Comparative In Vitro Activity of Semisynthetic Penicillins Against Proteae

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The in vitro susceptibilities of 181 isolates of precisely identified Proteae species to five semisynthetic penicillins were determined with low and high inocula. Significant differences in susceptibility patterns among various Proteae species to the penicillins examined were demonstrated. Providencia stuartii was clearly distinguished from Providencia alcalifaciens by its greater resistance to the antibiotics tested.

Carbenicillin and its related derivatives, the ureidopenicillins and aminobenzyl penicillins, are reported to have considerable activities against Proteae (1, 3, 6). Unfortunately, in studies of in vitro susceptibility, Proteae species are often split into Proteus, indole-positive Proteus, and Providencia spp. It has been recently shown that such broad groupings conceal major differences among species in susceptibility to aminoglycosides and cephalosporins (10).

In view of the potential use of carbenicillin-like semisynthetic penicillins against Proteae spp., we present here a study of the in vitro susceptibilities of precisely identified isolates from widely differing geographical locations. The minimal inhibitory concentration (MIC) endpoints were determined with a through-the-plate photometer connected to a microcomputer to provide rapid and easy analysis of results.

In this investigation, clinical isolates of Proteae from diagnostic laboratories in the United Kingdom and Canada were fully identified by the criteria suggested by Brenner et al. (2). Proteus penneri (not recognized at the time of that study) was identified by the biochemical tests described by Hickman et al. (5). Laboratory standards of the antibiotics used were obtained as gifts as follows: azlocillin and mezlocillin from Bayer Ltd., Haywards Heath, West Sussex, United Kingdom; ticarcillin and carbenicillin from Beecham Laboratories, Bristol, Tenn.; and piperacillin from Lederle Laboratories, Pearl River, N.Y. MICs were determined by a microdilution method with fully automated reading and data processing as described elsewhere (submitted for publication). Each batch of determinations was controlled by including susceptible control organisms whose MICs are known, i.e., Escherichia coli NCTC 10418 and Pseudomonas aeruginosa NCTC 10662. The medium used was brain heart infusion broth (Oxoid, Basingstoke, England). Two isolates of each Proteae species and the control strains were tested in Mueller-Hinton broth with both high and low inocula to determine the equivalency of results. MICs were within one dilution of those determined with brain heart infusion broth in 87% of the determinations. Tests were performed with two inocula, 10³ and 10⁵ CFU/ml, prepared by dilution of an overnight broth culture (in brain heart infusion broth) estimated by a serial dilution counting technique of those dilutions (9).

The susceptibilities of the Proteae species to the antibiotics carbenicillin, ticarcillin, piperacillin, azlocillin, and mezlocillin, using both high and low inocula, are shown in Table 1. The numbers of each species tested were as follows: Proteus mirabilis, 51; Proteus vulgaris, 19; Proteus penneri, 2; Morganella morganii, 16; Providencia rettgeri, 21; Providencia stuartii, 50; Providencia alcalifaciens, 22. Providencia stuartii generally was strikingly more resistant to the ureidopenicillins (azlocillin and mezlocillin) than to carbenicillin, ticarcillin, or piperacillin, but some strains that were highly resistant to carbenicillin and ticarcillin were susceptible to piperacillin. A similar differential susceptibility was seen with Providencia alcalifaciens, but Providencia stuartii was much more resistant to both piperacillin and azlocillin than was Providencia alcalifaciens. Proteus mirabilis was highly susceptible to all agents, with the exception of 6% of the isolates, which produced β-lactamases. At the lower inoculum, these isolates appeared to be susceptible to the ureidopenicillins and piperacillin, but at the higher inoculum, they were resistant to 256 μg of the antibiotics per ml. Proteus vulgaris had a susceptibility pattern similar to that of Proteus mirabilis, with the exception of a slightly increased resistance to the ureidopenicillins, carbenicillin and ticarcillin, at the higher inoculum. Although only two
isolates of *Proteus penneri* were available for testing, they seemed to be markedly more resistant to all the antibiotics tested than was *Proteus vulgaris*, within which this species has been included until recently (5). *Providencia rettgeri* was very similar to *Proteus mirabilis* in susceptibility pattern, except for a slightly increased resistance to azlocillin seen particularly with the higher inoculum. *M. morganii* had a susceptibility pattern very similar to that of the few *P. penneri* species tested.

Recent recommendations on the taxonomy of the *Proteaeae* have suggested that *Proteus morganii* be placed in its own genus and that *Morganella* and *Proteus rettgeri* be moved to *Providencia* (2). The expansion of the genus *Providencia* to three species (including the rarely differentiated *Providencia alcalifaciens*), the classification of which may be complicated by plasmid-mediated characteristics (4), suggests that the description *Providencia* sp. which is sometimes used in in vitro studies is inadequate. This has been confirmed for the aminoglycosides and cephalosporins by a recent study (10).

### TABLE 1. MICs of five semisynthetic penicillins inhibiting *Proteaeae*

<table>
<thead>
<tr>
<th>Antibiotic and organism tested</th>
<th><em>10&lt;sup&gt;3&lt;/sup&gt;</em></th>
<th><em>50%</em></th>
<th><em>90%</em></th>
<th><em>Range</em></th>
<th><em>10&lt;sup&gt;4&lt;/sup&gt;</em></th>
<th><em>50%</em></th>
<th><em>90%</em></th>
<th><em>Range</em></th>
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<td><strong>Mezlocillin</strong></td>
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<td></td>
<td>1</td>
<td>≤0.5-256</td>
<td>≤0.5</td>
<td></td>
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<td>2</td>
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</table>

* a 50% and 90%, MICs inhibiting 50 and 90% of *Proteaeae* isolates, respectively.
in which Providencia stuartii was found to be typically resistant to gentamicin and tobramycin, whereas Providencia alcalifaciens was susceptible. Providencia stuartii is known to commonly produce aminoglycoside 2′-N-acetyltransferase which is chromosomally mediated (8). The typical resistance pattern reported is accounted for by the substrate profile of 2′-N-acetyltransferase. In addition, marked differences between the susceptibilities of these two species to cephalosporins were reported. Again, Providencia alcalifaciens was the more susceptible of the two species (10). We have found that this difference also extends to all the modified penicillins that were tested in this study.

It has been reported on the basis of results obtained with 18 isolates that indole-positive Proteus and Providencia spp. are less susceptible to the ureidopenicillins than to carbenicillin (7). In this study, Providencia stuartii demonstrated a similar pattern of resistance; however, the closely related Providencia alcalifaciens shows this pattern to only a very slight extent, and the indole-positive Proteus, Proteus vulgaris, not at all. Interestingly, Proteus penneri, although on the basis of a very few isolates, is more resistant to the ureidopenicillins than is P. vulgaris, particularly when a high inoculum is used. This behavior is consistent with the antimicrobial susceptibilities to carbenicillin reported recently (5).

Major differences in susceptibility to carbenicillin-like β-lactam antibiotics among Proteae species have been demonstrated. The relative resistance of Providencia stuartii versus Providencia alcalifaciens described for aminoglycosides and cephalosporins has been confirmed for the ureidopenicillins and, to a lesser degree, the other β-lactam antibiotics reported in this study.

Accurate identification to species level of Proteae isolates in surveys of susceptibilities to antimicrobial agents is essential if potentially misleading results are not to be reported.

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LITERATURE CITED