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Original Article

Molecular epidemiology and phenotypes of invasive methicillin-resistant vancomycin-intermediate *Staphylococcus aureus* in Taiwan

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Abstract *Background:* Patients with invasive infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), especially those with an elevated minimal inhibitory concentration (MIC) of vancomycin (VA), are likely to have treatment failure and poor outcomes. The aim of this study was to delineate and correlate the genotypes and phenotypes of clinical VA-intermediate *S. aureus* (VISA) from invasive infections in Taiwan.
Methods: Between 2006 and 2010, a total of 670 non-duplicate MRSA isolates were collected from patients with invasive infections, mostly from blood, as part of a nationwide antimicrobial surveillance program named Tigecycline *in vitro* Surveillance in Taiwan. Among them, 10 (1.5%) VISA (VA MIC = 4 mg/L) isolates were identified. Molecular typing with staphylococcal cassette chromosome *mec* (SCC*mec*), multilocus sequence typing, staphylococcal protein A (*spa*), *mec*-associated hypervariable region (*dru*), accessory gene regulator (*agr*), and pulse-field gel electrophoresis, and phenotypic analysis including antibiotic susceptibility testing,

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gene encoding Panton-Valentine leukocidin (*pvl*), and superantigenic toxin profiles, were analyzed.
Results: All but one isolate was defined as molecular health-care-associated MRSA: 6 as SCC*mec*III-ST239-*spa* t037-*agr*I-*dru*7 (1 isolate) and *dru*14 (5 isolates), 2 as SCC*mec*II-ST5-*spa* t586-*agr*II-*dru*4, and one as SCC*mec*II-ST89-*spa* t3520-*agr*III-*dru*7. One isolate was defined as SCC*mec*IV-ST59-*spa* t437-*agr*I-*dru*8, which was categorized as molecular community-associated MRSA. Five pulsotypes were identified; only one had a positive D-test and 3 were insusceptible to daptomycin (MIC ≧1 mg/L). Five isolates possessed *sea-selk-selq*, among them 4 belonged to SCC*mec*III-ST239-*spa* t037-*agr*I.
Conclusion: In this study, VISA was rarely isolated from invasive MRSA infections, and most cases harbored limited genotypes and corresponding phenotypes.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), first identified in England in 1961, has rapidly disseminated worldwide, with several predominant clones circulating in health-care settings and communities.[1,2] Invasive infections, such as bacteremia and necrotizing pneumonia, which are caused by MRSA strains with a vancomycin (VA) minimal inhibitory concentration (MIC) exceeding 2 mg/L, are predisposed to treatment failure and cause notable mortality in patients treated with glycopeptides.[3,4] VA-intermediate *S. aureus* (VISA) was first identified and named as Mu50 in 1996 by Hiramatsu et al.[5] It was initially defined as having a VA MIC of 8—16 mg/L but then redefined as having a VA MIC of 4 to 8 mg/L in 2006.[6] VISA is not frequently isolated among MRSA by surveillance studies[7—9] and is often associated with previous exposure to glycopeptides.[10] The current study aimed to describe VISA prevalence in Taiwan via a 5-year nationwide surveillance study, and to delineate and correlate the VISA genotypes and phenotypes.

Materials and methods

Identification of bacterial strains and antimicrobial susceptibility testing

A nationwide, multicenter, prospective surveillance study, named Tigecycline *in vitro* Surveillance in Taiwan (TIST), was conducted in 22 medical centers and regional hospitals from 2006 to 2010. The study protocol was reviewed and approved by the Institutional Review Board of Tung's MetroHarbor General Hospital (TMHGH no. 102091). Clinical MRSA isolates were cultivated from sterile sites on patients diagnosed with invasive infections. All isolates were initially identified at the respective hospitals and then reconfirmed by the central laboratory at Taiwan National University Hospital.[11] In brief, bacterial identification of *S. aureus* was achieved using standard techniques and a phenotype of methicillin resistance was ascertained after screening with a 30-μg cefoxitin disc on Műeller-Hinton agar (MHA), according to the Clinical Laboratory Standards Institute (CLSI) protocol.[12] MRSA was confirmed by identifying *mecA* with polymerase chain reaction (PCR).[13] Antimicrobial susceptibility testing of *S. aureus* was performed by disc diffusion test with various antibiotics (erythromycin, clindamycin, tetracycline, oxacillin, VA, teicoplanin, fusidic acid, and tigecycline) and the D-test for the detection of inducible macrolide-lincosamide-streptogramin B resistance (MLSBi) were carried out according to CLSI.[14] Insusceptibility or resistance to ≥3 classes of antimicrobials was assigned as multidrug resistance. The MICs of *S. aureus* to VA were determined by agar dilution according to CLSI, and VISA was defined as isolates with a VA MIC of 4 to 8 mg/L.[14] Susceptibility testing of VISA to daptomycin and VA was performed by Etest.

