Journal of Microbiology, Immunology and Infection (2016) 49, 701—707

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Prevalence of vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogeneous VISA among methicillin-resistant *S. aureus* with high vancomycin minimal inhibitory concentrations in Taiwan: A multicenter surveillance study, 2012—2013

Sung-Hsi Huang [a], Yee-Chun Chen [a], Yin-Ching Chuang [b], Sheng-Kang Chiu [c], Chang-Phone Fung [d], Po-Liang Lu [e], Lih-Shinn Wang [f], Tsu-Lan Wu [g], Jann-Tay Wang [a,*]

[a] *Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[b] *Department of Medical Research, Chi Mei Medical Center, Tainan County, Taiwan*
[c] *Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*
[d] *Section of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, National Yan-Ming University, Taipei, Taiwan*
[e] *Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[f] *Department of Infectious Diseases, Buddhist Tzu Chi General Hospital, Hualien, Taiwan*
[g] *Department of Laboratory Medicine, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan*

Received 8 April 2015; received in revised form 12 June 2015; accepted 6 July 2015
Available online 31 July 2015

KEYWORDS
epidemiology;
heterogeneous vancomycin-intermediate

Abstract *Background/Purpose:* Intermediate-resistance and heteroresistance to vancomycin in methicillin-resistant *Staphylococcus aureus* (MRSA) is reported worldwide. A surveillance study in 2003 showed that the prevalence rates of vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) in Taiwan were 0.2% and 0.7%, respectively. This study aimed to investigate the updated prevalence of VISA and hVISA in Taiwan.

* Corresponding author. Department of Internal Medicine, National Taiwan University Hospital, Number 7, Chung-Shan South Road, Taipei, Taiwan.
E-mail address: wang.jt1968@gmail.com (J.-T. Wang).

http://dx.doi.org/10.1016/j.jmii.2015.07.003

Staphylococcus aureus;
Taiwan

Methods: MRSA isolates from sterile sites with minimal inhibitory concentrations (MICs) of 1 μg/mL or more to vancomycin were collected from 15 participating hospitals in Taiwan. Enrolled MRSA isolates were submitted to antimicrobial susceptibility testing, staphylococcal cassette chromosome *mec* (SCC*mec*) element typing, and multilocus sequence typing. Isolates with vancomycin MIC of 1 μg/mL or 2 μg/mL were screened for vancomycin heterogeneous resistance by Etest glycopeptide-resistance detection (GRD). Those with positive GRD screening results were then analyzed by modified population analysis profiling-area under the curve method for confirmation of vancomycin heteroresistance.
Results: Between 2012 and 2013, a total of 622 MRSA isolates from sterile sites with vancomycin MIC of 1 μg/mL or more were studied. The prevalence rates of hVISA and VISA among these isolates were 10.0% and 2.7%, respectively. The hVISA prevalence increased significantly compared to that in 2003. Compared with vancomycin-susceptible *S. aureus*, hVISA and VISA isolates were less susceptible to ciprofloxacin, clindamycin, daptomycin, gentamicin, rifampin, and trimethoprim/sulfamethoxazole, and are thus, more likely to have SCC*mec* II or III element. A twofold increase in either vancomycin or teicoplanin MIC doubled the probability of being hVISA.
Conclusion: Growing hVISA prevalence was highly suspected. Longitudinal surveillance of this phenomenon and monitoring of its clinical impact are necessary.

Introduction

Staphylococcus aureus is one of the most common human pathogens, which causes a broad spectrum of illnesses ranging from relatively mild skin infection to life-threatening septicemia.[1] As with other major human pathogens, antimicrobial resistance of *S. aureus* emerged along with the discovery and widespread use of different classes of antibiotics.[2] Among these, resistance to methicillin is of most concern because it leads to resistance to all β-lactams, the antibiotics most commonly used clinically; additionally, methicillin-resistant *S. aureus* (MRSA) infection is associated with significantly additional morbidity and mortality.[3] The prevalence of MRSA infection has increased during the past decades and in some areas its prevalence has been reported to be over 50%.[4] This has resulted in an increased use of glycopeptides such as vancomycin, which have been the treatment of choice for MRSA infection for decades.

