Molecular Typing and Resistance Profiles of Vancomycin-Intermediate *Staphylococcus aureus* in Korea: Results from a National Surveillance Study, 2007-2013

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Background: To investigate the national molecular epidemiology and resistance profiles of vancomycin-intermediate *Staphylococcus aureus* (VISA), we analyzed the characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) collected 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 a 12-week period in each year from 2007 to 2013.

Methods: VISA was defined by agar dilution, broth dilution and E-test methods with vancomycin minimum inhibitory concentrations of \( > 2 \mu g/mL \). All VISA isolates were characterized by multilocus sequence typing, staphylococcal cassette chromosome mec typing, spa typing, accessory gene regulator typing, Diversilab analysis, and antibiogram analysis.

Results: Of 109,345 MRSA isolates, 87,354 were screened and 426 isolates were identified as positive on brain heart infusion agar containing 4 \( \mu g/mL \) vancomycin (BHI-V4). Of 426 isolates, 76 isolates were identified as VISA. No VRSA isolates were detected among the isolates. Overall, a total of 6 genotypes were identified among VISA strains and the predominant clones were ST5-II-t2460, ST72-IV-t324, and ST239-III-t037 (44.7%, 15.8%, and 10.5%, respectively). Of note, ST72-IV-t324 clones are known to be a typical community-associated MRSA. ST239-III-t037 strains were more resistant to trimethoprim-sulfamethoxazole than any other type of strain. ST72-IV-t324 strains were susceptible to all of the antimicrobial agents tested except erythromycin and daptomycin. All of the VISA isolates were susceptible to linezolid and quinupristin-dalfopristin.

Conclusion: Although VRSA is still rare, continuous monitoring of VRSA occurrence is needed, as well as VISA prevalence, epidemic clonal shift, and antimicrobial resistance. (Ann Clin Microbiol 2016;19:88-96)

Key Words: Sequence type, Vancomycin-intermediate *Staphylococcus aureus*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the highly prevalent of hospital-associated and community-associated infections worldwide [1]. This has resulted in an increased use of vancomycin, which have been the treatment of choice for MRSA infection for decades. Unfortunately, this led worldwide to the development of *S. aureus* strains with reduced susceptibility to vancomycin [1].

The first strain of vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) was reported in Japan in 1997 [2]. Since then, clinical isolates of *S. aureus* with decreased susceptibility to vancomycin have been reported in many countries, including the United States [3,4], Canada [5], China [6], South Korea [6], United Kingdom [7,8], Italy [9], and France [10], and their occurrence has become a major concern throughout the world. Additionally, in 2002, the first vancomycin-resistant *S. aureus* (VRSA) strain was reported in the
United States [11]. Thirty five cases of VRSA infection have been reported worldwide by May 2016, including 1 from Pakistan, 3 from Iran, 14 from the USA, 16 from India, and 1 from Portugal [12-14].

Reduced susceptibility to vancomycin in S. aureus is complex and difficult to detect in clinical microbiology laboratories [15]. In 2006, to improve the correlation between in vitro susceptibility and clinical response, the Clinical and Laboratory Standard Institute (CLSI) redefined resistance breakpoints for vancomycin against S. aureus [15]. The current CLSI guidelines suggest that S. aureus strains should be categorized as susceptible, intermediate, or resistant when the vancomycin minimum inhibitory concentrations (MIC) is ≤2 μg/mL, 4 to 8 μg/mL, or ≥16 μg/mL, respectively.

Strains with reduced susceptibility to vancomycin have been associated with clinical treatment failure to vancomycin. A meta-analysis demonstrated that higher vancomycin MIC was significantly associated with mortality for MRSA infection. This mortality association was predominantly driven by bloodstream infections and isolates with a vancomycin MIC of 2 μg/mL by E-test [16].

In Korea, to monitor the emergence and spread of S. aureus strains with reduced susceptibility to vancomycin, we have been conducted a VISA/VRSA laboratory surveillance program in nationwide since 2001. In previous VISA/VRSA laboratory surveillance study, it showed that 33 hVISA/VISA strains through screening of 37,856 clinical isolates collected from 2001-2006 and VRSA was not detected [17].

In this study, to update the previous nationwide surveillance data, we investigated the prevalence and resistance profiles of VISA strains among clinical isolates of S. aureus collected in 2007-2013. We also characterized the genotypes of these strains using diverse genotyping methods such as MLST, SCCmec typing, spa typing, and rep-PCR-based typing.