Molecular-typing and phenotyping methods

Pulse-field gel electrophoresis (PFGE)

Genomic DNA was extracted from each isolate using the Genomic DNA Mini Kit (Geneaid, Taiwan) and digested with *SmaI* restriction enzymes (Promega Corp., Madison, Wis.). PFGE was then performed using a contour-clamped homogeneous electric field apparatus (DR-III, Bio-Rad, Hercules, CA).[15] Dendrograms were constructed using the Dice coefficient and the unweighted pair group method with an arithmetic average. Isolates were assigned as one pulsotype if the dendrogram of PFGE band patterns had estimates of percent similarity ≥70%.

SCC*mec* classification

Genomic DNA from MRSA as confirmed by the detection of *mecA*, was used as a template and the gene type encoding the cassette chromosome recombinase (*ccr*) complex and the *mec* gene complex were determined by multiplex PCR (M-PCR).[16] MRSA SCC*mec* types I to V were determined by comparing the M-PCR banding patterns of the isolates to those from the following reference strains: ATCC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), MW2 (SCC*mec* type IVa), WIS (SCC*mec* type V), and TSGH-17 (SCC*mec* type V_T).

Multilocus sequencing typing (MLST)

The following seven housekeeping genes of each *S. aureus* isolate were amplified and sequenced, as described

previously:[17]: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*) genes. The allelic number of each gene and the allelic profile of each isolate which make up the sequence type (ST), were determined by comparing the sequences to those of known alleles in the *S. aureus* MLST database (http://saureus.mlst.net/).

Polymorphism of the X region encoding protein A (*spa* typing)

The X region of the *spa* gene contains variable numbers of 21- to 27-base pair (bp) repeats, among which 24-bp repeats are most common.[18] The X region of each MRSA was amplified by PCR.[19] and the amplified products were then sequenced and analyzed by the Ridom StaphType software program (version 1.4; Ridom, GmbH, Wurzburg, Germany [http://spa.ridom.de/index.shtml]), which automatically determined the repeat profile and the *spa* type of each isolate.[20]

Polymorphism of the *mec*-associated hypervariable region

Amplification of the hypervariable region of SCC*mec*, which contains variable numbers of 40-bp direct repeat units (*dru*), was performed by PCR with orf145 and IS431*mec* primers.[21] The *dru* copy number in each MRSA was determined by the size of the amplified fragment according to the following equation: *dru* copy no. = (size of PCR product - 171) ÷ 40.[21]

Accessory gene regulator (*agr*) typing

The *agr* gene of each *S. aureus* isolate was amplified with specialized primers (Pan, agr1, agr2, agr3, and agr4).[22] These primers were designed to amplify a 441-bp DNA fragment from *agr* group I, a 575-bp fragment from *agr* group II, a 323-bp fragment from *agr* group III, and a 659-bp fragment from *agr* group IV strains.

Gene encoding Panton-Valentine leukocidin (*pvl*)

Amplification of the *pvl* gene was done using paired primers: luk-PV-1 and luk-PV-2.[23] The reference strains TSGH-17 and 85/2082 were used as positive and negative controls, respectively.

Superantigenic toxin gene profile

Each *S. aureus* isolate was examined for the presence of 18 genes encoding superantigenic toxins from *sea* to *selr* and *tsst-1* via four multiplex PCR reactions (M-PCR), with *femA* and *femB* as positive controls.[24] The toxin gene profile of each isolate was determined by toxin gene assembly.

