Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from blood cultures in Korea

Young Uh1*, Dong Hoon Shin2, In Ho Jang1, Gyu Yel Hwang1, Mi Kyung Lee1, Kap Jun Yoon1 and Hyo Youl Kim3

1Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Ilsan-dong 162, Wonju, Kangwon-do; 2Department of Laboratory Medicine, Hallym College of Medicine, Chuncheon; 3Department of Infectious Disease, Yonsei University Wonju College of Medicine, Wonju, South Korea

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Objectives: Our aim was to study the antimicrobial susceptibilities and macrolide resistance mechanisms of viridans group streptococci (VGS) in a Korean tertiary hospital.

Methods: MICs of five antimicrobials were determined for 106 VGS isolated from blood cultures. The macrolide resistance mechanisms of erythromycin non-susceptible isolates were studied by the double-disc test and PCR.

Results: In all, 42.4% of the isolates were susceptible to penicillin. Nine of 61 penicillin non-susceptible isolates were fully resistant (MIC ≥ 4 mg/L). Rates of non-susceptibility to erythromycin, clindamycin and ceftriaxone were 33.9%, 17.9% and 9.4%, respectively. Twenty-two (61.1%) of 36 erythromycin non-susceptible isolates expressed constitutive resistance to macrolide–lincosamide–streptogramin B antibiotics (a constitutive MLSB phenotype); 13 isolates (36.1%) expressed an M phenotype; and one isolate, a Streptococcus bovis isolate, had an inducible MLSB resistance phenotype. erm(B) was found in isolates with constitutive/inducible MLSB phenotypes, and mef(A) in isolates with the M phenotype. In three isolates (two isolates with a constitutive MLSB phenotype and in one isolate with the M phenotype), none of erm(A), erm(B), erm(C) or mef(A) was detected by PCR.

Conclusions: Penicillin non-susceptible VGS were more resistant to erythromycin, clindamycin and ceftriaxone than were penicillin-susceptible isolates. A constitutive MLSB phenotype associated with erm(B) was the predominant mechanism of macrolide resistance among erythromycin non-susceptible isolates from this Korean hospital.

Keywords: α-haemolytic streptococci, erythromycin resistance, MLSB phenotype, erm(B), mef(A)

Introduction

The viridans group streptococci (VGS) make up a heterogeneous group of streptococci that normally inhabit the mouth, gastrointestinal tract and female genital tract. They are often considered to be contaminants when isolated from blood cultures. However, they are also the leading cause of subacute bacterial endocarditis, and commonly cause catheter- and neutropenia-related bloodstream infections. β-Lactam agents are the treatment of choice for these infections, but increasing resistance to penicillin has recently been reported in many parts of the world.1 Macrolides and related drugs have been suggested as alternatives, but recent studies have shown that macrolide resistance may also be a problem.1

The objectives of the present study were to determine the incidence and patterns of antimicrobial resistance among VGS isolated from blood cultures in a Korean hospital and to clarify the macrolide resistance phenotypes and genotypes of erythromycin non-susceptible isolates.

Materials and methods

One hundred and six isolates of VGS isolated from blood cultures in 105 cases of bacteraemia were collected between May 2001 and August 2003 from the Wonju Christian Hospital, a 1000 bed teaching hospital in South Korea. Isolates were identified by standard methods and the API Rapid ID32 STREP system (bioMérieux, Marcy l’Étoile, France), and

*Corresponding author. Tel: +82-33-741-1592; Fax: +82-33-731-0506; E-mail: u931018@wonju.yonsei.ac.kr
were stored in brain–heart infusion broth plus 20% glycerol at −70°C until studied.

Susceptibility to penicillin G, erythromycin, clindamycin, ceftriaxone and vancomycin (Dae-
woong Lilly, Korea) was determined by an agar dilution method,2 and MICs were interpreted using NCCLS criteria.3 Macrolide resistance phenotypes of erythromycin non-susceptible isolates were determined using a double-disc test with erythromycin (15 μg) and clindamycin (2 μg) discs on Mueller–Hinton agar plates containing 5% sheep blood. Genomic DNA was extracted with an Easy-DNA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. The presence of erm (A) and mef class genes was determined by PCR amplification using previously described primers specific for erm(A), erm(B), erm(C) and mef(A).4,5

Results

Of the 106 VGS, 32 isolates were identified as Streptococcus mitis; other species included Streptococcus oralis (25 isolates), Streptococcus salivarius (nine isolates), Streptococcus anginosus (eight isolates), Streptococcus parasanguiinis (eight isolates), Streptococcus sanguinis (five isolates), Streptococcus constellatus (four isolates), Streptococcus vestibularius (four isolates), Gemella morbillorum (four isolates) and others (seven isolates). Using the agar dilution method, the rates of non-susceptibility were 58% for penicillin G (MIC ≥ 0.25 mg/L), 9% for ceftriaxone (MIC ≥ 2 mg/L), 18% for clindamycin (MIC ≥ 0.5 mg/L) and 34% for erythromycin (MIC ≥ 0.5 mg/L), whereas all isolates were susceptible to vancomycin. Of the 61 penicillin non-susceptible strains, nine were fully resistant to penicillin (range 4–16 mg/L) and 52 showed intermediate susceptibility (range 0.25–2 mg/L). The rates of susceptibility to erythromycin varied according to penicillin susceptibility: 87% in penicillin-susceptible isolates, 56% in penicillin-intermediate isolates and 22% in penicillin-resistant isolates. VGS non-susceptible to penicillin were also more resistant to clindamycin and ceftriaxone than penicillin-susceptible strains (Table 1).