2. Vancomycin agar screening and VISA confirmation test

Screening of MRSA with reduced susceptibility to vancomycin was performed using previously described [17]. A 10 μL drop of a 0.5 McFarland suspension was inoculated onto brain heart infusion agar supplemented with 4 μg/mL vancomycin (BHI-V4). After 24 and 48 h of incubation at 35°C, plates were examined for growth. A positive BHI-V4 screen result for VISA was defined as growth of two or more colonies from one droplet.

VISA was finally confirmed by agar dilution, broth dilution and E-test methods with vancomycin minimum inhibitory concentrations (MIC) of >2 μg/mL.

3. DNA extraction

Genomic DNA was extracted by using a Wizard genomic DNA preparation kit (Promega, Madison, WI, USA). According to manufacturer’s protocol for bacterial cells, we added lysostaphin at the final concentration of 30 μg/mL in lysis buffer and incubated 1 h at 37°C. For rep-PCR, genomic DNA from a 10-μL loopful of a S. aureus colony was extracted using an Ultra Clean microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions.

4. Molecular typing

All VISA isolates were determined molecular types. Five typing methods, i.e., MLST, SCCmec typing, spa typing, agr typing, and diversilab typing were used in the present study. MLST was carried out by PCR amplification and sequencing of seven housekeeping genes (arc, aroE, glpF, gmk, pta, tpi and yqiL) by using primer pairs as previously described [18]. The allelic profiles and sequence types (STs) were assigned by the MLST web site (http://saureus.mlst.net/). The spa typing was performed as previously described [19,20]. The spa types were determined by using Ridom SpaServer (http://spa.ridom.de/spatypes.shtml). SCCmec types were determined by the multiplex PCR method [21]. Strains COL, N315, and MW2 were included as controls for SCCmec type I, II, and IV, respectively. The agr grouping was performed by multiplex PCR as previously described [22]. For Diversilab (DL) typing, repetitive-sequence based PCR was performed using the DL Staphylococcus kit (bioMérieux, Marcy l’Etoile, France). The amplification products were separated using a DL DNA LabChip kit with microfluidic technology, as described previously [23]. The analysis was performed using DL software (version v.3.6). The data for each sample con-
sisted of a dendrogram, a vertical gel image (band pattern), a graph of fluorescence corresponding to each band pattern, and a similarity matrix. The relatedness was determined by cluster analysis and guidelines provided by manufacturer. Isolates were considered identical where there was >95% similarity and no differences in bands.

5. Antimicrobial susceptibility testing

All VISA isolates were tested for susceptibility to 14 antibiotics. The disk diffusion method was used to test of susceptibility to 10 antibiotics, including ce fazolin, clindamycin, erythromycin, gentamicin, ofloxacin, penicillin, quinupristin-dalfopristin, rifampicin, tetracycline, and trimethoprim-sulfamethoxazole. The susceptibilities of daptomycin, linezolid, teicoplanin and tigecycline were measured using the E-test (AB Biodisk, bioMérieux, Marcy-l'Etoile, France) according to the manufacturer’s instructions. *S. aureus* ATCC 29213 was used as the quality control strain.

**RESULTS**

1. Vancomycin agar screening

Among the 155,033 *S. aureus* isolates, 109,345 (70.5%) were identified as MRSA. The annual rate of MRSA isolation ranged from 69.5-72.3% (Table 1). The 426 (0.5%) MRSA were identified as positive on BHI-V4 screening of 87,354 MRSA tested. By E-test and dilution method, 148 out of 426 screening test positive isolates had vancomycin MIC of ≥2 μg/mL and 76 isolates were identified as VISA strains. An average of 60 hospitals participated in this study annually, and there was no difference in the prevalence of VISA among the participating hospitals. No VRSA isolates were detected among the isolates tested.

2. Method-specific vancomycin MIC values for VISA strains

The vancomycin MIC ranges were 1-8 μg/mL by broth microdilution and 1-4 μg/mL by agar dilution and 2-6 μg/mL by E-test, respectively. Vancomycin MICs generated by E-test were consistently one two-fold dilution higher than MICs determined by broth or agar dilution method. The 96.1% (73/76) of VISA isolates had vancomycin MICs 3 to 4 μg/mL by E-test, whereas only 38.2% (29/76) and 52.6% (40/76) of isolates had vancomycin MICs 4 μg/mL by broth microdilution and agar dilution (Table 2).