The first strain of MRSA with reduced vancomycin susceptibility was reported in Japan in 1997.[5] Vancomycin-resistant *S. aureus* (VRSA) infection remains rare to this date,[6,7] but vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) are encountered more commonly. In the initial Japanese study in 1997, 5–26% of MRSA isolates from various university hospitals were hVISA.[8] Later publications reported that the prevalence of hVISA ranged from 0% to 22.1% (of all MRSA isolates) with marked geographic variation.[9,10] By contrast, the VISA prevalence rate was generally lower than 1% across the globe.[11–14] Infections caused by hVISA and VISA were associated with vancomycin treatment failure and prolonged duration of treatment and hospitalization compared with those caused by vancomycin-susceptible *S. aureus* (VSSA).[15,16] The Clinical and Laboratory Standard Institute (CLSI) lowered the vancomycin minimal inhibitory concentration (MIC) breakpoint (from ≤4 μg/mL to ≤2 μg/mL for susceptible and from ≥32 μg/mL to ≥16 μg/mL for resistant strains) in 2006 to reflect the growing body of evidence that isolates with vancomycin MIC of 4 μg/mL or more are less likely to respond to vancomycin therapy.

There have been few studies investigating the epidemiology of hVISA and VISA in Taiwan: a network survey including 1000 MRSA isolates from 10 medical centers in 2003 showed that the prevalence rate of VISA and hVISA had been low,[11] whereas one small-sized single center study revealed an increasing burden of hVISA in 2009.[17] Using a larger sample collected from across Taiwan, we aimed to determine the recent prevalence of hVISA and VISA among MRSA isolates with vancomycin MICs of 1 μg/mL or more in Taiwan and identify the characteristics of these isolates with heteroresistance or intermediate resistance to vancomycin.

Methods

Bacterial isolates

The study period was from January 2012 to December 2013. In each year, MRSA isolates from sterile sites, including blood, cerebrospinal fluid, ascites, and pleural effusion, with vancomycin MIC of 1 μg/mL or more determined at their source hospitals, were collected consecutively since January 1 from 15 participating hospitals. The preset numbers of bacterial isolates enrolled each year was 300. When the total number of collected MRSA isolates reached 300 in each year, the participating hospital would be informed to stop submitting further samples for that year. Duplicated isolates would be excluded. The 15 participating hospitals included 12 medical centers and three regional hospitals distributed in northern (6 hospitals), central (2 hospitals), southern (5 hospitals), and eastern (2 hospitals)

Taiwan. The collected MRSA isolates were sent to the central laboratory located at National Taiwan University Hospital (NTUH). Only those isolates confirmed to be MRSA and had vancomycin MIC of 1 μg/mL or more by broth dilution method performed in the central laboratory were formally enrolled for subsequent microbiological studies. All isolates were preserved at −80°C before the microbiological studies.

Microbiological studies

Antimicrobial susceptibility testing by MIC

The MICs to 12 antibiotics (ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, oxacillin, rifampicin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole, and vancomycin) were determined by broth microdilution method using custom-designed 96-well panels (Sensititre Vizion Digital MIC Viewing System, Thermo Scientific, West Sussex, UK). The result was interpreted following the CLSI guidelines. Of note, isolates with vancomycin MIC of 4—8 μg/mL were defined as VISA.[18]

Etest glycopeptide-resistance detection

Each enrolled MRSA isolate with vancomycin MIC of 1 μg/mL or 2 μg/mL was screened for hVISA using Etest glycopeptide-resistance detection (GRD), which has been described earlier with 93% sensitivity compared with the population analysis profiling-area under the curve (PAP-AUC) method.[19] The test was performed according to the manufacturer's instruction (Etest GRD for screening of heterogeneous glycopeptide-intermediate *S. aureus*/glycopeptide-intermediate *S. aureus*, bioMérieux SA, Lyon, France). In brief, a bacterial suspension corresponding to a 0.5 McFarland standard was grown on a Müller—Hinton agar + 5% blood (MHB; Becton, Dickinson and Company, Sparks, MD, USA) plate. A GRD strip consisting of a double-sided gradient with vancomycin and teicoplanin was then applied to the MHB plate. The zone of the Etest GRD strip was read at complete inhibition of growth 24 hours and 48 hours after incubation. The test isolate was considered positive for hVISA if the Etest GRD strip result was 8 μg/mL or more for either vancomycin or teicoplanin.

Modified PAP-AUC

All isolates screened positive by E-test GRD were subsequently tested with modified PAP-AUC method as described previously.[20—22] In brief, a 0.5 McFarland standard suspension from an overnight culture of the isolate to be tested in Trypticase soy broth agar (Becton Dickinson and Company) was prepared. Serial dilutions (10^{-1} to 10^{-6}) were prepared in sterile saline, and 100 μL of 10^{-6} dilutions were plated by hand onto brain—heart infusion (BHI) agar (Becton, Dickinson and Company) to determine viable counts. Four 10-μL droplets from each of five dilutions (10^{-1} to 10^{-5}) were then added onto BHI agar plates containing increasing concentrations of vancomycin (0 mg/L, 0.25 mg/L, 0.5 mg/L, 0.75 mg/L, 1 mg/L, 1.5 mg/L, 2 mg/L, 3 mg/L, 4 mg/L, 6 mg/L, and 8 mg/L) and read after 24 hours and 48 hours of incubation at 35°C. All PAP-AUC methods were performed using Mu3 as a positive control. Interpretation of PAP-AUC was as follows: ratio of the AUC of the test isolate to Mu3 of 0.9 or more and less than 1.3 was considered hVISA,[23] ratio of the AUC of the test isolate to Mu3 of 1.3 or more was considered VISA, and the rest was considered VSSA.

