Current status of vancomycin susceptibility against methicillin resistant Staphylococcus aureus (MRSA) strains: A study at two tertiary care hospitals of Pakistan

Fatima Kaleem¹*, Javaid Usman¹, Abdul Sattar¹, Samina T. Amanat², Afrreenish Hassan¹, Maria Omair¹, Ali Khalid¹ and Muhammad Riaz²

¹Department of Microbiology, Army Medical College, National University of Sciences and Technology, Islamabad, Pakistan.
²Department of Microbiology, PAEC General Hospital, H-11/4, Islamabad.

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Vancomycin has been considered mainstay treatment of infections caused by methicillin-resistant Staphylococcus aureus. The reports of the emergence of vancomycin intermediate and vancomycin resistant S. aureus from various parts of the world have been of great clinical concern. This study was performed to monitor the status of glycopeptide susceptibility against methicillin resistant S. aureus in our set up. All non-duplicate methicillin resistant Staphylococcus aureus (MRSA) isolates recovered during the period of study from various wards of Military Hospital Rawalpindi and PAEC General Hospital Islamabad, were subjected to the detection of minimum inhibitory concentrations of vancomycin using E-strips. Results were analyzed to evaluate the possible presence of vancomycin intermediate and resistant strains in the set up. A total of 276 methicillin-resistant S. aureus isolates were studied. The range of vancomycin minimum inhibitory concentrations (MIC) was 0.19 to 3 ug/mL. MIC 50 came out to be 0.75 ug/mL whereas the MIC 90 was 1.5 ug/mL. 128 out of 276 (46%) isolates had vancomycin MIC equal to or greater than 1 ug/mL. Majority of the isolates (69%) were from pus samples. No vancomycin resistant or intermediate strain of MRSA was isolated during the study but there were a significant number of isolates having ≥ 1 µg/ml MIC of vancomycin.

Key words: E- test, methicillin resistant Staphylococcus aureus, minimum inhibitory concentrations (MIC), susceptibility, vancomycin.

INTRODUCTION

A clinical isolate of methicillin resistant Staphylococcus aureus (MRSA) showing in vitro intermediate susceptibility to vancomycin is called vancomycin-intermediate S. aureus (VISA), and it was first isolated from Japan in year 1996. Eight VISA infections had been documented in patients in the United States of America till June 2002. First clinical isolate of vancomycin resistant S. aureus (VRSA) was reported from a patient in Michigan (USA) in year 2002 (Chang et al., 2003). The term VRSA is based on the vancomycin minimum inhibitory concentration breakpoint of the Clinical and Laboratory Standards Institute (CLSI) where a strain for which the MIC is ≥ 16 ug/ml is defined as resistant (Wayne, 2010). It was reported for the first time in 2006 from Jordan (Azzam, 2006).

In Pakistan, the incidence of nosocomial infections caused by MRSA is continuously on the rise ranging from 5 to 61% (Qureshi et al., 2000; Hafiz et al., 2002; Hussain...
et al., 2005; Akhter et al., 2009). As the incidence of MRSA infections is rising, the use of glycopeptides (vancomycin and teicoplanin) is also increasing day by day. The glycopeptides antibiotic vancomycin was introduced clinically in year 1958 for the treatment of Gram-positive bacteria. Use of this agent has increased dramatically over the last 20 years. With the emergence of vancomycin resistant Enterococci, it was feared that this resistance might also spread to Staphylococci very soon and this became true when low-level vancomycin resistance in S. aureus was reported in 1996 (Hiramatsu et al., 1997). Such resistance, though rare, but it is an emerging threat because of its potential to disseminate very rapidly. The indiscriminate use of glycopeptides for suspected Gram positive bacterial infections/bacteremia in hospital settings has increased the chances of VISA and VRSA emergence in our set up.

The objective of this study was to determine the current status of Vancomycin susceptibility for the possible presence of vancomycin resistant and intermediate strains of S. aureus in our set up.

