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Glycopeptide and daptomycin susceptibility trends among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a tertiary care center in North India

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Summary Increased vancomycin minimum inhibitory concentration (MIC) levels in *Staphylococcus aureus* and their association with vancomycin treatment failure are well-known problems. Few studies have recognized progressive increases in glycopeptide MIC levels for *S. aureus* strains in recent years. This study determined glycopeptide and daptomycin susceptibility among methicillin resistant *S. aureus* (MRSA) strains. A total of 776 clinical isolates of MRSA recovered from 2009 to 2012 were studied for glycopeptide and daptomycin susceptibility using the E-test method. The vancomycin MIC geometric mean (GM) of the MRSA isolates was 0.923, 0.944, 1.134 and 1.294 mg/L in the years 2009, 2010, 2011 and 2012, respectively, and the trend significantly increased over the years ($P < 0.0001$). Similarly, the teicoplanin MIC GM was 1.47, 1.49, 1.8 and 2.04 mg/L in the years from 2009 to 2012, respectively ($P < 0.0001$). MIC shifts were not found for daptomycin ($P > 0.232$). A significant increase in the MIC for glycopeptides was observed among the clinical

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MRSA isolates at our center over a 4-year period. However, the daptomycin MIC did not increase in the observed MRSA isolates.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains with reduced susceptibility to vancomycin are emerging worldwide [1]. Several studies have reported elevated vancomycin MICs in MRSA isolates in which the MICs are at the upper end of the susceptibility range [2]. Although most of these strains have a vancomycin MIC within the susceptible range according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, some reports have shown a universal increase in the vancomycin MICs over time (also known as a MIC creep) [3]. Limited studies of the vancomycin MIC creep based on routine susceptibility testing in clinical laboratories are available. However, MIC creep is not global; some centers have observed no changes in vancomycin MICs over time [4,5]. The reports of vancomycin MIC creep in health care settings from different countries, including lower income countries, such as India, are inadequate because of insufficient microbiological facilities and the cost of MIC tests in many hospitals. This may be an important reason for the scant data on the MIC creeps in such countries. Several susceptibility tests are available, but CLSI recommends MIC testing for sensitivity to glycopeptide and daptomycin [6]. Although several MIC markers have been reported to detect changes in the MIC, the geometric mean MIC is considered to be a more sensitive marker [7].

The purpose of this study was to evaluate MIC trends among clinical MRSA isolates for glycopeptides and daptomycin over a 4-year period (2009—2012) by utilizing both a more sensitive MIC testing method (E-test) and a more sensitive susceptibility marker (geometric mean) to detect MIC changes over time.

Materials and methods

The present study was carried out in the Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow a 900 bedded tertiary care center of North India. A total of 776 MRSA strains isolated between 2009 and 2012 from different clinical specimens, such as blood, wounds, abscesses, sputum, urine and body fluids, were subjected to glycopeptide and daptomycin susceptibility tests. All of the isolates were stored in cryo vials at −80 °C until MIC testing was performed.

The MIC tests were performed using the standard E-test (BioMerieux India Pvt Ltd) procedure. Muller Hinton Agar (Oxoid Ltd., Basingstoke, Hampshire, England) plates were swabbed with 0.5 McFarland standard suspensions of test organisms and allowed to dry. Vancomycin, teicoplanin and daptomycin E-strips were placed in the plates, and then, the plates were incubated at 37 °C for 18 h.

MIC trends over 4 years were assessed using a non-parametric correlation (Spearman's *r*) test. One-way analysis of variance (ANOVA) with two-sided Bonferroni multiple comparison tests were performed to assess significance. Statistical significance was defined as P values <0.05. Statistical analyses were performed by using the Statistical Package for the Social Sciences version 15 (SPSS Inc., Chicago, IL).