3. Characterization of VISA by molecular types and antimicrobial susceptibility

The specimen sources of VISA isolates included sputum (25%, 19/76), pus (18.4%, 14/76), wound (9.2%, 7/76), blood (7.9%, 6/76), skin and soft tissue (6.6%, 5/76), urine (1.3%, 1/76), and other normally sterile sites (Table 3). A total of 4 STs were identified among the 76 VISA isolates (Table 3). One isolate was novel type with allele profile 1-4-1-8-4-99-3 which is a single-locus variant of ST72. The

| Table 2. Comparison of vancomycin MICs determined by broth microdilution, agar dilution, and E-test |
|---|---|---|---|
| Vancomycin MIC (μg/mL) | No. of isolates (%) with MIC (μg/mL) determined by Broth microdilution | Agar dilution | E-test |
| 1 | 1 (1.3) | 1 (1.3) | 0 (0) |
| 2 | 45 (59.2) | 35 (46.1) | 1 (1.3) |
| 3 | N/A | N/A | 44 (57.9) |
| 4 | 29 (38.2) | 40 (52.6) | 29 (38.2) |
| 6 | N/A | N/A | 2 (2.6) |
| 8 | 1 (1.3) | 0 (0) | 0 (0) |

Abbreviation: N/A, not applicable.
Table 3. Molecular characteristics of VISA isolates

<table>
<thead>
<tr>
<th>MLST (n)</th>
<th>spa type (n)</th>
<th>SCCmec type (n)</th>
<th>agr type (n)</th>
<th>Specimens (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST1 (3)</td>
<td>t286 (3)</td>
<td>IV (3)</td>
<td>III (3)</td>
<td>Pus (1), urine (1), others (1)*</td>
</tr>
<tr>
<td>ST5 (52)</td>
<td>t2460 (34)</td>
<td>II (34)</td>
<td>II (34)</td>
<td>Sputum (10), pus (5), blood (4), wound (3), catheter (3), tracheal aspirate (1), fluid (1), skin and soft tissue (1), others (6)*</td>
</tr>
<tr>
<td>t002 (10)</td>
<td>II (9), IV (1)</td>
<td>II (9), I (1)</td>
<td></td>
<td>Sputum (2), skin and soft tissue (2), fluid (2), pus (1), bone (1), lung PCD aspirate (1), others (1)*</td>
</tr>
<tr>
<td>t9353 (5)</td>
<td>II (5)</td>
<td>II (5)</td>
<td></td>
<td>Sputum (3), wound (1), bronchial aspirate (1)</td>
</tr>
<tr>
<td>ST72 (12)</td>
<td>t324 (10)</td>
<td>IV (10)</td>
<td>I (9), III (1)</td>
<td>Pus (5), sputum (2), eye discharge (1), blood (1), others (1)*</td>
</tr>
<tr>
<td>t601 (2)</td>
<td>II (2)</td>
<td>II (2)</td>
<td></td>
<td>Sputum (1), pus (1)</td>
</tr>
<tr>
<td>t264 (1)</td>
<td>II (1)</td>
<td>II (1)</td>
<td></td>
<td>Blood (1)</td>
</tr>
<tr>
<td>ST239 (8)</td>
<td>t037 (8)</td>
<td>III (8)</td>
<td>I (8)</td>
<td>Skin and soft tissue (2), wound (2), pus (1), sputum (1), others (2)*</td>
</tr>
<tr>
<td>Novel (1)</td>
<td>t324 (1)</td>
<td>IV (1)</td>
<td>I (1)</td>
<td>Wound (1)</td>
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<td></td>
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</tr>
<tr>
<td>ST239-III-t037</td>
<td>8 (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST239-IV-t324</td>
<td>2 (20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST1-IV-t286</td>
<td>3 (100)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Chemoport, percutaneous nephrostomy aspiration, tip, transtracheal aspirate.
Abbreviation: PCD, percutaneous catheter drainage.

Table 4. Antimicrobial resistance profiles of VISA clones

<table>
<thead>
<tr>
<th>ST-SCCmec-spa type</th>
<th>No. of isolates</th>
<th>No. (%) of isolates with resistance to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>C</td>
</tr>
<tr>
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<tr>
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<td>10</td>
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<td>5</td>
</tr>
<tr>
<td>ST239-III-t037</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>ST239-IV-t324</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>ST1-IV-t286</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviations: E, erythromycin; C, clindamycin; G, gentamicin; O, ofloxacin; R, rifampicin; S, trimethoprim-sulfamethoxazole; T, tigecycline; D, daptomycin L, linezolid; Q, quinupristin-dalfopristin.

