Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in a Korean hospital

Jung-A. Lim, Ae-Ran Kwon, Sook-Kyung Kim, Yunsop Chong, Kungwon Lee and Eung-Chil Choi

Materials and methods

Bacterial strains
A total of 1097 clinical isolates of Gram-positive cocci, comprising 467 methicillin-resistant *S. aureus* (MRSA), 169 methicillin-susceptible *S. aureus* (MSSA), 100 methi...
cillin-resistant CNS, 86 methicillin-susceptible CNS, 180 Enterococcus faecalis and 95 Enterococcus faecium, were collected from the Severance Hospital in Seoul, Korea between May 1999 and January 2000. Multiple isolates from the same patient were avoided. The strains were stored in brain heart infusion (BHI) broth plus 20% glycerol at −70°C until studied.

**Antibiotics**

Erythromycin and clindamycin were purchased from Sigma Chemical Co. (St Louis, MO, USA). The other antibiotics were obtained as follows: clarithromycin, Abbott Laboratories (Abbott Park, IL, USA); azithromycin, Pfizer Inc. (New York, NY, USA); josamycin, ICN Biomedicals (Costa Mesa, CA, USA) and pristinamycin, Rhône-Poulenc Rorer (Paris, France).

**Determination of MICs**

MICs were determined by a standardized agar dilution method with Mueller–Hinton (MH) agar for staphylococci and BHI agar for enterococci. The MIC resistance breakpoints used, based on the guidelines from the NCCLS and the French Society for Microbiology, were as follows: erythromycin, clarithromycin, azithromycin and josamycin ≥8 mg/L; clindamycin ≥4 mg/L and pristinamycin ≥2 mg/L. S. aureus ATCC25923 and E. faecalis ATCC29212 were used as controls in the MIC determinations. The bacterial suspensions (10⁴ cfu/spot) were inoculated using a microinoculator (Sakuma Co. Ltd, Tokyo, Japan).

**Determination of resistance phenotypes**

The resistance phenotypes of erythromycin-resistant isolates were determined by the double-disc test with erythromycin (15 μg) and clindamycin (2 μg) discs, as described previously. After 18 h incubation at 37°C, blunting of the clindamycin zone of inhibition proximal to the erythromycin disc indicated an inducible type of MLS resistance (IR), and resistance to both erythromycin and clindamycin indicated a constitutive type of MLS resistance (CR).

**Detection of resistance genotypes**

The presence of genes encoding MLS resistance due to alteration of the ribosome target site was determined by multiplex PCR amplification of *erm* genes using primers specific for *erm*(A), *erm*(B) and *erm*(C). In addition, the presence of the gene involved in the macrolide efflux system was determined by PCR with primers for *mef*. Genomic DNA was extracted as described previously, and was used as the template for amplification. Primers were designed from published GenBank sequences to provide specific PCR products (Table 1). We selected an

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
<th>Control strains</th>
<th>Location of gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>erm</em>(A)²⁰</td>
<td>5'-CTTCAAGAATATCTTACAGGACGACGC-3'</td>
<td>421</td>
<td>E. coli ATCC25522</td>
<td>pLS200⁶⁰</td>
</tr>
<tr>
<td><em>erm</em>(B)²⁷</td>
<td>5'-GGATCAGGAAAAGGACATTTTAC-3'</td>
<td>639</td>
<td>Bacillus subtilis 168</td>
<td>pAM77⁷⁴</td>
</tr>
<tr>
<td><em>erm</em>(C)²⁰</td>
<td>5'-CGTTAATATTGTTTAAATCGTCAATTCC-3'</td>
<td>572</td>
<td>B. subtilis BD170</td>
<td>PE194⁹⁷</td>
</tr>
<tr>
<td><em>mef</em>¹⁶</td>
<td>5'-GGATCAGGAAAAGGACATTTTAC-3'</td>
<td>348</td>
<td>Enterococcus isolate (this study)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Primer sequences and control strains used in the determination of resistance genotype

a Upper primer.
b Lower primer.
c The presence of *mef* was confirmed by Southern blot hybridization.
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Enterococcus isolate from this study as a control strain harbouring mef, and confirmed its presence by Southern blot hybridization using the mef PCR product as a probe. PCR was carried out on the 552 isolates (174 MRSA, 20 MSSA, 75 methicillin-resistant CNS, 50 methicillin-susceptible CNS and 233 enterococci) displaying resistance to erythromycin, as well as on the control strains for each genetic determinant (Table 1). All PCR amplifications consisted of an initial cycle of 5 min of denaturation at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 30 s of annealing at 54°C, 1 min of elongation at 72°C and one cycle of 5 min of extensive elongation at 72°C on a DNA thermal cycler (PTC-200; MJ Research, Inc., Watertown, MA, USA). After amplification, electrophoresis and visualization of the PCR products were carried out by established procedures.16

