Smith N, Lenhard JR, Bulitta JB, et al. Optimizing Polymyxin Combinations Against Resistant Gram-Negative Bacteria. Infect Dis Ther 2015;4:391–415.
[36] Gunderson BW, Ibrahim KH, Hovde LB, Fromm TL, Reed MD, Rotschafer JC. Synergistic activity of colistin and ceftazidime against multiantibiotic-resistant *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamic model. Antimicrob Agents Chemother 2003;47:905–9.
[37] Monogue ML, Nicolau DP. Antibacterial activity of ceftolozane/tazobactam alone and in combination with other antimicrobial agents against MDR *Pseudomonas aeruginosa*. J Antimicrob Chemother 2018;73:942–52.
[38] Rico Caballero V, Almarzoky Abuhussain S, Kuti JL, Nicolau DP. Efficacy of Human-Simulated Exposures of Ceftolozane-Tazobactam Alone and in Combination with Amikacin or Colistin against Multidrug-Resistant *Pseudomonas aeruginosa* in an *In Vitro* Pharmacodynamic Model. Antimicrob Agents Chemother 2018. https://doi.org/10.1128/AAC.02384-17.
[39] Ribera A, Benavent E, Lora-Tamayo J, Tubau F, Pedrero S, Cabo X, et al. Osteoarticular infection caused by MDR *Pseudomonas aeruginosa*: the benefits of combination therapy with colistin plus beta-lactams. J Antimicrob Chemother 2015;70:3357–65.
[40] Zusman O, Altunin S, Koppel F, Dishon Benattar Y, Gedik H, Paul M. Polymyxin monotherapy or in combination against carbapenem-resistant bacteria: systematic review and meta-analysis. J Antimicrob Chemother 2017;72:29–39.

OXFORD UNIVERSITY PRESS | Journal of Antimicrobial Chemotherapy

In vitro pharmacokinetics/pharmacodynamics of ceftazidime and its combination with colistin against *Pseudomonas aeruginosa* biofilm

Journal:	*Journal of Antimicrobial Chemotherapy*
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Gómez-Junyent, Joan; Monash University Monash Biomedicine Discovery Institute, Microbiology; Hospital Universitari de Bellvitge, Infectious Diseases Murillo, Oscar; Hospital Universitari de Bellvitge, Infectious Diseases Yu, Heidi; Monash University Monash Biomedicine Discovery Institute, Microbiology Azad, Mohammad; Monash University Monash Biomedicine Discovery Institute, Microbiology Wickremasinghe, Hasini; Monash University Monash Biomedicine Discovery Institute, Microbiology Rigo, Raül; Hospital Universitari de Bellvitge, Clinical Laboratory Benavent, Eva; Hospital Universitari de Bellvitge, Infectious Diseases Ariza, Javier; Hospital Universitari de Bellvitge, Infectious Diseases Li, Jian; Monash University Monash Biomedicine Discovery Institute, Microbiology
Keywords:	Pseudomonas aeruginosa, PK/PD, Colistin, Beta-lactams, In vitro

In vitro pharmacokinetics/pharmacodynamics of ceftazidime and its combination with colistin against *Pseudomonas aeruginosa* biofilm

Joan Gomez-Junyent[1,2], Oscar Murillo[2], Heidi H. Yu[1], Mohammad A. K. Azad[1], Hasini Wickremasinghe[1], Raul Rigo-Bonnin[3], Eva Benavent[2], Javier Ariza[2], Jian Li[1].

[1]Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Victoria, 3800, Australia; [2]Department of Infectious Diseases, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Spain; [3]Department of Clinical Laboratory, Hospital Universitari de Bellvitge, IDIBELL, Universitat de Barcelona, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Spain.

Corresponding author:

Oscar Murillo

Department of Infectious Diseases

Hospital Universitari de Bellvitge

IDIBELL, Universitat de Barcelona

Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain

Phone No. 34.93.260.76.25; Fax No. 34.93.260.76.37

E-mail address: omurillo@bellvitgehospital.cat

24 **Short running title:** *In vitro* PK/PD of ceftazidime with/without colistin against *P.*

25 *aeruginosa* biofilm

Synopsis

Objectives: The PK/PD of beta-lactams in continuous infusion (CI) for biofilm infections by *Pseudomonas aeruginosa* has not been defined. We evaluated the efficacy of several dosage regimens of CI ceftazidime, with or without colistin, an antibiotic with a potential anti-biofilm effect, against biofilm-embedded *P. aeruginosa*.

Methods: The reference strain PAO1 and a clinical isolate HUB8 (both ceftazidime- and colistin-susceptible) were investigated over 54 h using a dynamic CDC biofilm reactor. CI dosage regimens were ceftazidime monotherapy (4, 10, 20 and 40 mg/L), colistin monotherapy (3.50 mg/L), and combinations of colistin with ceftazidime (4 or 40 mg/L). Efficacy was evaluated by log changes and confocal microscopy.

Results: Against PAO1 at 54 h, the anti-biofilm activity of ceftazidime monotherapies was slightly higher for ceftazidime 20 mg/L (-2.84 $\log_{10}$cfu/mL) and 40 mg/L (-3.05), but there were no differences against HUB8. Ceftazidime-resistant colonies emerged with 4 mg/L regimens in both strains and with other regimens in PAO1. Colistin monotherapy had significant anti-biofilm activity against HUB8 (-3.07), but lower against PAO1 (-1.12), and colistin-resistant strains emerged. Combinations of ceftazidime-colistin at 54 h increased the killing compared to each monotherapy and prevented resistance emergence to both antibiotics; a higher killing was observed with ceftazidime 40 than 4 mg/L combinations (-4.19 vs -3.10 PAO1; -4.71 vs -3.44 HUB8).

Conclusions: This study demonstrated that, with %T>MIC=100%, CI ceftazidime displayed a concentration-dependent killing against *P. aeruginosa* biofilm, especially with colistin combination. Our results support using high-dosage regimens of CI ceftazidime with colistin against biofilm-associated infections by ceftazidime-susceptible *P. aeruginosa*.

Introduction

The antimicrobial treatment of foreign-body infections, including those involving orthopaedic devices, is particularly challenging due to the presence of bacterial biofilms.[1] The tolerance to antibiotics in biofilm-embedded bacteria limits the efficacy of these agents against biofilm-related infections.[2,3] Gram-negative bacilli are the second most frequent aetiology of orthopaedic device-associated infections and their incidence has increased in recent years;[4] in particular, *Pseudomonas aeruginosa* is a major pathogen often involved in such infections. Fluoroquinolones are the first-line antimicrobials for foreign-body infections by *P. aeruginosa*;[5] however, resistance is rapidly emerging.[4] Although the efficacy of beta-lactams in foreign-body infections has been questioned, they are often the main alternatives when fluoroquinolones are not available.