Nineteen (53%) of 36 erythromycin-resistant isolates were resistant to clindamycin. Isolates non-susceptible to both penicillin and erythromycin comprised 28% (n = 30) of all the isolates.

Among 36 erythromycin-resistant isolates, 22 isolates (61.1%) expressed a constitutive macrolide–lincosamide–streptogramin B (MLS_{B}) phenotype, 13 isolates (36.1%) expressed an M phenotype and one Streptococcus bovis isolate expressed an inducible MLS_{B} phenotype. The erm (B) gene was detected in most isolates with constitutive/inducible MLS_{B} phenotypes, while mef(A) was detected in most isolates with the M phenotype. None of erm(A), erm(B), erm(C) or mef(A) was detected in two isolates with a constitutive

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC_{50/90} ≤ 0.12 mg/L</th>
<th>MIC_{50/90} ≥ 2 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>0.06/2</td>
<td>0.06/16</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.06/0.12</td>
<td>0.06/16</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.12/0.25</td>
<td>0.25/1</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Species (no. tested)</th>
<th>Phenotype (no.)</th>
<th>Genotype (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mitis (14)</td>
<td>CR (9)</td>
<td>erm(B) (8), ND (1)</td>
</tr>
<tr>
<td>S. oralis (10)</td>
<td>CR (7)</td>
<td>mef(A) (5)</td>
</tr>
<tr>
<td>S. parasanguiinis (6)</td>
<td>CR (3)</td>
<td>erm(B) (3)</td>
</tr>
<tr>
<td>S. salivarius (3)</td>
<td>CR (2)</td>
<td>mef(A) (1)</td>
</tr>
<tr>
<td>S. bovis (2)</td>
<td>CR (1)</td>
<td>ND (1)</td>
</tr>
<tr>
<td>G. morbillorum (1)</td>
<td>M (1)</td>
<td>mef(A) (1)</td>
</tr>
</tbody>
</table>

| MLS_{B} phenotype, and in one isolate with the M phenotype (Table 2). Since strains positive for erm genes were not further tested for mef genes, we do not know how many isolates might have had both resistance mechanisms.

Discussion

Until the 1980s, VGS were considered to be uniformly susceptible to β-lactam antibiotics, but resistance spread rapidly in the 1990s. The prevalence of penicillin non-susceptible VGS varies between studies; in our study, the rate was 58% (intermediate 49% and resistant 9%). The susceptibility rate seen in this study is similar to that reported in the USA4 (44%), but lower than that of previously reported multicentre studies6 (61.9–70.3%). As reported in other studies,1,7 VGS that were not susceptible to penicillin also showed reduced susceptibility to ceftriaxone, erythromycin and clindamycin. Penicillin resistance in the VGS is mediated by the presence of the mosaic genes encoding altered penicillin-binding proteins with decreased affinity for β-lactam antibiotics. These determinants can be transferred between the VGS and Streptococcus pneumoniae.8 Therefore, the penicillin-resistant VGS constitute a potential pool of genes for the development of peni-
cillin resistance in *S. pneumoniae* and other streptococci. The genes conferring resistance to non-β-lactam antimicrobials, such as macrolides, ciprofloxacin, trimethoprim–sulfamethoxazole and chloramphenicol, are not known to be closely linked to the resistance determinant for penicillin.

Increased rates of macrolide resistance in blood isolates of the VGS have been reported in recent years, and the rate of erythromycin resistance noted in our study (34%) was similar to those reported among blood culture isolates in the USA (41%), Canada (38.1%) and northern Taiwan (35%). These rates limit the value of macrolides as prophylaxis in high-risk populations or as a treatment of viridans streptococcal bacteraemia or endocarditis.

The rate of clindamycin resistance in VGS has been determined mainly by the distribution of MLSB resistance phenotypes. In Taiwan, Teng et al. documented that all macrolide-resistant VGS were constitutively resistant. However, in studies reported by western countries, resistance rates to clindamycin were lower than that of erythromycin. In this study, a constitutive MLSB phenotype was the most frequent mechanism observed among erythromycin-resistant isolates (61.1%). The M phenotype was expressed by 36.1% of isolates; this rate is higher than that reported by Teng et al., but lower than that reported by Ioannidou et al. This difference of distribution of MLSB phenotypes may be explained by geographical variation and source of the isolates.

In conclusion, we found high rates of non-susceptibility to penicillin and macrolides among VGS in Korea, and found that a constitutive MLSB phenotype associated with *erm* (B) was dominant. The high frequency of non-susceptibility to penicillin and macrolides among the VGS limits the use of these drugs as therapeutic or prophylactic agents for bacteraemias caused by these organisms. Our findings indicate clearly that clinical microbiology laboratories should carry out periodic surveillance of antibiotic susceptibility among various species of VGS. Further studies are needed to determine the suitable antimicrobial agents for chemoprophylaxis.

**References**