Results

During the study period, a total of 776 clinical MRSA isolates were collected for analysis. The numbers of isolates tested each year were 131, 227, 207 and 211 in 2009, 2010, 2011 and 2012, respectively. The vancomycin MIC geometric means significantly increased from 0.923 to 1.294 mg/L over the study period (Fig. 1). The vancomycin MIC mode was 1.0 in 2009 but increased to 1.5 between 2010 and 2012 (Table 1). The teicoplanin geometric mean MIC ranged from 1.47 to 2.44 mg/L (Fig. 1), and a significant increase between 2009 and 2012 was observed ($P < 0.0001$). Teicoplanin and daptomycin MIC modes varied from 1.5 to 3.0 mg/L and 0.75 to 0.5 mg/L between 2009 and 2012, respectively (Table 1). A decreasing tendency was observed for the daptomycin MIC mode. The geometric mean of the daptomycin MIC ranged from 0.455 to 0.430 mg/L over the years, and a MIC shift was not observed for daptomycin ($P > 0.232$).

The MIC distributions for each drug from 2009 to 2012 are shown in Fig. 2. The most noticeable population shifts were seen with vancomycin and teicoplanin (Fig. 2a and b). The shifts in vancomycin MICs primarily occurred as a result of a decrease in the percentage of isolates with a MIC of 1 mg/L

Table 1 Susceptibility trends among MRSA strains isolated from 2009 to 2012.

Antibiotic	Year	Modal MIC	Median MIC	Geometric mean	Mean	%S/%R/%I
Vancomycin	2009	0.8	1.0	0.93	1.06	100/0/0
	2010	1.5	1.0	0.94	1.09	100/0/0
	2011	1.5	1.0	1.13	1.2	98/0/2
	2012	1.5	1.5	1.29	1.41	99/0/1
Teicoplanin	2009	1.5	1.5	1.47	2.01	100/0/0
	2010	1.5	1.5	1.49	2.04	100/0/0
	2011	3.0	2.0	1.8	2.56	100/0/0
	2012	2.0	2.0	2.04	3.01	100/0/0
Daptomycin	2009	0.75	0.38	0.465	0.53	100/0/0
	2010	0.5	0.38	0.4	0.46	100/0/0
	2011	0.5	0.38	0.35	0.40	98/0/2-NS
	2012	0.75	0.38	0.43	0.33	99/0/1-NS

NS: non-susceptible.

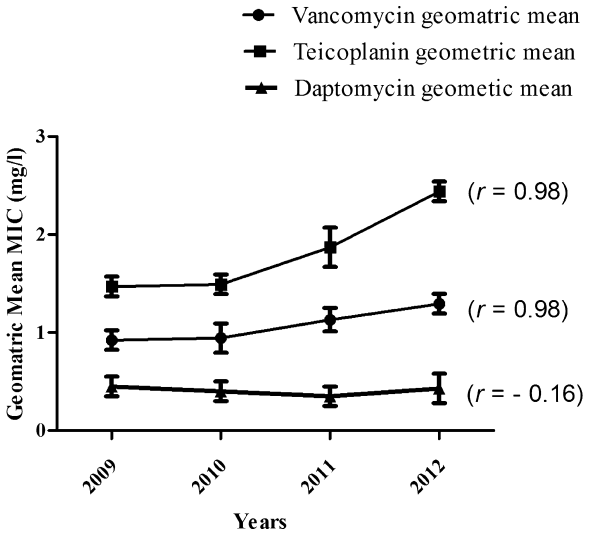

Figure 1 The geometric mean ± standard error of the vancomycin, teicoplanin and daptomycin MICs in methicillin-resistant *Staphylococcus aureus* isolated from 2009 to 2012.

(35.1% in 2009 to 20.8% in 2012) and an increase in the percentage of isolates with a MIC of 1.5 mg/L (22.1% in 2009 to 35.07% in 2012). There was no isolate with a vancomycin MIC ≥ 6 mg/L. Overall, the MICs increased for vancomycin and teicoplanin ($P < 0.0001$) and declined slightly for daptomycin ($P > 0.286$) over the study period. However, 3 isolates were non-susceptible to daptomycin using the current CLSI guidelines, and all of them were positive for hVISA by screening methods (data not given).

