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DNA Gyrase *gyrA* Mutations in Quinolone-Resistant Clinical Isolates of *Staphylococcus haemolyticus*

Staphylococci are significant human pathogens. Coagulase-positive *Staphylococcus aureus* causes a variety of infections. Recently, coagulase-negative staphylococci have been recognized as important pathogens. Fluoroquinolones have been widely used to treat staphylococcal infections. However, quinolone-resistant strains have been clinically isolated among *S. aureus* and *Staphylococcus epidermidis.*

One of the mechanisms of resistance to quinolones is an alteration of DNA gyrase. DNA gyrase contains the two subunits of GyrA and two subunits of GyrB encoded by the *gyrA* and *gyrB* genes, respectively. DNA gyrase catalyzes ATP-dependent supercoiling of DNA and is a target of the quinolones (3). Ser-84→Leu, Ser-84→Ala, Ser-85→Pro, Glu-88→Gly, and Glu-88→Lys changes were identified in *S. aureus* GyrA (1) and a Ser-84→Phe mutation was observed in *S. epidermidis* GyrA (2). These mutations fall within a small area of the N-terminal portion of the GyrA protein. Therefore, this region is designated the quinolone resistance-determining region (QRDR) of the *gyrA* gene (4).

Studies of quinolone resistance in the coagulase-negative staphylococci, except for *S. epidermidis*, are not advanced. In this study, we cloned and sequenced the QRDR of the *gyrA* genes from clinical isolates of coagulase-negative *Staphylococcus haemolyticus* and identified the mutation in quinolone-resistant strains.

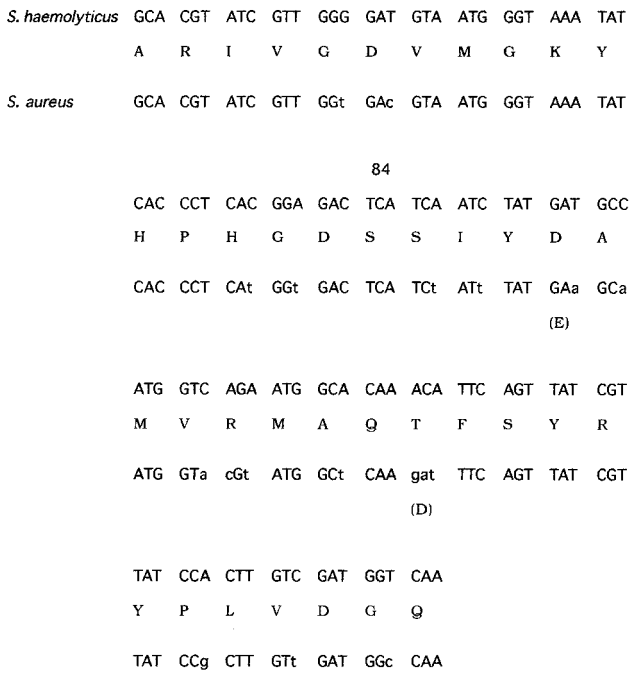

FIG. 1. Nucleotide sequence of the QRDR of *gyrA* and the deduced amino acid sequence from *S. haemolyticus* 11068 and *S. aureus.* Nucleotide differences are shown in lowercase type, and amino acid changes are indicated in parentheses. Ser-84 is shown according to the *S. aureus* coordinates.

Twenty-seven *S. haemolyticus* strains were clinically isolated from urine samples between 1991 and 1994. Among these strains, 15 were quinolone resistant (MIC of ciprofloxacin, ≧6.25 μg/ml). Chromosomal DNAs were prepared from quinolone-susceptible and -resistant *S. haemolyticus* strains, and PCR was performed to amplify the *gyrA* fragment, including the QRDR, with two nucleotide primers, 5′TTAAATGAACA AGGTATGAC3′ and 5′GCCATACCTACCGCGATACC3′, which were identical in sequence to nucleotide positions 157 to 176 or complementary in sequence to positions 520 to 539 of the *S. aureus gyrA* gene, respectively. PCR products were cloned in pT7Blue T-vector (Novagen, Madison, Wis.) and sequenced by the dideoxy chain termination method. The sequence of the QRDR in quinolone-susceptible *S. haemolyticus* 11068 (nucleotide sequence accession number D78568) showed 85% identity with that of *S. aureus.* Consequently, the deduced amino acid sequence of this region in *S. haemolyticus* differed at 2 positions from that in *S. aureus. S. aureus* had Glu and Asp residues at the NH_2-terminal 88th and 96th positions in GyrA, whereas *S. haemolyticus* had Asp and Thr at those positions, respectively (Fig. 1).

Table 1 shows MIC testing of the five clinical isolates. Four quinolone-resistant strains exhibited a fivefold or greater increase in the MICs of fluoroquinolones compared with those for susceptible isolates. The sequence of the QRDR in these four quinolone-resistant *S. haemolyticus gyrA* fragments differed at only 1 nucleotide position from that of the quinolone-susceptible strain 11068. The strains carried a C→T transition at the position corresponding to position 251 in *S. aureus gyrA*, resulting in a Ser-84→Leu mutation in GyrA. These results indicate that quinolone resistance in both *S. haemolyticus* and *S. aureus* is commonly associated with the Ser-84→Leu mutation in GyrA. Until now, this type of change has been most frequently found in *E. coli* and *S. aureus* (1). Changes in the amino acid at the equivalent position were also observed in other quinolone-resistant species. Our results add to the accumulating evidence that a change of Ser-84 in GyrA may be responsible in part of quinolone resistance.

TABLE 1. MICs of quinolones for five clinical isolates of *S. haemolyticus*

Strain no.	MIC (μg/ml)[a]			
	TFLX	NFLX	CPFX	OFLX
11068	0.05	0.39	0.10	0.20
11092[b]	3.13	25	6.25	6.25
11544[b]	12.5	>100	50	25
11549[b]	12.5	>100	50	25
11579[b]	12.5	>100	100	50

[a] TFLX, tosufloxacin; NFLX, norfloxacin; CPFX, ciprofloxacin, OFLX, ofloxacin.
[b] All showed the Ser-84→Leu change in GyrA.

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