Most prevalent types were ST5 (68.4%, 52/76), followed by ST72 (15.8%, 12/76), ST239 (10.6%, 8/76) and ST1 (3.9%, 3/76).

Three SCCmec types (type II to IV) were identified. The predominant types were SCCmec II (67.1%, 51/76), followed by SCCmec types IV (22.4%, 17/76) and III (10.5%, 8/76).

Ten spa types were identified including t2460, t324, t002, t037, t9353, t286, t601, t264, and t001. The spa type for one isolate was not identified. The most prevalent types were t2460 (44.7%, 34/76), followed by t324 (14.5%, 11/76), t002 (13.2%, 10/76), t037 (10.5%, 8/76), and t9353 (6.6%, 5/76).

Regarding agr groups, agr group II was predominant (67.1%, 51/76), and followed by agr group I (27.6%, 21/76), and agr group III (5.3%, 4/76). We found that ST5-II-t2460, ST72-IV-t324, and ST239-III-t037 were common strains of VISA isolates. These clones displayed different antimicrobial resistance profiles depending on the genotypes (Table 4). ST5-II-t2460, ST5-II-t002, ST5-II-t9353 and ST239-III-t037 strains were similarly resistant to most of the antimicrobial agents tested except rifampicin, and tigecycline, and trimethoprim-sulfamethoxazole. ST5-II strains were more resistant to rifampicin and tigecycline than ST239-III-t037 strains. ST239-III-t037 strains more resistant to trimethoprim-sulfamethoxazole than ST5-II-t2460, ST5-II-t002 and ST5-II-t9353 strains. ST72-IV-t324 strains were susceptible to all of the antimicrobial agents tested except erythromycin and daptomycin. ST1-IV-t286 strains were susceptible to most of the...
agents tested except erythromycin and gentamicin. ST5, ST239, and ST72 strains had similarly rate of resistance to daptomycin. Among ST5-II-2460 clones with nonsusceptible to daptomycin, two strains showed nonsusceptible to tigecycline simultaneously. All of the 76 VISA isolates were susceptible to linezolid and quinupristin-dalfopristin (Table 4).

Diversilab typing of the 76 VISA isolates revealed 4 distinct clusters (P1 to 4) and 1 singleton pattern (P5) (Fig. 1). The most common clones were P4 (ST5-SCC\textit{mec}II, \(n=52\)), followed by P2 (ST72-SCC\textit{mec}IV, \(n=12\)), P3 (ST239-SCC\textit{mec}III, \(n=8\)), P1 (ST1-SCC\textit{mec}IV, \(n=3\)), and P5 (new-SCC\textit{mec}IV, \(n=1\)). When comparisons were made to the DL MRSA library, it was found that P4 and P3 were closely related to the USA100 and Brazilian cluster, respectively. P1 showed 88% similarity with the CA-MRSA USA400 cluster. P2 and P5 did not match any isolates in the Diversilab library.

**DISCUSSION**

In the present study, during a seven-year period (2007-2013) we surveyed 155,033 \textit{S. aureus} isolates to determine the prevalence of VISA. The data showed that the prevalence of MRSA was 70.5% out of total \textit{S. aureus}. Among the MRSA, the rate of VISA was 0.07%. The previous surveillance study showed that 15 (0.04%) VISA and 18 (0.04%) hVISA strains through screening of 37,856 clinical isolates collected from 2001-2006 [16]. The prevalence of VISA was slightly higher than previous study. In the ANSORP surveillance study, total of 462 MRSA isolates obtained from 2004 to 2006 were investigated, and the hVISA rate was 7.0% in Vietnam (same as South Korea), 3.2% in Thailand, and 1.9% in Taiwan [24]. The result from the ANSORP study was higher than our two surveillance data. This differences in prevalence may be due to the period of the study or sample size. Additionally, a recent systematic review summarized 91 published studies from throughout the world comparing of incidence of \textit{S. aureus} with reduced susceptibility to vancomycin (hVISA/VISA) in different study year, locations, and types of clinical samples [25]. The prevalence of hVISA was 4.68% before 2006, 5.38% in 2006-2009, and 7.01% in 2010-2014. VISA prevalence was 2.05%, 2.63%, and 7.93%, respectively. The reason for such a discrepancy in prevalence could be attributed to several factors, including differences in test strategies, geographic regions, and study populations.

