Relationship between Cefamandole and Cefuroxime Activity against Oxacillin-Resistant *Staphylococcus epidermidis* and Oxacillin Resistance Phenotype

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The activity of cefamandole and cefuroxime against oxacillin-resistant *Staphylococcus epidermidis* was studied in vitro to determine whether there was any relationship between oxacillin resistance phenotypes and cephaparin activity. Oxacillin resistance phenotypes were determined by efficiency-of-plating studies on Mueller-Hinton agar containing oxacillin, with and without NaCl, and incubated at 30 and 35°C. On the basis of MIC and MBC determinations, cefamandole was more active than cefuroxime against oxacillin-resistant *S. epidermidis*. Although temperature had minimal effect on the activity of either cefamandole or cefuroxime, NaCl significantly decreased the activity of cefuroxime but not of cefamandole. Neither cephaparin consistently produced ≥99.9% bactericidal activity within 24 h in timed killing-curve studies. No consistent relationship was observed between cefamandole or cefuroxime activity and oxacillin resistance phenotype.

According to current standards for antimicrobial susceptibility tests of the National Committee for Clinical Laboratory Standards, all methicillin-resistant *staphylococci* should be considered resistant to cephaparins (8). In recent studies, however, cefamandole has been shown to be active in vitro against methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (3, 13). Furthermore, when compared with cefazolin for prophylaxis in cardiac surgery patients, both cefamandole (M. R. Petracek, A. B. Kaiser, J. W. Lea, D. S. Kernodle, and A. C. Roach, Clin. Res. 35:21a, 1987) and cefuroxime have been associated with significantly fewer postoperative staphylococcal infections (11). In contrast, cefamandole has not been consistently effective against methicillin-resistant *staphylococci* either in experimental models of infection (6) or in clinical studies (2, 4). These discrepant results may be due to differences in degrees of methicillin resistance among strains of *staphylococci* (7). Recently Hartman and Tomasz identified three phenotypes of methicillin-resistant *S. aureus*: homogeneous, heterogeneous, and thermostable heterogeneous (5). Studies identifying phenotypes of methicillin-resistant *S. epidermidis*, however, have not been done. The objectives of our study were first to determine whether methicillin (oxacillin) resistance phenotypes similar to those identified for methicillin-resistant *S. aureus* exist among oxacillin-resistant *S. epidermidis* and second to determine whether the resistance phenotype relates to the activity of cefamandole and cefuroxime against oxacillin-resistant *S. epidermi-

dis.

MATERIALS AND METHODS

Organisms. Blood culture isolates, identified specifically as *S. epidermidis*, were taken from 40 patients. Each isolate was initially characterized as being oxacillin resistant by disk diffusion susceptibility testing (16).

Antimicrobial agents and susceptibility testing. Oxacillin was obtained from Sigma Chemical Co., St. Louis, Mo.; cefamandole was obtained from Eli Lilly & Co., Indianapolis, Ind.; and cefuroxime was obtained from Glaxo Group Research Ltd., Greenford, England. The antibiotics were diluted in cation-supplemented Mueller-Hinton broth (MHB) (Difco Laboratories, Detroit, Mich.), both with and without 2% NaCl supplementation, and dispensed into microdilution trays, which were then stored frozen at −70°C. Concentrations tested were as follows: oxacillin, 0.25 to 512 μg/ml; and cefamandole and cefuroxime, 0.03 to 256 μg/ml.

Broth microdilution testing was performed by methods published by the National Committee for Clinical Laboratory Standards (8), except that the final inoculum was approximately 5 × 10^6 CFU/ml (5 × 10^2 CFU per well) and incubation temperatures were 30, 35, and 37°C for oxacillin and 30 and 35°C for cefamandole and cefuroxime. MICs were recorded after 24 h of incubation. *S. aureus* ATCC 29213 was used as the control strain for all MIC tests.

MBCs of cefamandole and cefuroxime were determined for all 40 strains at 30 and 35°C, both with and without 2% NaCl, by the method of Pearson et al. (9). A 0.1-ml transfer volume was used, as recommended by Shanholter et al. (10) for microdilution tests, and the ≥99.9% bactericidal endpoint was determined.