Since the efficacy of beta-lactams depends on the percentage of time that concentrations are above MIC (%T>MIC),[6] their use in continuous infusion (CI) has been proposed in order to optimize their pharmacokinetics/pharmacodynamics (PK/PD).[7,8] The use of CI beta-lactams can achieve %T>MIC of 100%, which may be beneficial against foreign-body infections. Moreover, maximum killing rates in *in vivo* studies are obtained with beta-lactam concentrations at 3-4 times the MIC (3-4xMIC).[7] However, our current knowledge of beta-lactam PK/PD is based on studies performed with planktonic bacteria, which may not necessarily reflect what occurs with biofilm-embedded bacteria. For instance, it is possible that beta-lactams may show concentration-dependent killing against *P. aeruginosa* biofilm for a certain concentration range, which might be related with drug diffusion through biofilms. Understanding the PK/PD characteristics of beta-lactams in biofilm-associated infections is crucial for optimising their use against foreign-body infections by *P. aeruginosa*, in order to obtain maximum killing and minimize emergence of resistance.

Recently, colistin has emerged as a last-line therapy for foreign-body infections by *P. aeruginosa*.[9] *In vitro* studies have found that colistin may have specific activity against deeper layers of *P. aeruginosa* biofilms.[10,11] PK/PD studies using *in vitro* dynamic models, such as the CDC Biofilm Reactor, have revealed synergistic effects of colistin combinations with beta-lactams, including carbapenems and cephalosporins, against biofilm infections by MDR and XDR *P. aeruginosa*.[12,13] Such combinations have been suggested as a valid therapeutic alternative for difficult-to-treat bone and joint infections by MDR/XDR *P. aeruginosa*.[14] However, it is unknown whether the addition of colistin can improve the PK/PD of beta-lactams against biofilm-embedded bacteria.

In the present study, we investigated the efficacy of several clinically achievable concentrations of CI ceftazidime, with and without colistin, against a biofilm infection by *P. aeruginosa* using an *in vitro* PK/PD model.

Materials and methods

Bacterial isolates

Two strains of *P. aeruginosa* were used in experiments with the CDC Biofilm Reactor, both of which were susceptible to ceftazidime and colistin, the reference strain PAO1 (American Type Culture Collection, Rockville, MD, USA) and a clinical isolate HUB8 from biofilm-related osteoarticular infections in Hospital Universitari de Bellvitge (Barcelona, Spain).

Static time-kill studies and antibiotics

Minimum inhibitory concentrations (MICs) of ceftazidime and colistin (sulphate) were determined by broth microdilution in cation-adjusted Mueller-Hinton broth (CAMHB; Ca^{2+} at 23.0 mg/L and Mg^{2+} at 12.2 mg/L; Oxoid, Basingstoke, UK). The susceptibility profile to both antibiotics in *P. aeruginosa* was defined according to EUCAST breakpoints.[15] Minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC) were determined in CAMHB using an MBEC™ device (Innovotech Inc., Edmonton, Canada).[16,17] All experiments were performed at least in triplicate. Biofilm forming ability was evaluated for each strain using a reported method.[18] Static time-kill experiments were also conducted to evaluate the antibacterial efficacy of ceftazidime alone (1X, 2X, 4X, 8X, 16X and 32XMIC).[19]

For MIC/MBIC/MBEC determinations and *in vitro* PK/PD studies, colistin (sulphate, BetaPharma, China) and ceftazidime (Sigma-Aldrich, Australia) were used. Colistin was used in the current study as it is the antibacterial entity formed *in vivo* after the administration of its inactive prodrug, sodium colistin methanesulfonate.[20] Stock solutions of colistin and ceftazidime were prepared immediately prior to each experiment

and sterilized by filtration with cellulose acetate syringe filters (Millipore, Bedford, MA, USA).

In vitro pharmacokinetics/pharmacodynamics biofilm model

A CDC Biofilm Reactor (BioSurface Technologies, USA) was used, which consisted of a glass vessel (350 mL) in continuous mixing by a magnetic baffled stir bar. Our protocol consisted of a biofilm conditioning phase, in which the biofilm was formed for 48 h followed by a therapeutic phase.[12,13,21] Briefly, the biofilm conditioning phase started with the bacterial inoculation inside the reactor (initial inoculum of 7 $\log_{10}$cfu/mL), followed by a 24-h batch culture at 37ºC in drug-free 20% cation-adjusted tryptone soy broth (20%-CATSB) (Oxoid, Basingstoke, UK). Then, fresh sterile 20%-CATSB was infused into the model for 24 h using a peristaltic pump (Masterflex, Cole-Parmer, USA) to achieve a bacterial residence time within the reactor shorter than the generation time for the suspended bacteria. Once the biofilm was formed, the therapeutic phase started (time zero, 0h) and fresh medium (20%-CATSB) was pumped at a flow rate (2 mL/min) reproducing the half-life of ceftazidime ($t_{1/2}$ = 2h).

Regimens evaluated were CI ceftazidime at clinically achievable concentrations (4, 10, 20 and 40 mg/L) and CI colistin (3.50 mg/L). For ceftazidime regimens, a bolus dose was injected at 0 h followed by infused medium with ceftazidime at the corresponding concentration. Colistin was pumped into the CDC Biofilm Reactor as a CI at 3.50 mg/L, which mimicked the unbound plasma steady-state concentration observed in certain patients receiving 6-9MU/day colistin methanesulfonate.[22,23] This was achieved by bolus administration at 0 h followed by infused medium with 3.50 mg/L colistin. In all cases, flow rates were calibrated prior to each experiment and monitored throughout to ensure optimal performance of the system. For both strains, the therapeutic regimens evaluated

were monotherapies of ceftazidime and colistin, 4 mg/L or 40 mg/L ceftazidime (in CI) in combination with colistin, and controls (no antibiotic). All the experiments were performed at least in duplicate.

Pharmacokinetic/Pharmacodynamic analysis

Colistin and ceftazidime concentrations were measured by HPLC.[24,25] Samples (1 mL) collected from the model at different timepoints were placed in 1.5-mL microcentrifuge tubes and immediately stored at -80ºC. Samples from medium (free-floating bacteria) and at least three coupons from a rod (biofilm-embedded bacteria) were collected at 0, 6, 24, 30, 48, and 54 h (two extra coupons were collected at 54 h). The removed coupons were processed as previously described;[12,13] biofilm-embedded and planktonic bacteria were serially diluted with sterile saline and 50 µL was spirally plated onto drug-free nutrient agar (NA) (Media Preparation Unit, Monash University, Victoria, Australia) using an automatic spiral plater (WASP, Don Whitley Scientific, West Yorkshire, UK). Serial 10-fold dilutions and spiral plating minimized antibiotic carryover. Colonies were counted after 24 h of incubation at 37ºC and 48 h for the plates with small colonies.

Bacterial counts were expressed as $\log_{10}$cfu/mL. The efficacy of a therapeutic regimen was evaluated against biofilm-embedded and planktonic bacteria ($\Delta\log_{10}$cfu/mL Xh-0h). Treatments were considered to be bactericidal (99.9% kill) when they led to a ≥3 $\log_{10}$cfu/mL reduction compared with the inocula at zero time. Monotherapy or combination regimens with a reduction of ≥1 $\log_{10}$cfu/mL at a specified time were considered active. Synergy was defined as ≥2 $\log_{10}$cfu/mL killing for the combination relative to the more active monotherapy at a specified time; additivity was defined as 1 to 2 $\log_{10}$cfu/mL greater killing for the combination.