Determination of staphylococcal cassette chromosome *mec* typing and multilocus sequence typing using polymerase chain reaction-based methods

All the studied isolates were submitted to staphylococcal cassette chromosome *mec* (SCC*mec*) typing and multilocus sequence typing (MLST) as described earlier.[24,25] MRSA isolates carrying type I, II, or III SCC*mec* element were classified as health-care-associated MRSA (HA-MRSA), and those carrying type IV or V as community-associated MRSA (CA-MRSA).[26]

Statistic analysis

The enrolled isolates were categorized into three groups, namely, VSSA, hVISA, and VISA. Their antibiotic susceptibility and molecular typing were compared. Statistical analyses were performed using SAS statistical software (Version 9.2, SAS Institute Inc., Cary, NC, USA). Continuous variables were compared by the Mann—Whitney *U* test or Student *t* test, and categorical variables were compared by Chi-square test or Fisher exact test. Selected variables, including year of the isolates; SCC*mec* II or III (indicating HA-MRSA); susceptibility to ciprofloxacin, clindamycin, daptomycin, gentamicin, rifampin, and trimethoprim/sulfamethoxazole; and MIC of vancomycin and teicoplanin in binary logarithm, were used for a stepwise backward regression analysis to determine the association with risk of hVISA and VISA. A *p* value less than 0.05 was considered significant. All tests were two-tailed.

Results

A total of 678 bacterial isolates were sent to the central laboratory at NTUH between 2012 and 2013. Among them, 39 isolates were reidentified as methicillin-susceptible *S. aureus*, 17 isolates were found to have vancomycin MIC of 0.5 μg/mL or less, and 13 isolates were VISA (vancomycin MIC = 4 μg/mL in 11 isolates and 8 μg/mL in 2 isolates). Methicillin-susceptible *S. aureus* and MRSA isolates with vancomycin MIC of 0.5 μg/mL or less were excluded. For the remaining 622 MRSA isolates with vancomycin MIC of 1 μg/mL or more, 386 came from the six hospitals in Northern Taiwan, 37 from the two hospitals in Central Taiwan, 179 from the five hospitals in Southern Taiwan, and 26 from the two hospitals in Eastern Taiwan. None of the isolates expressed full vancomycin resistance according to CLSI breakpoint.

Antimicrobial susceptibility testing on these isolates revealed that daptomycin, linezolid, and teicoplanin remained highly active against MRSA with susceptibility rate of 95%, 99.5%, and 99.5% respectively, whereas ciprofloxacin, clindamycin, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole had a susceptibility rate between 9% and 46.1% and were generally much less reliable (Table 1). The VISA isolates exhibited resistance to most tested antimicrobials but were universally susceptible to linezolid.

Table 1 Antimicrobial susceptibility of MRSA isolates categorized according to vancomycin susceptibility.

Antimicrobial agent	VSSA (*N* = 543)			hVISA (*N* = 62)			VISA (*N* = 17)			Total (*N* = 622)			*p**	*p***
	%S	%I	%R	%S	%I	%R	%S	%I	%R	%S	%I	%R		
Ciprofloxacin	29.7	4.6	65.7	9.7	1.6	88.7	11.8	0	88.2	27.2	4.2	68.6	0.001	<0.001
Clindamycin	27.1	5.7	67.2	12.9	4.8	82.3	5.9	0	94.1	25.1	5.5	69.5	0.02	0.003
Daptomycin***	96.7		3.3	88.7		11.3	64.7		35.3	95		5	<0.001	<0.001
Erythromycin	9.6	6.4	84	4.8	3.2	91.9	5.9	0	94.1	9	5.9	85	0.57	0.166
Gentamicin	20.3	3.9	75.9	8.1	1.6	90.3	5.9	0	94.1	18.6	3.5	77.8	0.056	0.005
Linezolid	99.8	—	0.4	98.4	—	1.6	100	—	0	99.5	—	0.5	0.339	0.355
Rifampin	82.5	11.4	6.1	66.1	19.4	14.5	52.9	17.6	29.4	80.1	12.4	7.6	0.001	<0.001
Teicoplanin	99.8	0	0.2	100	0	0	88.2	0	11.8	99.5	0	0.5	0.003	0.029
Tetracycline	31.5	1.1	67.4	19.4	0	80.6	17.6	0	82.4	29.9	1	69.1	0.195	0.052
Trimethoprim/ sulfamethoxazole	49.5	—	50.5	24.2	—	75.8	15.4	—	84.6	46.1	—	53.9	<0.001	<0.001

hVISA = heterogeneous vancomycin-intermediate *Staphylococcus aureus*; MRSA = methicillin-resistant *Staphylococcus aureus*; VISA = vancomycin-intermediate *Staphylococcus aureus*; VSSA = vancomycin-susceptible *S. aureus*.

