Epidemiology and antibiotic resistance of group A streptococci isolated from healthy schoolchildren in Korea

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Objectives: To assess the epidemiology, antibiotic resistance and genotypic characterization of group A streptococci (GAS) in Jinju, Korea.

Methods: Isolates were characterized in terms of their antibiotic resistance, the phenotypes of erythromycin resistance, the frequencies of \textit{erm}(B), \textit{erm}(A) and \textit{mef}(A) genes, and by \textit{emm} genotyping or M typing. The data were compared with those from 85 GAS strains collected during 1995 in the same area.

Results: A total of 98 (16.9%) of 581 healthy schoolchildren yielded GAS from throat swab culture during 2002. The most frequent \textit{emm} types were \textit{emm}12 (34.4%), followed by \textit{emm}75 (10.4%), \textit{emm}18 (9.4%), \textit{emm}22 (8.3%) and \textit{emm}1 (7.3%) in 2002, whereas M12 (21.2%) and M22 (14.1%) were common in 1995. The resistance rates to erythromycin and clindamycin in 2002 were 51.0% and 33.7%, respectively, compared with 29.4% and 10.1% in 1995. Among the erythromycin-resistant strains, constitutive resistance, inducible resistance, and the M phenotype were observed in 61.2%, 2.0% and 36.7% in 2002, compared with 64.0%, 0% and 36.0% in 1995, respectively, which correlated with the presence of resistance genes. Most of the \textit{emm}12 strains showed constitutive resistance, whereas \textit{emm}18 and \textit{emm}75 showed the M phenotype. The organisms with other \textit{emm} genotypes were susceptible to both erythromycin and clindamycin.

Conclusions: Erythromycin and clindamycin resistance increased markedly during the period 1995–2002 in Korea. Constitutive resistance is more common than the M phenotype, with inducible resistance occurring rarely. The phenotypes of erythromycin resistance seem to be associated with certain \textit{emm} genotypes.

Keywords: \textit{emm} genotype, \textit{erm}(B), \textit{erm}(A), \textit{mef}(A), erythromycin resistance

Introduction

Humans are the natural reservoir for group A streptococci (GAS), which is the most common cause of paediatric bacterial pharyngitis. This organism is also the causative agent of severe life-threatening diseases and non-suppurative sequelae. The prevalence of GAS in the throat is known to be highest in school-aged children, but it varies geographically in different countries and also by year. Therefore, continuous epidemiological surveillance is important in the community.

The \textit{emm} gene encoding the M protein has highly heterogeneous 5'-ends, and \textit{emm} genotyping using PCR followed by automatic sequencing has become an alternative means of M typing in epidemiological studies.

Penicillin is the drug of choice for GAS infection as penicillin resistance has not been encountered. However, macrolides such as erythromycin have been more commonly used for the treatment of GAS infection, especially for those who are allergic to penicillins. Recently, the prevalence of erythromycin resistance has increased in several countries, although it remains low in others. In Korea, increased resistance to erythromycin in GAS has been detected in the last decade, but there are scanty data on the epidemiology, antibiotic resistance and genotypic characterization of isolates. Therefore, we carried out an epidemiological...
investigation to determine emm genotyping or M typing, antibiotic resistance, and the phenotypes and genes of erythromycin resistance in GAS recovered from healthy schoolchildren during 2002 and compared the results with those obtained from a similar survey undertaken in 1995.

Materials and methods

**Bacterial isolates**

Throat cultures were carried out in 581 schoolchildren during 2002, and 476 children during 1995, in Jinju, which is located in the southern part of Korea. Any children with symptoms or signs of upper respiratory tract infection were excluded from the study. The study was approved by the Committee for Clinical Research, Gyeongsang National University Hospital, and informed consent was obtained from each child’s guardian. Each throat swab was inoculated on a blood agar plate with a bacitracin (0.04 U) disc. After 16–18 h of incubation at 35°C, grey-white colonies with β-haemolysis were subjected to latex agglutination testing with the Seroiden Strepto Kit (Eiken Co., Tokyo, Japan). All the isolates identified were frozen at −70°C until they were processed for other tests.

