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Original Article

Microbiology Section

Reduced Daptomycin Susceptibility in Clinical MRSA Isolates Showing Vancomycin MIC Creep Phenomenon

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ABSTRACT

Introduction: Infections caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, longer antimicrobial therapy, etc. First option for treating invasive MRSA infections is glycopeptide vancomycin. Daptomycin, a lipopeptide rapidly bactericidal invitro against MRSA, is an acceptable alternative.

Aim: To identify MRSA isolates from clinical specimens and assess their vancomycin and daptomycin susceptibility pattern.

Materials and Methods: The cross-sectional study was conducted over a period of six months (January to June 2019) on 90 clinical samples in a rural teaching hospital in Pune, Maharashtra, India, including all samples except sputum received in the Microbiology laboratory. MRSA isolates were tested for vancomycin and daptomycin susceptibility by Epsilometer (E) test Minimum Inhibitory Concentration (MIC) method. Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 16.0 software.

Results: Among 90 MRSA isolates, most were from pus 51 (56.7%) followed by urine 23 (25.5%), blood 9 (10%), followed by miscellaneous samples 7 (7.7%). All MRSA isolates in this study were susceptible to daptomycin with MIC in the range of 0.25-1 µg/mL with maximum isolates (39) with MIC of 0.38 µg/mL. Vancomycin MIC creep phenomenon was observed in 68 isolates. All these isolates also showed reduced susceptibility to daptomycin.

Conclusion: MRSA in hospital set up mandates strict infection control practices in place. Daptomycin can be a good therapeutic alternative to treat infections caused by MRSA keeping in mind its therapeutic limitations and prior vancomycin usage in the same patient. Empirical therapy should always be based on antibiogram pattern. Adherence to hospital antibiotic policy and constant surveillance of antimicrobial resistance is the need of the hour.

Keywords: Empirical therapy, Epsilometer test, Methicillin resistant *Staphylococcus aureus*, Minimum inhibitory concentration

INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the most common pathogens causing severe infections. Infections caused by MRSA are associated with increased morbidity, longer antimicrobial therapy, increased healthcare costs, prolonged hospital stay and increased risk of death [1]. The first option for treating invasive MRSA infections is glycopeptide vancomycin, which continues to be the reference standard approach [2]. Use of vancomycin has been increasing since the mid-1980s, which results in the emergence of MRSA with reduced susceptibility to vancomycin [3]. Vancomycin MIC creep phenomenon is described as increase in the MIC of vancomycin for a particular isolate though within the susceptible range [1,4].

Linezolid has a broad spectrum of activity against gram positive bacteria including multiple drug resistant isolates. Linezolid is a bacteriostatic agent which inhibits bacterial protein synthesis by binding to the 50s ribosomal subunit near to the interface with the 30s subunit, causing inhibition of 70S initiation complex formation [5]. Daptomycin is a cyclic lipopeptide antibiotic that is bactericidal, invitro against a broad spectrum of gram positive bacteria, including MRSA. Current published evidence suggests that daptomycin may be an acceptable alternative to vancomycin for MRSA infections, especially MRSA bacteremia involving isolates with vancomycin MIC values of 1.5 to 2 µg/mL [6].

Daptomycin was found to be at par with the standard antimicrobial therapy used currently and hence was approved in a dose of 4 mg/kg for treating complicated skin and soft tissue infections and at a dose of 6 mg/kg for treating *S. aureus* bacteremia and right-sided endocarditis. Clinical observations have showed that daptomycin can be a treatment option for gram positive bone and joint infections. Even the multidrug-resistant gram positive microorganisms have demonstrated high daptomycin susceptibility as stated in a few large international studies. Thus, the possible indication for the use of daptomycin may be in the treatment of infections caused by drug resistant gram positive cocci [7].

The activity of daptomycin is strictly dependent on the physiological levels of calcium ions, which induce conformational changes in daptomycin [8,9]. These conformational changes are believed to increase the exposure of hydrophobic moieties in daptomycin molecule which in turn expedite daptomycin oligomerisation and membrane insertion [9-11]. Also, daptomycin is being used with increasing frequency as a primary agent for the treatment of *S. aureus* bacteremia, particularly for persistent bacteremia in which vancomycin MICs are 2 µg/mL. Two studies showed that daptomycin may be more efficacious than vancomycin for the treatment of such bacteremias [12,13]. These two studies showed daptomycin is associated with decreased 60 day or 30 day mortality and fewer instances of persistent bacteremia [12,13].

Similarly, daptomycin is commonly employed for the treatment of difficult Vancomycin Resistant *Enterococcus* (VRE) infections, such as bacteremia, based on invitro activity and data from individual cases reports, despite the lack of clinical trial data [14]. Many studies have reported invitro susceptibility to daptomycin and linezolid against gram positive bacterial isolates [15-18].

