Antimicrobial Susceptibility of *Streptococcus pneumoniae* Isolated from Patients at Siriraj Hospital

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Abstract

Eighty-five strains of *Streptococcus pneumoniae* isolated from various sites in different patients at Siriraj Hospital from August 1986 to October 1987 were tested by agar dilution method for susceptibility to eight antimicrobial agents: penicillin G, ampicillin, cefuroxime, vancomycin, erythromycin, chloramphenicol, co-trimoxazole and gentamicin. In addition, all strains were tested for beta-lactamase production by chromogenic cephalosporin method. Seven (8.2%) and two (2.4%) strains of tested isolates were relatively resistant and completely resistant to penicillin G respectively. Only one (1.2%) strain was resistant to ampicillin with MIC of 4 mg/l. Five (5.9%) and six (7.1%) strains were relatively resistant to ampicillin and chloramphenicol. All strains were susceptible to cefuroxime, vancomycin, erythromycin and co-trimoxazole. In contrast, gentamicin had poor activity against this organism. All strains showed no beta-lactamase activity.
INTRODUCTION

Streptococcus pneumoniae is one of the leading causes of significant clinical infections, especially community acquired pneumonia, acute otitis media, sinusitis, meningitis, septicemia, and primary peritonitis. Its susceptibility to penicillin was remarkably stable for the long period. However, in 1965-1967 pneumococcal strains that were isolated from Australian natives and other patients had elevated penicillin susceptibility levels. Later, reports of this relative resistance have come from many parts of the world including: New Guinea, Great Britain, Switzerland, Canada, United States, and South Africa. Moreover, since 1977, when Streptococcus pneumoniae resistant to penicillin and chloramphenicol was first reported from Africa, multiple resistant strains, exhibiting resistance to penicillin, chloramphenicol, erythromycin, tetracycline, clindamycin and co-trimoxazole, have occurred worldwide.

In 1985, 10 and 8 per cent of penicillin resistant and moderately resistant pneumococcal isolates were found at Bacteriological laboratory, Siriraj Hospital by modified Kirby-Bauer method using 1 μg oxacillin disc. This high incidence prompted us to determine minimal inhibitory concentration (MIC) to various antimicrobial agents of pneumococci isolated from clinical specimen. Beta-lactamase production of these isolates was also tested.

MATERIALS AND METHODS

Organism: A total of 85 clinical isolates of Streptococcus pneumoniae were obtained from different patients at Siriraj Hospital during August 1986 – October 1987. The sources of isolates included blood (44), sputum (19), cerebro-spinal fluid (4), pus (11), pleural fluid (4) and miscellaneous sources (3). Pneumococci were identified by their colonial morphology on blood agar plates and optochin susceptibility. Staphylococcus aureus ATCC 29213 and Streptococcus faecalis ATCC 29212 were used as control organisms for susceptibility testing. All strains were incubated at 37°C overnight in an atmosphere of 5 per cent CO₂ and 95 per cent room air. MIC was defined as the lowest concentration of antimicrobial agent that would inhibit visible growth disregarding a very fine, barely visible haze, or growth of a single colony. Susceptibility tests were performed in duplicate.

Beta-lactamase determinations

All strains were tested for production of beta-lactamase, using a rapid chromogenic cephalosporin method.

RESULTS

The MIC results are listed in Table 1. Penicillin susceptibilities were defined as follows: susceptible, MIC ≤ 0.06 mg/l; relatively resistant, MIC = 0.1 to 1 mg/l; and resistant, MIC ≥ 2 mg/l. Of the 85 strains tested, two strains (2.4%) were resistant to penicillin which had MICs of 2 mg/l. They were isolated from sputum and pus from eye. Seven strains (8.2%) were relatively resistant to penicillin. All isolates tested were beta-lactamase negative. Cross resistance between penicillin G and ampicillin was observed. One (1.2%) and 5 (5.9%) strains were resistant and relatively resistant to ampicillin, respectively. Most of susceptible isolates showed low penicillin and ampicillin MICs, which were still equal or less than 0.03 mg/l. None of strains tested were resistant to cefuroxime, vancomycin, erythromycin and co-trimoxazole. Six (7.1%) isolates exhibited moderate resistance to chloramphenicol, with MICs 16 mg/l. A total of 68 strains (80%) were resistant to gentamicin (Table 2).

