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Increased Vancomycin Minimum Inhibitory Concentrations of Methicillin-Resistant *Staphylococcus aureus* Nosocomial Isolates in Southwestern Saudi Arabia

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Abstract This study aimed to determine the distribution of vancomycin (VAN) MIC values and antimicrobial resistance patterns of MRSA nosocomial isolates from a Saudi tertiary care hospital and evaluate the presenting clinical and demographic features of different infections caused by these isolates. A total of 104 non-duplicating MRSA nosocomial strains were isolated. VAN MICs were determined by standard Etest and the Etest macromethod (MET). Among all isolates, 7.7% had a MIC = 2 µg/ml, 70.2% had a MIC = 1 µg/ml and 22.1% had a MIC = 0.5 µg/ml. No heterogeneous VAN-intermediate *S. aureus* (hVISA) were detected. Patients infected with high VAN MRSA nosocomial isolates were of significantly older age (p = 0.035), presented more often with bacteraemia (p = < 0.0001) and had longer hospital stays (p = < 0.0001). The presence of high VAN MICs of some MRSA isolates in our hospital is worrying and a cause for concern due to the possibility of the potential failure of treatment of these isolates. Moreover, accurate MIC testing using MET simultaneously or as a supplement to automated systems (i.e. Vitek) is important.

Keywords: *MRSA, vancomycin, MIC, Etest macromethod, nosocomial*

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1. Introduction

Staphylococcus aureus is commonly associated with hospital and community-acquired infections. Currently, measures to control *S. aureus* infections are challenged by a large and continuing increase in the prevalence of methicillin-resistant *S. aureus* (MRSA) worldwide [1,2], the spread of highly virulent community-associated MRSA [3], and the emergence of *S. aureus* with reduced susceptibility to vancomycin (VAN) and other glycopeptides [4,5,6].

Vancomycin remains the antibiotic of choice to treat many MRSA infections. In recent years, new anti-staphylococcal antibiotics, such as linezolid or daptomycin, have been developed, but glycopeptides remain the first-line therapeutic option [7]. However, due to the dramatic rise in MRSA infections and widespread use of VAN, which is known to have marginal tissue penetration and slow bactericidal activity, MRSA strains with reduced susceptibility to VAN are emerging [8,9]. Although most of these strains have a VAN MIC within the susceptible range, according to the Clinical and Laboratory Standards Institute; CLSI [10], some reports have shown a generalized increase in VAN MIC over time, also known as MIC creep [5,11,12].

Associated with this issue is the presence of heterogeneous VAN-intermediate *S. aureus* (hVISA). These organisms are described as being susceptible to VAN but contain a subpopulation that possesses a thicker cell wall and expresses resistance to VAN [13].

Emerging data suggest that VAN may be less effective against serious MRSA infection with MIC values at the higher end of the susceptibility range. Although the CLSI susceptibility breakpoint has been reduced to 2 ug/ml (previously 4 ug/ml), the increased rate of failures reported for MRSA infection at 2 µg/ml and 1.5 or 1 µg/ml has prompted a debate about whether the MIC breakpoints should be decreased even further [14,15].

The ability to determine which patients are likely to be infected with MRSA strains that have elevated MICs to VAN should be useful clinically. This knowledge could lead to earlier recognition of patients with increased potential for VAN treatment failure, allowing for the early use of alternative therapy in appropriate situations [4, 5].

Most hospitals report estimated VAN MICs through automated methods. These MIC values do not accurately reflect those produced with other standardized methods, such as the population analysis profile method using the

area under the concentration-time curve (PAP-AUC), Etest, microbroth dilution, macromethod Etest (MET) and glycopeptide resistance detection (GRD) Etest techniques, on which most outcomes data are based [6,9,12,13,16]. Up to 90% of MRSA isolates with an MIC of 2 µg/ml are missed by the automated systems [9].

With the development of the various detection methods, the accurate detection of VAN MICs and the prevalence of hVISA have been reported worldwide. However, little information from Saudi Arabia has been available [17,18].