Population analysis profiling (PAP)

Isolates assigned as VISA underwent population analysis to identify subpopulations of cells that were susceptible or resistant to VA.[25] Briefly, 50 μl of the starting cell suspension of each isolate and its serial dilutions was spread on BHI agar plates containing 1 to 10 μg/ml VA in 1-μg/ml increments. The starting cell suspension was prepared by diluting the fresh bacterial culture in BHI broth to an optical density of 580 nm (OD580) of 0.3. The plates were incubated at 37 °C for 48 h, and the number of VA-resistant cells in each of the 50-μl aliquots was plotted semi-logarithmically. Heterogenous VA-intermediate *S. aureus* (hVISA) was defined as a population analysis profiling—area under the curve (PAP-AUC) ratio of 0.9 and 1.3 compared with the Mu3 reference strain.[25]

Results

MRSA and VISA collection, identification, and antimicrobial susceptibility testing

During the 5-year (2006—2010) study period, a total of 670 non-duplicate *mec*A-positive MRSA isolates, which were resistant to cefoxitin as determined by the 30-μg cefoxitin disk diffusion test, were collected from patients with invasive infections, among which most (628, 93.7%) were due to bacteremia.[26] All except one had a VA MIC ≥1 mg/L, range 0.5—4 mg/L. Among them, only 10 (1.5%) isolates, all cultivated from blood, were defined as VISA (VA MIC = 4 mg/L) by agar dilution. The VISA isolates were equally isolated from each year except in 2008, when none was isolated.

The antimicrobial susceptibility test results of the 10 VISA isolates are listed in Table 1. Disk diffusion with seven antibiotic discs was applied on MHA, which revealed that all VISA isolates were multidrug resistance (i.e., resistant to ≥3 classes of antibiotics). All VISA isolates defined by agar dilution were susceptible by disk diffusion to vancomycin and teicoplanin but resistant to oxacillin and erythromycin, and most were resistant or insusceptible to tetracycline (80%) and clindamycin (70%). Only one strain (H13-303), which was susceptible to clindamycin by disk diffusion, was found to have a phenotype of inducible clindamycin resistance (i.e., a positive D test). Only one strain (H09-113) (10%) was revealed to be insusceptible to tigecycline. The Etest showed that all 10 isolates harbored an increased VA MIC from 2 to 4 mg/L, and 3 (H06-318, H06-322, and H07-036) were revealed to have a daptomycin insusceptible phenotype (≥1 mg/L). With agar dilution, all 10 isolates showed visible bacterial growth on MHA with VA 2 mg/L but no growth with 3 mg/L, and they were assigned as VISA with a VA MIC of 4 mg/L according to CLSI.[12]

Results of various molecular typing methods

Table 2 shows the results of various molecular typing methods for the 10 VISA isolates. Six isolates (60%) were assigned as SCC*mec*III-ST239-*spa* t037 (or t3524)-*agr*I-*dru*7 (1 isolate), and *dru*14 (5 isolates); 2 were SCC*mec*II-ST5-*spa* t586-*agr*II-*dru*4, and each one for SCC*mec*II-ST89-*spa* t3520-*agr*III-*dru* 7 and SCC*mec*IV-ST59-*spa* t437-*agr*I-*dru*8. Fig. 1 shows the PFGE patterns of the 10 VISA blood isolates and the 6 consecutive VISA isolates (96b, 97b, 98p, 98b, 99p, and 99b) with a VA MIC of 4 mg/L from one patient diagnosed with MRSA vertebral osteomyelitis. There were 5 pulsotypes noted among the 10 VISA blood isolates and 6 VISA isolates from the soft tissue. Two VISA blood isolates

Table 1 Phenotypes including antimicrobial susceptibility and superantigenic toxin gene profiles of the 10 VISA blood isolates with a VA MIC of 4 mg/L.

