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ORIGINAL ARTICLE

Molecular epidemiology and clinical characteristics of hetero-resistant vancomycin intermediate *Staphylococcus aureus* bacteremia in a Taiwan Medical Center

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Staphylococcal cassette chromosomes

Background: Hetero-resistant vancomycin intermediate *Staphylococcus aureus* (hVISA) emerges worldwide in recent decade. The purpose of this study was to investigate the glycopeptide usage trend, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced vancomycin susceptibility, the susceptible rates to newer antimicrobials, molecular epidemiology, clinical characteristics, as well as patient outcome among *S. aureus* bacteremia cases in a Taiwanese medical center.
Methods: From March to December 2009, among 118 *S. aureus* blood isolates in a Taiwanese medical center, 62 MRSA isolates were screened for hVISA by Etest macromethod and further confirmed with modified population analysis profiling method. Molecular typing of hVISA isolates was performed.
Results: Five (4.2%) isolates were hVISA. Compared with non-hVISA MRSA, hVISA isolates had higher resistant rates to ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole, and

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tetracycline. Among the MRSA infected, patients infected with hVISA had a higher in-hospital mortality rate than non-hVISA group (60% vs. 17.5%, $p = 0.025$). All hVISA isolates were nosocomial and had different pulsed field gel electrophoresis pulsotype. Four hVISA isolates carried type III staphylococcal cassette chromosome *mec* (SCCmec) and the remaining isolate carried SCC*mec* type II. Three of the 5 hVISA isolates belonged to sequence type 239, which is the most common type in Taiwan. Glycopeptide usage increased in the study hospital; however, these hVISA-infected patients did not receive glycopeptide treatment in the recent 6 months.
Conclusion: Our results suggested hVISA might have disseminated in the hospital before we observed this highest hVISA rate in Taiwan and increasing glycopeptide usage might serve as selection pressure. Measures to prevent the transmission of MRSA with reduced vancomycin susceptibility and to treat such infection were urgently needed.

Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin have emerged. Very rarely vancomycin resistant *S. aureus* (VRSA; Minimal inhibitory concentration (MIC) $\geq$16 μg/ml) was isolated. The more commonly encountered were vancomycin-intermediate *S. aureus* (VISA; MIC: 4—8 μg/ml) and hetero-resistant vancomycin-intermediate *S. aureus* (hVISA) whose vancomycin MIC was no more than 2 μg/ml but with subpopulations of MRSA with intermediate vancomycin resistance. Although not all clinical studies about hVISA revealed its significant impact on mortality outcome,[1,2] hVISA causes concerns in treatment failure in glycopeptide therapy and the increased medical cost.[3,4]

The incidence of hVISA among MRSA has increased during the past two decades from 2.2% to 8.3% in Detroit[5] while the hVISA frequency was stable in other United States studies.[6,7] The rate of hVISA decreased in a France study from 2000 to 2007.[8] The above revealed the trend of hVISA differed in different areas. An Asia surveillance study on MRSA from 1997 to 2000 reported that hVISA rate was 4.3% (ranged from 0 to 8.2% among countries)[9] that indicated the hVISA prevalence varied in different Asia countries. The rate of hVISA among MRSA in China in 2005 was as high as 15.7%.[10] Differently, hVISA had been first isolated in Taiwan in 2002.[11] The prevalence rates of VISA and hVISA among MRSA were 0.7% and 0.2%, respectively, in Taiwan in 2003.[12] To understand the updated epidemiology of MRSA with reduced vancomycin susceptibility in Taiwan,we investigated the glycopeptide usage trend, prevalence of MRSA with reduced vancomycin susceptibility, the susceptibility rates to newer antimicrobials, molecular epidemiology, clinical characteristics as well as patient outcome among *S. aureus* bacteremia cases in a Taiwanese medical center.