Population analysis profiles of colistin and resistance

Baseline heteroresistance to colistin was studied with population analysis profiles (PAPs).[26] In order to evaluate the emergence of ceftazidime and colistin resistance, both biofilm-embedded and planktonic bacterial samples were additionally plated onto NA containing 16 mg/L and 4 mg/L of ceftazidime and colistin, respectively (Media Preparation Unit). Colonies able to grow on plates containing ceftazidime 16 mg/L were then subcultured and MICs were determined by broth microdilution. For all treatment regimens containing colistin, PAPs of biofilm-embedded and planktonic bacteria recovered at the end of experiments were also analysed.

Confocal laser scanning microscopy (CLSM)

Coupons were evaluated by CLSM to confirm biofilm infection (0 h) and treatment activity (54 h). Images of the biofilms stained with LIVE/DEAD BacLight Bacterial Viability Kit (ThermoFisher Scientific, USA) were acquired using a Nikon Eclipse Ti confocal laser scanning microscope (Nikon Instruments Inc., Japan) equipped with a 488-nm argon laser and 561-nm He/Ne laser (Monash Micro Imaging) using a 20x dry objective (0.75 numerical aperture). Different image stacks were acquired with a 3-micron distance between planes and the pinhole size was kept at 1.2 AU. The number of total planes was calculated according to the thickness of each biofilm and different stacks of each coupon were obtained randomly. Selected fields were acquired with an image resolution of 1024x1024 pixels. The images obtained were processed with IMARIS software (Bitplane AG, Switzerland).

Statistical analysis

Data were analysed using Stata 13.1 (Stata Corporation, USA). An analysis of variance

with Tukey´s post hoc test was performed for each treatment regimen to evaluate changes in $\log_{10}$cfu/mL for biofilm-embedded and planktonic bacteria. A p value of ≤ 0.05 was considered statistically significant.

Results

MICs, MBICs, MBECs and biofilm formation of PAO1 and HUB8 are summarized in Table 1. Both strains were strong biofilm formers and had high MBECs of ceftazidime and colistin. Baseline PAPs of both strains revealed heteroresistance to colistin prior to the treatment (Figure 1). Static time-kill assays showed that concentrations of ceftazidime at 4XMIC and above did not result in greater killing (Supplementary Figure 1). In the dynamic CDC Biofilm Reactor experiments, achieved colistin concentration (mean±SD) was 3.03±1.66 mg/L (targeted value 3.5 mg/L) and measured ceftazidime concentrations and ratios of these concentrations to the MIC and MBIC are shown in Table 2. Log changes of biofilm-embedded and free-floating bacteria from the reactor experiments in the presence or absence of antibiotics are illustrated in Figure 2. For planktonic bacterial cells at 54 h, non-bactericidal killing was observed for all the treatments evaluated. The combination of colistin plus 40 mg/L ceftazidime was the most active against PAO1 ($\Delta\log_{10}$cfu/mL=-2.61) and HUB8 ($\Delta\log_{10}$cfu/mL=-2.06).

Colistin monotherapy resulted in rapid initial killing against biofilm-embedded bacterial cells of PAO1, but regrowth was observed after 24 h with mild efficacy at 54 h ($\Delta\log_{10}$cfu/mL=-1.12). Substantial killing by colistin was also observed against biofilm-embedded bacterial cells of HUB8 at 24 h without regrowth, and colistin was the only bactericidal monotherapy ($\Delta\log_{10}$cfu/mL=-3.07). Biofilm-embedded bacteria growing on 4 mg/L colistin plates were observed in all timepoints with colistin monotherapy against both strains; PAPs of bacteria recovered at 54 h after this treatment showed significantly greater proportions of colonies growing at ≥2 mg/L colistin, compared to the baseline (Figure 3).

Ceftazidime monotherapy resulted in >2 $\log_{10}$ reduction at 54 h against biofilm-embedded cells of both strains (Figure 2A). Against PAO1, greater activities were observed with

ceftazidime at both 40 mg/L (Δlog_{10}cfu/mL=-3.05) and 20 mg/L (Δlog_{10}cfu/mL=-2.84). Actually, 20 and 40 mg/L of ceftazidime were significantly more active than 4 and 10 mg/L against PAO1 ($p<0.001$). While against HUB8, no significant differences were observed when comparing the high and low ceftazidime concentrations ($p=0.424$). Well-formed biofilm prior to the treatment was observed with CLSM for both strains. Monotherapies with 4 or 40 mg/L ceftazidime and colistin resulted in the appearance of a mixed staining in green and red fluorescence (Figure 4), showing the presence of live and damaged bacteria within the biofilm structure. Monotherapies with ceftazidime also altered the cell shape, which appeared enlarged. With both strains, resistance to ceftazidime among biofilm-embedded bacteria emerged in the 4 mg/L ceftazidime group at 24-30 h after treatment. Ceftazidime resistance also emerged at 54 h in PAO1 with 10, 20 and 40 mg/L ceftazidime, but not in HUB8. Ceftazidime MICs of resistant clones ranged from 32 to 128 mg/L. Ratios of ceftazidime concentrations to MBIC above 5 protected against the emergence of ceftazidime resistance in HUB8 at 54 h, which was achieved with concentrations higher than 4 mg/L. Against PAO1, ratios of ceftazidime concentrations to MBIC above 5 were not achieved with any concentration evaluated; however, there was a lower percentage of ceftazidime-resistant isolates recovered at 54 h after treatment with 20 and 40 mg/L ceftazidime than 4 and 10 mg/L (Table 2).

The addition of colistin (3.5 mg/L) to 4 or 40 mg/L ceftazidime increased the killing activity of monotherapies at 54 h against biofilm-embedded bacteria of both isolates. The combination of colistin plus 4 mg/L ceftazidime resulted in significantly greater bactericidal activity than 4 mg/L ceftazidime monotherapy against PAO1 (-3.10 vs -2.27; $p<0.001$) and HUB8 (-3.44 vs -2.50; $p<0.001$). This combination was synergistic compared to colistin monotherapy against PAO1 ($p<0.001$), but not significantly more effective against HUB8 ($p=0.344$). The activity of colistin plus 40 mg/L ceftazidime

239 resulted in the highest killing against both strains at 54 h (Δlog_{10}cfu/mL=-4.19 for PAO1;
240 Δlog_{10}cfu/mL=-4.71 for HUB8) ($p<0.001$ in all comparisons). Colistin plus 40 mg/L
241 ceftazidime resulted in >1 log_{10} reduction at 54 h compared to the combination of colistin
242 plus 4 mg/L ceftazidime against both isolates ($p<0.001$). No colistin- or ceftazidime-
243 resistant strains emerged with combinations throughout treatment. Colistin PAPs of
244 recovered colonies at 54 h showed that combinations of colistin with 4 or 40 mg/L
245 ceftazidime resulted in lower heteroresistance, compared to the baseline (Figure 3).
246 CLSM images revealed a greater red fluorescence and more severely damaged bacteria
247 with the combination therapies of ceftazidime and colistin, in particular with 40 mg/L
248 ceftazidime (Figure 4).