* Fisher exact test for distribution of susceptibility between VSSA, hVISA, and VISA.

** Fisher exact test for distribution of susceptibility between vancomycin-susceptible (VSSA) and nonsusceptible isolates (hVISA and VISA).

*** The number of percentage is reported as %R, which represents nonsusceptible rate.

Etest GRD was performed on all the 609 isolates with vancomycin MICs of 1 μg/mL or 2 μg/mL. Among these isolates, 150 had either vancomycin or teicoplanin MIC of 8 μg/mL or more and were further examined by modified PAP-AUC method. Finally, 62 isolates (10%) were identified as hVISA as they had AUC of the test isolate to Mu3 ratio of 0.9 or more and less than 1.3, compared with the Mu3 reference strain. Of note, four isolates examined by the PAP-AUC method had AUC of the test isolate to Mu3 ratio of 1.3 or more of the Mu3 strain. These isolates were reclassified as VISA in the subsequent analysis. Among the 62 hVISA isolates, 48 had a vancomycin MIC of 2 μg/mL (48/270, 17.8%) and 14 had a vancomycin MIC of 1 μg/mL (14/355, 4.2%).

All 622 isolates underwent SCC*mec* element typing and MLST genotyping. Of the 561 isolates with typable SCC*mec*, more than half (312/561, 55.6%) had SCC*mec* III, whereas SCC*mec* II, SCC*mec* IV, and SCC*mec* V accounted for 9.8%, 16.2%, and 18.4% of the isolates, respectively. Of the 499 VSSA isolates with typable SCC*mec*, 61.7% of the isolates belonged to molecularly defined HA-MRSA strains (carrying SCC*mec* II and III). In comparison, 12 of 15 (80.0%) VISA isolates and 47 of 57 (82.5%) hVISA isolates belonged to molecularly defined HA-MRSA strains. The result from MLST genotyping showed that the most common sequence type (ST) among MRSA isolates was ST239 (50.9%), followed by ST59, ST5, and ST45. Seventeen other minor STs, including ST508, ST188, ST573, ST6, ST97, ST1149, ST15, ST22, ST72, ST241, ST338, ST672, ST1454, and ST1598, accounted for the rest 11.9% of the isolates. The ST distribution did not differ significantly among VSSA, hVISA, and VISA isolates ($p = 0.181$).

Upon comparing drug susceptibilities between VSSA ($n = 543$), hVISA isolates ($n = 62$), and VISA ($n = 17$), the VSSA isolates were significantly more susceptible to ciprofloxacin, clindamycin, daptomycin, gentamicin, rifampin, and trimethoprim/sulfamethoxazole (Table 1). Linezolid and daptomycin were highly active *in vitro* against most MRSA isolates, including the hVISA isolates. However, 35.3% of VISA isolates were nonsusceptible to daptomycin. The distribution of teicoplanin MICs stratified by vancomycin susceptibility is shown in Figure 1. The MRSA isolates with higher teicoplanin MICs, especially those with MICs of 2 μg/mL or more, were more likely to exhibit heteroresistance and intermediate resistance to vancomycin (Figure 2).

In the attempts to identify factors associated with hVISA and VISA that were identified by the PAP-AUC method, the MRSA isolates with vancomycin MICs of 1 μg/mL and 2 μg/mL (609 isolates) were included in a multivariate logistic regression analysis. The result showed that a twofold

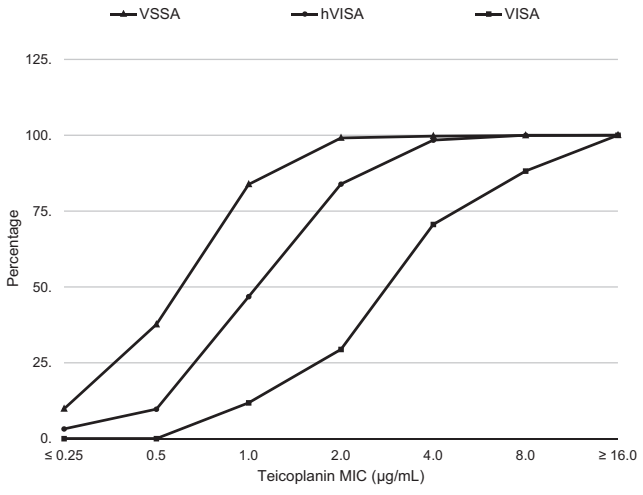

Figure 1. Distribution of teicoplanin minimum inhibitory concentrations (MICs) in vancomycin-susceptible *Staphylococcus aureus* (VSSA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and vancomycin-intermediate *S. aureus* (VISA) isolates.

