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We investigated the prevalence and the molecular characteristics of vancomycin-intermediate Staphylococcus aureus (VISA) among methicillin-resistant Staphylococcus aureus (MRSA) strains isolated from clinical samples at tertiary or general hospitals participating in a nationwide surveillance program for VISA and vancomycin-resistant Staphylococcus aureus (VRSA) in Korea during an 8-week period in each year from 2001 to 2006. Of 41,639 MRSA isolates, 37,856 were screened and 169 grew on brain heart infusion agar supplemented with 4 µg/ml vancomycin. A vancomycin MIC of 4 µg/ml was confirmed for 33 VISA isolates of the 169 isolates. Eighteen of the 33 isolates were classified as hetero-VISA (hVISA) by the population analysis profile (PAP) method. All VISA isolates were susceptible to linezolid, tigecycline, and quinupristin−dalfopristin. Most VISA isolates (MIC 4 µg/ml) showed a PFGE C pattern with sec, seg, and sei enterotoxin genes, including ST5-SCCmec type II, or a PFGE A pattern with sec, including ST239-SCCmec type III.

Keywords: Staphylococcus aureus, surveillance, vancomycin-intermediate

Staphylococcus aureus is one of the leading causes of life-threatening infections in hospital settings and is increasingly a cause of disease in the community. Glycopeptides, such as vancomycin, are effective against methicillin-resistant S. aureus (MRSA). Although vancomycin resistance was first reported for enterococci in 1988, the first glycopeptide-intermediate S. aureus strain was isolated in France in 1995 [27]. The first documented infection caused by vancomycin-intermediate S. aureus (VISA) was reported from Japan in 1996 [15]. The strain, known as Mu50, had a minimum inhibitory concentration (MIC) to vancomycin of 8 µg/ml. Since then, there have been further reports of VISA in the United States, Germany, and Korea [3, 4, 19, 32]. In addition to VISA and another type of vancomycin-resistant bacteria, a hetero-VISA (hVISA), known as Mu3, has been described [14]. This strain appears to be borderline susceptible to vancomycin (MIC 2–4 µg/ml) but exhibits low-level subpopulation, at a frequency of 10⁶ or greater, and is able to grow at vancomycin concentrations of 4–8 µg/ml. hVISA may be a precursor of VISA [16, 33] and may be associated with treatment failure [11, 14, 25, 37]. Methicillin resistance is prevalent (about 70%) among S. aureus isolates from tertiary hospitals in Korea [21]. Therefore, vancomycin used for the treatment of MRSA infections is increasing. Moreover, with the revised Clinical and Laboratory Standards Institute (CLSI) guidelines [7], the isolates with MIC values for vancomycin of 4 µg/ml are classified as intermediate. Thus, many of the isolates originally described as vancomycin-susceptible S. aureus are now classified as VISA strains. The possibility that VISA/VRSA may be on the rise necessitates periodic nationwide surveillance for these S. aureus strains. In this study, we conducted a nationwide survey to investigate the prevalence and molecular characterization of VISA in Korea from 2001 to 2006.

Materials and Methods

Bacterial Isolates
During an 8-week period from 2001 to 2006, 58,501 S. aureus strains isolated from clinical samples at general and tertiary hospitals participating in a nationwide laboratory surveillance program for VISA/VRSA in Korea were collected. MRSA were defined as S. aureus that grew on mannitol salt agar with 6 µg/ml oxacillin and confirmed by meca gene PCR in our laboratory. S. aureus ATCC...
Table 1. Screening of resistance to vancomycin of clinical MRSA isolates from 2001 to 2006 in Korea.

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participating hospitals</td>
<td>27</td>
<td>42</td>
<td>40</td>
<td>49</td>
<td>52</td>
<td>45</td>
<td>37,856</td>
</tr>
<tr>
<td>No. of MRSA screened</td>
<td>3,764</td>
<td>5,057</td>
<td>5,506</td>
<td>7,372</td>
<td>7,748</td>
<td>8,409</td>
<td>37,856</td>
</tr>
<tr>
<td>No. of screening-test positive (%)</td>
<td>43 (1.1)</td>
<td>16 (0.3)</td>
<td>41 (0.7)</td>
<td>24 (0.3)</td>
<td>28 (0.3)</td>
<td>17 (0.2)</td>
<td>169 (0.4)</td>
</tr>
</tbody>
</table>
body fluids. Their susceptibilities, by the disk diffusion or E-test, against 15 antibiotics are listed in Table 3. Most of the isolates were resistant to 10 antibiotics, at a range 21% to 100%. All the isolates were susceptible to linezolid, quinupristin-dalfopristin, and tigecycline. Some isolates (15/33, 45%) were nonsusceptible to daptomycin, as E-test results of daptomycin showed MICs of >1 µg/ml.