Discussion

Biofilm complicates the antimicrobial management of foreign-body associated infections by *P. aeruginosa*.[2] In an era of rapidly increasing antibiotic resistance, it is crucial to optimize treatment strategies to achieve the best possible outcomes in these difficult-to-treat scenarios.[27] Beta-lactams are still one of the most frequently used antibiotics for the treatment of foreign-body associated infections by *P. aeruginosa*, although their anti-biofilm activity has been questioned.[28] As traditional PK/PD principles are mainly based on the killing against planktonic bacterial cells, understanding the PK/PD associated with the anti-biofilm efficacy is key in optimising the treatment for biofilm-related infections. In the present study, using a biofilm PK/PD model with *P. aeruginosa,* we examined several dosage regimens of CI ceftazidime, which optimized the time-dependent activity of beta-lactams by achieving %T>MIC≈100[7] while also evaluated the effect of ceftazidime concentration on the killing. Our findings are of particular interest to clinicians, as we investigated clinically achievable concentrations of both ceftazidime and colistin. Although the CDC Biofilm Reactor is a validated tool for investigating the PK/PD of antibiotics, biofilm structure may be different *in vivo* and host-pathogen interactions were not taken into account in our study. In this study, CI ceftazidime monotherapy at several concentrations was associated with a notable efficacy after 54 h of treatment (killing >2 $\log_{10}$cfu/mL). Our results suggest the importance of administering high concentrations of ceftazidime for longer periods in order to improve its anti-biofilm activity against *P. aeruginosa*. In our study, the %T>MIC during CI ceftazidime was optimized to maximum in all experiments (%T>MIC=100%) and it was evident that higher concentrations of ceftazidime were associated with greater anti-biofilm activity against PAO1. This was not so evident against HUB8, with similar efficacy between ceftazidime regimens.

To our knowledge, no previous *in vitro* studies have evaluated the strategy of CI ceftazidime against *P. aeruginosa* biofilms or have compared its efficacy with that of intermittent infusion. Our findings are consistent with previous experimental studies which showed that the time-dependent killing of beta-lactams on planktonic bacteria was complemented with a dose-dependent killing against biofilms in *P. aeruginosa*.[29-31] Hengzhuang *et al*. used an *in vitro* time-kill study to examine the anti-biofilm effect of imipenem against planktonic and biofilm bacteria with a non-mucoid PAO1 strain and its isogenic mucoid variant strain; imipenem exhibited time-dependent activity against the biofilm of both strains, but higher concentrations for longer treatment periods (above 64 mg/L for at least 6 h) were required against the biofilm, than for planktonic cells.[30] Results from a neutropenic mouse model of biofilm lung infection with *P. aeruginosa* PAO1 further validated their *in vitro* findings, and showed a concentration-dependent killing of colistin against the biofilms and a time-dependent killing by imipenem against biofilm-embedded cells.[31] Interestingly, the AUC/MIC index correlated well with the anti-biofilm efficacy of imipenem against biofilm-embedded cells.[31] The PK/PD of ceftazidime and imipenem was investigated in three different *in vitro* biofilm infection models with *P. aeruginosa* PAO1 and its beta-lactamase overproducing mutant.[29] Similar results regarding PK/PD for imipenem were found; however, a concentration-dependent killing of ceftazidime was observed against the beta-lactamase overproducing mutant, which the authors associated to beta-lactamases potential accumulation within biofilms. Overall, it was proposed that biofilm-related PK/PD parameters (e.g. the time exceeding the MBIC [%T>MBIC], AUC/MBIC or C_{max}/MBIC complementing %T>MIC) can be optimized by the administration of CI beta-lactams for the treatment of biofilm-related infections by *P. aeruginosa*.[29-31]

In the present study, the efficacy of ceftazidime monotherapies at 54 h might have been interfered by the emergence of resistant subpopulations, which were indeed observed in a higher proportion in PAO1 compared to HUB8 and using lower ceftazidime concentrations in both strains (Table 2). Ceftazidime resistance is extensively related to β-lactamase overproduction.[32,33] Tam *et al*. examined the ability of different beta-lactam dosage regimens to suppress the emergence of resistance using a hollow-fibre PK/PD model with wild-type and drug-resistant clinical isolates of *Klebsiella pneumoniae* and *P. aeruginosa* (harbouring extended-spectrum beta-lactamase and AmpC-overexpression, respectively).[34] They evaluated standard clinical doses of ceftazidime, cefepime and meropenem administered every 8 h, and showed that beta-lactam resistance was prevented by ensuring C_{min}/MIC≥3.8. In our present study, although only two *P. aeruginosa* strains (a wild-type and clinical isolate) were examined, we observed minimal resistance with higher ratios of ceftazidime concentration (in CI) to MBIC, which were achieved against HUB8 strain using 10, 20 and 40mg/L ceftazidime. Although more studies are needed, these results may have clear implications for ceftazidime dosing in clinical practice, supporting the use of high doses in CI.

Our results also support previous studies that showed a synergistic effect of the combination of beta-lactams plus colistin against biofilms by *P. aeruginosa*.[12,13] In line with the PK/PD activity of ceftazidime monotherapy, we also noted that the greatest bactericidal efficacy against both strains was achieved with the higher concentrations (e.g. 40 mg/L) of CI ceftazidime plus colistin. Confocal microscopy imaging results also support the synergy of the combination of beta-lactams and colistin against *P. aeruginosa* biofilms. Ceftazidime plus colistin combinations were associated with a greater red fluorescence (damaged cells) across all biofilm layers, in contrast to monotherapies, in which a mix of live and damaged bacteria were generally found. Our study also highlights

the importance of colistin and beta-lactam combinations for minimizing the emergence of colistin resistance (Figure 3), which can have an important impact on the efficacy of the combination. In contrast, colistin monotherapy modified the initial heteroresistance profile of both strains, with higher proportion of colonies able to grow at concentrations of ≥2 mg/L at 54 h compared to baseline, a finding that was prevented with both combinations of ceftazidime plus colistin. Previous PK/PD *in vitro* studies, including *P. aeruginosa* biofilms or infections with planktonic bacteria, have also shown the benefits of colistin plus beta-lactam combination therapy for preventing the emergence of colistin-resistant strains.[12,13,26,35]

The mechanism involved in the synergistic efficacy with the combination of colistin and ceftazidime is not well known. It has been reported that colistin is active against bacteria with low metabolic profiles within the biofilms;[10,11,36,37] in contrast, beta-lactams can show predominant killing against bacteria present in the outer layers of biofilm, as these are more metabolically active.[38,39] We also hypothesized that higher ceftazidime concentrations may result in greater ceftazidime diffusion through the heterogeneous structure of biofilms, where bacterial subpopulations with different metabolic status and antibiotic tolerance might be present.[40] The addition of colistin made the combination more effective by disrupting the biofilm and facilitating the access of beta-lactams to subpopulations within biofilm layers.[10,37] Previous studies with MDR *A. baumannii* in an early logarithmic growth phase revealed time-dependent synergistic killing by colistin - beta-lactam combinations. Colistin initially disorganized the bacterial cell envelope followed by the inhibition of cell wall biosynthesis by beta-lactams via multiple metabolic pathways.[41,42] Thus, the activity of ceftazidime and colistin on different cellular targets and biofilm layers may explain the synergy observed with the combination.