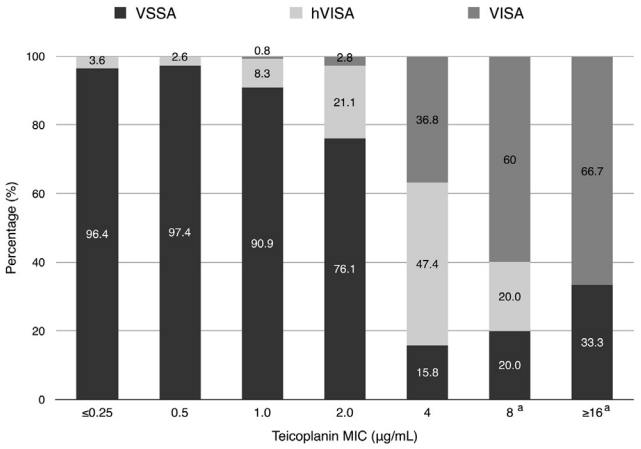

Figure 2. Proportion of vancomycin-intermediate *Staphylococcus aureus* (VISA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and vancomycin-susceptible *S. aureus* (VSSA) in methicillin-resistant *S. aureus* (MRSA) isolates with different teicoplanin minimum inhibitory concentrations (MICs). [a]There were only a total of five and three isolates with teicoplanin MIC of 8 μg/mL and 16 μg/mL or more, respectively.

Table 2 Logistic regression model for factors associated with hVISA.

	Odds ratio (95% confidence interval)	*p*
MIC of vancomycin in binary logarithm	2.274 (1.140–4.539)	0.02
MIC of teicoplanin in binary logarithm	2.397 (1.660–3.460)	<0.001

hVISA, heterogenous vancomycin-intermediate *Staphylococcus aureus*; MIC = minimum inhibitory concentration.

increase in MIC of teicoplanin or vancomycin doubled the risk for the isolate to be identified as hVISA (Table 2). Despite the association, incorporating both vancomycin and teicoplanin MIC of 2 μg/mL or more as a screening tool did not significantly improve the value in predicting hVISA and VISA compared with using vancomycin MIC of 2 μg/mL or more alone (Table 3).

Discussion

In this surveillance study, we systemically collected MRSA isolates from sterile sites with vancomycin MICs of 1 μg/mL or more in a 2-year period and applied standard methods to detect hVISA and VISA for all enrolled isolates. The isolates came from 15 different medical centers in four different parts of Taiwan. Thus, our results should be more representative of local epidemiology in Taiwan. Among the 622 MRSA isolates with vancomycin MIC of 1 μg/mL or more, 17 (2.7%) VISA isolates and 62 (10.0%) hVISA isolates were identified.

A wide range of prevalence of hVISA has been reported and the prevalence varied between different geographic area, source of isolates, and detection methods. hVISA isolates generally accounted for a substantial portion of MRSA among Asian countries (16.5% from 830 blood-borne MRSA isolates in Japan, 11.1% from 1175 various clinical isolates in 14 Chinese hospitals, and 22.1% from 184 sterile site isolates in China).[10,27,28] One previously published epidemiological study in Taiwan reported an hVISA prevalence of only 0.7% in 2003.[11] Another previous single-center observation study from Southern Taiwan showed the prevalence rate of 4.2% among MRSA blood isolates in 2009. Our study only enrolled MRSA isolates with vancomycin MIC of 1 μg/mL or more, and the result showed that the hVISA prevalence among them was 10.0%. Between 2006 and 2010, the proportion of MRSA with vancomycin MIC of 0.5 μg/mL or less among all MRSA isolates from sterile sites was reported to be 2.4%.[29] Accordingly, the estimated hVISA prevalence, based on available data, among MRSA isolates with vancomycin MIC of 1 μg/mL or more in 2003 and 2009 would be approximately 0.7% and 4.3%, respectively, which were still lower than 10.0%. In addition, our data showed an alarming VISA prevalence rate of 2.7%, which is higher than that reported worldwide (<1%).[11–14] While the difference could be related to different enrollment criteria or different screening methods, an evolution of the pathogen to become more resistant to vancomycin is also highly possible. The increase of hVISA prevalence rate and reduced vancomycin susceptibility among MRSA isolates should raise caution.