Methods

From March to December, 2009, patients with *S. aureus* bacteremia at a Taiwanese medical center were reviewed for their medical history and clinical characteristics. Prior antimicrobial drug exposure was traced up to 6 months before bacteremia. Healthcare associated infection and community acquired infection were defined according to the U.S. Centers for Disease Control and Prevention definition.[13] Comorbidity was assessed by the Charlson comorbidity index.[14] Persistent MRSA bacteremia was defined by positive cultures at least one week after glycopeptide treatment. The glycopeptide use data in the hospital from 2003 to 2009 was expressed as defined daily dose (DDD) per 1000 patient-days.[15]

Antimicrobial susceptibility testing

All *S. aureus* isolates were tested for susceptibility to antimicrobial agents using the Vitek 2 system. The interpretation of susceptibility followed Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] The vancomycin and daptomycin MIC of all MRSA isolates were determined using standard Etest methods (protocol EAS 003 on AB Biodisk website). Isolates with vancomycin by Etest (AB Biodisk, Solna, Sweden) MIC more than 2 μg/ml were further tested with broth microdilution method. The susceptibility interpretation for fusidic acid is according to the European Committee on Antimicrobial Susceptibility Testing (ECAST) clinical MIC breakpoints that >1 μg/ml is resistant.

Method of hVISA screening with Etest macromethod

Colonies isolated from overnight growth on trypticase soy agar plate containing 5% sheep blood were inoculated into brain heart infusion (BHI) broth to achieve 2 McFarland turbidity. A total of 200 μl of the suspension was dispensed onto BHI agar. Vancomycin and teicoplanin Etest strips were placed on the plate surface incubated at 35 °C and read at 24 and 48 hours. hVISA was suspected when vancomycin and teicoplanin MICs $\geq$ 8 μg/ml or teicoplanin MIC $\geq$ 12 μg/ml. Controls run with each procedure included *S. aureus* strains American Type Culture Collection (ATCC) 29213 (vancomycin susceptible) and ATCC 700698 (hVISA; Mu3).[17]

Modified population analysis profiling—area under the curve method

All MRSA isolates were assessed by the modified population analysis profiling—area under the curve (PAP-AUC) method

Table 1 Antimicrobial resistance rates of MSSA and MRSA among *Staphylococcus aureus* blood isolates

	MSSA ($n = 56$)	MRSA ($n = 62$)	p value
Ciprofloxacin	2 (3.6%)	29 (46.8%)	<0.0001
Fusidic acid	0 (0%)	1 (1.6%)	1.000[a]
Gentamicin	2 (3.6%)	30 (48.4%)	<0.0001
Erythromycin	2 (3.6%)	56 (93.3%)	<0.0001
Moxifloxacin	2 (3.6%)	23 (37.1%)	<0.0001[a]
Rifampicin	1 (1.8%)	6 (9.7%)	0.117[a]
Ampicillin/sulbactam	2 (3.6%)	61 (98.4%)	<0.0001
Trimethoprim/sulfamethoxazole	2 (3.6%)	19 (30.6%)	<0.0001
Tetracycline	32 (57.1%)	30 (48.4%)	0.342
Teicoplanin	0%	0%	-
Vancomycin	0%	0%	-
Linezolid	0%	0%	-
Daptomycin	0%	0%	-

MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-sensitive *Staphylococcus aureus*.
[a] By Fisher's exact test.

in duplicate.[18] Isolates were defined as hVISA if they had a PAP-AUC ratio between 0.9 and 1.3 compared with the Mu3 reference strain (ATCC 700698).

Staphylococcal cassette chromosome *mec*, multilocus sequence typing, and pulsed field gel electrophoresis typing

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed as previously described.[19] Multilocus sequence typing (MLST) and *spa* typing by analysis of the polymorphic X-region of the protein A gene were performed on hVISA isolates.[20,21] Molecular typing of hVISA strains were performed by using pulsed field gel electrophoresis (PFGE) with the restriction enzyme *Sma*I.[22]

Statistical analysis

P values were calculated by Chi-Square test and Fisher's exact test for categorical variables. Linear regression analysis was used for the trend of glycopeptides usage (DDD/1000 patient-days) with time. A p value < 0.05 was considered significant.

Results

Among 118 patients having *S. aureus* bacteremia, 62 patients (52.5%) were infected by MRSA. The MICs of vancomycin by Etest were $\leq$ 1 μg/ml for 53 (45%) isolates, 2 μg/ml for 62 (52.5%) isolates, and 3 μg/ml for three (2.5%) isolates. No VISA and VRSA was identified as the three isolates with vancomycin MIC of 3 μg/ml by Etest were tested to have MIC 2 μg/ml with broth microdilution method. Seven isolates were preliminarily suspected hVISA by the Etest macromethod (EMM). Among them, five isolates were further confirmed to be hVISA with modified PAP-AUC method. The percentage of hVISA among MRSA was 8.1% (5/62). The percentage of hVISA among *S. aureus* blood isolates was 4.2% (5/118). All hVISA isolates' MICs to vancomycin were 2 μg/ml. The difference of antimicrobial