Further studies are warranted to elucidate the spatiotemporal activity of colistin and beta-lactam combinations on *P. aeruginosa* biofilm and the mechanism of their synergy.

In summary, our findings suggest a potential dose-dependent killing of CI ceftazidime against *P. aeruginosa* biofilms; thus, with maximum %T>MIC of ceftazidime, other biofilm-related PK/PD parameters (e.g. AUC/MBIC) should also be examined. Ceftazidime concentrations of 40 mg/L in CI provided greater anti-biofilm benefits in combination with colistin and may minimise the emergence of resistance to both antibiotics in foreign-body associated infections caused by *P. aeruginosa.* These findings provide important PK/PD information for ceftazidime dosing against biofilm-related infections by *P. aeruginosa* in clinical practice, supporting its use in CI at high concentrations and combination with colistin.

Acknowledgements

The support and guidance by staff at Monash Micro Imaging (MMI) of Monash University (Melbourne, Australia), especially Dr Alex Fulcher, is gratefully acknowledged. We are also indebted to Dr Fe Tubau (Department of Microbiology, Hospital Universitari de Bellvitge, Spain) for kindly providing the isolates used in these experiments.

Part of this study was presented at the 29th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, Netherlands, 13-16 April 2019.

Funding

This study was partly supported by a research grant from the Spanish Ministry of Health, Instituto de Salud Carlos III, Spain (PI14/00511). J. G-J was supported by a grant from the Spanish Ministry of Education (FPU14/03124) and by travel grants by the Spanish Ministry of Education (EST17/00672) and Universitat de Barcelona.

Transparency declarations

None to declare.

References

1 Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004; **351**: 1645-54.

2 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999; **284**: 1318-22.

3 Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2004; **2**: 95-108.

4 Benito N, Franco M, Ribera A et al. Time trends in the aetiology of prosthetic joint infections: a multicentre cohort study. *Clin Microbiol Infect* 2016; **22**: 732.e1-8.

5 Rodriguez-Pardo D, Pigrau C, Lora-Tamayo J et al. Gram-negative prosthetic joint infection: outcome of a debridement, antibiotics and implant retention approach. A large multicentre study. *Clin Microbiol Infect* 2014; **20**: O911-9.

6 Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998; **26**: 1-10; quiz 1-2.

7 Mouton JW, Vinks AA. Continuous infusion of beta-lactams. *Curr Opin Crit Care* 2007; **13**: 598-606.

8 Roberts JA, Webb S, Paterson D et al. A systematic review on clinical benefits of continuous administration of beta-lactam antibiotics. *Crit Care Med* 2009; **37**: 2071-8.

9 Li J, Nation RL, Turnidge JD et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 2006; **6**: 589-601.

10 Pamp SJ, Gjermansen M, Johansen HK et al. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. *Mol Microbiol* 2008; **68**: 223-40.

399 **11** Pletzer D, Hancock RE. Antibiofilm Peptides: Potential as Broad-Spectrum
400 Agents. *J Bacteriol* 2016; **198**: 2572-8.
401 **12** Gomez-Junyent J, Benavent E, Sierra Y et al. Efficacy of ceftolozane/tazobactam,
402 alone and in combination with colistin, against multidrug-resistant *Pseudomonas*
403 *aeruginosa* in an *in vitro* biofilm pharmacodynamic model. *Int J Antimicrob Agents* 2019;
404 **53**: 612-9.
405 **13** Lora-Tamayo J, Murillo O, Bergen PJ et al. Activity of colistin combined with
406 doripenem at clinically relevant concentrations against multidrug-resistant *Pseudomonas*
407 *aeruginosa* in an *in vitro* dynamic biofilm model. *J Antimicrob Chemother* 2014; **69**:
408 2434-42.
409 **14** Ribera A, Benavent E, Lora-Tamayo J et al. Osteoarticular infection caused by
410 MDR *Pseudomonas aeruginosa*: the benefits of combination therapy with colistin plus
411 beta-lactams. *J Antimicrob Chemother* 2015; **70**: 3357-65.
412 **15** The European Committee on Antimicrobial Susceptibility Testing. Breakpoint
413 tables for interpretation of MICs and zone diameters, version 9.0. 2019.
414 **16** Ceri H, Olson ME, Stremick C et al. The Calgary Biofilm Device: new technology
415 for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin*
416 *Microbiol* 1999; **37**: 1771-6.
417 **17** Moskowitz SM, Foster JM, Emerson J et al. Clinically feasible biofilm
418 susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic
419 fibrosis. *J Clin Microbiol* 2004; **42**: 1915-22.
420 **18** Stepanovic S, Vukovic D, Hola V et al. Quantification of biofilm in microtiter
421 plates: overview of testing conditions and practical recommendations for assessment of
422 biofilm production by staphylococci. *Apmis* 2007; **115**: 891-9.

423 **19** Abdul Rahim N, Cheah SE, Johnson MD et al. Synergistic killing of NDM-
424 producing MDR *Klebsiella pneumoniae* by two 'old' antibiotics-polymyxin B and
425 chloramphenicol. *J Antimicrob Chemother* 2015; **70**: 2589-97.
426 **20** Bergen PJ, Li J, Rayner CR et al. Colistin methanesulfonate is an inactive prodrug
427 of colistin against Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2006; **50**:
428 1953-8.
429 **21** Goeres DM, Loetterle LR, Hamilton MA et al. Statistical assessment of a
430 laboratory method for growing biofilms. *Microbiology* 2005; **151**: 757-62.
431 **22** Garonzik SM, Li J, Thamlikitkul V et al. Population pharmacokinetics of colistin
432 methanesulfonate and formed colistin in critically ill patients from a multicenter study
433 provide dosing suggestions for various categories of patients. *Antimicrob Agents*
434 *Chemother* 2011; **55**: 3284-94.
435 **23** Plachouras D, Karvanen M, Friberg LE et al. Population pharmacokinetic analysis
436 of colistin methanesulfonate and colistin after intravenous administration in critically ill
437 patients with infections caused by gram-negative bacteria. *Antimicrob Agents Chemother*
438 2009; **53**: 3430-6.
439 **24** Rigo-Bonnin R, Cobo-Sacristan S, Padulles A et al. Measurement of ceftazidime
440 concentration in human plasma by ultra-performance liquid chromatography-tandem
441 mass spectrometry. Application to critically ill patients and patients with osteoarticular
442 infections. *Biomed Chromatogr* 2016; **30**: 410-8.
443 **25** Li J, Milne RW, Nation RL et al. Stability of colistin and colistin
444 methanesulfonate in aqueous media and plasma as determined by high-performance
445 liquid chromatography. *Antimicrob Agents Chemother* 2003; **47**: 1364-70.
446 **26** Bergen PJ, Tsuji BT, Bulitta JB et al. Synergistic killing of multidrug-resistant
447 *Pseudomonas aeruginosa* at multiple inocula by colistin combined with doripenem in an