Discussion

Reduced susceptibility to glycopeptides in *S. aureus* has been a major medical concern over the past decade. Previous studies have reported elevated vancomycin MICs in MRSA isolates in which the MICs are at the higher end of the susceptibility range [8—10]. As decreases in vancomycin susceptibility appear to occur, close monitoring of vancomycin susceptibility trends is necessary, especially in countries where the inappropriate use of antibiotics is common [11] and the bacterial disease burden is high [12]. Recently, the UK and Ireland reported a significant increase in vancomycin MICs for *S. aureus* isolates, and the analysis was based on the changes in the geometric mean of the MICs [2]. Similarly, in the present study, the geometric mean for vancomycin was 0.923 in 2009 and increased to 1.294 in 2012 (1.38-fold), while the teicoplanin MIC increased from 1.47 in 2009 to 2.44 in 2012 (1.65-fold). Previous reports from other centers and the USA showed a significant increase (1.5—3.5-fold) in the vancomycin MIC geometric means over a time period [3,13]. In the present study, the daptomycin geometric mean remained unchanged over time and actually declined slightly in 2012 (0.93-fold) from the 2009 MIC geometric mean. Consistent with our findings, one previous study from the USA also revealed a 0.90-fold decrease in the daptomycin MIC geometric mean in 2007 [13]. Although there was a decrease in the daptomycin MIC geometric mean over the study period in our center, 3 strains were found to be non-susceptible to daptomycin, and all of these isolates were identified as hVISA. This observation is of clinical concern and suggests the need to monitor the emergence of resistance to daptomycin in *S. aureus* strains and also highlights the need for the judicious use of this drug to retain its long-term effect. In accordance with previous observations [14], we also recommend the administration of other agents, such as daptomycin, linezolid and rifampicin, alone or in combination with vancomycin, in patients infected

