Resistance Phenotypes and Genotypes of Erythromycin-Resistant Streptococcus pneumoniae Isolates in Beijing and Shenyang, China

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Of a total of 192 Streptococcus pneumoniae isolates, 149 (77.6%) were not susceptible to erythromycin. Of these 149 isolates, 117 (79.1%) contained the erm(B) gene, 16 (10.8%) contained the mef(A) gene, and 15 (10.1%) harbored both the erm(B) and mef(A) genes.

The burgeoning problem of resistance to antibiotics in Streptococcus pneumoniae has attracted the attention of researchers all over the world. The resistance of S. pneumoniae to macrolides is becoming increasingly severe in China (11, 21). Two principal mechanisms of macrolide resistance have been described. Target modification is mediated by a RNA erythromycin resistance methylase and coded by the erm[erm(B) or erm(TR)] gene (3, 10, 20). These organisms express the macrolides-lincosamides-streptogramin B resistance (MLSb) phenotype and produce cross-resistance to macrolides, lincosamides, and streptogramin B antibiotics. Resistance can be expressed either constitutively (cMLSb phenotype) or inducibly (iMLSb phenotype). The M phenotype involves an active efflux pump, which removes both 14-membered and 15-membered macrolides from the bacterial cell. The determinant was identified to be the mef(A) gene. Isolates showing the M phenotype are susceptible to 16-membered macrolides, lincosamides, and streptogramin B (19). In this study, we observed the prevalence of these phenotypes in S. pneumoniae in two big cities in northern China and correlated it with the presence of the erm and mef genes.

A total of 192 strains of S. pneumoniae isolated from the respiratory tract (multiple isolates from the same patient were avoided) were collected between 2001 and 2002 from patients in the Chinese PLA General Hospital and Beijing Union Medical College Hospital (both located in Beijing, China) and the Second Affiliated Hospital of China Medical University in Shenyang, China (about 700 km from Beijing). MICs for the 192 clinical isolates were determined by the broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (15). S. pneumoniae ATCC 49619 was used as a reference for quality control.

Resistance phenotypes were observed by the triple-disk test by the method of Morosini et al. (14) using disks (Oxoid) containing erythromycin (15 μg), clindamycin (2 μg), and spiramycin (100 μg). After 20 h of incubation at 37°C, the absence of a zone of inhibition around the three disks indicated constitutive resistance (cMLSb phenotype), blunting of the clindamycin or spiramycin zone of inhibition proximal to the erythromycin disk indicated inducible resistance (iMLSb phenotype), and susceptibility to clindamycin and spiramycin with no blunting of the zone of inhibition around the two disks indicated the M phenotype. The presence of erythromycin resistance genes was detected by PCR. Primer pairs specific for the detection of erm(B), mef(A), and erm(TR) (expected PCR product sizes of 639, 348, and 530 bp, respectively) were used by the method of Sutcliffe et al. (18) and Kataja et al. (9). S. pneumoniae ATCC 49619 and five other pneumococcal strains susceptible to erythromycin were used as negative controls in PCR experiments.

Of the 192 isolates investigated, 42.7% were found to be nonsusceptible to penicillin and 77.6% were nonsusceptible to erythromycin. The rates of susceptibility to levofloxacin, gatifloxacin, and moxifloxacin were 91.7, 92.7, and 94.3%, respectively (Table 1).

The resistance phenotypes of 149 pneumococcal strains that were not susceptible to erythromycin (MIC of ≥0.5 μg/ml) were observed by the triple-disk test. In the test, only one strain was susceptible to erythromycin and the other 148 pneumococcal strains displayed resistance phenotypes. The results showed that most isolates (89.2%) expressed the MLSb phenotype (cMLSb [85.1%] and iMLSb [4.1%]). The 148 strains that were not susceptible to erythromycin displayed three re-

TABLE 1. In vitro activities of antimicrobial agents against S. pneumoniae isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>% Isolates</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Penicillin</td>
<td>57.3</td>
<td>31.8</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>22.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>33.3</td>
<td>5.7</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>91.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>92.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>94.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

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* 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.
TABLE 2. Relationship between the presence of macrolide resistance genes and the phenotypes of 148 erythromycin-resistant S. pneumoniae isolates

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%) of phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cMLS&lt;sub&gt;B&lt;/sub&gt;</td>
</tr>
<tr>
<td>+ +</td>
<td>112 (75.7)</td>
</tr>
<tr>
<td>+ +</td>
<td>14 (9.5)</td>
</tr>
<tr>
<td>+ −</td>
<td>16 (10.8)</td>
</tr>
</tbody>
</table>

Total 126 (85.1) 6 (4.1) 16 (10.8) 148 (100.0)

Resistance genotypes: 79.1% had the erm(B) gene, 10.1% carried erm(B) and mef(A) genes simultaneously, and 10.8% carried mef(A) genes (Table 2). No strain had the erm(TR) gene. We selected two samples with positive PCR products for the erm(B) gene and another two samples with positive PCR products for the mef(A) gene for sequence analysis (using an ABI PRISM 377 DNA sequencer). The results revealed that their sequences were identical to the sequences of erm(B) (GenBank accession number AY355404) (22) and mef(A) (GenBank accession number AF376746) (5) genes in the gene bank.

The MICs of erythromycin for erm(B)-positive strains were higher than those for mef(A)-positive strains. The MICs of erythromycin for 74.4% of erm(B)-positive strains were >16 μg/ml, and the MICs of erythromycin for mef(A)-positive strains ranged from 0.5 to 4 μg/ml. The MICs of erythromycin for erm(B)- and mef(A)-positive strains were >16 μg/ml.

Since penicillin-nonsusceptible S. pneumoniae strain was first isolated in 1967 in Australia (6), the prevalence of pneumococcal resistance to antibiotics, especially to penicillin and erythromycin, has been increasing worldwide. In China, the resistance rate of S. pneumoniae to penicillin and erythromycin is great. The resistance rate of new fluoroquinolones is higher than that in other countries or areas except Hong Kong (7), which is possibly a result of selective pressure due to the increased use of quinolones in China.

The prevalence of the MLS<sub>B</sub> and M phenotypes varies geographically. In Italy, Spain, and South Africa, the most prevalent phenotype is the MLS<sub>B</sub> phenotype (12, 13, 16). On the other hand, the M phenotype predominates in the United States and Canada (8, 17). In our study, the erm(B) gene was the most prevalent genotype.

S. pneumoniae is the most common cause of community-acquired pneumonia (2, 4). In the Infectious Disease Society of America guidelines (1), macrolide antibiotics remain a viable first choice for empirical treatment of community-acquired pneumonia in outpatients. Our study shows that in China, the resistance rate of S. pneumoniae to erythromycin is great and that target modification is the main resistance mechanism of erythromycin. Therefore, caution is necessary when macrolides are used empirically in suspected cases of pneumococcal pneumonia.

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REFERENCES