448 i*n vitro* pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2011;
449 **55**: 5685-95.
450 **27** Boucher HW, Talbot GH, Bradley JS et al. Bad bugs, no drugs: no ESKAPE! An
451 update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**: 1-12.
452 **28** Gilbert P, Brown MR. Biofilms and beta-lactam activity. *J Antimicrob Chemother*
453 1998; **41**: 571-2.
454 **29** Hengzhuang W, Ciofu O, Yang L et al. High beta-lactamase levels change the
455 pharmacodynamics of beta-lactam antibiotics in *Pseudomonas aeruginosa* biofilms.
456 *Antimicrob Agents Chemother* 2013; **57**: 196-204.
457 **30** Hengzhuang W, Wu H, Ciofu O et al. Pharmacokinetics/pharmacodynamics of
458 colistin and imipenem on mucoid and nonmucoid *Pseudomonas aeruginosa* biofilms.
459 *Antimicrob Agents Chemother* 2011; **55**: 4469-74.
460 **31** Hengzhuang W, Wu H, Ciofu O et al. *In vivo*
461 pharmacokinetics/pharmacodynamics of colistin and imipenem in *Pseudomonas*
462 *aeruginosa* biofilm infection. *Antimicrob Agents Chemother* 2012; **56**: 2683-90.
463 **32** Cabot G, Zamorano L, Moya B et al. Evolution of *Pseudomonas aeruginosa*
464 Antimicrobial Resistance and Fitness under Low and High Mutation Rates. *Antimicrob*
465 *Agents Chemother* 2016; **60**: 1767-78.
466 **33** Feng Y, Jonker MJ, Moustakas I et al. Dynamics of Mutations during
467 Development of Resistance by *Pseudomonas aeruginosa* against Five Antibiotics.
468 *Antimicrob Agents Chemother* 2016; **60**: 4229-36.
469 **34** Tam VH, Chang KT, Zhou J et al. Determining beta-lactam exposure threshold to
470 suppress resistance development in Gram-negative bacteria. *J Antimicrob Chemother*
471 2017; **72**: 1421-8.

472 **35** Bergen PJ, Forrest A, Bulitta JB et al. Clinically relevant plasma concentrations
473 of colistin in combination with imipenem enhance pharmacodynamic activity against
474 multidrug-resistant *Pseudomonas aeruginosa* at multiple inocula. *Antimicrob Agents*
475 *Chemother* 2011; **55**: 5134-42.
476 **36** Brochmann RP, Toft A, Ciofu O et al. Bactericidal effect of colistin on planktonic
477 *Pseudomonas aeruginosa* is independent of hydroxyl radical formation. *Int J Antimicrob*
478 *Agents* 2014; **43**: 140-7.
479 **37** Kolpen M, Appeldorff CF, Brandt S et al. Increased bactericidal activity of
480 colistin on *Pseudomonas aeruginosa* biofilms in anaerobic conditions. *Pathog Dis* 2016;
481 **74**: ftv086.
482 **38** Haagensen J, Verotta D, Huang L et al. Spatiotemporal pharmacodynamics of
483 meropenem- and tobramycin-treated *Pseudomonas aeruginosa* biofilms. *J Antimicrob*
484 *Chemother* 2017; **72**: 3357-65.
485 **39** Haagensen JA, Verotta D, Huang L et al. New *in vitro* model to study the effect
486 of human simulated antibiotic concentrations on bacterial biofilms. *Antimicrob Agents*
487 *Chemother* 2015; **59**: 4074-81.
488 **40** Hoiby N, Bjarnsholt T, Givskov M et al. Antibiotic resistance of bacterial
489 biofilms. *Int J Antimicrob Agents* 2010; **35**: 322-32.
490 **41** Han ML, Liu X, Velkov T et al. Comparative Metabolomics Reveals Key
491 Pathways Associated With the Synergistic Killing of Colistin and Sulbactam
492 Combination Against Multidrug-Resistant *Acinetobacter baumannii*. *Front Pharmacol*
493 2019; **10**: 754.
494 **42** Han ML, Liu X, Velkov T et al. Metabolic Analyses Revealed Time-Dependent
495 Synergistic Killing by Colistin and Aztreonam Combination Against Multidrug-Resistant
496 *Acinetobacter baumannii*. *Front Microbiol* 2018; **9**: 2776.

497 **Table 1.** MICs, MBICs, MBECs (mg/L) and biofilm formation assays for the *P. aeruginosa* strains examined in this study.

498

Isolate	MIC		MBIC		MBEC		Biofilm formation	
	Ceftazidime	Colistin	Ceftazidime	Colistin	Ceftazidime	Colistin	Index (OD/ODc)	Category
PAO1	2	1	8	32	>512	>512	5.2	Strong
HUB8	1	1	2	32	>512	128	13.1	Strong

499 Index and category of biofilm formation assays are based on the methodology described by Stepanovic *et al.*[18] MIC: Minimum inhibitory
500 concentration. MBIC: Minimum biofilm inhibitory concentration. MBEC: Minimum biofilm eradication concentration.

