In Vitro Activities of Six Quinolones and Mechanisms of Resistance in *Staphylococcus aureus* and Coagulase-Negative Staphylococci

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Of 94 clinical isolates of *Staphylococcus aureus* (n = 51) and coagulase-negative staphylococci (CNS) (n = 43), mutations in the quinolone resistance-determining region of topoisomerases GrlA, GrlB, GyrA, and GyrB together with MICs of six quinolones were analyzed. Amino acid substitutions at identical residues (GrlA residues 80 and 84; GyrA residues 84 and 88) were found in *S. aureus* and CNS. Active eflux, as suggested by blocking by reserpine, contributed substantially to the resistance phenotype in some strains. Among ciprofloxacin, clinafloxacin, levofloxacin, nalidixic acid, trovafloxacin, and sparfloxacin, a 0.5-μg/ml concentration of sparfloxacin discriminated best between strains with two or three mutations and those with no mutations.

Considerable information about the mechanisms of quinolone resistance is available for *Staphylococcus aureus* (2, 5, 7, 12, 16, 17, 20, 23); however, less is known for *Staphylococcus epidermidis* (9, 19) and other coagulase-negative staphylococci (*Staphylococcus* (12, 16, 17, 20, 23)); however, less is known for *Staphylococcus epidermidis* (9, 19) and other coagulase-negative staphylococci (*Staphylococcus* strains) and to the combinations of ciprofloxacin (CIP)-reserpine (RES) and trovafloxacin (TVA)-RES for these isolates. The MICs were correlated with mutations in the quinolone-resistance determining region (QRDR) of the grlA and gyrA genes (all strains) and grlB and gyrB genes (*S. aureus* and *S. epidermidis*).

All strains (except methicillin-resistant [Met']) *S. aureus* were consecutive isolates collected from individual patients at the Institute for Medical Microbiology, University of Regensburg, between 1995 and 1998 and included 27 methicillin-susceptible (Met') *S. aureus* isolates, 24 (Met') *S. aureus* isolates (each with a unique pattern in pulsed-field gel electrophoresis [24]), 12 Met' *S. epidermidis* isolates, 19 Met' *S. epidermidis* isolates, 8 Met' CNS (1 *Staphylococcus haemolyticus* isolate, 5 *Staphylococcus hominis* isolates, and 2 *Staphylococcus capitis* isolates), and 4 Met' CNS (3 *S. haemolyticus* isolates and 1 *Staphylococcus simulans* isolate). CNS were isolated from normally sterile sites. Isolates were identified by a latex agglutination test (Slidex Staph-Kit; bioMérieux sa, Marcy-l’Étoile, France) and by biochemical reactions (ID 32 STAPH; bioMérieux sa). Antimicrobial agents were provided by the manufacturers: CIP (Bayer AG, Leverkusen, Germany), clinafloxacin (Parke-Davis Pharmaceutical Research, Freiburg, Germany), levofloxacin (Hoechst Marion Roussel, Frankfurt, Germany), sparfloxacin (SPF) (Rhône-Poulenc-Rohrer, Köln, Germany), and TVA (Pfizer, Karlsruhe, Germany). Nalidixic acid was purchased from Sigma (Deisenhofen, Germany) (catalog no. N8878). MICs were determined by the agar dilution method on Mueller-Hinton agar (Oxoid, Wesel, Germany) according to NCCLS guidelines (13). In two determinations the effect of RES (catalog no. R0875; Sigma) on MICs of TVA and CIP was evaluated on Mueller-Hinton agar plates with and without RES (20 μg/ml) and Etest strips (AB BIODISK, Solna, Sweden) and was expressed as the change of dilution steps. If the MIC exceeded the maximum concentration on the strip, double the concentration was arbitrarily used for further calculations. Protocols for the amplification of grlA, grlB, gyrA, and gyrB of *S. aureus* and grlA of CNS as published previously were used (3, 21, 26). Primers and PCR conditions for amplification of grlB, gyrA, and gyrB genes of CNS are listed in Table 1.

