In vitro Activities of Penicillin G, Cefotaxime, Fosfomycin, Fusidic Acid and Vancomycin Against Streptococcus pneumoniae

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ABSTRACT

Penicillin G, cefotaxime, fosfomycin, fusidic acid and vancomycin were studied by E-test method for their antibacterial activities against 53 consecutive clinical isolates of Streptococcus pneumoniae in Songklanagarind Hospital, Songkhla, Thailand during 2001 and 2002. Penicillin-sensitive S. pneumoniae (PSSP), penicillin-intermediate S. pneumoniae (PISP) and penicillin-resistant S. pneumoniae (PRSP) accounted for 24.5 percent, 56.6 percent and 18.9 percent, respectively, of all isolates. All isolates were susceptible to vancomycin and fosfomycin but 54.7 percent and 15.1 percent were susceptible to cefotaxime and fusidic acid, respectively. Five of six isolates (83.4%) from cerebrospinal fluid were susceptible to cefotaxime while one isolate was highly resistant. Isolates from other specimens were less susceptible to cefotaxime and fusidic acid. All of 10 PRSP isolates were resistant to fusidic acid and only 1 isolate was susceptible to cefotaxime. In every case of pneumococcal meningitis, penicillin and cefotaxime susceptibilities should be determined for minimal inhibitory concentrations. (J Infect Dis Antimicrob Agents 2004;21:41-6.)

INTRODUCTION

Streptococcus pneumoniae is one of the most important pathogens of respiratory tract and systemic infections. Penicillin has been accepted as the drug of choice for treating pneumococcal infections for a long time.1 Over the past two decades, there has been a dramatic increased incidence of infections caused by pneumococcal strains with resistance to penicillin and other antimicrobial agents including cefotaxime.1-3 This has resulted in poor responses to therapy and increased mortality from pneumococcal infections.4,5 Therefore, it has been necessary to consider alternative agents to penicillin for the treatment of pneumococcal infections particularly meningitis.3-7 The optimal therapy for pneumococcal meningitis is still not known, but cefotaxime or ceftriaxone with or without vancomycin are the recommended choices.5-7 Fosfomycin and fusidic acid have also activities against S. pneumoniae. Fosfomycin is a unique bactericidal antibiotic that exhibits good in vitro and in vivo activities against...
both penicillin-sensitive (PSSP) and penicillin-resistant S. pneumoniae (PRSP), and fusidic acid has activities against a variety of gram-positive cocci.

This study aims to determine the susceptibility of S. pneumoniae, isolated from clinical specimens at Songklanagarind Hospital, Songkla, Thailand to penicillin G, cefotaxime, vancomycin, fosfomycin and fusidic acid.

MATERIALS AND METHODS
Fifty-three consecutive clinical isolates of S. pneumoniae obtained from hospitalized patients in Department of Medicine during 2001 and 2002 were studied. Each specimen was obtained from each patient. Thirty-nine isolates were recovered from sputum, and 14 from sterile sites [6 from cerebrospinal fluid (CSF), 4 from blood and 4 from pleural fluid].

Susceptibility testing
All five antimicrobial agents including penicillin G, cefotaxime, vancomycin, fosfomycin and fusidic acid were tested by Epsilon (E)-test method (AB Biodisk®, Sweden). Quantitative susceptibilities were determined by gradient diffusion methods as described by the manufacturer (AB Biodisk®, Sweden). S. pneumoniae was grown on Mueller-Hinton agar (MHA) supplemented with 5 percent sheep blood. E-test strips were applied to the dry surface of media overlayed by each bacterial isolate. They were incubated at 35°C in 5 percent CO₂ incubator for 20 hours. S. pneumoniae ATCC 49619 was used as the quality control.

Analysis
The minimal inhibitory concentrations (MICs) were used as the end point of determination. These were read at the ellipse intersects on the scale on E-test strips. The range of MICs was reported. The MIC₅₀ and MIC₉₀ were calculated. The percentage of susceptibility was determined using the following breakpoint concentrations: penicillin ≤ 0.06 mg/L and vancomycin ≤ 1 mg/L, according to the current standardized method (the National Committee for Clinical Laboratory Standards, NCCLS 2002). The cefotaxime MIC breakpoint of S. pneumoniae was classified into meningitis and non-meningitis groups with the MICs of ≤ 0.5 and ≤ 1 mg/L, respectively. The MIC breakpoint for susceptibility of fosfomycin is ≤ 64 mg/L and fusidic acid is ≤ 2 mg/L. Penicillin MICs of S. pneumoniae were grouped as: susceptible (PSSP, MIC of ≤ 0.06 mg/L); intermediately resistant (PISP, MIC of > 0.06 and < 2 mg/L) and highly resistant (PRSP, MIC of ≥ 2 mg/L).