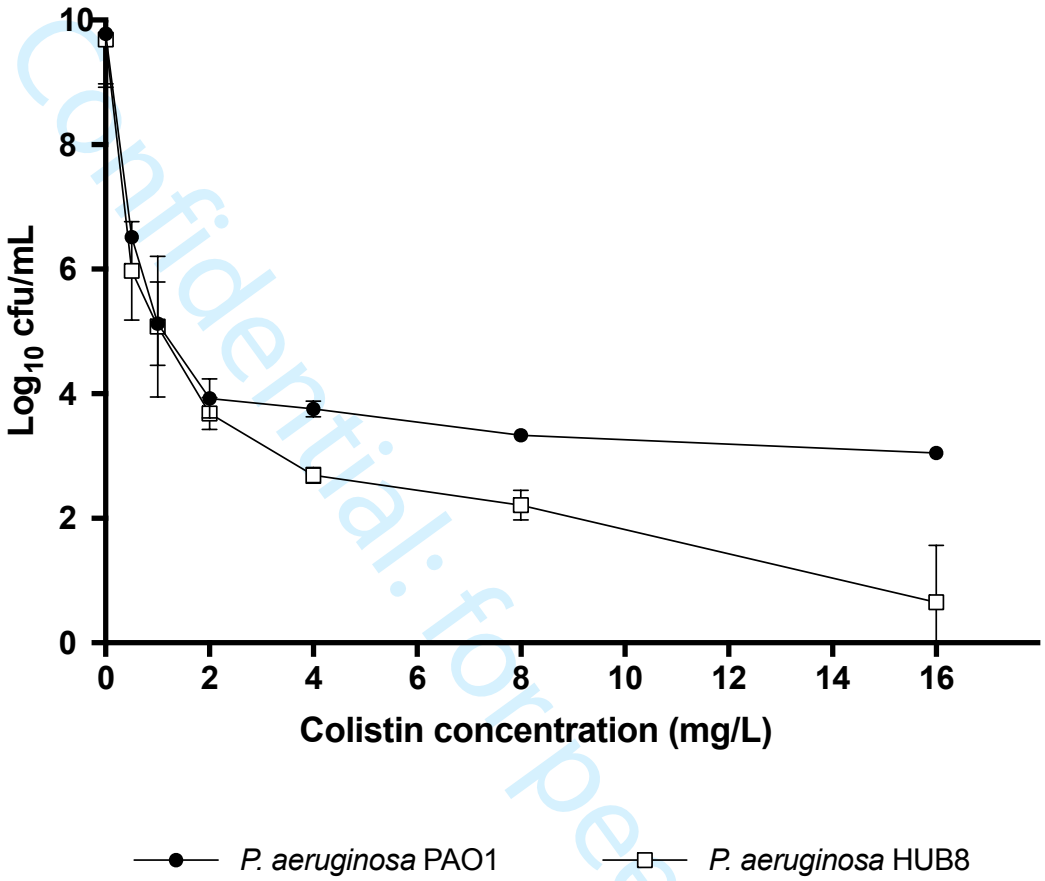
Table 2. Observed mean ceftazidime concentrations and ratios to MIC and MBIC during the CDC Biofilm Reactor experiments and emergence of ceftazidime resistance at 54h.

Therapeutic group	PAO1				HUB8			
	CAZ concentration (mg/L)[1]	CAZ concentration/ MIC	CAZ concentration/ MBIC	CAZ resistance (%)[2,3]	CAZ concentration (mg/L)[1]	CAZ concentration/ MIC	CAZ concentration/ MBIC	CAZ resistance (%)[2,3]
CAZ4	4.55±0.6	2.27	0.57	Yes (66)	4.01±1.1	4.01	2	Yes (40)
CAZ10	9.51±0.5	4.77	1.18	Yes (66)	10.3±2.8	10.3	5.15	No (0)
CAZ20	18.0±3.0	9.01	2.25	Yes (40)	18.84±1.1	18.8	9.42	No (0)
CAZ40	34.5±5.0	17.2	4.31	Yes (26)	35.17±4.8	35.2	17.5	No (0)
CAZ4 + CST	3.33±0.2	1.67	0.42	No (0)	3.26±0.9	3.26	1.63	No (0)
CAZ40 + CST	38.67±4.1	19.3	4.83	No (0)	40.13±3.8	40.1	20.1	No (0)

[1]All measurements are expressed as mean ± standard deviation. [2]Percentage of ceftazidime 16 mg/L containing plates at 54 h showing the presence of *P. aeruginosa* colonies. [3]At 54 h, the proportion of ceftazidime-resistant isolates increased from $1x10^{-3}$ in CAZ20 and CAZ40 experiments to $1x10^{-1}$ – $1x10^{-2}$ in CAZ4 and CAZ10 experiments against PAO1, and was $1x10^{-2}$ – $1x10^{-3}$ in CAZ4 experiments against HUB8.

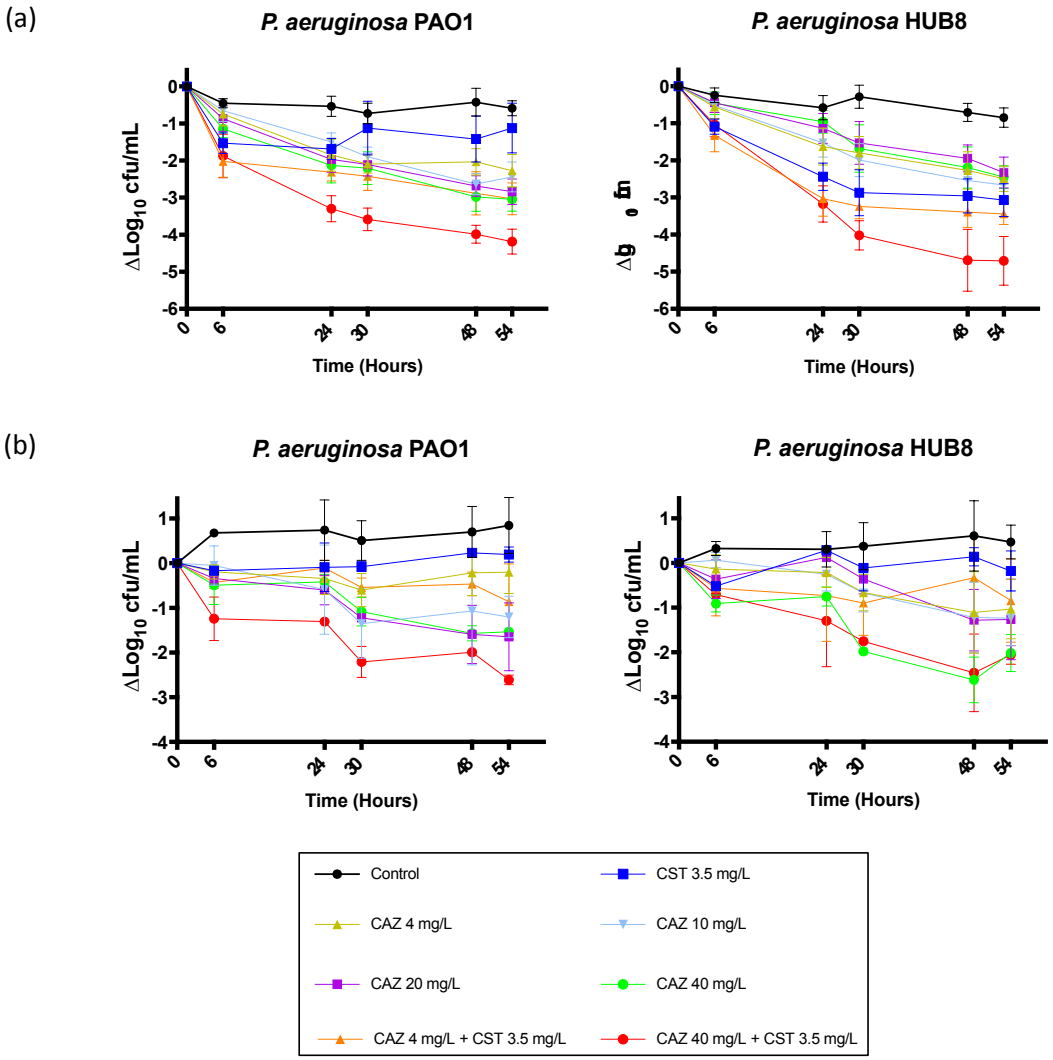
506 MIC: Minimum inhibitory concentration. MBIC: Minimum biofilm inhibitory concentration. CAZ: Ceftazidime. CST: Colistin. CAZ4:
507 Ceftazidime 4 mg/L. CAZ10: Ceftazidime 10 mg/L. CAZ20: Ceftazidime 20 mg/L. CAZ40: Ceftazidime 40 mg/L.
508

509 **Figure 1.** Baseline population analysis profiles of the *P. aeruginosa* strains evaluated in
510 this study. Data are presented as mean±standard deviation (n=3).



