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NOTE

Emergence of Vancomycin-Intermediate *Staphylococcus aureus* from Predominant Methicillin-Resistant *S. aureus* Clones in a Korean Hospital

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The genetic and epidemiological features of four vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates obtained from a Korean hospital were evaluated in this study. The VISA isolates were genotyped as sequence type (ST) 5-staphylococcal cassette chromosome *mec* (SCC*mec*) II variant (n=2) and ST239-SCC*mec* III (n=2), which were derived from the predominant methicillin-resistant *S. aureus* (MRSA) clones in Korean hospitals. One VISA isolate was acquired during vancomycin treatment, whereas three VISA isolates were obtained from the patients who had not previously been exposed to glycopeptides. As VISA is likely to arise from the predominant MRSA clones and may then possibly spread between patients, the emergence of VISA should be monitored with great care in hospitals.

***Keywords*:** MRSA, VISA, sequence type, SCC*mec*

The high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has led to the frequent prescription of glycopeptides in clinical settings. The use of vancomycin has resulted in the emergence of MRSA with reduced vancomycin susceptibility (Hiramatsu *et al*., 1997). Vancomycin-resistant *S. aureus* (VRSA) strains possessing the enterococcal vancomycin-resistant *vanA* gene have been quite rare until now, whereas vancomycin-intermediate *S. aureus* (VISA) strains with minimal inhibitory concentrations (MICs) between 4-8 μg/ml and heterogeneous VISA (hVISA) strains that are susceptible to vancomycin, but contain a subpopulation of bacteria with reduced susceptibility to vancomycin (MICs≥4 μg/ml) have been increasingly reported worldwide (CLSI, 2006; Neugen *et al*., 2009). As VISA/hVISA strains are potentially associated with the failure of vancomycin treatment, the emergence of VISA/hVISA is a matter of great concern in hospitals. The frequency of MRSA among *S. aureus* isolates is currently estimated to be in excess of 70% in Korean hospitals (Kim *et al*., 2003; Cha *et al*., 2005), but true VISA infections have been reported only rarely in Korea (Kim *et al*., 2000, 2006). In this study, we screened VISA among MRSA isolates, and determined their genetic and epidemiological features.

A total of 448 MRSA isolates were collected between 2001 and 2007: 331 hospital-acquired MRSA isolates were obtained from Kyungpook National University Hospital in Daegu, Korea between 2001 and 2005 and 117 community-acquired MRSA isolates were obtained from patients with no history of hospitalization or admission to a long-term care facility within one year in the primary clinics in Daegu, Korea between 2005 and 2007. *S. aureus* was identified using an API-Staph kit (bioMérieux, France). MRSA was initially screened by phenotypic resistance to oxacillin according to the CLSI (2006) and genotypically confirmed by polymerase chain reaction (PCR) amplification specific to the *nuc* and *mecA* genes (Aires de Sousa *et al*., 2000; Pérez-Roth *et al*., 2001). All MRSA isolates were assessed for growth on brain-heart infusion agar (Difco, USA) containing 4 μg/ml of vancomycin (Sigma, USA), using 10 μl of a bacterial suspension adjusted with densities equivalent to a 0.5 McFarland turbidity standard in saline. The plates were incubated for 48 h at 35°C. The isolates were identified as potential VISA if confluent growth was observed within 24 h. The potential VISA isolates were further tested to determine the MICs of vancomycin by the agar dilution method in accordance with the guidelines established by the CLSI (2006). *S. aureus* Mu50 and AMC11094 for VISA strains, Mu3 for a hVISA strain, and ATCC 29213 for a vancomycin-susceptible strain were utilized as controls. A population analysis profile (PAP) of the screening-positive isolates was conducted to confirm VISA as compared with Mu50 (Walsh *et al*., 2001).

PCRs were conducted in order to detect *vanA*, *vanB*, *vanC1*, and *vanC2/3* (Oh *et al*., 2007). The MICs of the antimicrobial agents, which included oxacillin, teicoplanin, vancomycin, gentamicin, tobramycin, chloramphenicol, erythromycin, clindamycin, tetracycline, rifampin, and ciprofloxacin, were determined by the agar dilution method according to the guidelines of the CLSI (2006). To determine the genotypes of the VISA isolates, staphylococcal cassette chromosome *mec* (SCC*mec*) type (Oliveira and Lencastre, 2002), multilocus sequence typing (MLST) (Enright *et al*., 2000), *spaA* typing (Shopsin *et al*., 1999), and pulsed-field gel electrophoresis

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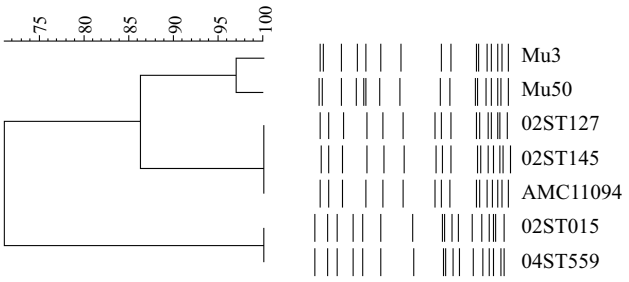

Fig. 1. Pulsed-field gel electrophoresis patterns of 5 Korean VISA isolates. The dendrogram is based on a cluster analysis of the unweighted-pair group method with average linkages. Mu50 and Mu3 are Japanese VISA and hVISA strains, respectively.