There is evidence that hVISA and VISA are associated with vancomycin treatment failure, persistent bacteremia, longer hospital stay, and adverse final clinical outcome.[15,30,31] While glycopeptides remained the first-line treatment for MRSA

Table 3 Sensitivities and specificities of different strategies incorporating vancomycin and teicoplanin MIC in prediction of hVISA.

	Sensitivity	Specificity	PPV	NPV	Sensitivity + specificity
Vancomycin MIC ≥ 2 μg/mL	78.8%	59.1%	19%	95.8%	1.379
Teicoplanin MIC ≥ 2 μg/mL	53%	83.8%	28.5%	93.6%	1.368
Either vancomycin or teicoplanin MIC ≥ 2 μg/mL	83.3%	55.6%	18.6%	96.5%	1.389
Both vancomycin and teicoplanin MIC ≥ 2 μg/mL	48.5%	87.3%	31.7%	93.3%	1.358
Comparison					
Etest GRD at 48 h[19]	93%	82%			
Macro Etest at 48 h[19]	83%	94%			

GRD = glycopeptide-resistance detection; hVISA = heterogenous vancomycin-intermediate *Staphylococcus aureus*; MIC = minimal inhibitory concentration; NPV = negative predictive value; PPV = positive predictive value.

infection, more judicious use of vancomycin and teicoplanin, along with appropriate dosing regimen, regular therapeutic drug monitoring, adequate infection source control, and strategies to prevent further nosocomial transmissions would be required to prevent treatment failure in an increasingly vancomycin hetero-resistant microenvironment.[32]

It is well-known that HA-MRSA is generally more resistant to multiple non-β-lactams antimicrobials compared with CA-MRSA. Most hVISA and VISA isolates in this study belonged to molecularly defined HA-MRSA and were highly resistant to a broad spectrum of antimicrobials compared with VSSA, consistent with prior publications.[17,21,33] The current mainstream theory postulates that heterogeneous and intermediate vancomycin resistances evolve in a stepwise process and are related to higher background antibiotic selection pressure and infection sites that are more difficult to treat.[34] In this context, it is reasonable that the hVISA and VISA isolates in this study were found to be more likely to be molecularly defined HA-MRSA.

Still, a certain percentage of hVISA isolates in our study belonged to molecularly defined CA-MRSA. This phenomenon has not been shown in a prior Taiwanese epidemiological study,[17] but a prior large-scale study in Chicago did demonstrate that CA-MRSA can harbor vancomycin heteroresistance.[30] Therefore, heteroresistance to vancomycin should also be taken into consideration when the clinical response is inadequate while using vancomycin to treat CA-MRSA infection, and the use of linezolid or daptomycin should be considered in this situation. We also noticed a decreased daptomycin susceptibility in hVISA and VISA isolates compared with VSSA isolates. Similar observations have been reported and current hypothesis suggests that this phenomenon could be related to the thickening of bacterial cell wall.[34,35] The resistance to daptomycin should thus be continuously monitored.

The standard method to detect hVISA, the PAP-AUC method, is time consuming, labor intensive, and not routinely available and thus has its limitation. Microbiology laboratories use different screening strategies, including Etest macromethod, Etest GRD, and BHI screening agar containing 3—4 μg/mL of vancomycin to identify hVISA. All of the screening methods still required extra work, man power, and training. In one report, Etest macromethod and Etest GRD had good specificity but limited sensitivity (57%), whereas methods using BHI screening were highly sensitive but less specific (67—94%).[20] In this study, the test using simple screening methods such as teicoplanin and vancomycin MIC failed to provide convincing prediction of hVISA. A simpler, but yet more accurate, diagnostic method still awaits discovery.

This study had several limitations. First, the study was aimed to provide epidemiological surveillance data, and thus, it lacked important clinical information outside of microbiology laboratories. Second, despite good sensitivity of Etest GRD,[19] certain portion of hVISA would still be false negative during screening process. Furthermore, although it has been reported that MRSA isolates with vancomycin MIC of 0.5 μg/mL or less accounted for only a small portion of all MRSA isolates in Taiwan,[29,36] and the prevalence of hVISA among these isolates is expected to be quite low,[37,38] our study only enrolled MRSA isolates with vancomycin MIC of 1 μg/mL or more, which would likely overestimate the hVISA prevalence among all MRSA isolates. Therefore, our result should be interpreted and applied conservatively.