Population Analysis Profiling

Eighteen of the 33 VISA strains were identified as hVISA, and 15 were identified as VISA (Table 2). The PAP–AUC ratio of these isolates ranged from 0.9 to 1.2 (hVISA) and from 1.3 to 1.7 (VISA) (data not shown).

Table 2. MICs of vancomycin and teicoplanin against 33 VISA isolates.

<table>
<thead>
<tr>
<th>PAP (No. of isolates)</th>
<th>MICs (µg/ml)</th>
<th>Agar dilution method</th>
<th>Broth dilution method</th>
<th>E-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>VAN⁰</td>
<td>TEC⁰</td>
<td>VAN</td>
</tr>
<tr>
<td>Range</td>
<td>2–4</td>
<td>1–16</td>
<td>1–4</td>
<td>0.5–16</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>4</td>
<td>8</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

⁰VAN: vancomycin, ⁰TEC: teicoplanin.

Table 3. The antimicrobial susceptibilities of 33 VISA strains isolated from 2001 to 2006.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
</tr>
<tr>
<td>OX</td>
<td>33 (100)</td>
</tr>
<tr>
<td>AM</td>
<td>33 (100)</td>
</tr>
<tr>
<td>P</td>
<td>31 (94)</td>
</tr>
<tr>
<td>E</td>
<td>29 (88)</td>
</tr>
<tr>
<td>CZ</td>
<td>28 (85)</td>
</tr>
<tr>
<td>CC</td>
<td>25 (76)</td>
</tr>
<tr>
<td>Te</td>
<td>24 (73)</td>
</tr>
<tr>
<td>OFX</td>
<td>21 (64)</td>
</tr>
<tr>
<td>GM</td>
<td>21 (64)</td>
</tr>
<tr>
<td>SXT</td>
<td>19 (58)</td>
</tr>
<tr>
<td>RA</td>
<td>7 (21)</td>
</tr>
<tr>
<td>LINE</td>
<td>0 (0)</td>
</tr>
<tr>
<td>SYN</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TIGE</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DAP</td>
<td>15 (45)</td>
</tr>
</tbody>
</table>

⁰OX, oxacillin; P, penicillin; AM, ampicillin; E, erythromycin; CC, clindamycin; CZ, ceftazolin; OFX, ofloxacin; Te, tetracycline; RA, rifampin; GM, gentamicin; SXT, trimethoprim–sulfamethoxazole; LINE, linezolid; SYN, quinupristin–dalfopristin; the susceptibilities were measured by the disk-diffusion method. DAP, daptomycin; TIGE, tigecycline; the susceptibilities were measured by the E-test method (AB biodisk).

Contribution of Various Genotypes

For 33 VISA isolates, detection of staphyloccocal enterotoxin genes and typing of STs, SCCmec, PFGE were characterized, as shown in Fig. 1. All the strains were identified as follows: 14 isolates hold enterotoxin genes of sec, seg, and sei with SCCmec II and ST5; sea with SCCmec III or IIa and ST239 (12 isolates); seg and sei with SCCmec IV and ST72 (5 isolates); and seh with SCCmec IV and ST1 (1 isolate). One VISA strain isolated in 2006 was identified as sea with SCCmec III and a new MLST (2-3-1-new-4-4-3), showing 99% similarity to the gmk-1 allele. Analysis of the PFGE band patterns for the 33 isolates revealed three different groups: pattern A with A1 to A5 subtypes, pattern B with B1 to B2 subtypes, and pattern C with C1 to C7 subtypes, with a Dice coefficient of 80% cut-off.

DISCUSSION

After the first VRSA appeared in 2002 in Michigan, U.S.A., an additional two cases occurred in 2007 and a total of nine cases with the vanA gene have been reported to date in the U.S.A. [5, 10]. VRSA have also been reported in other countries such as India (5 VRSA including 1 vanA-positive isolate) and Iran (2 VRSA including 1 vanA-positive isolate) [1, 31, 35]. In Korea, S. aureus with reduced susceptibility to vancomycin was reported for the first time in 2000 [19], but VRSA has not appeared to date. Since its first appearance, some surveillance studies [18, 20] reported that numerous VISAs (MIC of 4 µg/ml) and hVISAs had been isolated from specimens from hospitalized patients. The incidence of VISAs and VRSA is in an increasing trend globally, including in Korea, and thus the necessity of information sharing through an international surveillance system is increasing further.