**M typing**

M protein typing was carried out by a serological method at the WHO Collaborating Center for Reference and Research on Streptococci (University of Minnesota, Minneapolis, MN, USA) for the strains isolated in 1995. M type was identified by either double immunodiffusion with anti-M sera or opacity factor inhibition with anti-OF sera.

**emm genotyping**

The DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, USA) was used to isolate bacterial DNA according to the manufacturer’s instructions. PCR was carried out using the AccuPower PCR PreMix Kit (Bioneer, Chungwon, Korea) and emm primers.7 The PCR mixture contained 1 μL (100 pmol) of each primer, 4 μL of DNA solution and 44 μL of distilled water. Samples were amplified for 35 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C, and the PCR products were examined as described earlier.

**Phenotypes and genes of erythromycin resistance**

Susceptibility to erythromycin, clindamycin, tetracycline and ofloxacin was determined by disc diffusion (BBL Sensi-Disc; Becton-Dickinson Microbiology Systems, Cockeysville, MD, USA) as recommended by the NCCLS.8 Discs of erythromycin and clindamycin were inoculated at a distance of 1 cm and incubated at 35°C for 16–18 h. The phenotype of erythromycin resistance was determined as constitutive resistance, the M phenotype, or inducible resistance.

The frequencies of ermA(B), ermA(A) and mef(A) genes were determined by PCR. The DNeasy Tissue Kit and the AccuPower PCR PreMix Kit were used as described earlier. One microlitre (100 pmol) of each primer, 2 μL of DNA solution, and 16 μL of distilled water were mixed together. Samples were amplified for 30 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C, and the PCR products were examined as described earlier.

**Results**

**Epidemiological characteristics of GAS**

The isolation rates of GAS were 16.9% in 2002, and 17.9% in 1995, respectively. emm12 (34.4%) was most frequent, followed by emm75 (10.4%), emm18 (9.4%), emm22 (8.3%) and emm1 (7.3%) in 2002. M12 (21.2%), M22 (14.1%) and M28 (9.4%) were common in 1995.

**Phenotypes and genes of erythromycin resistance**

The rates of resistance to erythromycin and clindamycin in 2002 were 51.0% and 33.7%, respectively, which were higher than the rates of 29.4% and 10.1% seen in 1995. Resistance to tetracycline Table 1. Phenotypes of erythromycin resistance according to emm gene (2002) and M protein type (1995)

<table>
<thead>
<tr>
<th>emm gene or M protein type</th>
<th>No. (%) in 2002</th>
<th>No. (%) in 1995</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no.</td>
<td>CR</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>33*</td>
<td>28</td>
</tr>
<tr>
<td>18</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>NT</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>NA</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Other types</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td>30</td>
</tr>
</tbody>
</table>

CR, constitutive resistance; M, M phenotype; NT, not typeable; NA, not available (emm or M typing was not undertaken).

*One strain of emm12 showed the inducible resistance. 

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and ofloxacin were 38.8% and 4.1% in 2002, compared with 35.3% and 0% in 1995, respectively. Among the erythromycin-resistant strains, constitutive resistance, inducible resistance, and the M phenotype were observed in 61.2%, 2.0% and 36.7% in 2002, compared with 64.0%, 0% and 36.0% in 1995. The \text{erm(B)}, \text{erm(A)} and \text{mef(A)} genes were present in the same percentage as each phenotype of erythromycin-resistant strains, respectively.

The phenotypes of erythromycin resistance were also analysed according to the \text{emm} genotype (Table 1). \text{emm} types 12, 18 and 75 showed high rates of erythromycin resistance. Most of the \text{emm12} strains showed constitutive resistance, whereas \text{emm18} and \text{emm75} showed the M phenotype. Most of the other \text{emm} or M type strains were susceptible to both erythromycin and clindamycin.