Table 2 Antimicrobial resistance rates of hVISA and non-hVISA among MRSA isolates

	Non-hVISA MRSA ($n = 57$)	hVISA ($n = 5$)	p value
Ciprofloxacin	24 (42.1%)	5 (100%)	0.018[a]
Fusidic acid	1 (1.8%)	0 (0%)	1.000[a]
Gentamicin	25 (43.9%)	5 (100%)	0.022[a]
Erythromycin	51 (89.5%)	5 (100%)	1.000[a]
Moxifloxacin	19 (33.3%)	4 (80%)	0.059[a]
Rifampicin	4 (7.0%)	2 (40%)	0.069[a]
Ampicillin/sulbactam	56 (98.2%)	5(100%)	1.000[a]
Trimethoprim/sulfamethoxazole	15 (26.3%)	4 (80%)	0.028[a]
Tetracycline	25 (43.9%)	5 (100%)	0.022[a]
Teicoplanin	0%	0%	
Vancomycin	0%	0%	
Linezolid	0%	0%	
Daptomycin	0%	0%	

hVISA = hetero-resistant vancomycin intermediate *Staphylococcus aureus*; MRSA = methicillin-resistant *Staphylococcus aureus*.
[a] By Fisher's exact test.

Table 3 Comparison of characteristics of hVISA and non-hVISA among MRSA isolates

Characteristic	hVISA	non-hVISA MRSA	*p* value
	N = 5	*N* = 57	
Mean age (y) ± SD	72.8 ± 14.5	65.3 ± 20.0	0.41
Charlson index (age unadjusted)	3.6 ± 1.7	4.0 ± 2.5	0.74
Charlson index (adjusted)	6.6 ± 2.5	6.3 ± 2.7	0.80
Underlying disease			
Heart failure	2 (40%)	16 (28.1%)	0.573
End stage renal disease	0 (0%)	8 (14.0%)	0.369
Diabetes	3 (60%)	25 (43.9%)	0.487
Liver cirrhosis	1 (20%)	8 (14.0%)	0.717
Malignancy	1 (20%)	14 (24.6%)	0.819
Prior *S. aureus* infection	0 (0%)	6 (10.5%)	0.445
Prior MRSA infection	0 (0%)	8 (14.0%)	0.369
Outcome			
ICU stay	4 (80%)	22 (38.6%)	0.07
Hospital duration (d)	32.2 ± 23.9	32.3 ± 43.5	0.99
Persistent bacteremia (> 7 d)	1 (20%)	8 (14%)	0.717
In-hospital mortality	3 (60%)	10 (17.5%)	0.025

hVISA = hetero-resistant vancomycin intermediate *Staphylococcus aureus*; ICU = intensive care unit; MRSA = methicillin-resistant *Staphylococcus aureus*; SD = standard deviation.

resistance rates between MRSA and Methicillin-sensitive *Staphylococcus aureus* (MSSA) isolates was presented in Table 1. All these isolates were susceptible to vancomycin, teicoplanin, daptomycin and linezolid. Only one (1/118, 0.85%) isolate is resistant to fusidic acid. Significant differences in antimicrobial resistant rates to ciprofloxacin, gentamicin, erythromycin, moxifloxacin, ampicillin/sulbactam, and trimethoprim/sulfamethoxazole were observed between MRSA and MSSA (Table 1). Among MRSA isolates, hVISA had significantly higher resistant rates to ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole and tetracycline (Table 2).

Patients with hVISA bacteremia were not significantly different from those with non-hVISA bacteremia in terms of age, underlying diseases and Charlson comorbidity index. Among MRSA patients, hVISA patients had a higher in-hospital mortality rate compared these with non-hVISA (Table 3). All the five hVISA infections were nosocomial. None of the five hVISA cases had prior *S. aureus* or MRSA infection and no glycopeptide exposure in 6 months before the bacteremia occurred. All the hVISA isolates from the same patient had the same PFGE pulsotype. These five hVISA strains from five patients had five different pulsotypes (Fig. 1). Four of the five hVISA strains belonged to SCC*mec* type III and the other is SCC*mec* type II. The only SCC*mec* II strain is of MLST type ST5. Among the four SCC*mec* III strains, two are of ST239, one is of ST239 single locus variant (SLV, glpF1:262 T to C), one is of ST900. Except that one of the isolate's *spa* type is not typable, three of four SCC*mec* III strains are of *spa* type 037 and the only SCC*mec* II strain is of *spa* type 002.