EOP. Organisms were grown in MHB at 35°C to mid-logarithmic phase and then adjusted to a density equal to that of a no. 0.5 McFarland standard. Serial 10-fold dilutions in saline and quantitative colony counts were performed. From the dilution yielding 30 to 300 colonies per plate, 0.1 ml was subcultured in duplicate on each of the following: (i) antibiotic-free Mueller-Hinton agar (MHA) with and without 4% NaCl supplementation (control), (ii) MHA containing 4 μg of oxacillin per ml with and without 4% NaCl, and (iii) MHA containing 64 μg of oxacillin per ml with and without 4% NaCl. One of each pair of plates was incubated at 30°C, the other was incubated at 35°C, and colonies were counted at 48, 72, and 96 h. To ensure that survivors were not missed by this method, additional lesser dilutions were tested with

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strains that initially did not grow on MHA containing 64 µg of oxacillin per ml with and without 4% NaCl. Testing different dilutions did not alter the final results. The efficiency of plating (EOP) was calculated as follows: EOP (%) = (number of CFU on oxacillin-containing plates/number of CFU on control plates) × 100. To determine the reproducibility and accuracy of the method and to determine what difference between colony counts was statistically significant, 10 replicate quantitative studies were performed with three strains on MHA under each of the following set of conditions: (i) antibiotic-free MHA with and without 4% NaCl and 30 and 35°C, and (ii) MHA containing 64 µg of oxacillin per ml with and without 4% NaCl at 30 and 35°C. Data were analyzed by using analysis of variance and Duncan’s multiple range test (12).

**Timed killing-curve studies.** Organisms were inoculated into brain heart infusion broth and incubated at 35°C to obtain log-phase growth (2 to 3 h). The turbidity of each culture was adjusted to equal that of a no. 1 McFarland standard and diluted 1:10 to yield a final inoculum of between 10⁶ and 10⁷ CFU/ml. A 1:10 dilution of this inoculum was made in cation-supplemented MH8 containing cefamandole or cefuroxime at 1X, 2X, and 4X the MIC for the strain tested and incubated at 30 and 35°C. One strain was also tested at 16X the cefamandole MIC at 35°C. Quantitative subcultures were performed at 0, 3, 6, and 24 h and inoculated at 30 and 35°C for 48 h. Samples (100 µl) were plated directly for colony counts, as were 100-µl samples of 1:10 dilutions thereof. The lower limit of sensitivity was approximately 10 CFU/ml.

**RESULTS**

The activity of oxacillin against the 40 strains of *S. epidermidis* is shown in Table 1. Incubation at 35°C decreased the activity of oxacillin compared with incubation at either 30 or 37°C. Salt supplementation decreased the activity of oxacillin at all three temperatures.

On the basis of oxacillin MIC results at 30 and 35°C with and without 2% NaCl, five groups of oxacillin resistance were defined: (i) MIC at 30°C/MIC at 35°C ≥ 4 (3 strains), (ii) MIC at 35°C/MIC at 30°C ≥ 4 (6 strains), (iii) high-grade resistance (MIC ≥ 32 µg/ml) at both temperatures (6 strains), (iv) low-grade resistance (MIC = 2 to 4 µg/ml) at both temperatures (6 strains), and (v) MIC with 2% NaCl/MIC without 2% NaCl ≥ 4 at 30 and/or 35°C (17 strains). Two strains did not fit exactly into any one of these five groups.

Within groups 1 through 4, salt decreased the activity of oxacillin against some strains; however, the effect of temperature or the level of resistance appeared to be more important.

From each of the above five groups, three representative strains were selected for EOP study. Statistical analysis of the replicate sampling data showed no significant difference in the variability of the colony counts observed under each of the various conditions tested either within or between isolates. There were, however, significant differences under different conditions for specific organisms (P < 0.05), and overall there was a significant difference between growth and no growth on MHA containing 64 µg of oxacillin per ml (P < 0.001). On the basis of these analyses, the 15 organisms were recategorized as shown in Table 2. Two major phenotype groups were identified: (i) low-grade oxacillin resistant, defined as no growth on MHA containing 64 µg of oxacillin per ml (5 strains), and (ii) high-grade oxacillin resistant, defined as growth on MHA containing 64 µg of oxacillin per ml (10 strains). Within each of these two major groups, organisms were further characterized according to the influence of temperature of incubation and salt supplementation of MHA. EOPs of temperature-stable strains did not differ significantly at 30 and 35°C, whereas EOPs of temperature-sensitive strains at 30°C were either higher (three low-grade oxacillin-resistant strains) or lower (one low-grade and two high-grade oxacillin-resistant strains) than their respective EOPs at 35°C. Salt supplementation (4%) enhanced growth (two strains), inhibited growth (six strains), had a variable effect at different temperatures and/or different concentrations of oxacillin (six strains), or had no effect (one strain).