Strain No.	Antimicrobial susceptibility test (disc diffusion)							MIC (mg/L)			D-test	*pvl*	Superantigenic toxin gene profiles
								agar dilution	Etest				
	CLI	ERY	TET	VA	TEI	OXA	TIG	VA	VA	DPC			
H05-033	S	R	R	S	S	R	S	4	3	0.19	—	—	*sea-selk-selq*
H06-318	I	R	I	S	S	R	S	4	4	2	—	—	*sec-seg-sei-sell-selm-seln-selo-selq-tst1*
H06-322	S	R	S	S	S	R	S	4	4	1.5	—	—	*sec-seg-sei-sell-selm-seln-selo-selq-tst1*
H07-036	I	R	R	S	S	R	S	4	4	1	-(HD)	—	*sea-selk-selq*
H09-113	I	R	R	S	S	R	I	4	3	0.38	—	—	*sea-selk-selq*
H13-303	S	R	R	S	S	R	S	4	3	0.5	+ (D+)	—	*seb-selk-seln-selp-selq*
H13-315	I	R	S	S	S	R	S	4	2	0.25	-(HD)	—	*selm-seln-selo*
H15-105	R	R	R	S	S	R	S	4	3	0.75	—	—	*sea-selk-selq*
H15-157	I	R	R	S	S	R	S	4	3	0.38	-(HD)	—	*selk-selq*
H20-083	R	R	R	S	S	R	S	4	3	0.75	—	—	*sea-selk-selq*

Abbreviations: VISA, vancomycin-intermediate *Staphylococcus aureus*; CLI, clindamycin; ERY, erythromycin; VA, vancomycin; TEI, teicoplanin; OXA, oxacillin; TIG, tigecycline; S, susceptible; I, intermediate; R, resistant; D-test, inducible clindamycin resistance test (MLS_B); *pvl*, gene encoding Panton-Valentine leukocidin; HD, hazard D phenotype.

Table 2 Molecular typing results of the 10 VISA[a] blood isolates.

Strain No.	Year of isolation	SCC*mec*[1]	MLST	MLST allelic profiles	*spa*	*spa* allelic profiles	*agr*	*dru*
H05-033	2008	III	239	2-3-1-1-4-4-3	t037	15-12-16-2-25-17-24	I	14
H06-318	2007	II	5	1-4-1-4-12-1-10	t586	26—16	II	4
H06-322	2007	II	5	1-4-1-4-12-1-10	t586	26—16	II	4
H07-036	2008	III	239	2-3-1-1-4-4-3	t037	15-12-16-2-25-17-24	I	14
H09-113	2007	III	239	2-3-1-1-4-4-3	t3519	15-12-16-2-25-17-17-24	I	14
H13-303	2007	IV	59	19-23-15-2-19-20-15	t437	4-20-17-20-17-25-34	I	8
H13-315	2007	II	89	1-26-28-18-18-33-50	t3520	49-13-23-5-34	III	7
H15-105	2006	III	239	2-3-1-1-4-4-3	t037	15-12-16-2-25-17-24	I	14
H15-157	2007	III	239	2-3-1-1-4-4-3	t037	15-12-16-2-25-17-24	I	11
H20-083	2006	III	239	2-3-1-1-4-4-3	t037	15-12-16-2-25-17-24	I	7

Abbreviation: VISA, vancomycin-intermediate *Staphylococcus aureus*.

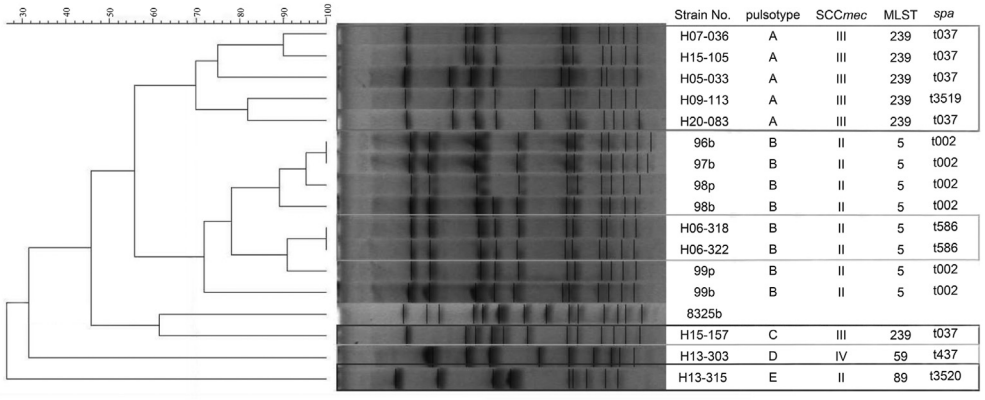

Fig. 1. Pulsotypes and corresponding molecular types (SCC*mec*/MLST/*spa*) of the 10 vancomycin-intermediate *Staphylococcus aureus* (VISA) blood isolates with a VA MIC of 4 mg/L from TIST and 6 consecutive MRSA isolates (96b, 97b, 98p, 98b, 99p, and 99b) with a VA MIC of 4 mg/L from one patient with vertebral osteomyelitis (b, blood culture; p, pus culture).

