In Vitro Activities of the Newer β-Lactam and Quinolone Antimicrobial Agents against *Pseudomonas pseudomallei*

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Received 16 March 1988/Accepted 14 June 1988

Imipenem was highly active and bactericidal against all 100 strains of *Pseudomonas pseudomallei* tested, followed in activity by piperacillin, carumonam, ceftazidime, cefotaxime, and ceftriaxone. The addition of clavulanic acid significantly increased the activities of both amoxicillin and ticarcillin. Ciprofloxacin and norfloxacin showed poor activity against test strains.

Tetracycline and its analogs, chloramphenicol, and sulfa-methoxazole-trimethoprim are the antimicrobial agents most commonly used for the treatment of melioidosis, an infectious disease endemic in Southeast Asia and northern Australia (6, 7). Provided that the causative bacterium, *Pseudomonas pseudomallei*, is recovered, identified, and adequately treated early in the illness, the prognosis of patients with chronic or subacute melioidosis is quite good, although recrudescence of infection is not uncommon (2, 7). However, the mortality associated with the acute stage of the infection, even with early, active antibiotic cover, is as high as 75% (6, 9). Consequently, there has long been a need for potent antimicrobial agents against *P. pseudomallei*; previously available agents have been only moderately active in vitro and unavailing in most cases of acute infections. The newer β-lactam and quinolone antibiotic agents, in contrast to those mentioned above, are essentially bactericidal in their activity, and they may therefore have an important role in the treatment of acute or recalcitrant cases of melioidosis (10, 11). However, there is little information concerning the in vitro activities of these newer drugs against *P. pseudomallei*, particularly strains found in Australia.

The aim of this study was to investigate the in vitro activities of various penams (amoxicillin-clavulanic acid [Augmentin; Beecham Pharmaceuticals, Brentford, United Kingdom], azlocillin, piperacillin, and ticarcillin-clavulanic acid [Timentin; Beecham]), cephems (cefotaxime, ceftriaxone, and cefixime), a carbapenem (imipenem), monobactams (aztreonam and carumonam), and quinolones (ciprofloxacin and norfloxacin) against 100 strains of *P. pseudomallei*. All the strains were unique clinical isolates from human patients residing in northern Australia, and all the strains were identified as *P. pseudomallei* by colonial morphology, biochemical reactions, and agglutination with specific antisera (1).

The MICs for the *P. pseudomallei* strains were determined by the microdilution broth technique (8). Briefly, twofold dilutions of antibiotics in Mueller-Hinton broth (BBL Microbiology Systems) were dispensed in sterile U-well microdilution plates. The inoculum, derived from a Mueller-Hinton broth culture in logarithmic phase, was diluted to correspond to a 0.5 McFarland turbidity standard and then was further diluted so that the final concentration of bacteria in each well was approximately 5 × 10^4 CFU. The concentrations of antibiotic after inoculation ranged from 64 to 0.016 μg/ml. Recommended reference strains were used to control the test (8). The MIC was taken as the lowest dilution of antimicrobial agent that showed no turbidity after overnight incubation. The MBCs were determined in a similar manner by using an initial inoculum of 1.5 × 10^6 CFU and by culturing onto nutrient agar 0.01 ml of the broth from wells which showed no turbidity. The MBC was defined as the lowest dilution of antibiotic that completely inhibited growth on nutrient agar after a further overnight incubation.

Table 1 summarizes the inhibitory and bactericidal activities of the 12 antibiotics against the 100 isolates of *P. pseudomallei*. Imipenem showed the highest activity in vitro with an MIC and MBC for 90% of strains of 1 and 2 μg/ml, respectively. The β-lactamase-resistant ureidopenicillins (azlocillin and piperacillin), the cephems, and carumonam were also highly potent in vitro against test strains of *P. pseudomallei*, while aztreonam was found to be variable in its activity, with MICs and MBCs ranging from 2 to 16 and 4 to 32 μg/ml, respectively. In contrast to the newer β-lactam drugs under study, the quinolones, ciprofloxacin and norfloxacin, were considerably less active against *P. pseudomallei*, with MICs and MBCs ranging from 0.5 to 32 and 1 to 32 μg/ml, respectively.