In conclusion, the prevalence of hVISA and VISA among MRSA isolates from sterile sites and with vancomycin MIC of 1 μg/mL or more in Taiwan was 10.0% and 2.7%, respectively. The hVISA prevalence probably increased compared with prior reports. In addition, heteroresistance to vancomycin was noted among CA-MRSA isolates in our study. The burden and clinical impact of hVISA and VISA should be closely and continuously monitored.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

We thank our colleagues in the Departments of Laboratory Medicine at the 15 hospitals for their excellent work in primary isolation, identification, and preservation of the bacterial isolates. This study was supported by grants from the Centers for Disease Control, Taiwan (Grant Nos. CM-DOH101-DC-1204-04 and CM-DOH102-DC-1508-04). The funding source had no role in study design, study conduction, data collection, data analysis, data interpretation, the writing of the manuscript, or the decision to submit it for publication.

References

1. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med* 1998; **339**:520—32.
2. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest* 2003;**111**:1265—73.
3. Turnidge JD, Kotsanas D, Munckhof W, Roberts S, Bennett CM, Nimmo GR, et al. *Staphylococcus aureus* bacteraemia: a major cause of mortality in Australia and New Zealand. *Med J Aust* 2009;**191**:368—73.
4. Loffler CA, Macdougall C. Update on prevalence and treatment of methicillin-resistant *Staphylococcus aureus* infections. *Expert Rev Anti Infect Ther* 2007;**5**:961—81.
5. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997;**40**:135—6.
6. Limbago BM, Kallen AJ, Zhu W, Eggers P, McDougal LK, Albrecht VS. Report of the 13th vancomycin-resistant *Staphylococcus aureus* isolate from the United States. *J Clin Microbiol* 2014;**52**:998—1002.
7. Friães A, Resina C, Manuel V, Lito L, Ramirez M, Melo-Cristino J. Epidemiological survey of the first case of vancomycin-resistant *Staphylococcus aureus* infection in Europe. *Epidemiol Infect* 2015;**143**:745—8.
8. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* 1997;**350**:1670—3.
9. Gallon O, Lamy B, Laurent F, Reverdy ME, Doucet-Papulaire F, Decousser JW, et al. Antimicrobial susceptibility profiles of *Staphylococcus aureus* isolated in 2007 from French patients with bloodstream infections: goodbye hVISA, welcome Geraldine? *J Antimicrob Chemother* 2010;**65**:1297—9.