This study aimed to determine the distribution of VAN MIC values and antimicrobial resistance patterns of MRSA nosocomial isolates from a Saudi tertiary care hospital and evaluate the presenting clinical and demographic features of different infections caused by these isolates to create a predictive tool to help guide clinical decision-making in this context.

2. Materials and Methods

This study was conducted in King Khalid hospital, a 350-bed tertiary care hospital in Najran, southwestern Saudi Arabia during the period from November 2012 to September 2013. Clinical MRSA isolates were collected from different specimens of patients, who were hospitalized for ≥48 hours. Only one isolate per patient was included in the study. For patients with more than one isolate, only the first isolate was tested. Identification of the infection focus was based on clinical, bacteriological and radiological investigations, and was defined according to the CDC/NHSN criteria [19]. The clinical and socio-demographic data for each patient were recorded using a standardized questionnaire, including age, sex, pre-existing co-morbidities, type of infection, history of stay in intensive care unit (ICU), history of prior VAN therapy, history of recent hospitalization and recent surgery.

All *S. aureus* isolates were identified according to conventional standardized laboratory methods (Gram stain, colony morphology, slide and/or tube coagulase and DNAse tests) and by Vitek 2 semi-automated system (bioMerieux, Marcy l'Etoile, France). Isolates resistant to oxacillin (1 ug) and cefoxitin (30 ug) by the disk diffusion technique were confirmed as MRSA by PCR detection of the *mec*A gene according to CLSI guidelines and as previously described [20,21].

2.1. Antimicrobial Susceptibility Testing

The susceptibility testing of MRSA isolates was determined by broth microdilution method, using Vitek 2 semi-automated system (bioMerieux) in the hospital's laboratory, as recommended by CLSI. The antimicrobial agents used included ciprofloxacin, levofloxacin, clindamycin, doxacycline, erythromycin, gentamycin, rifampin, vancomycin and trimethoprim-sulfamethoxazole Quality control was performed by using CLSI-recommended reference strains [10,20].

2.2. Vancomycin MIC Analysis

2.2.1. Standard Etest

Vancomycin MICs were determined by standard Etest methods (AB-Biodisk, Solna, Sweden) using a 0.5 McFarland standard inoculum on Mueller-Hinton agar plates (Remel, Lenexa, KA), according to the manufacturer's instructions.

2.2.2. Macromethod Etest

The MET was performed in the microbiology department of the Najran University College of Medicine. Briefly, the method included a 2.0 McFarland inoculum on brain heart infusion agar plates (Difco, Becton Dickinson and Company, USA) using VAN and teicoplanin Etest strips (AB-Biodisk), as previously described [22]. MET assays were performed on the same day and with an inoculum from the same initial culture as that used for the standard Etest. Heteroresistance by the MET was defined as MICs for VAN and teicoplanin of ≥8 µg/ml or a teicoplanin MIC of ≥12 µg/ml regardless of the VAN MIC.

The isolates with VAN MIC = 2 µg/ml were designated the "high MIC" group, while those with MIC = 1 µg/ml or 0.5 µg/ml were considered to be the "low MIC" group [14].

2.3. Statistical Analysis

Percentages were used for all categorical variables. For univariate analysis, the high MIC and low MIC groups were compared using the chi-square or the Student t-test or Fisher's exact test, as appropriate. Statistical significance was defined as a *p* value less than 0.05. All analyses were performed with the Statistical Package for the Social Sciences (SPSS), Version 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1. Demographic and clinical characteristics of the study patients by vancomycin MIC