RESULTS
The results of MIC determination were summarized in Table 1 and 2. Of all isolates, there were 24.5 percent, 56.6 percent and 18.9 percent of PSSP, PISP and PRSP, respectively. The MIC₅₀ was chosen for comparing the antibacterial activities of the studied drugs because it correlates well with

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Range (mg/L)</th>
<th>MIC₅₀ (mg/L)</th>
<th>MIC₉₀ (mg/L)</th>
<th>Susceptibility No (%)</th>
<th>Susceptibility breakpoint (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>0.06–&gt;32</td>
<td>0.75</td>
<td>2</td>
<td>13/53 (24.5)</td>
<td>≤ 0.06</td>
</tr>
<tr>
<td>Cefotaxime (meningitis)</td>
<td>0.008–4</td>
<td>0.12</td>
<td>0.5</td>
<td>5/6 (83.4)</td>
<td>≤ 0.5</td>
</tr>
<tr>
<td>Cefotaxime (non-meningitis)</td>
<td>0.012–&gt;32</td>
<td>0.25</td>
<td>1.5</td>
<td>24/47 (51.1)</td>
<td>≤ 1</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>1.64–8</td>
<td>8</td>
<td>24</td>
<td>53/53 (100)</td>
<td>≤ 64</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>1.5–6</td>
<td>4</td>
<td>12</td>
<td>8/53 (15.1)</td>
<td>≤ 2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.016–0.5</td>
<td>0.38</td>
<td>0.5</td>
<td>53/53 (100)</td>
<td>≤ 1</td>
</tr>
</tbody>
</table>
Table 2. MIC range of penicillin G, cefotaxime, fosfomycin, fusidic acid and vancomycin for penicillin-sensitive 
*S. pneumoniae* (PSSP); intermediately resistant (PISP) and highly resistant *S. pneumoniae* (PRSP).

<table>
<thead>
<tr>
<th>Antimicrobial Agents</th>
<th>PSSP n=13</th>
<th>PISP n=30</th>
<th>PRSP n=10</th>
<th>Susceptibility breakpoint (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>0.006-0.047</td>
<td>0.125-1.5</td>
<td>2-&gt;32</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>1-24</td>
<td>3-64</td>
<td>8-32</td>
<td>≤64</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>2-12 (S=4)</td>
<td>1.5-16 (S=4)</td>
<td>3-8 (S=0)</td>
<td>≤2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0.016-0.5</td>
<td>0.125-0.5</td>
<td>0.19-0.5</td>
<td>≤1</td>
</tr>
<tr>
<td>Cefotaxime (meningitis, n=6)</td>
<td>0.008-0.023 (n=4)</td>
<td>0.25 (n=1)</td>
<td>4.0 (n=1)</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Cefotaxime (non-meningitis, n=47)</td>
<td>0.012-1 (n=9)</td>
<td>0.094-3 (n=29)</td>
<td>0.5 ≤32 (n=9)</td>
<td>≤1</td>
</tr>
</tbody>
</table>

Note: S=susceptible, n=number

Table 3. The cefotaxime susceptibility of penicillin-sensitive (PSSP), penicillin-intermediate (PISP) and penicillin-resistant *S. pneumoniae* (PRSP) isolated from different clinical specimens.

<table>
<thead>
<tr>
<th>Clinical specimen (n)</th>
<th>PSSP</th>
<th>PISP</th>
<th>PRSP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Cefo-S</td>
<td>n</td>
<td>Cefo-S</td>
</tr>
<tr>
<td>Non–sterile site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum (39)</td>
<td>9</td>
<td>8</td>
<td>22</td>
<td>10</td>
</tr>
</tbody>
</table>

Sterile site

Non-meningitis

| Blood (4)           | 0    | 0     | 3    | 2     | 1     | 0     | 4    | 2 (50) |
| Pleural fluid (4)   | 0    | 0     | 4    | 3     | 0     | 0     | 4    | 3 (75) |
| Total (8)           | 0    | 0     | 7    | 5     | 1     | 0     | 8    | 5 (62.5) |

Meningitis

| CSF (6)             | 4    | 4     | 1    | 1     | 1     | 0     | 6    | 5 (83.4) |
| Total of isolates from sterile site | 4 | 4 | 8 | 6 | 2 | 0 | 14 | 10 (71.4) |

Total (53) | 13 | 12 | 30 | 16 | 10 | 1 | 53 | 29 (54.7) |

Note: PSSP=penicillin-sensitive *S. pneumoniae*, PISP=penicillin-intermediate *S. pneumoniae*, PRSP=penicillin-resistant *S. pneumoniae*, n=number, Cefo-S=cefotaxime-susceptible, CSF=cerebrospinal fluid

an intrinsic activity of the drug, and the results showed vancomycin was the most active agent (Table 1). All isolates were susceptible to vancomycin and fosfomycin. Only 54.7 percent and 15.1 percent of isolates were susceptible to cefotaxime and fusidic acid, respectively. Five of six meningeal isolates were susceptible to cefotaxime and one isolate was PRSP with cefotaxime MIC of 4 mg/L (Table 3). Only 9 of 39 isolates (23.0%) from sputum and 4 (from CSF) of 14 isolates (28.6%) from sterile sites were PSSP. All but 1 of these PSSP isolates were also susceptible to cefotaxime (Table 3). All of 10 PRSP isolates were resistant to fusidic acid and only 1 isolate was susceptible to cefotaxime.
DISCUSSION

