In Vitro Activity of Piperacillin against 497 Gram Negative Bacilli Isolated from Blood Cultures

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Abstract

Agar dilution sensitivity tests to piperacillin were performed on 497 strains of gram negative bacilli isolated from blood cultures of patients admitted to Maharaj Nakorn Chiang Mai Hospital. Piperacillin was found to have significant activity against Pseudomonas aeruginosa, Aeromonas spp and Enterobacter spp. A substantial proportion of Escherichia coli, Klebsiella pneumoniae and Acinetobacter anitratus were not inhibited by the antibiotic at achievable concentrations. The resistant strains probably produced beta-lactamases, to which piperacillin is susceptible.

INTRODUCTION

Although much attention has been focused on the new cephalosporin-class antibiotics, there has also been progress made over the last few years in developing new, broad-spectrum, semisynthetic penicillins, namely azlocillin, mezlocillin and piperacillin. Piperacillin has been registered with the Royal Thai Ministry of Public Health and will be marketed in June, 1985. In vitro studies with piperacillin have shown it to have a wide antibacterial spectrum and to be more active against many gram negative organisms than currently available penicillins.1,2 Because it inhibits many Pseudomonas aeruginosa strains in low concentration and because of its useful activity against Klebsiella pneumoniae, piperacillin may be used in therapeutic situations where carbenicillin and ticarcillin are presently used. The object of this study was to assess the future usefulness of this antibiotic in septicemic patients at Maharaj Nakorn Chiang Mai Hospital, and to identify possible limitation to its use.

MATERIALS AND METHODS

Agar dilution sensitivity tests to piperacillin were performed on 497 strains of gram negative bacilli. Identification of the species of these bacilli was...
carried out using standard diagnostic microbiology techniques. Two-hundred-and-one, 132, 29, 18 and 83 strains of Escherichia coli, Klebsiella pneumoniae, Enterobacter spp, Aeromonas spp, Acinetobacter anitratus and Pseudomonas aeruginosa respectively were studied. They were isolated from blood culture of patients admitted to Maharaj Nakorn Chiang Mai Hospital in the period between October 1982 and December 1984. Each strain was the only representative of its species isolated from a given patient. The sensitivity tests were performed using serial 2-fold dilution of piperacillin in Mueller-Hinton agar. Final concentrations of the antibiotic ranged from 1 to 1024 mg/L. Overnight broth cultures of organisms were diluted so that inocula of $10^4$ organisms can be delivered by the tines of the multipoint inoculator used. Twenty-five inocula were used on a 9-cm-diameter plate. Following inoculation, plates were incubated at 35°C for 18 hours before being read by eye. The minimal inhibitory concentration (MIC) was taken as the lowest concentration showing no growth, as compared with an antibiotic-free control plate. Strains of organisms were defined as susceptible if their MIC’s to piperacillin were less than or equal to 128 mg/L. $\text{MIC}_{50}$ and $\text{MIC}_{90}$ were the concentrations of piperacillin that inhibited 50 per cent and 90 per cent of the strains respectively. E. coli ATCC 25922 and Ps. aeruginosa ATCC 27853 were included in every test as control organisms. The MIC of E. coli ATCC 25922 was consistently within one dilution of 1 mg/L. The MIC of Ps. aeruginosa ATCC 27853 was consistently 4 mg/L.

**RESULTS**

Table 1 shows the species (or genus) of 497 isolates of gram negative bacilli tested as well as their susceptible percentage at 128 mg/L of piperacillin, MIC range, $\text{MIC}_{50}$ and $\text{MIC}_{90}$. The susceptible percentage ranged from 43.5 per cent for A. anitratus to 100 per cent for Aeromonas spp. The $\text{MIC}_{50}$ ranged from 1.1 mg/L for Aeromonas spp to 160 mg/L for A. anitratus and the $\text{MIC}_{90}$ ranged from 3.5 mg/L for Aeromonas spp to 736 mg/L for A. anitratus.