The annual DDD of glycopeptide in the hospital increased from 296 in 2004 to 385 in 2009. The DDD/1000 patient-days of glycopeptide increased significantly from 0.71 in 2004 to 0.95 in 2009 (*p* value = 0.023). These results are shown in Fig. 2.

Discussion

The various prevalence rates of hVISA and VISA in Asian countries were summarized in Table 4. The highest hVISA prevalence rate among MRSA blood isolates in Asia was 13.1% in China, followed by 12.5% in Singapore. Our results revealed the rate of hVISA to be 8.1% among MRSA blood isolates (4.2% of *S. aureus* blood isolates) in 2009. To our

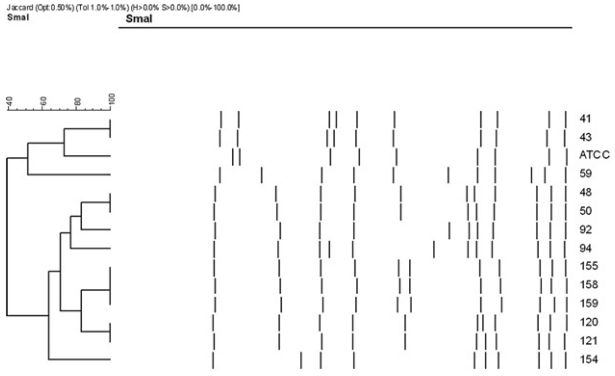

Figure 1. Pulsotypes of the hVISA isolates. Molecular typing with pulsed field gel electrophoresis. Pulsotypes were analyzed to deduce a dendrogram with the unweighted pair group method using the arithmetic average clustering technique after calculation of similarities using Pearson correlation coefficient between every pairs. Isolates from five hVISA cases: isolate number 48 and 50 were from the same case; 120 and 121 (the same case); 155, 158 and 159 (the same case); 59; and 94. Isolate numbers 41 and 43 (the same case), 92, and 154 were non-hVISA isolates randomly selected from the study. ATCC: *S. aureus* strain ATCC 29213; hVISA = hetero-resistant vancomycin intermediate *Staphylococcus aureus*.

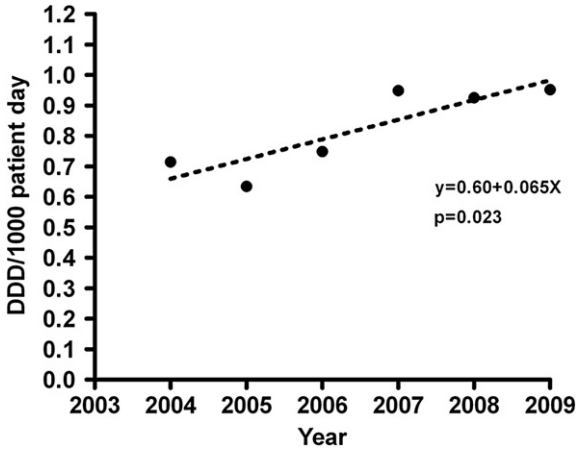

Figure 2. The glycopeptide use per 1000 patient-days each year in the study hospital from 2003 to 2009.

knowledge, it is the latest and highest rate of hVISA in Taiwan that suggests the emergence of hVISA in Taiwan.

In this study, the five hVISA isolates possess either SCC*mec* III or II that are more common in nosocomial MRSA strains.[22,23] Reviewing these five cases' medical history also proved the nosocomial infections. We observed the trend of hVISA with more ICU stay and higher mortality. Although a higher prevalence of hVISA may be related to nosocomial clonal spread,[24] molecular typing with PFGE identified no closely related pulsotypes of hVISA in the hospital during the study period. Different from that hVISA in a Singapore hospital had closely related pulsotypes,[25] hVISA isolates in the study had unrelated pulsotypes. Because of the increasing frequency of MRSA infection in Taiwan,[23] glycopeptide use increased in recent years. When we observed the emergence of hVISA in the hospital, we noticed the increased glycopeptide use that may served as selection pressure (Fig. 2). However, our five hVISA patients did not have glycopeptide exposure in the recent 6 months, which suggested these hVISA strains already circulated in the hospital and patients' hVISA might be from unidentified hVISA colonizers. The threat of hVISA is therefore not limited to those patients with glycopeptides use. The findings also emphasize the need of infection control measures to prevent MRSA transmission.