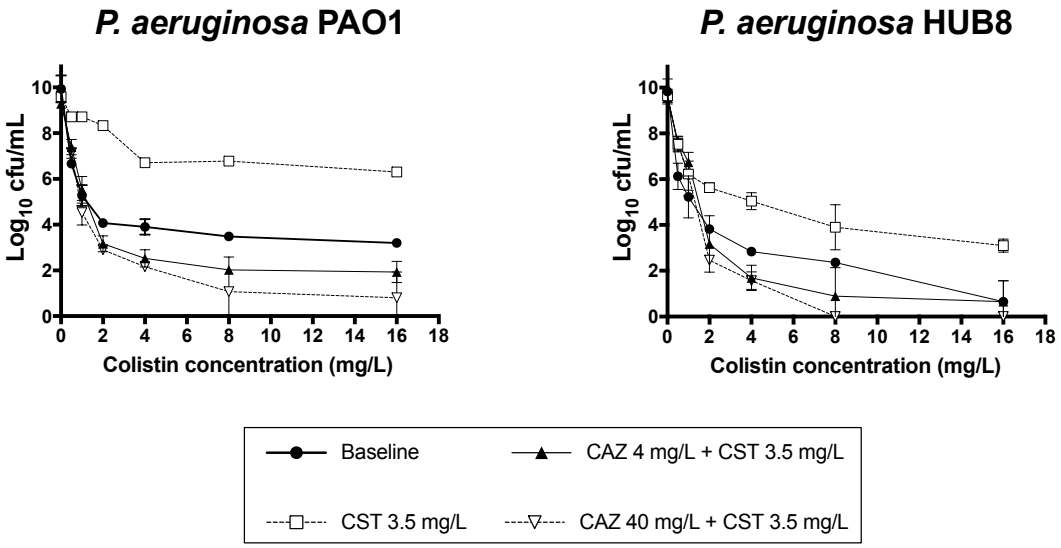
511
512
513

514 **Figure 2.** Bacterial killing by different treatments against biofilm-embedded (a) and
515 planktonic (b) cells of *P. aeruginosa* evaluated with the CDC Biofilm Reactor. Bacterial
516 killing is expressed using the log change. Data are presented as mean ±SD (n=24). CAZ:
517 Ceftazidime. CST: Colistin.



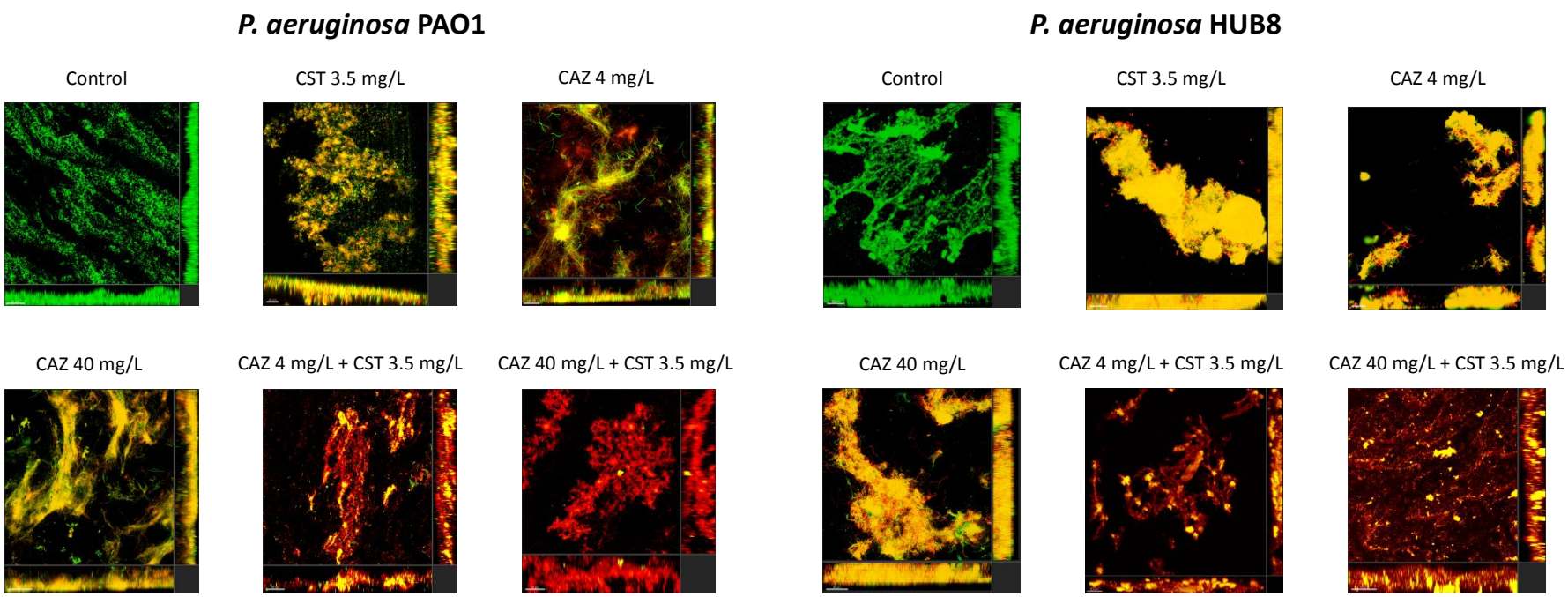
518

519 **Figure 3.** Population analysis profiles of biofilm-embedded bacteria of *P. aeruginosa*
520 evaluated with the CDC Biofilm Reactor after 54 h of treatment. Data are presented as
521 mean ±SD (n=10). CAZ: Ceftazidime. CST: Colistin.
522



523
524
525

Figure 4. Confocal laser scanning microscopy images of biofilm-embedded bacteria of *P. aeruginosa* evaluated with the CDC Biofilm Reactor after 54 hours of treatment. Live bacteria stained with Syto 9 are green, whereas dead bacteria stained with propidium iodide are red; yellow represents a mixture of live and dead cells. Maximum intensity projection of confocal images of total biofilm thickness is represented as central image. Rectangle images below and to the right of the projection correspond to the XZ and YZ planes, respectively. CAZ: Ceftazidime. CST: Colistin.



Supplemental Figure 1. Time-kill curves of *P. aeruginosa* strains evaluated in the CDC Biofilm reactor exposed to different concentrations of ceftazidime. MICs are 2 mg/L for PAO1 and 1 mg/L for HUB8.