<table>
<thead>
<tr>
<th>Of isolates</th>
<th>Susceptible strains (%)</th>
<th>MIC range (mg/L)</th>
<th>$\text{MIC}_{50}$</th>
<th>$\text{MIC}_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>201</td>
<td>62.5</td>
<td>$&lt; 1 - &gt; 1024$</td>
<td>29</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>132</td>
<td>58.1</td>
<td>$2 - &gt; 1024$</td>
<td>6.2</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>34</td>
<td>75.8</td>
<td>$&lt; 1 - &gt; 1024$</td>
<td>13.5</td>
</tr>
<tr>
<td>Aeromonas spp</td>
<td>29</td>
<td>100</td>
<td>$&lt; 1 - 32$</td>
<td>1.1</td>
</tr>
<tr>
<td>A. anitratus</td>
<td>18</td>
<td>43.5</td>
<td>$4 - &gt; 1024$</td>
<td>160</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>83</td>
<td>86.2</td>
<td>$4 - &gt; 1024$</td>
<td>40</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 1 Cumulative frequency (in per cent) of the MIC’s of the 497 strains of gram negative bacilli.
showing cumulative frequency (in per cent) of the MIC's of the 497 isolates tested, broken down by species (or genus) are shown in Figure 1. Figure 2 shows the same graph for the 132 isolates of K. pneumoniae plotted in isolation to more clearly illustrate the biphasic distribution of the MIC's. A few other species (or genus) tested also showed similar biphasic distribution (Figure 1).

**DISCUSSION**

Isolates of gram negative bacilli in this study were significantly less susceptible to piperacillin when compared to values of MIC50 and MIC90 given in the manufacturer's hospital formulary monograph. They were isolated from blood culture of hospitalized patients, compared to the "clinical" isolates referred to in the hospital formulary monograph. The majority of our isolates were from patients with nosocomial infection. When species-matched collections of bacteria were compared, inpatient organisms were found to be significantly more resistant to piperacillin than outpatient organisms. In addition, if those inpatient organisms were nosocomial isolates, they would be more likely to be resistant to piperacillin. The majority of gram negative bacilli resistant to gentamicin (i.e. nosocomial isolates) were found to be resistant to piperacillin in one medical center where the antibiotic had never been used.

The results of this study were comparable to those reported by others. Nosocomial isolates of E. coli were reported to be more frequently resistant to piperacillin. Thirty seven per cent of E. coli isolates were resistant to piperacillin in this study. From 40 to 80 per cent of isolates of Klebsiella spp were found to be susceptible to piperacillin at achievable concentrations. Fifty eight per cent of our K. pneumoniae isolates were susceptible to piperacillin. Piperacillin inhibited approximately 75 per cent of Enterobacter spp and approximately 90 per cent of Ps. aeruginosa isolates, including some strains resistant to other antipseudomonal penicillins. In our study, 75.8 and 86.2 per cent of Enterobacter spp and Ps. aeruginosa respectively were inhibited by piperacillin at achievable concentrations. Piperacillin was found to have no significant advantage over older drugs against A. anitratus. Only 43.5 per cent of A. anitratus isolates were susceptible to piperacillin in this study. On the other hand, piperacillin was found to have excellent activity against Aeromonas spp isolates, while Fainstein et al reported that piperacillin had no appreciable activity against 16 clinical isolates of Aeromonas hydrophila obtained from cancer patients with septicemia.

Our results suggest that piperacillin, because of its relatively greater activity, may eventually replace carbenicillin and ticarcillin for many therapeutic purposes. However, piperacillin is susceptible to the plasmid-mediated TEM betalactamases, which are the most frequently occurring betalactamases among resistant Enterobacteriaceae. Because of the uncontrolled pattern of antibiotic usage in Thailand, resistant strains of gram negative bacilli has already been prevalent. Piperacillin has never been used at Maharaj Nakorn Chiang Mai Hospital, but gram negative bacilli isolated from blood culture of patients.
admitted to the hospital already show considerable resistance to it. The importance of beta-lactamase in determining resistance to piperacillin is suggested by the high MIC90 of certain species compared to its MIC90 found in this study. The discrepancy is caused by the biphasic distribution of the susceptibility of these species to piperacillin. Organisms that do not produce beta-lactamases are highly susceptible to piperacillin and those that produce beta-lactamases require a higher concentration of piperacillin for inhibition. Figure 2 demonstrates this biphasic distribution for the 132 isolates of *K. pneumoniae* tested. Other species or genus showing the same pattern of distribution can also be seen in Figure 1.

Because of its intrinsic activity against *Ps. aeruginosa*, piperacillin may be especially useful against infection due to this organism. However, because of the substantial proportion of resistant strains among the important pathogens, piperacillin may not always be effective as therapy for serious infection of unknown cause or antibiotic susceptibility. In these clinical circumstances, piperacillin should be used in combination with an agent giving reasonable assurance of activity, such as an aminoglycoside or third-generation cephalosporin. It is also important to note that unless a more rational pattern of antibiotic usage is established among the Thai medical community, potentially useful new antibiotics, such as piperacillin will have significant drawback even before they are marketed.

REFERENCES