The prevalence of *S. pneumoniae* with reduced penicillin susceptibility from many hospitals in Thailand has been shown to be more than 50 percent, and this rate of PRSP has increased rapidly from 0 to 23 percent within 2 years from 1995 to 1997 at Siriraj Hospital.\(^6\) The antibiotic susceptibility data in each hospital is needed for the selection of empirical antibiotic for pneumococcal infections. Study in 13 provinces in Southern Thailand (92 isolates) showed that the prevalence of PSSP, PISP and PRSP were 43.5 percent, 48.9 percent and 7.6 percent, respectively (K. Uranakarn, Master of Pharmacy Thesis in Clinical Pharmacy, Prince of Songkla University, 2002). In our study, the percentage of PSSP, PISP and PRSP were 24.5 percent, 56.6 percent and 18.9 percent, respectively.

In 2002, the NCCLS based on M100-512 document\(^{14}\) recommended a cefotaxime interpretative MIC breakpoint for non-meningeal isolates of *S. pneumoniae* separately from meningeal isolates because it correlates better with the clinical outcome of treatment than the previous recommended MIC breakpoint.\(^{19}\)

The *S. pneumoniae* isolates obtained from various clinical specimens had different susceptibilities to penicillin and cefotaxime. The CSF isolates are great of concern with the susceptibility due to the difficulty in treatment of meningitis. In our study, 4 of 6 CSF isolates were PSSP and 5 isolates (83.4%) were susceptible to cefotaxime. All 8 isolates from blood and pleural fluid were penicillin-nonsusceptible and only 5 (62.5%) were susceptible to cefotaxime. This finding is compatible with Uranakarn’s study which showed that of 10 CSF isolates, there were 7 PSSP and 3 PISP and all isolates were susceptible to cefotaxime. However, ANSORP study reported 16.7 percent of penicillin-nonsusceptible *S. pneumoniae* (PNSP) among isolates from CSF.\(^1\) In the United States, 36 percent of cases, of pneumococcal meningitis were caused by PNSP with PRSP accounting for 14 percent.\(^{20}\) Cefotaxime alone is ineffective in treatment of meningitis due to relatively resistant pneumococci.\(^{21}\) Thus, cefotaxime in combination with vancomycin are recommended for empirical therapy of pneumococcal meningitis in hospitals with high prevalence of *S. pneumoniae* with reduced susceptibility to both penicillin and cephalosporin.\(^7,21\)

Our study demonstrates good activities of vancomycin and fosfomycin against *S. pneumoniae*, regardless of penicillin susceptibility. Vancomycin penetrates the CSF poorly,\(^{22}\) however bactericidal levels (from less than 1 to 7 mg/L) have been demonstrated in the CSF of most but not all patients with meningitis.\(^{22}\) Therapeutic failures have been documented in meningitis, and this problem has led some clinicians to use simultaneous intrathecal administration in the past.\(^{23}\) CSF penetration of vancomycin can be improved with CSF/blood concentration ratio reaching up to 0.48, provided that high dosages are continuously infused and meningeal inflammation is present.\(^{24}\)

Most of our isolates were resistant to fusidic acid. Fusidic acid is a bacteriostatic antibiotic that did not show good activity against pneumococci either alone\(^{12}\) or in combination with levofloxacin.\(^3\)

Fosfomycin is a bactericidal antibiotic with some favorable characteristics including small molecular weight, minimal toxicity, wide antimicrobial spectrum, low plasma protein binding and great CSF penetration.\(^8,25\) Several authors have reported an *in vitro* synergy between cefotaxime or ceftriaxone\(^8,9\) and fosfomycin against PRSP strains. Amoxicillin\(^{10}\) or cefotaxime\(^{11}\) in combination with fosfomycin increased bacterial reduction and delayed the time of regrowth of PRSP in rabbit models. Fosfomycin may be a potentially useful drug for a combination treatment with cefotaxime of severe PRSP infections, particularly in meningitis since the concentration approximates to clinically-achievable levels in blood and CSF.\(^{25}\) In a rabbit model of experimental pneumococcal meningitis, fosfomycin was less active than ceftriaxone and coadministration of both drugs tended to be more active than either each drug alone.\(^{26}\) The combination of
In vitro activities of antibiotics against pneumococci: Chayakul P & Hortiwakul R.

Cefotaxime and fosfomycin has been used in France since 1987 for the empirical treatment of nosocomial meningitis because of their effectiveness against the majority of staphylococci and Enterobacteriaceae encountered.25

In conclusion, our study reports a high prevalence of PNSP. Vancomycin and fosfomycin show good in vitro activities against PSSP and PNSP, while most of these isolates were resistant to fusidic acid. Fosfomycin might be a potential alternative drug to vancomycin in combination with cefotaxime for treatment of pneumococcal meningitis particularly caused by resistant strains. However, the proper clinical trials are still needed.

References