In the view of a few published studies from India on the antimicrobial susceptibility to daptomycin against MRSA isolates showing vancomycin creep phenomenon [5], the study was undertaken with the aim of identifying isolates of MRSA from clinical specimens and assessing the vancomycin and daptomycin susceptibility pattern of MRSA isolates and to find out the existence of any association between MICs of these two crucial antimicrobials.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of six months from January to June 2019, in a tertiary care hospital in Pune,

Maharashtra, India, after obtaining the approval from Institutional Ethics committee (approval no. IEC/315). The informed consent was obtained from patients after admission to the hospital for all the investigations to be conducted on the patient, as per hospital policy. All the isolates who were fulfilling inclusion criteria were included in the study. The current study was a part of author's earlier study on vancomycin MIC creep phenomenon [19].

Inclusion criteria: Only one isolate per patient was included in this study. For patients with more than one isolate, only the first isolate was tested. All nonrepetitive MRSA isolates from different clinical specimen, received in the Microbiology laboratory during the study period were included in the study.

Exclusion criteria: Repetitive MRSA isolates from a patient and MRSA from sputum samples were excluded.

Study Procedure

All isolates were identified as *S. aureus* using routine bacteriological procedures (Gram's stain microscopic examination, catalase test and coagulase test, mannitol fermentation test). Susceptibility testing for cefoxitin was performed using the disk diffusion method, in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI). Isolates which showed resistance to cefoxitin were labelled as MRSA [20].

MIC determination for vancomycin and daptomycin: MIC value for vancomycin was determined by using the E-test (Hi media) [21]. Minimum Inhibitory Concentration (MIC) value for daptomycin was determined by Epsilometer test using the E-test MIC strip (Hi media) method, in which daptomycin E-test strips (Himedia) were applied onto Muller Hinton agar plates supplemented with calcium with 0.5 McFarland suspension of microorganisms. The daptomycin E-test contained a concentration gradient of daptomycin throughout the strip.

All plates were incubated at 35°C for 24 hours. The MIC values for vancomycin and daptomycin were measured as the zone of inhibition that corresponds to a concentration gradient on the E-test strips, as per the manufacturer's instructions and interpretation made as per CLSI criteria. Susceptibility breakpoint for daptomycin was considered as <1 µg/mL for staphylococci, as recommended by the CLSI [20].

STATISTICAL ANALYSIS

Statistical analysis was done by SPSS (version 16.0) statistical software (SPSS Inc., IBM), and descriptive statistics was used.

RESULTS

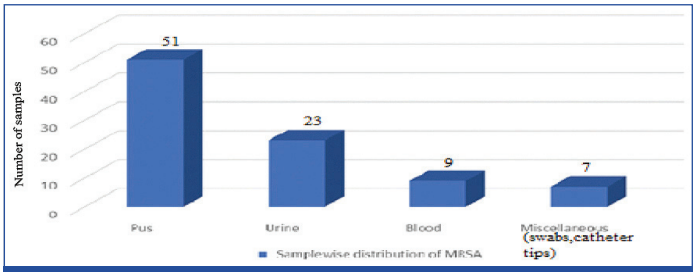
In this study, 90 isolates of MRSA were included. More number of MRSA was isolated from male patients 51 (56.7%) as compared to female patients 39 (43.3%). We included MRSA isolated from Inpatient Department (IPD) samples 81 (90%) and Intensive Care Unit (ICU) 9 (10%).

Distribution of MRSA in different types of samples: Among the total 90 samples, MRSA was isolated most commonly from pus 51 (56.7%) followed by urine 23 (25.5%), blood 9 (10%) and miscellaneous samples which included swabs 5 (5.5%) and catheter tips 2 (2.2%) [Table/Fig-1].

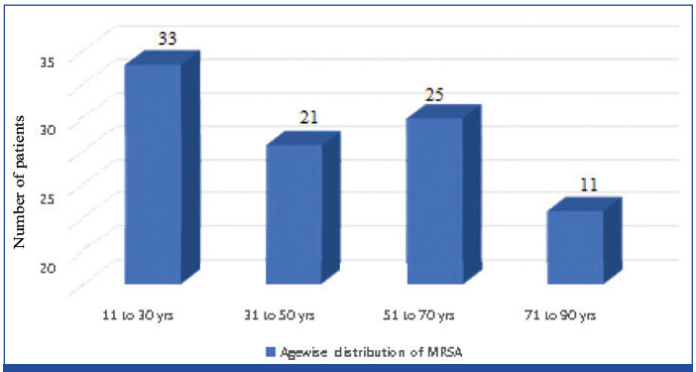
Distribution of MRSA as per age of patients: MRSA was most commonly isolated from the age group 11-30 years (33 isolates) and least number of cases were seen in the age group of 71-90 years (11 isolates) [Table/Fig-2].