Results and discussion

Susceptibility testing and MLS resistance phenotypes

The MIC range and the MIC50 and MIC90 values for the three groups of test organisms are displayed in Table 2. The S. aureus and CNS strains were mostly resistant to the macrolide and lincosamide antibiotics. The MIC90 values of pristinamycin for S. aureus and CNS strains were relatively low. However, the high rates of resistance to pristinamycin in S. aureus and CNS do not support pristinamycin being regarded as a first-line agent for staphylococcal infections. The enterococcal isolates were also resistant to MLS antibiotics. Pristinamycin was ineffective against the E. faecalis isolates because of natural resistance to streptogramins, but the MIC50 of pristinamycin for E. faecium was lower (1 mg/L) than those of the other macrolide and lincosamide antibiotics (≥64 mg/L). MLS antibiotics cannot be considered as efficient therapeutic agents for enterococcal infections in Korea. Antibiotic resistance, including MLS, is usually closely related to the extent to which these agents are used, and some reports have shown that a decrease in the use of these antibiotics has led to a decrease in the prevalence of resistance.17–19 The relatively low incidence of resistance to pristinamycin may be related to the low usage of this antibiotic in Korea.

The overall extent and frequency of resistance to MLS were high compared with those determined in other countries.20,21 This seems to be influenced by the fact that the Severance Hospital, which participated in this study, is an institution classified as the final level of medical treatment in Korea.

The resistance phenotypes of 851 erythromycin-resistant isolates (comprising 493 S. aureus, 125 CNS and 233 enterococci) were determined according to the results of double-disc tests. The IR phenotype was demonstrated in 72 S. aureus isolates (14.6%) and 12 CNS (9.6%), with the

<table>
<thead>
<tr>
<th>Table 2. Antibacterial activities of macrolide, lincosamide and streptogramin against three groups of Gram-positive cocci</th>
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</thead>
<tbody>
<tr>
<td>Organism (no. isolates)</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>S. aureus (636)</td>
</tr>
<tr>
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<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>CNS (186)</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Enterococci (275)</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
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<tr>
<td>E. faecalis (180)</td>
</tr>
<tr>
<td>E. faecium (95)</td>
</tr>
</tbody>
</table>
Table 3. Distribution of the *erm* genes and the *mef* gene among erythromycin-resistant Gram-positive cocci

<table>
<thead>
<tr>
<th>Gene</th>
<th>S. aureus (n = 194)</th>
<th>CNS (n = 125)</th>
<th>Enterococci (n = 233)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>MSSA</td>
<td>total</td>
</tr>
<tr>
<td><strong>erm(A)</strong></td>
<td>147</td>
<td>13</td>
<td>160 (82.5)</td>
</tr>
<tr>
<td><strong>erm(B)</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(C)</strong></td>
<td>2</td>
<td>3</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td><strong>mef</strong></td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>erm(A) + erm(B)</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(A) + erm(C)</strong></td>
<td>15</td>
<td>1</td>
<td>16 (8.2)</td>
</tr>
<tr>
<td><strong>erm(A) + mef</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(B) + erm(C)</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(B) + mef</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(C) + mef</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>erm(A) + erm(B) + mef</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Not detected</strong></td>
<td>10</td>
<td>3</td>
<td>13 (6.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>174 (89.7)</td>
<td>20 (10.3)</td>
<td>194 (100)</td>
</tr>
</tbody>
</table>

Abbreviations: MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; MRCNS, methicillin-resistant coagulase-negative staphylococci; MSCNS, methicillin-susceptible coagulase-negative staphylococci
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remained exhibiting the CR phenotype. The IR phenotype in enterococci was rare, being found in only two of 233 isolates. In conclusion, the resistance rate to MLS antibiotics of the Gram-positive cocci isolated from the Severance Hospital was very high, and the most prevalent MLS resistance phenotype of the three Gram-positive groups was CR.