(H06-3181 and H06-3221) had similar PFGE patterns to the 6 VISA isolates from one patient with vertebral osteomyelitis, which were collected in sequence from 2007 to 2010.

Other phenotypes and their corresponding molecular types

The phenotypes of the 10 VISA isolates are listed in Table 1. No VISA isolate harbored the gene encoding Panton-Valentine leukocidin (*pvl*). The superantigenic toxin gene profiles showed that 5 isolates (50%) possessed *sea-selk-selq*, which belongs to molecular type SCC*mec*III-ST239-*spa* t037 (or t3524)-*agr*I-*dru*7. There were 2 (H06-3181 and H06-3221) with *sec-seg-sei-sell-selm-seln-selo-selq-tst1*, both of which were assigned as SCC*mec*II-ST5-*spa* t586-*agr*II-*dru*4. The other toxin gene profiles were listed as follows: one (H15-157) harbored *selk-selq*, which was assigned as SCC*mec*III-ST239-*spa* t037-*agr*I-*dru*11; one (H13-315) harbored *selm-seln-selo*, which was assigned as SCC*mec*II-ST89-*spa* t3520-*agr*III-*dru7*; and one (H13-303) harbored *seb-selk-seln-selp-selq*, which was assigned as SCC*mec*IV-ST59-*spa* t437-*agr*I-*dru*8.

All 10 *mecA* + MRSA isolates were able to grow on MHA with a VA concentration of 2 mg/L, but no growth was seen on MHA with a VA concentration of 3 or 4 mg/L. According to the CLSI definition, these 10 MRSA isolates were defined as having a VA MIC of 4 mg/L and were classified as VISA. The population analysis of the 10 VISA blood isolates and 3 reference strains (ATCC 10442, VSSA; Mu3, hVISA; Mu50, VISA) is shown in Fig. 2. After 48 h incubation, all were CLSI-defined as VISA but one strain (H06-322), ATCC 10442, and Mu3 were completely inhibited at a VA concentration of 4 mg/L. Strain H06-322 and Mu50 were inhibited at a VA concentration of 5 and 6 mg/L, respectively. According to the PAP-AUC criteria by Wootton et al.,[25] all but two behaved like hVISA with heterogeneous resistance. The PAP-AUC ratios of H06-322 and H15-157 were 1.37 and 0.74, respectively.

Discussion

MRSA, a leading clinical pathogen, has successfully colonized and transmitted between communities and health-care settings for six decades, and the epidemiology of antibiotic resistance surveillance for MRSA has always been a concerning worldwide issue.[2,10] Treatment failure and an increased mortality rate have been reported in patients with invasive MRSA infections, such as bacteremia and endocarditis with a VA MIC exceeding 1 mg/L.[3,4,27] VISA was first reported in Japan in 1997 as having a VA MIC of 8 mg/L,[5] and has now been redefined as having a VA MIC of 4 to 8 mg/L by broth dilution or agar dilution methods according to CLSI.[14] We found that only 10 (1.5%) of the 670 non-duplicate *mecA* + MRSA isolates from sterile sites were defined as VISA, which was comparable to previous studies with a range from 0.2% to 6.1%.[7,8,28] Our epidemiologic data revealed similar findings to previous studies, as VISA isolates were not frequently isolated from patients with invasive MRSA infections.