<table>
<thead>
<tr>
<th>Target</th>
<th>Oligonucleotide (5' → 3')</th>
<th>Positiona</th>
<th>Tm (°C)</th>
<th>MgCl2 (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>grlA (S. epidermidis, CNS)</td>
<td>GTTTAAGACGTAACAGGGG</td>
<td>2141–2159</td>
<td>49</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>CGCAATGTGACTGTCGAGTTG</td>
<td>2418–2430</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grlB (S. epidermidis)</td>
<td>AAGCGACAAAGCGGGGCTCTG</td>
<td>1526–1549</td>
<td>65</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TTTAAACGCACCTACACCAA</td>
<td>1850–1873</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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* Numbering according to *S. aureus* sequence.

b Annealing temperature.
Three units of Expand High Fidelity Taq polymerase (pre-cast solution; Roche Molecular Biochemicals, Mannheim, Germany) was used for all amplifications. The PCR products were purified with a PCR purification kit (QIAQuick; Qiagen, Hilden, Germany). Complementary strands were sequenced on a 310 DNA sequencer (Perkin-Elmer, Foster City, Calif.) using PCR primers (6 μmol). Sequences were compared with published wild-type sequences of S. aureus (gyrA and gyrB: GenBank accession number M86227; grlA and grlB: GenBank accession number D67075). SPSS 10.0 for Windows was used for calculation of the chi-square and Mann-Whitney U test results. The partial sequences of the grlA gene of S. haemolyticus, S.
TABLE 2. Degree of similarity of nucleotide and amino acid sequences in the QRDRs of GyrA, GyrB, GrlA, and GrlB of staphylococci.

<table>
<thead>
<tr>
<th>Species</th>
<th>Gyra % Similarity to S. epidermidis sequence</th>
<th>GyrB nt</th>
<th>GyrB aa</th>
<th>GrlA nt</th>
<th>GrlA aa</th>
<th>GrlB nt</th>
<th>GrlB aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. haemolyticus</td>
<td>85.8</td>
<td>95</td>
<td>—</td>
<td>90.8</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S. hominis</td>
<td>86.7</td>
<td>95</td>
<td>—</td>
<td>87.5</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S. capitis</td>
<td>90.5</td>
<td>100</td>
<td>—</td>
<td>83.3</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S. simulans</td>
<td>87.5</td>
<td>100</td>
<td>—</td>
<td>89.2</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>S. aureus</td>
<td>83.3</td>
<td>100</td>
<td>83</td>
<td>87.5</td>
<td>97.5</td>
<td>97</td>
<td>97</td>
</tr>
</tbody>
</table>

a nt, nucleotide.  
b aa, amino acid.  
c —, not done.

hominis, S. capitis, and S. simulans and of the gyrA gene of S. hominis, S. capitis, and S. simulans appear in the GenBank nucleotide sequence database under accession numbers AF159150, AF159151, AF159152, AF159153, AF159154, AF159155, and AF159156, respectively. Partial sequences of the grlB and gyrB genes of S. epidermidis are listed under accession numbers AF314403 and AF314404, respectively.

The MICs at which 50 and 90% of S. aureus and CNS strains tested were inhibited were comparable to those found in previous studies (1, 4, 8, 18). The distribution of the MICs for the different groups of staphylococci is shown in Fig. 1. In both S. aureus and CNS, resistance to methicillin and to quinolones was highly correlated (P < 0.0001 [chi-square test]).

In S. aureus, resistance to quinolones was correlated with the number of point mutations in gyrA and gyrB, leading to amino acid changes in residues Ser80 and/or Glu84 of GyrA and Ser84 and/or Glu88 of GyrB. The association of the GrlB432 alteration with resistance is unclear (seen in two strains; MICs of SPX for these strains were 0.06 to 0.25 μg/ml, indicating no apparent effect of the Glu88Asp change in GyrA of S. haemolyticus or S. hominis.