**MLS resistance genotypes**

The distribution of resistance genes among 552 isolates resistant to erythromycin (194 *S. aureus*, 125 CNS and 233 enterococci) as determined by PCR amplification of the genes *erm*(A), *erm*(B), *erm*(C) and *mef*, is displayed in Table 3. The most prevalent resistance determinant in *S. aureus* was *erm*(A), which was detected in 82.5% of the isolates. The *erm*(C) determinant was found as a single MLS resistance gene in five (2.6%) isolates while 16 isolates contained both *erm*(A) and *erm*(C). No *S. aureus* harbouring different mechanisms of resistance (i.e. a methylase and a macrolide efflux protein) was found. Neither *erm*(B) nor *mef* was identified in *S. aureus*. The distribution of resistance determinants in *S. aureus* was less complex than those in the CNS and enterococcal strains. This point could be explained by the selection of a high proportion of clonal MRSA in this study. One hundred and seventy-four of 194 erythromycin-resistant *S. aureus* isolates selected from 260 clinical isolates were methicillin resistant (89.7%).

Among 125 erythromycin-resistant CNS isolates, 64% were methicillin resistant. In these CNS isolates, the *erm*(C) gene was detected alone in 47.2% (59 of 125) of the isolates, and with the *erm*(A) (15.2%) or *mef* gene less frequently (3.2%). Five CNS strains harbouring two determinants encoding different mechanisms (*erm* and *mef*) were detected. There were four strains that harboured the *erm(B)* gene alone, and this *erm(B)* determinant was not detected in combination with any other resistance genes in CNS. The *erm*(B) amplicons in CNS were sequenced to confirm the results, and 98% similarity with *erm*(B) of *E. faecalis* and *Staphylococcus intermedius* (GenBank accession no. AF9773) was identified. When a single resistance determinant was present in staphylococci, the *erm*(A) gene was more common in *S. aureus*, whereas *erm*(C) was predominant in CNS. These results confirmed a previous report describing the predominance of *erm*(C) in a large series of clinical and commensal CNS.

*erm*(B) was the most prevalent gene in enterococci, whereas it was rarely identified in *Staphylococcus* spp.. It was detected alone in 55.4% of the isolates and with *erm*(A) (5.2%) or *mef* less frequently (3.5%). The *erm* gene has previously been demonstrated to be involved in macrolide resistance in various Gram-positive bacteria, such as *Enterococcus, S. pneumoniae, S. pyogenes* and *S. aureus*. Our results support the fact that the *erm(B)* gene is most frequently found among the enterococci. In contrast, the *erm*(C) gene was not detected in enterococci, whereas it was the most prevalent resistance genotype in CNS. This result is consistent with other reports of enterococci lacking *erm*(C). In general, erythromycin-resistant enterococci have a more complex distribution of resistance genotypes than staphylococci. For example, three enterococcal isolates were detected that each harboured the three genetic determinants, *erm*(A), *erm*(C) and *mef*.

The *mef* determinant was found, either alone or with the *erm* gene, in CNS and enterococci, but was not detected in the *S. aureus* isolates. The *mef* gene has also been detected in a variety of Gram-positive genera, including *S. pyogenes, S. pneumoniae* and *Streptococcus agalactiae*, as well as in *Micrococcus luteus, Corynebacterium jeikeium, Corynebacterium* spp. and viridans streptococci, indicating wide-spread distribution.

In 13 *S. aureus*, 22 CNS and 51 enterococci that were macrolide resistant, *erm* and *mef* genes were not detected. It is likely that these isolates harbour other resistance genes, such as *msrA/B*, *linA/A*, *vga*, *vgb* or *vat*. The *msrA/B* (macrolide and streptogramin resistance) efflux pump gene differs from the *mef* gene, in that *msrA/B* confers resistance to both macrolide and streptogramin antibiotics. Lincomycin nucleotidyltransferase (*linA/A*), virginiamycin factor A (*vga*), virginiamycin factor B hydrolyase (*vgb*) and virginiamycin factor A acetyltransferase (*vat*) have been identified previously in several Gram-positive bacteria.

In conclusion, the three groups of Gram-positive cocci used in this study showed a relatively high rate of resistant to MLS antibiotics. The MLS resistance genes were species specific with *erm(A)* dominant in *S. aureus*, *erm(B)* in enterococci and *erm(C)* in CNS.

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