Vancomycin MICs: Out of 90 isolates, isolate no. 1 had vancomycin MIC 0.5 µg/mL while for second isolate MIC was 0.6. For isolates no. 3-22 MIC was 0.75 and isolate no. 23-46 showed MIC value of 1. MIC value for the isolate no.47-78 was 1.5. Five isolates in this study (no.79-83) showed MIC 1.75 and remaining seven isolates (no. 84-90) demonstrated MIC value of 2 µg/mL [Table/Fig-3].

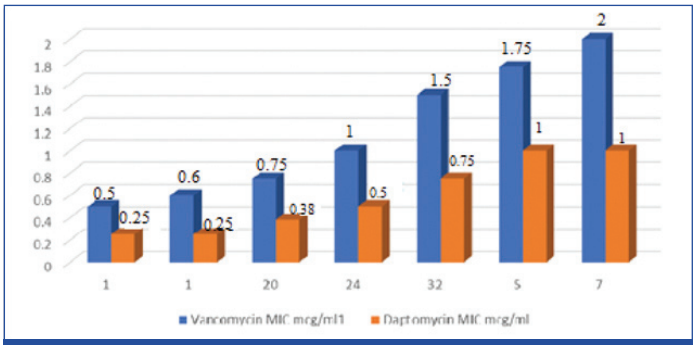
Daptomycin MICs: Out of 90 isolates included in the study, isolate no. 1 and 2 had Daptomycin MIC 0.25 µg/mL. For isolates no. 3-22 MIC was 0.38 and isolate no. 23-46 showed MIC value of 0.5. MIC value for the isolate no. 47-78 was 0.75. Last 12 isolates in this study (no. 79-90) demonstrated MIC value of 1 µg/mL [Table/Fig-3].



[Table/Fig-1]: Sample wise MRSA distribution.



[Table/Fig-2]: Age wise distribution of MRSA isolates.



[Table/Fig-3]: Vancomycin and daptomycin MIC range distribution in MRSA isolates. X axis shows MRSA isolates and Y axis shows vancomycin and daptomycin MICs

A 68 MRSA isolates showed vancomycin MIC creep phenomenon. These results suggested that the MRSA isolates which showed increased MICs for vancomycin also showed corresponding increase in daptomycin MICs.

DISCUSSION

Though, all MRSA isolates in this study were susceptible to daptomycin, a few isolates showed increase in MIC in the susceptible range, which well correlated with isolates showing vancomycin MIC creep. Daptomycin susceptibility results for MRSA from this study correlate well with other similar studies conducted in India which also reported 100% daptomycin susceptibility [5]. A study group from Asia pacific region reported >99% susceptibility of daptomycin against staphylococci [16]. Another study from India has also reported daptomycin MIC in the range of 0.047-1 ug/mL [22].

The MIC for daptomycin among MRSA, in present study was in the range of 0.25 1 µg/mL with most of the isolates of MRSA having an MIC of 0.38-0.75 µg/mL and a few isolates having MIC of 1 µg/mL. The MIC of daptomycin for MRSA in present study however was relatively higher than in study by Yousuf T et al., in Kashmir in 2015. In their study, maximum MRSA isolates were reported with MIC value of 0.128 µg/mL [23].

Another study conducted by Chitnis S et al., showed majority of MRSA isolates had MIC values between 0.19-0.5 µg/mL with maximum isolates having MIC value of 0.5 µg/mL [5]. MIC values

reported in present study was comparatively higher than Chitnis S et al., study [5].

In this study, association was found between MICs of vancomycin and daptomycin. The isolates showing increased vancomycin MICs (vancomycin MIC creep) also showed increase in MICs of daptomycin. These findings was in concordance with the study by Kelly P et al., [24]. In a study by Sakoulas G et al., they also supported the evidence of reduced activity of daptomycin against MRSA which exhibit a reduced susceptibility to vancomycin, especially if a patient is treated earlier heavily with vancomycin. The authors in their study, also concluded that MRSA infections treated with daptomycin in early phases, instead of vancomycin can have better potential of cure of these infections [25]. The same can be true for our patients also, as most patients had received vancomycin empirically, even before antimicrobial susceptibility test report is available which must have led to the obvious decrease in susceptibility to daptomycin.

Further studies are needed especially from Indian set up, to throw light upon the probability of development of daptomycin non susceptibility in MRSA showing reduced vancomycin susceptibility.

Limitation(s)

Sample size was small in present study. Also, only E-test method was used for detection of vancomycin and daptomycin MICs instead of more better and gold standard methods like MIC broth microdilution.

CONCLUSION(S)

Strict infection prevention and control measures need to be emphasised to control the prevalence of MRSA in healthcare setup. Daptomycin can be a good therapeutic alternative to the vancomycin to treat infections caused by MRSA in present setup. The selection of antibiotic should be based on invitro susceptibility, antibiogram pattern of microorganisms in a particular healthcare setting and history empirical use of vancomycin especially while considering treatment with daptomycin.

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