Resistance patterns of pneumococcal isolates in this study were exhibited in Table 3. Thirteen (15.3%) isolates were susceptible to all drugs tested. The number of isolates exhibiting resistance to penicillin or chloramphenicol or penicillin-chloramphenicol-gentamicin or ampicillin-chloramphenicol-gentamicin was 1 for each pattern. Three isolates showed resistance to penicillin-ampicillin-gentamicin.
### Table 1 Susceptibility of 85 pneumococci to eight antimicrobial agents

<table>
<thead>
<tr>
<th>Drug*</th>
<th>≤ 0.03</th>
<th>0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
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<tbody>
<tr>
<td>P</td>
<td>68</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Am</td>
<td>72</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CFX</td>
<td>65</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>66</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>59</td>
<td>26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>31</td>
<td>20</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>11</td>
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<td>3</td>
<td>11</td>
<td>36</td>
<td>32</td>
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</tr>
</tbody>
</table>

*P = Penicillin G; Am = ampicillin; CFX = cefuroxime; V = vancomycin; E = erythromycin; C = chloramphenicol; SXT = co-trimoxazole; G = gentamicin

### Table 2 Percentage of strains susceptible to each antimicrobial agent

<table>
<thead>
<tr>
<th>Agent*</th>
<th>Susceptible</th>
<th>Intermediate resistant</th>
<th>Resistant</th>
</tr>
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<tbody>
<tr>
<td>P</td>
<td>89.4</td>
<td>8.2</td>
<td>2.4</td>
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<tr>
<td>Am</td>
<td>92.9</td>
<td>5.9</td>
<td>1.2</td>
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<tr>
<td>CFX</td>
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<tr>
<td>V</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>92.9</td>
<td>7.1</td>
<td>0</td>
</tr>
<tr>
<td>SXT</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>20</td>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>

*See Table 1 for abbreviation

### Table 3 Resistance patterns of strains studied

<table>
<thead>
<tr>
<th>Resistant to*</th>
<th>NONE</th>
<th>P</th>
<th>C</th>
<th>G</th>
<th>PAm</th>
<th>PG</th>
<th>CG</th>
<th>PAmG</th>
<th>PCG</th>
<th>AmCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of strains</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>58</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Percentage of strains</td>
<td>15.3</td>
<td>1.2</td>
<td>1.2</td>
<td>68.2</td>
<td>2.4</td>
<td>2.4</td>
<td>3.5</td>
<td>3.5</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*See Table 1 for abbreviation.

**DISCUSSION**

Antimicrobial susceptibility testing of *Streptococcus pneumoniae* particularly with isolates from meningitis or bacteremia is now essential to monitor for the presence of resistance to antimicrobial agents such as the penicillins, macrolides, chloramphenicol, co-trimoxazole. It would allow rapid selection of the best secondary antimicrobial for the penicillin allergic patients and for those infections with penicillin resistant pneumococcal strains. The Microbiology Survey of the
College of American Pathologists (CAP) found that 1-
μg oxacillin disk was the best for performing disk
diffusion test to identify relatively resistant and resist­
ant to penicillin of this organism. So, this method
was used for screening penicillin susceptibility of pneu­
ococcal isolates in this study. In addition, agar dilu­
tion method was used for quantitative susceptibility
testing to penicillin and other drugs in order to determine
the present prevalence of resistance and hence to estab­
lish empirical therapeutic guidelines.

Data from epidemiological survey collected by
Handwerger S. and Tomasz A.17 and reports from
others5–6,18 showed that the incidence of penicillin-resis­
tant pneumococci varies greatly from one part of the
world to another ranging from 1-53 per cent. They also
reported that strains isolated from children are more
resistant than those from adults. In contrast, result from
this study showed no significant difference in resistance
between these two age groups. Seven (8.2%) strains
were relatively resistant (MIC 0.25 mg/l) and 2 (2.4%)
strains were resistant (MIC 2 mg/l) to penicillin, respec­
tively. Patients with pneumonia caused by the first
group may respond to conventional therapy with high
dose of penicillin, whereas patients with meningitis
have responded irregularly.18 All isolates tested do not
produce β-lactamase. So, the mechanism of penicillin
resistance in these isolates do not due to the production
of this enzyme. However, it was suggested elsewhere17
that penicillin resistance in pneumococci occurs as a
result of sequential, cumulative alterations in the PBPs
1 and/or 2. So, the selection by penicillin during
treatment and/or prophylaxis may be a major factor in
the upward shift in MICs of this drug for clinical
isolates.

Chloramphenicol resistance was found in 7.1 per
cent of strains tested. This incidence is lower than those
finding in Spain that reported 45 per cent11 and 47.3 per
cent19 incidence of resistance. However, the separation
of susceptible from resistant strains was more reliable
by disk diffusion than by MIC determination.11 The
reason for this is linked to the production of inducible
chloramphenicol acetyltransferase in resistant strains.20,21
The higher MICs of resistant isolates were
obtained after incubation in a subinhibitory concen­
tration of the drug.

All strains tested were susceptible to erythromy­
cin, cefuroxime, co-trimoxazole and vancomycin.
The excellent results obtained with these drugs make them
possible alternatives for the treatment of serious Strepto­
coccus pneumoniae infections. In contrast with gentamycin and all aminoglycoside group, these drugs are
not effective for this organism.

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