10. Liu C, Chen ZJ, Sun Z, Feng X, Zou M, Cao W, et al. Molecular characteristics and virulence factors in methicillin-susceptible, resistant, and heterogeneous vancomycin-intermediate *Staphylococcus aureus* from central-southern China. *J Microbiol Immunol Infect* 2014:1—7. http://dx.doi.org/10.1016/j.jmii.2014.03.003 [Epub ahead of print].
11. Ho CM, Hsueh PR, Liu CY, Lee SY, Chiueh TS, Shyr JM, et al. Prevalence and accessory gene regulator (agr) analysis of vancomycin-intermediate *Staphylococcus aureus* among methicillin-resistant isolates in Taiwan—SMART program, 2003. *Eur J Clin Microbiol Infect Dis* 2010;**29**:383—9.
12. Richter SS, Satola SW, Crispell EK, Heilmann KP, Dohrn CL, Riahi F, et al. Detection of *Staphylococcus aureus* isolates with heterogeneous intermediate-level resistance to vancomycin in the United States. *J Clin Microbiol* 2011;**49**:4203—7.
13. Hu J, Ma XX, Tian Y, Pang L, Cui LZ, Shang H. Reduced vancomycin susceptibility found in methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* clinical isolates in Northeast China. *PLoS One* 2013;**8**:e73300.
14. Song JH, Hiramatsu K, Suh JY, Ko KS, Ito T, Kapi M, et al. Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Antimicrob Agents Chemother* 2004;**48**:4926—8.
15. van Hal SJ, Paterson DL. Systematic review and meta-analysis of the significance of heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. *Antimicrob Agents Chemother* 2011;**55**:405—10.
16. Khatib R, Jose J, Musta A, Sharma M, Fakih MG, Johnson LB, et al. Relevance of vancomycin-intermediate susceptibility and heteroresistance in methicillin-resistant *Staphylococcus aureus* bacteraemia. *J Antimicrob Chemother* 2011;**66**:1594—9.
17. Lin SY, Chen TC, Chen FJ, Chen YH, Lin YI, Siu LK, et al. Molecular epidemiology and clinical characteristics of heteroresistant vancomycin intermediate *Staphylococcus aureus* bacteremia in a Taiwan Medical Center. *J Microbiol Immunol Infect* 2012;**45**:435—41.
18. *Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: twenty-forth informational supplement*. 100th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. p. 68—75.
19. Leonard SN, Rossi KL, Newton KL, Rybak MJ. Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides. *J Antimicrob Chemother* 2009;**63**:489—92.
20. Satola SW, Farley MM, Anderson KF, Patel JB. Comparison of detection methods for heteroresistant vancomycin-intermediate *Staphylococcus aureus*, with the population analysis profile method as the reference method. *J Clin Microbiol* 2011;**49**:177—83.
21. Rybak MJ, Leonard SN, Rossi KL, Cheung CM, Sader HS, Jones RN. Characterization of vancomycin-heteroresistant *Staphylococcus aureus* from the metropolitan area of Detroit, Michigan, over a 22-year period (1986 to 2007). *J Clin Microbiol* 2008;**46**:2950—4.
22. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. *J Antimicrob Chemother* 2001;**47**:399—403.
23. Walsh TR, Bolmström A, Qwärnström A, Ho P, Wootton M, Howe RA, et al. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. *J Clin Microbiol* 2001;**39**:2439—44.
24. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005;**43**:5026—33.
25. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;**38**:1008—15.
26. Wang JT, Wang JL, Fang CT, Chie WC, Lai MS, Lauderdale TL, et al. Risk factors for mortality of nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection: with investigation of the potential role of community-associated MRSA strains. *J Infect* 2010;**61**:449—57.
27. Hanaki H, Cui L, Ikeda-Dantsuji Y, Nakae T, Honda J, Tanagihara K, et al. Antibiotic susceptibility survey of blood-borne MRSA isolates in Japan from 2008 through 2011. *J Infect Chemother* 2014;**20**:527—34.
28. Chen H, Liu Y, Sun W, Chen M, Wang H. The incidence of heterogeneous vancomycin-intermediate *Staphylococcus aureus* correlated with increase of vancomycin MIC. *Diagn Microbiol Infect Dis* 2011;**71**:301—3.
29. Chen YH, Liu CY, Ko WC, Liao CH, Lu PL, Huang CH, et al. Trends in the susceptibility of methicillin-resistant *Staphylococcus aureus* to nine antimicrobial agents, including ceftobiprole, nemonoxacin, and tyrothricin: results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) study, 2006—2010. *Eur J Clin Microbiol Infect Dis* 2014;**33**:233—9.
30. Casapao AM, Leonard SN, Davis SL, Lodise TP, Patel N, Goff DA, et al. Clinical outcomes in patients with heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) bloodstream infection. *Antimicrob Agents Chemother* 2013;**57**:4252—9.
31. Bae IG, Federspiel JJ, Miró JM, Woods CW, Park L, Rybak MJ, et al. Heterogeneous vancomycin-intermediate susceptibility phenotype in bloodstream methicillin-resistant *Staphylococcus aureus* isolates from an international cohort of patients with infective endocarditis: prevalence, genotype, and clinical significance. *J Infect Dis* 2009;**200**:1355—66.
32. Chen YS. Guidelines for the treatment of methicillin-resistant *Staphylococcus aureus* infections in Taiwan. *J Microbiol Immunol Infect* 2013;**46**:147—50.
33. Di Gregorio S, Perazzi B, Ordoñez AM, De Gregorio S, Foccoli M, Lasala MB, et al. Clinical, microbiological, and genetic characteristics of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia in a teaching hospital. *Microb Drug Resist* 2015;**21**:25—34.
34. Howden BP, Peleg AY, Stinear TP. The evolution of vancomycin intermediate *Staphylococcus aureus* (VISA) and heterogenous-VISA. *Infect Genet Evol* 2014;**21**:575—82.
35. van Hal SJ, Paterson DL, Gosbell IB. Emergence of daptomycin resistance following vancomycin-unresponsive *Staphylococcus aureus* bacteraemia in a daptomycin-naïve patient—a review of the literature. *Eur J Clin Microbiol Infect Dis* 2011;**30**:603—10.
36. Tsao SM, Wang WY, Ko WC, Huang CH, Lu CT, Chuang YC, et al. Trend in vancomycin susceptibility and correlation with molecular characteristics of methicillin-resistant *Staphylococcus aureus* causing invasive infections in Taiwan: results from the Tigecycline in vitro Surveillance in Taiwan (TIST) study, 2006—2010. *Diagn Microbiol Infect Dis* 2014;**80**:162—7.
37. Sancak B, Yagci S, Gür D, Gülay Z, Ogunc D, Söyletir G, et al. Vancomycin and daptomycin minimum inhibitory concentration distribution and occurrence of heteroresistance among methicillin-resistant *Staphylococcus aureus* blood isolates in Turkey. *BMC Infect Dis* 2013;**13**:583.
38. Musta AC, Riederer K, Shemes S, Chase P, Jose J, Johnson LB, et al. Vancomycin MIC plus heteroresistance and outcome of methicillin-resistant *Staphylococcus aureus* bacteremia: trends over 11 years. *J Clin Microbiol* 2009;**47**:1640—4.