In the present study, we determined the proportion of VISA isolates evolved from MRSA, yet several studies for the preva-
lence of hVISA/VISA from MSSA were reported. Hu et al. [26] reported that the proportion of hVISA among MSSA isolates was 4.1% in 2007-2010 period and increased significantly year-by-year in Northeast China. Liu and Chambers [27] found that the prevalence of hVISA was 0.05% in 1,868 MSSA isolates. Additionally, seven isolates (0.3%) of 2,300 S. aureus were MSSA-hVISA in French hospital [10] and 2 isolates of 171 S. aureus were MSSA-VISA in Iran [28]. This suggests that it is as important to detect reduced vancomycin susceptibility in MSSA isolates as in MRSA, and there is an alarming need to pay attention to the occurrence of MSSA-hVISA/VISA.

Many studies have concerned on VISA strains isolated from blood, whereas in this study, we identified VISA strains from diverse sources, such as sputum, pus, wound, blood, skin and soft tissue, and others. Therefore, we recommend that S. aureus isolates from diverse sources should be tested for reduced vancomycin susceptibility to prevent the emergence and spread of vancomycin resistance.

The results of molecular typing showed that ST5-SCCmecII (68.4%) was predominant clone, followed by ST72-SCCmecIV-t324 (15.8%), ST239-SCCmecIII-4037 (10.5%), ST1-SCCmecIV-t286 (3.9%). Interestingly, the proportion of ST239-SCCmecIII clones was reduced, while ST5-SCCmecII clones was increased compared with previous surveillance data [17]. Because of this change, ST72-SCCmecIV was the second predominant clone. In recent year, community-associated MRSA (CA-MRSA) has emerged as an important cause of infection with geographical differences among strains: ST1 (USA400) and ST8 (USA300) exist in North America, ST80 is found in Europe, ST59 is found in the Asia-Pacific region and ST30 is noted worldwide [29,30]. The ST72 which is major CA-MRSA clone in South Korea is distinct from those that have spread throughout Asia (ST30, ST59, and ST338) or internationally [30]. The Korean CA-MRSA strain has emerged in the community and has recently been spreading in healthcare settings [31,32]. The ST72 may play a role as revolving door that spread of multidrug resistant bacteria both community and hospital. Therefore, it suggests that continuously molecular surveillance of VISA is needed.

The antimicrobial susceptibility assay revealed all VISA isolates were fully susceptible to the linezolid. ST5 and ST239 strains shared the same resistance profile (erythromycin, clindamycin, gentamicin, oxolinicin) except to trimethoprim-sulfamethoxazole and tigecycline. ST239 strains more resistant to trimethoprim-sulfamethoxazole than ST5 strains (87.5% compared with 5.9%). Nonsusceptible to the tigecycline was observed only in ST5 strains. In addition, ST5 and ST239 clones differed from ST72 and ST1 clones by an overall higher frequency of drug resistance to multiple antibiotics, including erythromycin, clindamycin, oxolinicin, and rifampicin. These finding suggests that the clinical selection of antibiotic according to molecular type information is advantageous for the treatment.

In this study, we found one novel ST. It is likely that novel ST evolved from ST72 by change of single locus allele number in tpi, as these two sequence type share the same genetic background (spa type t324, SCCmec IV, pvl negative). This novel type isolated in 2010 from wound. In addition to, this strain had a teicoplanin MIC for 48 μg/mL and distinct diversilab pattern. These suggest that continuously monitoring to new clone emergence and antibiotic resistance of VISA is needed.