According to the antimicrobial disc diffusion tests, all 10 VISA isolates belonged to multidrug-resistant strains with resistance to at least 3 classes of antibiotics tested. A previous multicenter surveillance study showed that VISA accounted for 2.7% of MRSA cases, and harbored phenotypes with multiresistance to ciprofloxacin, clindamycin, gentamicin, rifampin, and TMP/SXZ.[29] In the current study, there was some inconsistency in the VA MIC results between the agar dilution and the Etest. Only 3 VISA isolates had a VA MIC of 4 mg/L while the other 7 had a VA MIC less than 4 mg/L, as determined by the Etest. The VA MIC

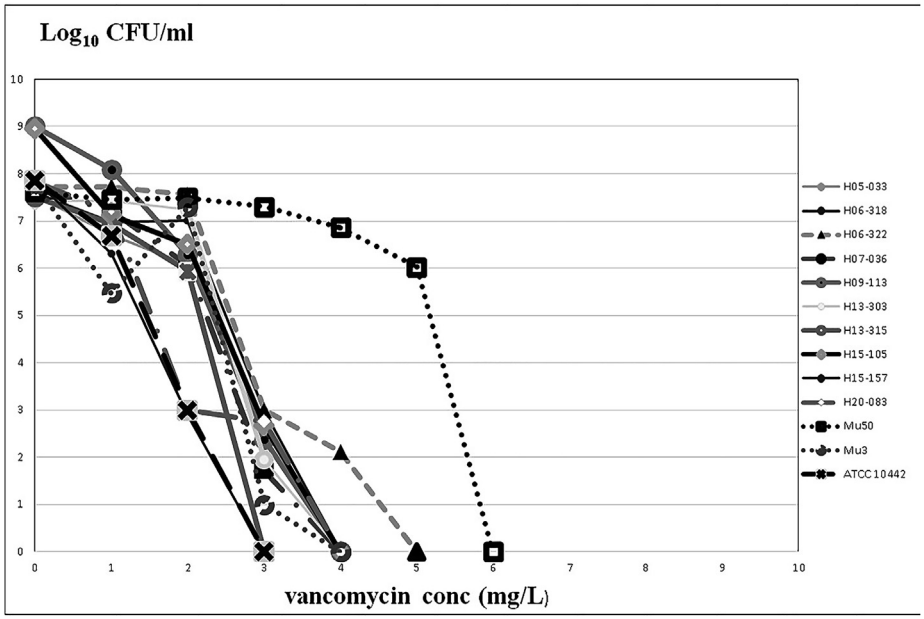

Fig. 2. Population analysis of the 10 vancomycin-intermediate *Staphylococcus aureus* (VISA) blood isolates with a VA MIC of 4 mg/L (strain no. ATCC 10442, MRSA with SCC*mec*I and VSSA; Mu3, hVISA; Mu50, VISA).

results by Etest were generally equal to or below those of the dilution tests, which was comparable with one previous study.[7]

The D-test, used to detect inducible resistance to clindamycin (MLSBi), was only found to be positive in 1 (10%, H13-303) of the 10 VISA isolates, which belonged to SCC*mec*IV-ST59-*spa* t037-*agr*II-*dru*8. We previously reported that only 8.3% of the 157 MRSA blood isolates harbored a positive D-test phenotype, which was not associated with a particular molecular type.[30] After adjusting for the results of the D-test, only 2 (20%) VISA isolates were susceptible to clindamycin. This result reveals that clindamycin is not appropriate for empirical treatment of invasive VISA infections, and that MRSA with a phenotype of clindamycin susceptibility by disk diffusion should undergo a D-test for further confirmation.

Daptomycin has been used as an alternative treatment for invasive MRSA infections, such as bacteremia and right side infective endocarditis.[31] Better outcomes are observed with daptomycin treatment for those with MRSA bacteremia with a VA MIC >1 mg/L.[32] We determined that 3 (30%) VISA (H06-318, H06-322, and H07-036) harbored a phenotype of insusceptibility to daptomycin (DAP MIC ≥ 1 mg/L). The authors previously demonstrated that there was a moderate degree of correlation between DAP MIC and VA MIC,[30] and that VISA was less susceptible to daptomycin.[29] The authors recommended that daptomycin insusceptibility should be considered and DAP MIC should be performed while treating patients with invasive VISA infections.

S. aureus produces lots of toxins, including Panton-Valentine leukocidin (PVL), staphylococcal enterotoxins (SEs), and toxic shock syndrome toxin-1 (TSST-1). We did not find any VISA harboring genes encoding PVL, and there were 5 VISA harboring toxin profiles of *sea-selk-selq*, which all belonged to molecular type of SCC*mec*III-ST239-*spa* t037 (or t3519)-*agr* group I. This finding is compatible with previous literature, in which 95 blood MRSA and VSSA isolates harbored the same molecular type and toxin profile as our 5 VISA.[30] Based on the evidence from the molecular and superantigenic toxin profiles, we propose that VISA evolves from VSSA under certain conditions (e.g., glycopeptides exposure).