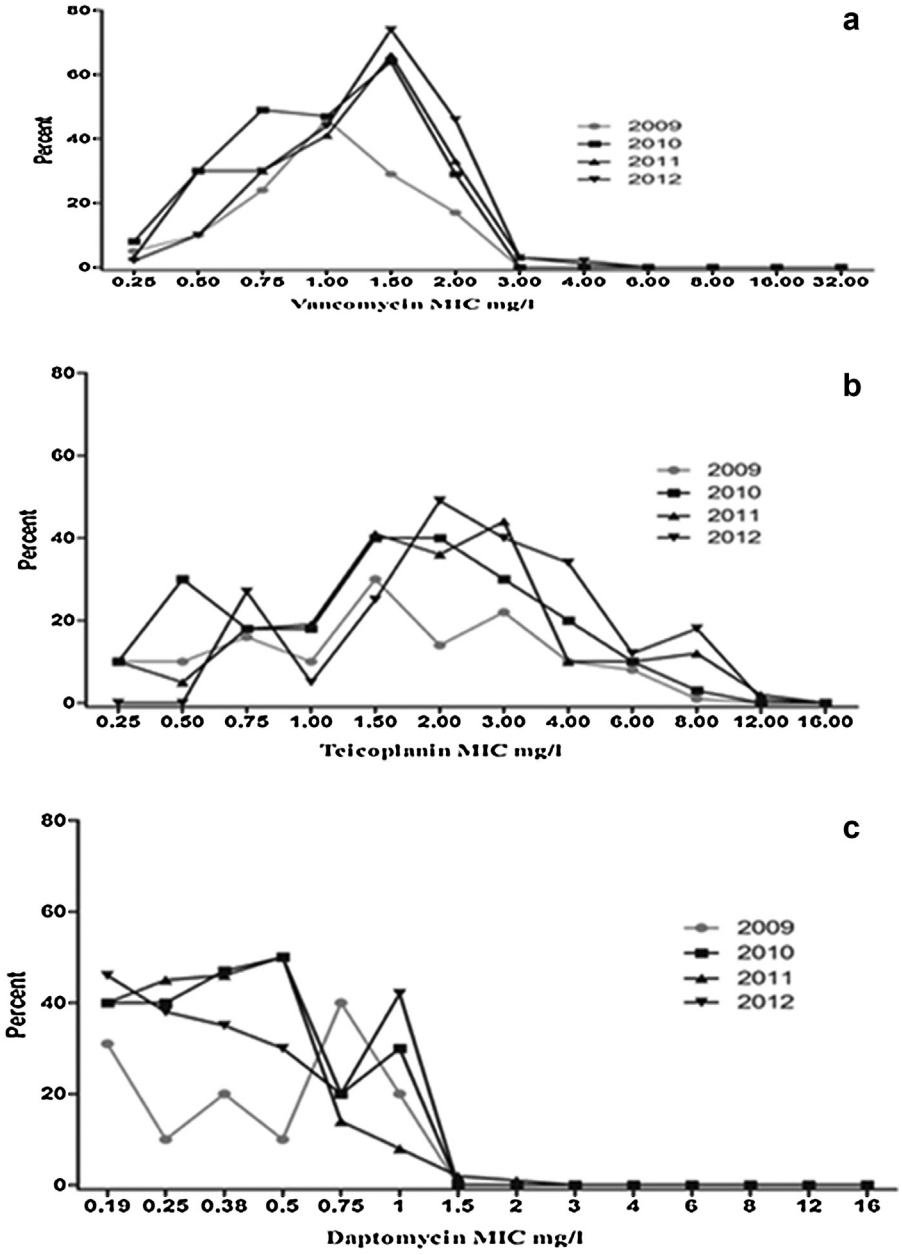

Figure 2 The population distribution of vancomycin, teicoplanin and daptomycin from 2009 to 2012 (a: the vancomycin MIC population distribution 2009—12, b: the teicoplanin MIC population distribution 2009—12, c: the daptomycin MIC population distribution 2009—12).

with high glycopeptide MIC strains. However, linezolid is not licensed for the treatment of bacteremia/infective endocarditis and daptomycin is not used for respiratory infections [15]. The vancomycin monitoring guidelines recommend higher troughs (15—20 μg/mL) for serious infections, such as bacteremia, endocarditis, osteomyelitis, meningitis and hospital-acquired pneumonia, caused by *S. aureus* and the use of an alternative agent when the MIC is ≥2.0 μg/mL [16]. Strains with elevated glycopeptide MICs require aggressive vancomycin dosing to achieve troughs between 15 and 20 μg/mL. However, therapeutic drug monitoring of vancomycin is required to reduce the risk of toxicity (ototoxicity and nephrotoxicity). It has also been observed that serum unbound vancomycin trough concentrations at 4—5 times the MIC or a 24-h AUC/MIC ratio of 400 are optimal for bacterial eradication and clinical success [14].

A shift in MICs over time may accelerate the development of resistance because of suboptimal drug exposure resulting from vancomycin dosing regimens [17]. The factors involved in the development of reduced susceptibility to vancomycin and

the subsequent ''glycopeptide MIC creep'' are not entirely known, but this phenomenon is of clinical concern because poorer treatment outcomes have been associated with higher vancomycin MICs [18]. Therefore, the recognition of this phenomenon is important because it may be a precursor to hVISA and VISA. To the best of our knowledge, this is the first report of a daptomycin-non-susceptible hVISA infection in India.

Conclusion

Glycopeptide MIC increases within the susceptible range were observed among the clinical MRSA isolates at our center over a 4-year period. While no daptomycin MIC creep was observed in the MRSA isolates, the emergence of daptomycin non-susceptible hVISA strains calls for constant monitoring.

Conflict of interest

All of the authors confirm that they have no conflict of interest.

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