Characteristic	Vancomycin MIC		*P* value
	MIC <2 µg/ml	MIC = 2 µg/ml	
Age in years, mean	46.1 ±18.6	60.5 ±12.9	0.035
Male gender (%)	57 (59.4%)	5 (62.5)	0.952
Type of infection			
UTI	33 (37.5)	0 (0)	0.564
RTI	32 (33.3)	1 (12.5)	0.431
SSI	23 (20.8)	0 (0)	0.195
Primary bacteraemia	8 (8.4)	7 (87.5)	<0.0001
Co-morbidities			
Diabetes	20 (20.8)	2 (25)	0.675
Hypertension	25 (26)	2 (25)	0.962
Malignancy	12 (12.5)	1 (12.5)	0.972
Recent surgery	20 (20.8)	3 (37.5)	0.869
ICU admission	39 (40.6)	6 (75)	0.074
Hospitalization days, mean	13 ±1.8	17.9 ±4.5	<0.0001
Empirical glycopeptides use <48 hours	67 (60.4)	5 (62.5)	0.67

Abbreviations: UTI, urinary tract infection; RTI, respiratory tract infection; SSI, skin and soft tissue infection.

A total of 104 non-duplicating MRSA nosocomial isolates were collected during the study period. Of these isolates, 7.7% had a MIC = 2 µg/ml, 70.2% had a MIC = 1 µg/ml and 22.1% had a MIC = 0.5 µg/ml. No hVISA or VISA were detected. Table 1 compares groups infected with high (MIC = 2 µg/ml) versus low (< 2 µg/ml) VAN MIC strains based on demographic and clinical data. Univariate analysis indicated that patients with MRSA nosocomial infection who were of old age; > 60 years (*p* =

0.035) or presented with primary bacteraemia (p = <0.0001) or who had been hospitalized for a long time; > 15 days, (p = <0.0001) were more likely to be infected with MRSA strains with a high VAN MIC. The age, sex ratio, types of infection other than primary bacteraemia, associated co-morbidities, and percentage of early empirical use of VAN were similar (not significantly different) in both groups.

Table 2. Number and percentages of *S. aureus* isolates resistant to non-glycopeptide antimicrobial agents at different vancomycin MICs

Antimicrobial agent	Vancomycin MIC	
	MIC < 2 µg/ml	MIC = 2 µg/ml
Ciprofloxacin	79 (82.3)	7 (87.5)
Clindamycin	77 (80.2)	7 (87.5)
Daptomycin	19 (9.4)	0 (0)
Erythromycin	77 (80.2)	8 (100)
Gentamycin	28 (29.2)	6 (75)
Moxifloxacin	40 (41.7)	4 (50)
Linezolid	0 (0)	0 (0)
Rifampin	0 (0)	0 (0)
TMP/SMX	59 (61.5)	5 (62.5)

Abbreviations: TMP/SMX, trimethoprim/sulfamethoxazole.

The difference of antimicrobial resistance rates between high versus low VAN MIC MRSA nosocomial isolates was presented in Table 2. The MRSA isolates with VAN MIC of 2 µg/ml were more frequently resistant to most non-glycopeptides antimicrobial agents. However, they were fully susceptible to Daptomycin, Linezolid and rifampin.

4. Discussion

Reduced susceptibility to VAN in *S. aureus* has been a major medical concern for over a decade. The interest in analyzing VAN MICs not only originates from concern that a further creep could shift MICs into the non-susceptible zone providing a precursor to hVISA and VISA, but there have been several reports of treatment failure against VAN susceptible isolates [5,11,12,14,23]. In this study, no hVISA were detected by using the MET. In a previous study investigated 1357 MRSA isolates from 12 Asian countries [24], Hetero-intermediate resistance to VAN was found among MRSA isolates from Japan (8.2%), India (6.3%), South Korea (6.1%), the Philippines (3.6%), Vietnam (2.4%), Singapore (2.3%), and Thailand (2.1%), but it was not found among strains from Saudi Arabia, China, Indonesia, Sri Lanka, or Taiwan. Based on the published reports, the prevalence rate of hVISA in other hospitals has varied, ranging from 0 to 50% [6,24,25,26].