The activities of cefamandole and cefuroxime against the 40 oxacillin-resistant strains of *S. epidermidis* are shown in Table 1. With MIC breakpoints for both antibiotics of ≤8 and ≥32 µg/ml representing susceptibility and resistance, respectively, no strains were resistant to cefamandole. The number of strains resistant to cefuroxime varied from 7 (17.5%) at 30°C without 2% NaCl to 35 (87.5%) at 35°C with 2% NaCl. Incubation at 30°C did not significantly decrease the activity of either antibiotic compared with incubation at 35°C. In fact, cefuroxime appeared more active at the lower temperature. Salt supplementation of MH8 only slightly decreased the activity of cefamandole against oxacillin-resistant *S. epidermidis*, primarily affecting the MBC (Table 3). In contrast, the activity of cefuroxime was significantly decreased in the presence of 2% NaCl, at both 30 and 35°C (Table 3). MBC/MIC ratios for cefamandole and cefuroxime
are shown in Table 4. The majority of cefamandole MBC/MIC ratios were 2 or 4; however, two strains had a ratio of 16 (one at 35°C without 2% NaCl and one at 30°C with and without 2% NaCl). Of the cefuroxime MBC/MIC ratios that could be determined, most were >4 and several were as high as 128 to 256.

MICs and MBCs of cefamandole and cefuroxime were determined at 30 and 35°C with and without 2% NaCl by using colonies from strains growing on MHA containing 64 μg of oxacillin per ml. In this instance, survivors were directly suspended in MHB and the density was adjusted to match that of a no. 0.5 McFarland standard. Overall, the MICs and MBCs of both antibiotics against these highly oxacillin-resistant subpopulations were either unchanged or differed by 1 dilution from those of the parent strains. A greater degree of change was observed under the following conditions: (i) MBCs of cefamandole at 35°C with 2% NaCl, which in two instances were fourfold higher, and (ii) MICs of cefuroxime at 30°C without 2% NaCl, which in three instances were actually lower against the highly oxacillin-resistant subpopulations than against parent strains.

Timed killing rates of cefamandole were determined with one representative strain from each of the five initial groups (based on MICs under the various test conditions) (Fig. 1). Because MICs of cefuroxime for two of these five strains were lower than 0.05 μg/ml, only the time-kill results for cefamandole were included.

Table 4 shows the MBC-to-MIC ratios of 40 isolates of oxacillin-resistant S. epidermidis to cefamandole and cefuroxime at 30 and 35°C with and without 2% NaCl. The majority of MBC/MIC ratios were <4.

**TABLE 2. EOP values for selected strains of oxacillin-resistant S. epidermidis**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Oxacin resistance group</th>
<th>EOP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxacin at 4 μg/ml</td>
<td>Oxacin at 64 μg/ml</td>
</tr>
<tr>
<td></td>
<td>NaCl</td>
<td>NaCl</td>
</tr>
</tbody>
</table>

**TABLE 3. Effect of 2% NaCl on MICs and MBCs of cefamandole and cefuroxime against 40 isolates of oxacillin-resistant S. epidermidis**

| Antibiotic | No. of isolates with: | 0.5 | 1 | 2 | 4 | >4 | 1<sup>#</sup> | 0.5 | 1 | 2 | 4 | >4 | 1<sup>#</sup> |
|------------|------------------------|-----|---|---|---|----|-----|-----|---|---|---|---|----|-----|
| Cefamandole | | | | | | | | | | | | | | | |
| 30°C       | | | | | | | | | | | | | | | |
| 35°C       | | | | | | | | | | | | | | | |
| Cefuroxime | | | | | | | | | | | | | | | |
| 30°C       | | | | | | | | | | | | | | | |
| 35°C       | | | | | | | | | | | | | | | |

<sup>a</sup> MIC-NaCl<sup>-</sup>/MIC-NaCl<sup>-</sup> ratio of<sup>+</sup>
<sup>b</sup> MIC-NaCl<sup>-</sup>/MIC-NaCl<sup>-</sup> ratio of<sup>+</sup>

<sup>1</sup> Ratio indeterminate (MIC and MBC > 256 μg/ml).
<sup>2</sup> For one isolate MIC = 256 μg/ml and MBC > 256 μg/ml.