Certain HA-MRSA clones, such as SCC*mec*II-ST5 (CC5) and SCC*mec*III-ST239 (CC8), and CA-MRSA clones, including SCC*mec*IV and V-ST59 (CC59), have been previously found in a 2002 nationwide antimicrobial resistance surveillance program named Taiwan Surveillance of Antimicrobial Resistance (TSAR)[28] and in the 2003 Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) program.[30] Most (9, 90%) of the VISA in the current study were defined as HA-MRSA, including SCC*mec*III-ST239-*spa* t037 or t3524-*agr*I (6, 60%), SCC*mec*II-ST5 or ST89-*spa* t586-*agr*II (1, 10%), and SCC*mec*II-ST89-*spa* t3520-*agr*III (1, 10%). Our molecular typing results confirmed the hypothesis that those VISA were indigenous MRSA clones, which was supported by findings from Khatib et al. in the United States and Hu et al. in Northeast China, where VISA was significantly associated with SCC*mec*II and *agr* group II.[7,33] The authors considered that VISA mainly originated from MRSA following exposure to glycopeptides instead of inherent VA resistance.[7,10,34] This could explain why VISA accounts for such a small number of MRSA isolates and does not disseminate widely between countries.[10]

PFGE has been used for clone identification and outbreak investigation.[15] We found 5 pulsotypes in these 10 VISA, of which pulsotype A contained 5 VISA (H05-033, H07-036, H09-113, H15-105, and H20-083) collected from different hospitals. Interestingly, these 5 VISA with the same pulsotype also harbor almost the same molecular types [SCC*mec*III-ST239-*spa* t037 (or t3519)-*agr* group I]. Another finding was that two VISA from blood (H06-318 and H06-322 from one medical center in northern Taiwan) and a series of 6 VISA from one patient with vertebral osteomyelitis in our hospital (one medical center in central Taiwan coding H12) were collected from different hospitals. These eight VISA shared similar PFGE patterns (pulsotype B) and were of the same molecular type (SCC*mec*II-ST5-*spa* t586-*agr* group II). Both findings support the theory that limited VISA clones circulate in Taiwan hospitals.

PAP has been proposed as the most precise method for determining heterogeneous resistance (hVISA) and homogeneous resistance (VISA).[35] hVISA, within a susceptible rage (VA MIC ≤ 2 mg/L), carries a proportion of *S. aureus* cells (usually at a frequency of 10^{-5} to 10^{-6}) within a VA intermediate range (4—8 mg/L). Wooden et al. established criteria for hVISA as a PAP/AUC ratio between 0.9 and 1.3 when compared with Mu3, the prototype for hVISA.[25] Although 10 VISA strains in the current study harbored VISA phenotypes with a VA MIC of 4 mg/L by agar dilution, 8 of them behaved like Mu3 with a PAP/AUC ratio ranging from 0.9 to 1.3. The PAP result indicates that VISA is not always homogeneously resistant, but to a great extent, heterogeneously resistant to VA. This finding was similar to a study by Musta et al., which showed that the frequency of hVISA in MRSA blood isolates was VA MIC dependent.[27] We suggest that most VISA possess heterogeneous resistance to VA, and further studies are required to clarify the impact of this on clinical practice.

The present study had several limitations. First, our VISA sample size was relatively small, and therefore there was limited ability to detect significant molecular and phenotypic differences between VISA and VSSA. Second, because of the lack of clinical profiles, we could not compare the outcomes of patients infected by VISA with those infected by VSSA. In summary, VISA accounted for very few of the MRSA isolates taken from patients with invasive infections. VISA from invasive infections were mostly determined as molecularly HA-MRSA, and belonged to a few pandemic clones which had multidrug resistance phenotypes and certain toxin profiles.

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Ethical approval

This study was approved by the research ethics committees or institutional review boards of the 22 participating hospitals.

Declaration of competing interest

None declared.

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