MATERIALS AND METHODS

Study design

The study was a non-interventional, descriptive cross sectional study. It was conducted at the Department of Microbiology, Army Medical College, Rawalpindi, National University of Sciences and Technology, Islamabad, Pakistan and PAEC General Hospital Islamabad from December 2009 to June 2011.

Two hundred and seventy six clinical isolates of MRSA from various clinical specimens sent for culture and sensitivity to the Department of Microbiology of Army Medical College, Rawalpindi were included in the study.

Clinical specimens of the patients like pus, blood, sputum, tissue, body fluids, urine, pus swabs and catheter tips received for culture and sensitivity from various wards and outpatient departments of Military Hospital, Rawalpindi and PAEC General Hospital Islamabad, were processed by Gram staining and culture on appropriate media and incubated for 24 to 48 h at 37°C to get bacterial growth. Isolates were identified as S. aureus by colony morphology, microscopy of Gram’s stained smears, catalase, coagulase and DNase tests (Bannerman, 2003). Isolates were subjected to routine antimicrobial susceptibility testing. For identification of S. aureus as MRSA, according to the CLSI guidelines S. aureus isolates were tested for methicillin resistance by modified Kirby-Bauer disk diffusion technique using 1 µg oxacillin disk and 30 µg cefoxitin disk (Oxoid, Basingstoke, UK) and Mueller-Hinton agar (Oxoid, Basingstoke, UK) containing 2% NaCl. The plates were incubated at 35°C for 24 h. Susceptibility to oxacillin and cefoxitin was interpreted as per CLSI criteria (Wayne, 2010).

MIC was done using E-strips according to the guidelines provided by the CLSI (Wayne, 2010). Bacterial suspensions were made in normal saline, matching 0.5 McFarland turbidity standards. A sterile swab was dipped into the inoculum suspension, excess fluid was removed by pressing the swab against the inside wall of the test tube. The entire Mueller Hinton agar surface was swabbed three times by rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. Excess moisture was allowed to be absorbed for about 15 min so that the surface was completely dry before applying the E-test vancomycin strip.

The strip was gripped with a pair of sterile forceps, and placed on to the inoculated agar surface. It was made sure that the whole length of the strip was in complete contact with the agar surface. The plates were incubated at 37°C for 16 to 18 h. When bacterial growth was clearly visible, the MIC values were read where the respective inhibition ellipses intersected the strip. Growth along the entire gradient that is no inhibition ellipse indicated that the MIC is greater than the highest value on the reading scale. An inhibition ellipse below the gradient indicated a MIC greater than the lowest value on the scale. When mutant colonies were present in the inhibition ellipse, the MIC was read where those colonies were inhibited. MSSA ATCC 25923, MRSA 33591 were used as control strains.

Statistical analysis

The numerical data consisting of vancomycin disc diffusion zone size and MIC were entered in statistical programme for social sciences (SPSS) version 17.0 software. Frequency of the identified vancomycin resistant, intermediate and sensitive isolates was calculated in percentage out of total number of MRSA isolates. MIC 50 and MIC 90 were calculated.

RESULTS

A total of 276 MRSA isolates were studied. The range of vancomycin MIC was 0.19 to 3 µg/mL. MIC 50 came out to be 0.75 µg/mL whereas the MIC 90 was 1.5 µg/mL (Table 1). 128 out of 276 (46%) isolates had vancomycin MIC equal to or more than 1µg/mL. Majority of isolates (69%) were isolated from pus and pus swab, and remaining were isolated from respiratory secretions including sputum, nasobronchial and bronchoalveolar lavage (18%), catheter tips (5%), blood (3%), urine (2%) body fluids (2%) and nasal swab (1%) (Table 2). 33% of MRSA isolates were recovered from patients of OPD, while the remaining 67% were from patients admitted in different wards including Medicine, Surgery, Dermatology, Paediatrics Medicine, Ophthalmology, intensive care unit (ICU), Dialysis unit and Obs and Gyné (Figure 1). Mean age of the patients was 43±2 years (mean± SD), range was from new born to elderly and median age of the patients was 45 years. Seventy six (72%) MRSA isolates were recovered from male patients and the remaining twenty four (28%) were from female patients (M: F: 3:1).