In this study, 8% of MRSA isolates had VAN MIC of 2 µg/ml. In a previous epidemiologic study performed on 512 MRSA strains isolated from six major hospitals in Riyadh, Saudi Arabia, 12.7% of the isolates displayed VAN MIC of 2 µg/ml and 80.5% of isolates yielded MICs in the range of 1.0 to 1.5 µg/ml [17]. Several studies have reported elevated VAN MICs in MRSA isolates where the MICs were at the upper end of the susceptibility range [4,6,8,9]. However, the variability in VAN MIC testing methods should be considered, and the accuracy of susceptibility testing methods is debatable [9]. In this study the MET was used. This is a much less labor-intensive and less costly procedure than the standard broth microdilution method and maintains excellent sensitivity and specificity [8,15,22]. In a recent Saudi study, Al-Obeid et al reported the first case of VAN treatment failure in a male patient with sever sepsis caused by an MRSA isolate with VAN MIC of 3 and 2 µg/ml, as determined by Etest and microdilution methods, respectively [18]. Even though in this study we did not correlate the clinical outcome of MRSA nosocomial infections, the possibility of VAN treatment failures in our hospital settings could not be ruled out and is an area of concern.

Clinical factors associated with an elevated VAN MIC are similar to those associated with the development of hVISA. Previous studies showed that infections caused by MRSA with higher VAN MIC are seen in patients with recent exposure to VAN within one month of the current infection, prior recent hospitalization, surgery within last 6 months, chronic liver disease, cardiovascular disease, presence of non-tunneled central venous catheter and those with blood stream infections prior to admission in ICUs [4,15,27]. Our study demonstrated that patient factors, such as old age, patients with invasive infections due to primary bacteraemia and prolonged hospital stay are epidemiological predictors of acquisition of nosocomial infection by an MRSA isolate with high VAN MIC in our institution and therefore help in selecting the early and empiric effective anti-MRSA therapy.

In this study, similar to previous reports, MRSA isolates with high MICs were more resistant to other antimicrobial agents, but they were fully susceptible to linezolid and daptomycin [14,28,29]. Following the advice in the Infectious Diseases Society of America (IDSA) MRSA guidelines would mean that clinicians would start therapy with VAN while anticipating the possibility of an elevated VAN MIC and potential clinical failure, but with a plan to then escalate therapy [30]. However, increasing the dosage of VAN given to MRSA patients with elevated VAN MICs does not seem to improve outcome; instead, it may impair renal function, particularly when patients are concurrently taking a nephrotoxic medication [31]. Therefore, recently published guidelines suggest considering alternative antibiotics such as daptomycin or linezolid in complicated MRSA infections when the isolates are found to have MICs ≥ 2 µg/ml [32]. Our result indicated that daptomycin might be an optimal therapeutic alternative to treat some MRSA nosocomial infections, especially primary bacteremia caused by MRSA isolates, even with a VAN MIC of 2 µg/ml.

Limitations to the current study exist and should be noted. First, the MRSA nosocomial isolates were collected from a single tertiary care hospital. Institutional differences in patients' characteristics, antibiotic prescribing patterns and resistance profiles may affect the applicability of these results to other institutions. Another limitation is the lack of clinical information about patient outcome, infection-related mortality and VAN dosing information and serum concentrations. Nevertheless, this is not a clinical trial but is rather an epidemiological study analyzing VAN MICs. Finally, the study period was not sufficiently long to analyze the VAN MICs trends and VAN creep. Therefore, a larger study will be needed to follow the changing patterns of VAN MICs among nosocomial MRSA isolates and evaluate whether any effect of increasing VAN MICs on clinical characteristics and outcomes of serious MRSA infections becomes apparent.

5. Conclusion

The presence of high VAN MICs (2 µg/ml) in 8% of MRSA nosocomial isolates in our hospital is worrying and a cause for concern due to the potential failure of treatment of MRSA isolates with increased MICs. Moreover, accurate MIC testing using MET simultaneously or as a supplement to automated systems (i.e. Vitek) is important and will help clinicians to select appropriate empirical choice of therapy for managing serious MRSA nosocomial infections, particularly for high-risk.

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