The results of this study are similar to those obtained in previous studies with the agar dilution technique and strains isolated from various regions of Asia (4, 5). Although the methods varied, these studies suggest that there is little difference in patterns of susceptibility to the newer antibiotics among *P. pseudomallei* strains from different geographic areas.

Generally, the difference in MICs and MBCs was a twofold dilution for both the β-lactam antibiotics and the quinolones. Azlocillin, piperacillin, cefotaxime, ceftazidime, ceftriaxone, imipenem, and carumonam were bactericidal in vitro against all 100 strains of *P. pseudomallei* at concentrations readily achievable in the sera of humans. On the basis of their in vitro activities alone, any one of these drugs would be suitable for the empirical or specific treatment of melioidosis; and these antimicrobial agents are therefore candidates for clinical trials of serious infections with *P. pseudomallei*, particularly in view of the success, in isolated cases, of the broad-spectrum cephalosporins in controlling *P. pseudomallei* infection (10, 11). Which drug to use would probably depend more on cost, availability, and pharmacokinetic properties relevant to the clinical situation rather than antimicrobial activity per se.

It was not surprising that all strains of *P. pseudomallei* were found to be resistant to amoxicillin and ticarcillin, since
**TABLE 1. Comparison of MICs and MBCs of various β-lactam and quinolone antibiotic agents for 100 strains of *P. pseudomallei***

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)a</th>
<th>MBC (µg/ml)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td><strong>Penams</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>2&lt;-&gt;64</td>
<td>2</td>
</tr>
<tr>
<td>Azlocillin</td>
<td>2-16</td>
<td>2</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>1-4</td>
<td>1</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Ticarcillin-clavulanic acid</td>
<td>4-32</td>
<td>16</td>
</tr>
<tr>
<td><strong>Cephems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.25-2</td>
<td>0.5</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1-8</td>
<td>2</td>
</tr>
<tr>
<td><strong>Carbenem (imipenem)</strong></td>
<td>2-8</td>
<td>4</td>
</tr>
<tr>
<td><strong>Monobactams</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aztreonam</td>
<td>2-16</td>
<td>4</td>
</tr>
<tr>
<td>Carumomam</td>
<td>1-8</td>
<td>2</td>
</tr>
<tr>
<td><strong>Quinolones</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ciprofloxacin</td>
<td>0.5-16</td>
<td>2</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>1-32</td>
<td>4</td>
</tr>
</tbody>
</table>

a 50% and 90%, MIC for 50 and 90% of strains tested, respectively.

b 50% and 90%, MBC for 50 and 90% of strains tested, respectively.

*P. pseudomallei* is known to be a β-lactamase-producing organism (3). When these drugs were combined with the β-lactamase-inhibitor clavulanic acid, they were considerably active in vitro. Except for three strains which were totally resistant, amoxicillin-clavulanic acid showed inhibitory and bactericidal activities in vitro against all *P. pseudomallei* strains at 4 and 8 µg/ml, respectively. The successful therapeutic management of clinical cases of melioidosis requires a long-term, high-dose regimen which traditionally has involved the oral administration of tetracycline or one of its analogs or sulfamethoxazole-trimethoprim or both (2, 6). Patients should be regularly monitored during treatment because of the problems associated with long-term use of these drugs (2). Unfortunately, no clearly effective, alternative regimen has been defined for patients who are unable to tolerate prolonged treatment or for those who fail to respond to treatment with these antibiotics. The newer β-lactam drugs are available only for parenteral use and are therefore generally unsuitable for long-term therapy. However, amoxicillin-clavulanic acid is available as an oral preparation, and based on clinical experiences it appears that it is a safe drug for oral use. Because of its in vitro bactericidal activity against *P. pseudomallei* at therapeutic concentrations, it may prove useful, and clinical trials are certainly warranted to determine its efficacy for the prolonged oral treatment of melioidosis. There is a major need for critical evaluation of new drugs and drug combinations that may prove effective in vivo against *P. pseudomallei*, particularly for patients with acute and chronic, recalcitrant infections.

**LITERATURE CITED**