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**References**

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FIG. 1. Timed killing-curve studies of cefamandole against five isolates of S. epidermidis. Isolate numbers (Table 2) with corresponding cefamandole MICs (without NaCl) at 30 and 35°C, respectively, are: 14948, 2 and 2 \( \mu \)g/ml (A); 16345, 1 and 2 \( \mu \)g/ml (B); 15771, 4 and 4 \( \mu \)g/ml (C); 15652, 1 and 1 \( \mu \)g/ml (D); and 15668, 2 and 1 \( \mu \)g/ml (E).

exceeded 256 \( \mu \)g/ml, timed killing rates were determined for cefuroxime with only three strains (Fig. 2). Cefamandole at a concentration of 4\( \times \) MIC at 30 and 35°C resulted in \( \geq 99.9\% \) killing of three strains within 24 h, as defined by a decrease from the initial inoculum of \( \geq 3 \times \log_{10} \) CFU/ml (Figure 1A to C). Although \( \geq 99.9\% \) killing of the other two strains was not achieved by 4\( \times \) the MIC of cefamandole, \( \geq 99.9\% \) killing of one of these two strains was attained at 16\( \times \) its MIC at 35°C (Figure 1E). Although the MICs and MBCs for two strains (Fig. 1B and E) were unaffected by incubation temperature, incubation for timed killing-curve studies at 30°C decreased the activity of cefamandole against both strains compared with those at 35°C. With cefuroxime, 4\( \times \) MIC achieved \( \geq 99.9\% \) kill of only one strain (Fig. 2B, 30°C). Incubation at 30°C compared with 35°C had variable effects on the activity of cefuroxime: activity was decreased against the strain in Fig. 2A, increased against the strain in Fig. 2B, and unchanged at 24 h against the strain in Fig. 2C. Similar temperature effects on the MICs and MBCs for these strains were not observed. Among the four highly oxacillin-resistant strains tested, \( \geq 99.9\% \) killing of two strains was achieved with cefamandole. Timed killing rates were determined for cefuroxime with two of these highly oxacillin-resistant strains, and \( \geq 99.9\% \) killing did not occur for either strain at 24 h.

DISCUSSION

On the basis of EOP studies, we identified two major phenotypes of oxacillin-resistant S. epidermidis, low-grade and high-grade resistance, suggesting that S. epidermidis does not conform to the resistance phenotypes identified in
methicillin-resistant *S. aureus* by Hartman and Tomasz (5). We did, however, find both temperature-stable and temperature-sensitive strains within each group. Moreover, in contrast to the observations by Hartman and Tomasz regarding methicillin-resistant *S. aureus* (5), we did observe a significant effect of salt supplementation of MHA. For some strains in our study the EOP phenotype correlated with the initial MIC-based grouping, but for other strains there was no correlation. Particularly striking was the influence of salt. In our EOP studies, the growth of several strains of oxacillin-resistant *S. epidermidis* was actually inhibited on MHA containing both oxacillin and 4% NaCl, whereas the MICs for the same strains were either unaffected or increased by 2% NaCl supplementation of MHB. One of the currently recommended methods for detecting methicillin-resistant *S. aureus* is a screening procedure involving the use of MHA containing 6 μg of oxacillin per ml and 4% NaCl (14). Our data indicate that use of this method with isolates of *S. epidermidis* would misidentify some strains as susceptible. We therefore believe that the recommendation to supplement the agar used for the oxacillin screen with 4% NaCl should be evaluated specifically for *S. epidermidis*.

Menzies et al. observed that cephalosporin resistance in any coagulase-negative staphylococcal isolate was dependent on both the proportion of cells resistant to methicillin and the degree of methicillin resistance (7). We observed no consistent correlation between the oxacillin resistance phenotype based on EOP and the activity of the two cephalosporins tested against oxacillin-resistant *S. epidermidis*. Cefamandole appeared more active against oxacillin-resistant *S. epidermidis* than did cefuroxime on the basis of MICs and MBCs. In contrast to data regarding cephalothin (1, 15), incubation at 30°C did not significantly decrease the activity of either cefamandole or cefuroxime against oxacillin-resistant *S. epidermidis*. In fact, the activity of cefuroxime was greater at 30°C than at 35°C. Salt supplementation had minimal effect on the activity of cefamandole against oxacillin-resistant *S. epidermidis*, but significantly decreased the activity of cefuroxime. The clinical relevance of this finding, however, is unknown. Although cefamandole was more active than cefuroxime against oxacillin-resistant *S. epidermidis* on the basis of conventional MIC and MBC test results, cefamandole inconsistently achieved ≥99.9% kill in timed killing-rate studies within 24 h. This suggests that the MBC may not accurately reflect the activity of the antibiotic in certain infections in which high bactericidal activity is required for cure.

**ACKNOWLEDGMENTS**

This study was supported by grants from the Lilly Research Laboratories and Glaxo Inc. We thank Faith Cumberledge for her secretarial assistance, Lynn Atkins for her technical assistance, and Gerald Beck for his help with statistical analysis.

**LITERATURE CITED**