The identification of VISA in the clinical laboratory depends on standard susceptibility testing (agar dilution and broth microdilution) and optional E-test method. In this study, the 96.1% (73/76) of VISA isolates had vancomycin MICs 3 to 4 μg/mL by E-test, whereas only 38.2% (29/76) and 52.6% (40/76) of isolates had vancomycin MICs 4 μg/mL by broth microdilution and agar dilution. Comparing MIC values determined by these three methods, our results are similar to other studies which found that MICs of vancomycin generated by E-test are one two-fold dilution higher than MICs determined by broth microdilution or agar dilution. Prakash et al. [33] analyzed 101 strains and found that 89-98% of vancomycin MICs were between 1.5 or 2.0 μg/mL by E-test, but only 3-12% were 2.0 μg/mL when determined by broth microdilution or agar dilution. Sader et al. [34] analyzed 1800 strain samples and 90.4% of them exhibited vancomycin MICs 1.5 or 2.0 by E-test, whereas 96.9% were ≤ 1.0 μg/mL by microdilution. It is suggested that a single dilution difference in the vancomycin MIC in the range of 2 to 4 μg/mL would have significant to diagnosis of VISA. Further studies are needed to determine which method better reflects the VISA phenotype. Additionally, the subpopulations comprising the hVISA phenotype that typically low frequencies (≤10^{-2}-10^{-4}) are not likely to detect in test method using relatively low inoculums of 10^5 CFU as is used with the dilution methods. The standard method to detect hVISA, the PAP-AUC method, is time-consuming, labor-intensive, and not routinely available and thus has its limitation. Although different screening strategies are existed to detect hVISA including macromethod E-test, E-test GRD, and BHI screening agar containing 3-4 μg/mL of vancomycin, all of these methods still required extra work, man power, and training. A simpler, but yet more accurate, diag-
nosing method should be developed.

In summary, this study conducted in South Koera from 2007 to 2013 shows that 76 VISA strains were observed, but no VRSA isolates were detected. In addition, we observed that the major clones are ST5-II-2460, ST5-II-2002, ST72-IV-2324, and ST239-III-4037. Currently, CA-MRSA strains widely spread community and hospitals. The increase of various CA-MRSA and HA-MRSA clones is thought to be continuing. This will also lead to abuse of glycopeptides cause an increase of VISA and VRSA. Although VRSA is still rare, it is likely to emergence of VRSA in the future considering that most of the identified VRSA acquired the vancomycin resistance gene cluster vanA from vancomycin-resistant Enterococcus, which widespread in hospitals of South Korea. Therefore, we recommend ongoing monitoring to demonstrate VRSA occurrence, trends in VISA prevalence, epidemic clonal shift, new clone emergence, and antimicrobial resistance pattern.

ACKNOWLEDGMENTS

We thank those who provided bacterial strains to the Nationwide Laboratory Surveillance Program for VISA/VRSA in Korea. This study was supported by a grant from the Korea Centers for Disease Control and Prevention (2016-NI44001-00). The authors declare no conflicts of interest.

REFERENCES


=국문초록=

2007년부터 2013년까지 국내에서 분리된 반코마이신 중등도 내성 황색포도알균의 특성

질병관리본부 국립보건연구원 약제내성과
김정욱, 강기수, 유재일, 김화수, 이영선, 유재연, 이광준, 박찬, 김일환


방법: 반코마이신 4 μg/mL가 함유된 Brain heart infusion agar 선별배지에서 양성인 분리주를 대상으로 하였으며 반코마이신에 대한 MIC는 한천배지 희석법, 액체배지 미량희석법 및 E-test 시험법으로 확인하였다. 3가지 시험법 중 1가지 이상에서 MIC >2 μg/mL이면 VISA로 확인하였다. VISA 분리주의 분자생물학적 특성을 확인하기 위해 staphylococcal cassette chromosome mec typing, spa typing, accessory gene regulator typing를 실시하였고, VISA간의 분자역학적 유연관계를 분석하기 위하여 7개의 house keeping gene (arcC, arOE, glpF, gmk, pta, tpi, yqiL)을 이용하여 multilocus sequence type를 분류하고 repetitive sequence-based PCR을 이용한 diversilab analysis를 실시하였다.

결과: 총 109,345 MRSA 중 426주가 선별검사 양성으로, 76주가 VISA로 확인되었으며 VRSA는 확인되지 않았다. 분리된 VISA는 6가지 유전형으로 나타났고, ST5-II-t2460 (44.7%), ST72-IV-t324 (15.8%), ST239-III-t037 (10.5%)가 가장 많이 확인되었다. 특히, community-associated MRSA 대표적 유형인 ST72-IV가 증가하고 있어 국내 healthcare-associated MRSA 분자역학이 변하고 있음을 확인하였다. 항균제에 감수성 시험결과 모든 VISA 분리주는 linezolid와 quinupristin-dalfopristin에 감수성을 나타냈고, ST239-III-t037 유전형을 갖는 VISA 분리주는 다른 유전형의 VISA 보다 trimethoprim-sulfamethoxazole에 대한 내성률이 높았으며, ST72-IV-t324 분리주는 erythromycin과 daptomycin이외의 항균제 대해 모두 감수성을 보였다.


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