Discussion

The isolation rates of GAS were similar in 1995 and 2002. \text{emm} types 12, 75, 18, 22 and 1 were common in 2002, whereas M types 12, 22 and 28 were the most common in 1995. \text{emm75} and \text{emm18} were not detected in 1995. Prevalence of common \text{emm} types had varied in Jinju during several years.

Macrolides including erythromycin have been widely used for treatment of acute pharyngitis, and clindamycin has been recommended for the treatment of invasive infection of GAS. Susceptibility tests to these antibiotics are important especially because some GAS are susceptible to 16-membered macrolides or clindamycin in spite of resistance to erythromycin.\textsuperscript{10–12} Recently, a high frequency of resistance to erythromycin in GAS has been reported, particularly in countries where antibiotics are overused. Spanish data showed a resistance rate to erythromycin of 1.2% in 1990, which increased to 34.8% in 1995, which correlated with the total amount of macrolides used.\textsuperscript{13} In Italy, the resistance rate to erythromycin was less than 10% until 1993, but it increased up to 30.7% in 1995 and 35.8% in 2002.\textsuperscript{14,15} In the USA, the resistance rate to erythromycin was only 2.6% for GAS isolates during the period 1994–1997, but it increased up to 9.6% in a recent report.\textsuperscript{16,17} In Ontario, Canada, the erythromycin resistance rate was 2.1% in 1997, but 14.4% in 2001.\textsuperscript{18,19} In Asia, a high incidence of resistance in Taiwan has been recognized since the mid-1990s, and the resistance rate ranged from 60% to 70%.\textsuperscript{20} Although erythromycin resistance in Japan was reported up to 70% in the late 1970s and early 1980s, a decline in erythromycin resistance was reported with decreased consumption of erythromycin in the 1990s.\textsuperscript{21,22} In Korea, the resistance rate to erythromycin was reported as only 2% in 1994 but 41.3% in 1998.\textsuperscript{23,24} In this study, the resistance rates to erythromycin and clindamycin increased further to 51.0% and 33.7% in 2002 from 29.4% and 10.1% in 1995, respectively. However, no dramatic change was observed in the resistance rate to tetracycline (38.8% in 2002 from 35.3% in 1995) and ofloxacin (4.1% in 2002 from 0% in 1995). Given the relatively small number of isolates in this study, however, the increase in resistance could also be confirmed by comparing recent data from the clinical isolates.

The phenotypic expression of macrolide resistance is classified as constitutive, inducible, or M phenotype, according to the results of susceptibility to erythromycin and clindamycin. Erythromycin resistance is mediated by genes named \text{erm(B)}, \text{erm(A)} and \text{mef(A)}.\textsuperscript{13,25,26} In our study, inducible resistance was very rare (only one strain in 2002), and the distribution of phenotypes of macrolide resistance has almost not changed during the period 1995–2002. In Korea, the positive rates of \text{erm(B)} and \text{mef(A)} were reported as 62.5% and 31.3% in 1999.\textsuperscript{27}

There is debate as to whether serotypes of GAS are related to antibiotic resistance or not.\textsuperscript{28,29} We compared the resistance phenotypes to erythromycin with each \text{emm} genotype. Most \text{emm12} and \text{M28} isolates belonged to \text{erm(B)}-positive, constitutive resistance. Most \text{emm18} and \text{emm75} isolates belonged to the \text{mef(A)}-positive, M phenotype. However, the erythromycin resistance rates of \text{emm} types 2, 4, 5, 6, 9, 11, 13, 22, 49, 50 and 78 were all 0%. In recent reports, \text{emm} genotypes were suggested to be related to erythromycin resistance or its phenotypes,\textsuperscript{30,31} which may possibly reflect the spread of resistant clones. Therefore, PFGE of erythromycin-resistant GAS is needed to better define the status of resistance in a future study.

In summary, a marked increase in resistance to erythromycin and clindamycin was noted during the period 1995–2002 in Jinju, Korea. The phenotypes of erythromycin resistance had not changed during the period. Constitutive resistance is more common than the M phenotype, however inducible resistance is hardly observed. The phenotypes of erythromycin resistance seem to be associated with certain \text{emm} genotypes.

References

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