Identical to S. aureus, elevated MICs for CNS were found in strains with amino acid changes in residues Ser80 and/or Asp84 of GrlA and residues Ser84 and Glu88 of GyrA. In GrlA of S. epidermidis only Ser80Phe or Ser80Tyr changes were found by Li et al. (9) and in the present study, while S. hominis and S. haemolyticus had Ser80Val or Ser80Leu amino acid exchanges (21). No mutations in gyrB or grlB in any strain of S. epidermidis were found. In CNS other than S. epidermidis, only the gyrA and gyrB genes were analyzed. Different primers designed for amplification of the grlB and gyrB genes of S. aureus or S. epidermidis, tested under various nonstringent conditions, did not yield any product in S. capitis, S. hominis, S. haemolyticus, and S. simulans.

In strains with identical amino acid changes but different MICs, additional resistance mechanisms may be active. We investigated whether inhibition of efflux pump systems (presumably NorA [11, 14; F. J. Schmitz, B. Hertel, B. Hoffmann, S. Scheuring, J. Verhoef, A. C. Fluit, H. P. Heinz, K. Körner, and M. E. Jones, Letter, J. Antimicrob. Chemother., 42:561–

TABLE 3. Changes in MICs of CIP and TVA for S. aureus and CNS on Mueller-Hinton agar with RES (20 μg/ml)

<table>
<thead>
<tr>
<th>Strains</th>
<th>No.</th>
<th>Change in MIC (dilution steps) of:</th>
<th>CIP</th>
<th>TVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Range</td>
</tr>
<tr>
<td>All</td>
<td>94</td>
<td>1.4</td>
<td>1</td>
<td>4–6</td>
</tr>
<tr>
<td>S. aureus</td>
<td>51</td>
<td>1.9</td>
<td>2</td>
<td>1–4.4</td>
</tr>
<tr>
<td>CIP-S</td>
<td>23</td>
<td>3</td>
<td>2</td>
<td>2–4.4</td>
</tr>
<tr>
<td>CIP-R</td>
<td>28</td>
<td>1.1</td>
<td>1</td>
<td>1–4.4</td>
</tr>
<tr>
<td>CNS</td>
<td>43</td>
<td>0.8</td>
<td>1</td>
<td>4–6</td>
</tr>
<tr>
<td>CIP-S</td>
<td>20</td>
<td>0.9</td>
<td>1</td>
<td>0–3</td>
</tr>
<tr>
<td>CIP-R</td>
<td>23</td>
<td>0.7</td>
<td>1</td>
<td>4–6</td>
</tr>
</tbody>
</table>

a r², linear regression coefficient (chi-square test).  
b P < 0.001 (Mann-Whitney test).

glutamate present in S. aureus. This has also been reported for S. epidermidis by Li et al. (9). In the QRDR of GyrA, S. epidermidis, S. capitis, and S. simulans had amino acid sequences identical to that of S. aureus. Both S. haemolyticus and S. hominis differ from the other CNS at codon 88 in that they have a conservative change from glutamate to aspartate (21). MICs of SPX for these strains were 0.06 to 0.25 μg/ml, indicating no apparent effect of the Glu88Asp change in GyrA of S. haemolyticus or S. hominis.

In strains with identical amino acid changes but different MICs, additional resistance mechanisms may be active. We investigated whether inhibition of efflux pump systems (presumably NorA [11, 14; F. J. Schmitz, B. Hertel, B. Hoffmann, S. Scheuring, J. Verhoef, A. C. Fluit, H. P. Heinz, K. Körner, and M. E. Jones, Letter, J. Antimicrob. Chemother., 42:561–
The results of typing Met’ S. aureus by pulsed-field gel electrophoresis were kindly provided by Michaela Metz. We thank Markus Bollwein and Christine Irtenkauf for excellent technical assistance. Nucleotide sequence determination was performed by Holger Melzl and Josef Köstler.

REFERENCES