DISCUSSION

Our study indicates that there is emergence of increased vancomycin resistance among MRSA strains in our setup. Tough there was no VISA or VRSA strain detected but a large number of isolates turned out to be having vancomycin MIC -> 1 µg/mL. A study conducted at the Armed Forces Institute of Pathology Rawalpindi in the year 2003 to 2004 indicated that, there was no reduced susceptibility of vancomycin against studied MRSA isolates as indicated in our study as well but 4% VISA
Table 1. Range, MIC 50 and MIC 90 of vancomycin against MRSA isolates.

<table>
<thead>
<tr>
<th>Range</th>
<th>MIC 50</th>
<th>MIC 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19 to 3 µg/ml</td>
<td>0.75 µg/ml</td>
<td>1.5 µg/ml</td>
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Table 2. Distribution of samples from which MRSA were isolated.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of MRSA isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus</td>
<td>69%</td>
</tr>
<tr>
<td>Respiratory samples</td>
<td>18%</td>
</tr>
<tr>
<td>Catheter tips</td>
<td>5%</td>
</tr>
<tr>
<td>Blood</td>
<td>3%</td>
</tr>
<tr>
<td>Urine</td>
<td>2%</td>
</tr>
<tr>
<td>Body Fluids</td>
<td>2%</td>
</tr>
<tr>
<td>Nasal Swabs</td>
<td>1%</td>
</tr>
</tbody>
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![Figure 1](image-url) Percentage of isolates from outpatient department and different wards.

strains were detected in a study carried out in King Edward Medical College Lahore in 2004. In the year 2007, 13% VISA strains were detected from 2 civil hospitals of Karachi but no VRSA strain was detected (Mehmood et al., 2007; Bukhari et al., 2004; Hakim et al., 2007). Regional studies show following VISA incidence, 3.3% in Srinagar Kashmir 2003, 6% in India 2007 and 7.5% in Iran 2008 (Assadullah et al., 2003; Mehdinejad et al., 2008; Horieh et al., 2005).

Recently Pallazo et al. (2005) have also reported some vancomycin resistant strains of CoNS in Brazil. More recently, Bathaineh (2006) has reported VRSA strains from Jordan. Asadullah et al. (2003) have reported some strains of VISA from India. Song et al. (2004) have also reported the emergence of heterogeneous VRSA strains from India and its neighboring countries. The current vancomycin resistant Staphylococci in hospital as well as in community are alarming situation to the clinicians. The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to its easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries (Holloway, 2000). This emergence of VRSA/VISA may be due to building of selective pressure of vancomycin. Vancomycin a glycopeptide is currently the main antimicrobial agent available to treat life-threatening infections with MRSA. Widespread use of vancomycin to treat infections caused by MRSA and other gram-positive cocci has led to the emergence of vancomycin resistance. The large scale of development and subsequent spread of resistance to vancomycin has been perceived as a fearsome threat to the already challenging therapy of MRSA (Marchese et al., 2000). Although the emergence of vancomycin-intermediate S. aureus strains and that of vancomycin-resistant S. aureus strains are reasons for concern, these organisms still are extremely rare. The E-test method is an attractive option for alternative vancomycin testing since it is easy to perform and cost-effective for testing only one drug-bug combination.

CONCLUSION AND RECOMMENDATIONS

No vancomycin resistant or intermediate strain of MRSA was isolated during our study but there are significant numbers of isolates having ≥ 1 µg/ml MIC of vancomycin. Effective as well as cost effective antimicrobials should be prescribed for MRSA infections. Prescribing antibiotics other than glycopeptides for MRSA infections will minimize the chances of emergence of VRSA. Good hospital infection control measures prove to be the main stay against these infections.

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REFERENCES