In this study, hVISA was not associated with persistent bacteremia as previously reported,[2] which may be owing to the small case number of hVISA in the study that made us unable to observe a significant difference. Although the age in both hVISA and non-hVISA groups were not significantly different, the trend to be more aged of hVISA group may affect the clinical outcome. In our study, hVISA infection was associated with a higher in-hospital mortality rate and a higher rate of ICU stay among MRSA isolates. However, the impact of hVISA on mortality is controversial.[1,3,4,12] Among *S. aureus*, a meta-analysis revealed that hVISA was associated with a similar 30-day mortality compared to vancomycin-susceptible *S. aureus* infections.[26] As the hVISA rate among *S. aureus* is relatively low, a large case number is required to identify its impact on mortality among *S. aureus*.

Multiple drug resistance raised a serious concern for MRSA when compared with MSSA. Among MRSA blood isolates, hVISA isolates had higher resistance rates to ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole and tetracycline that indicated the more limited antimicrobial options for hVISA. Our results revealed these hVISA isolates were all susceptible to daptomycin, linezolid and tigecycline; those could be treatment options.

ST239 MLST type is the most predominant MRSA clone in Taiwan and ST59-SCC *mec* IV or ST59-SCC*mec*V is the second.[23,27] In the study, three of the 5 hVISA isolates belong to ST239 and had SCC*mec* III. The appearance of hVISA in the major MLST clone warrants close monitor in fear of the possible expanding hVISA epidemic in Taiwan.

The limitations of our study include: (a) because of the retrospective design, we did not categorize all cases of *S. aureus* infection to be of community-acquired infection or healthcare associated infection. The number of glycopeptide exposure would be underestimated because some patients may have been exposed to it in other hospitals, and (b) hVISA isolates in the study were of ST5 and ST239 that both usually were resistant to ciprofloxacin and

Table 4 Prevalence rates of hVISA and VISA among MRSA isolates in Asia

Country	Year	No. of MRSA isolates	Specimen	hVISA among MRSA	VISA among MRSA	hVISA among *S. aureus*	VISA among *S. aureus*	Reference
Asia	1997–2000	1349	ND	4.3%	0	NA	NA	9
Japan	1997	6625	ND	0	0	0	0	28
Hong Kong	1997–1998	52	Blood	5.8%	0	1.8%	0	29
India	1999	120	Various	ND	33.3%	ND	ND	30
Korea	1999–2001	439	Various	0	0	0	0	31
China	2002–2007	200	Blood	13.1%	0.05%	0	0	10
Taiwan	2003	1000	Various	0.7%	0.2%	0	0	32
Singapore	2005–2006	56	Blood	12.5%	5.4%	0	0	1
Thailand	2002–2003	533	ND	0.8%	0	0	0	33
	2006–2007	361		2.2%		0	0	
Southern India	ND	102	ND	ND	0.9%	ND	0.7%	34
Taiwan	2009	62	Blood	8.1%	0	4.2%	0	This study

hVISA = hetero-resistant vancomycin intermediate *Staphylococcus aureus*; NA = not applicable; ND = no data; MRSA = methicillin-resistant *Staphylococcus aureus*.

gentamicin in Taiwan and China.[10,35] Although we observed hVISA isolates were more resistant to ciprofloxacin, gentamicin, trimethoprim/sulfamethoxazole, and tetracycline than non-hVISA isolates, the resistant pattern may be related to the characteristics of MLST types.

In conclusion, 8.1% MRSA blood isolates in the Taiwan hospital were hVISA that caused nosocomial infection with a higher in-hospital mortality rate. Increasing glycopeptide usage may serve as selection pressure for the emergence of hVISA. That hVISA isolates were not from patients with prior glycopeptide use and not of closely related pulsotype suggest the existence and transmission of hVISA before this surveillance. Measures to prevent the expanding transmission of MRSA with reduced vancomycin susceptibility and to treat the related infections are urgently needed.

Acknowledgments

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