(PFGE) (Murchan *et al*., 2003) were performed. The medical records of the patients with VISA were reviewed.

Among the 448 MRSA isolates tested, 55 (12.3%) were grown with variable colonies on screening agar plates containing 4 μg/ml of vancomycin. Four out of the 55 screening-positive MRSA isolates were identified as VISA based on the PAP and MICs to vancomycin. Four VISA isolates, three from 2002 and one from 2004, evidenced confluent growth on the screening agar plate, and their MICs to vancomycin were between 4-8 μg/ml (Table 1). All VISA isolates originated from hospital-acquired MRSA. No VRSA was detected on the basis of the MICs to vancomycin and PCR amplification of *van* genes.

Four VISA isolates were resistant to clindamycin, erythromycin, aminoglycosides, ciprofloxacin, and tetracycline, but were susceptible to teicoplanin, rifampin, and chloramphenicol. Based on the results of molecular typing, four VISA isolates were classified into two genotypes: 02ST127 and 02ST145 were sequence type (ST) 5, SCC*mec* II variant, *spa* type of TJMBBMDMGMK, and arbitrarily PFGE pattern A (Fig. 1). The remaining two VISA isolates, 02ST015 and 04ST559, were ST239, SCC*mec* III, *spa* type of WGKAOMQ, and arbitrarily PFGE pattern B. Interestingly, the genotype of the first Korean VISA strain, AMC11094 from Seoul, Korea in 1997 (Kim *et al*., 2000), was identical to that of 02ST127 and 02ST145 isolates in this study (Table 1). Among the four patients harboring VISA, one patient with 04ST559 was diagnosed with soft tissue infection, whereas 02ST127 and 02ST145 in two patients and 02ST015 in one patient were identified as colonizers and a contaminant, respectively. The three patients colonized or contaminated with VISA had not been previously exposed to any glycopeptide, whereas 04ST559 was obtained from a patient that had received prolonged vancomycin treatment for >60 days. The patient colonized with 02ST015 died as the result of the underlying disease, whereas the remaining three patients survived.

Since the first emergence of the VISA strain in Korea in 1997 (Kim *et al*., 2000), two additional VISA infections have been reported in Korean hospitals (Kim *et al*., 2006; Hong *et al*., 2008). However, the genetic characteristics of the Korean VISA isolates have yet to be clearly determined. The present study demonstrated that four VISA isolates originated from two predominant hospital-acquired MRSA clones, ST5 and ST239, in Korea. Three VISA isolates, including the first Korean VISA strain, originated from ST5, a pandemic MRSA clone. The genotype of these VISA isolates was identical to that of the first VISA isolate, Mu50 from Japan, with regard to ST5 and SCC*mec* II (Enright, 2003), but the PFGE pattern differed between the Korean and Japanese strains of ST5 VISA (Fig. 1). Two VISA isolates, 02ST015 and 04ST559, originated from the ST239 clone, which was the most common MRSA clone in the study hospital between 2001 and 2004 (Cha *et al*., 2005). Although the ST239-VISA-III was recently reported in Taiwan (Wang *et al*., 2009), the emergence of ST239-VISA-III was relatively uncommon. The emergence of the VISA isolate 04ST559 was associated with prolonged vancomycin exposure, whereas the remaining three VISA isolates were obtained from the patients who had not been previously exposed to glycopeptides, suggesting the acquisition of VISA isolates from other patients or the general hospital environment. Although the genetic and epidemiological features of VISA isolates from other countries remain to be clarified, our preliminary findings indicate that VISA is likely to arise from the predominant hospital-acquired MRSA clones in the hospitals, and then possibly spread to other patients.

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Table 1. Bacteriological characteristics of 5 Korean VISA isolates

Isolate No.	Isolation year	Patient sex/age	Isolation ward	Specimen	MIC (μg/ml) to vancomycin	Sequence type	SCC*mec* type	*spa* type	PFGE pattern
02ST127	2002	Male/42	Internal medicine	Sputum	8	5	II variant	TJMBBMDMGMK	A
02ST145	2002	Female/65	Rheumatology	Vaginal swab	8	5	II variant	TJMBBMDMGMK	A
02ST015	2002	Male/77	General surgery	Blood	4	239 slv[a]	III	WGKAOMQ	B
04ST559	2004	Female/56	Plastic surgery	Wound	4	239	III	WGKAOMQ	B
AMC11094[b]	1997	Male/45	Hematology/oncology	Blood	8	5	II variant	TJMBBMDMGMK	A

[a] slv, single locus variant in *pta* gene
[b] This is the first Korean VISA isolate from Seoul, Korea in 1997 (Kim *et al*., 2000)

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