During the period of this study (2001–2006), VISAs were identified in 33 out of a total of 37,856 MRSA isolates, with vancomycin MIC of 4 µg/ml, and their prevalence was shown to be 0.09%. In France, the France Teaching Hospital showed a VISA prevalence of 0.07% [29], which is similar to the result of this study, and in the case of the U.S.A., VISA prevalence in hospitals in the Detroit metropolitan area has been 0.3–2.3% for 22 years [30]. In Asia, VISA prevalence’s in Thailand (0.8%, 3/361 MRSAs) and Japan (0.24%, 6/2,446 MRSAs) were slightly higher than the result of this study [13, 22].

In this study, about 70% of all S. aureus isolated from participating hospitals in Korea were methicillin-resistant. Because vancomycin is one of the frequently prescribed antimicrobial agents for treatment of these MRSA infections, the appearance of VISA is expected to increase. In addition, to reduce the rate of failure in treating MRSA patients along with VISA patients, hVISA should also be investigated. In this study, the PAP–AUC method was used.
to screen VISA and hVISA from specimens; the PAP is being suggested as a method to identify hVISA and VISA in spite of controversies over the clinical importance of hVISA strains and hVISA detection methods [9, 12]. In this study, 18 out of 33 VISA strains were identified as hVISA by PAP-AUC ratio. In the U.S.A., 112 hVISA (7.5%) strains were identified out of 1,499 S. aureus strains, and, of these, 84.6% showed a vancomycin MIC of 4 µg/ml [30]. In the case of Japan, hVISA prevalence was shown to be 3% (34/1,149 MRSAs) in one study, but no hVISA was found in any other study conducted in the same year [14, 17]. In Italy, Germany, France, and The Netherlands, the prevalence of hVISA in MRSA was 1.1%, 0.21%, 0.6%, and 6%, respectively [3, 23, 28, 36]. Thus, hVISA prevalence appears to occur with slight differences among countries, although this is partially due to detection methods. Nevertheless, some actual differences among countries do occur.

Currently, the CDC in the U.S.A. uses BHI agar containing 6 µg/ml of vancomycin as a vancomycin-screening medium, and CLSI criteria for VISA were changed, which means there is a possibility of missing VISA. When 33 VISA strains confirmed in our study were investigated for growth on BHI supplemented with 6 µg/ml vancomycin, only 14 strains grew on screening plate of 6 µg/ml vancomycin. Therefore, we propose a routine use of screening plate containing 4 µg/ml vancomycin to reinforce the screening of reduced susceptibility to vancomycin.

Thirty-three VISA isolates were tested for susceptibility to daptomycin, and, based on the results, 15 isolates were nonsusceptible. This reduced susceptibility of VISA strains to daptomycin was reported in 2006 by Cui et al. [8], and
the report was shown to be consistent with the result that the reduced susceptibilities of VISAs to vancomycin and daptomycin are correlated with each other.

The domestic epidemiology of VISAs was identified through PFGE and MLST, and, as a result, a lineage with three patterns of independent genetic traits could be identified. These isolates are PFGE A with SCCmec III and ST239, PFGE B with SCCmec IV and ST72, and PFGE C with SCCmec II and ST5. Given that genetic similarities were shown among the restriction patterns of PFGE, even though the strains were isolated from different hospitals and from different specimens, it is possible that the prevalent clones in hospitals in Korea, such as ST5, ST239, and ST72, obtained resistance to vancomycin or that clonal transmissions occurred among VISA isolates in the hospital. Moreover, it is assumed that these VISAs had reduced susceptibility to vancomycin as a result of clinical administrations of vancomycin. Kim et al. [18] reported that out of 12 vancomycin (4 µg/ml) patients, 11 patients had been exposed to glycopeptide antibiotics for long periods (56 days on average).

In conclusion, 33 VISA strains with vancomycin MIC of 4 µg/ml were reported in Korea, but no VRSA was detected. Since most VISA strains showed the characteristics of epidemic strains found in hospitals in Korea, we assume that VISAs were exposed to vancomycin, and this subsequently reduced their susceptibility to vancomycin, enabling them to spread in some hospitals. Therefore, the prudent use of antibiotics is very important in order to suppress the appearance of VISAs/VRSAs. Continued surveillance and infection control are essential in preventing the occurrence and spreading of VISAs/VRSAs.

Acknowledgments

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