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Prevalence of two different genes encoding NorA in 23 clinical strains of *Staphylococcus aureus*

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Sir,

The mechanism of resistance to quinolones in *Staphylococcus aureus* results from the presence of several mutations in genes encoding DNA gyrase and topoisomerase IV or from overexpression of efflux pumps, such as NorA.[1,2] The most important point mutations associated with the acquisition of quinolone resistance are in the *gyrA* and *grlA* genes,[1] which encode the A subunit of DNA gyrase and topoisomerase IV, respectively. NorA plays an important role in the acquisition of resistance to hydrophilic quinolones, such as norfloxacin, but does not affect the MIC of more hydrophobic quinolones.[2] Some mutations have been reported in *norA* and have been associated with increased levels of expression of NorA.[3]

The DNA and the amino acid sequences of NorA have been reported by two groups, first by Yoshida *et al.*[2] (accession number D90119) and later by Kaatz *et al.*[4] (accession number M97169). The DNA sequences determined by these two groups differed by approximately 8.82%, while the amino acid sequence differed by about 4.88%. The majority of the amino acid changes were located in four regions: (i) between amino acids 87 and 93; (ii) between positions 183 and 186; (iii) between 277 and 297; and (iv) at the end of the protein, between positions 385 and 389. The promoter region also showed differences between these two forms of the *norA* gene.[5] The main aim of this study was to design two sets of primers for the specific amplification of each gene and establish the prevalence of these two forms of *norA*.

We amplified these two *norA* sequences with the same set of primers, NorA1 (5′-TTCACCAAGCCATCAAAAAG-3′) as the upper primer and NorA2 (5′-GCACATCAAATAACGCACCT-3′) as the lower primer, and obtained a 705 bp PCR product. To differentiate between these two sequences, two different primers were designed, YonorA (5′-ATATTCAGTTGTTGTCTTAATAT-3′) and KanorA (5′-ATATTCAGTTATTGTATTAGTGC-3′). Both are upper primers, based on the third variable region mentioned above, and used with NorA2 as lower primer to amplify a 230 bp fragment (Figure). The specificity of the PCR products was confirmed by DNA sequencing. We used these two sets of specific primers (YonorA and NorA2 or KanorA and NorA2) in each strain studied and found that in each case only one set could amplify a PCR product (Figure). Twenty-three clinical isolates of *S. aureus* were studied to establish the prevalence of these two *norA* genes. With the first set of primers (NorA1 and NorA2) a product was amplified from all strains, so all had a *norA* gene. However, when the specific primers were used, DNA from 18 strains could be amplified with the YonorA primer and only five could be amplified with the KanorA primer. We did not find any strain that had both genes. Schmitz *et al.*,[5] analysed the DNA sequence of the *norA* promoters in 42 strains of *S. aureus* and found two types of promoters: most of the strains (39) had a promoter identical to that seen by Yoshida *et al.*,[2] while the other three strains had a promoter similar to that reported by Kaatz *et al.*[4] This study is in accordance with our results.

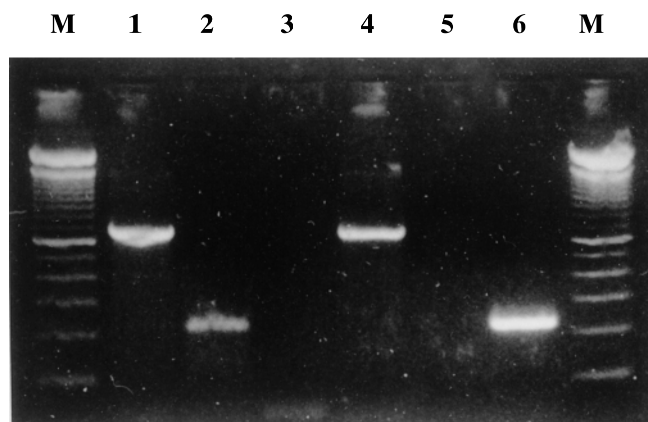

Figure. Amplification of the *norA* gene in two strains carrying different *norA* sequences: strain 4-32 (like that of Yoshida *et al.*[2]) and strain 4-2 (like that of Kaatz *et al.*[4]). Lane M, molecular weight marker (100 bp; Gibco-BRL, Gaithersburg, MD, USA); lanes 1, 2 and 3, strain 4-2; lanes 4, 5 and 6, strain 4-32. Lanes 1 and 4 were amplified with NorA1 and NorA2 primers, lanes 2 and 5 were amplified with KanorA and NorA2 primers, and lanes 3 and 6 were amplified with YonorA and NorA2 primers.

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Other efflux pumps in *S. aureus*, such as QacA and QacB, which differ only in seven amino acids,[6] with different affinity for the same substrate have been found.[6] A similar scenario may be expected with these two forms of NorA. Further studies are needed to establish whether these two sequences are two different genes or alleles and their involvement in the development of quinolone resistance.

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