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Original Article

Emergence of Vancomycin Intermediate Staphylococcus aureus (VISA) in a Tertiary Care Hospital in Lahore: An Alarming Situation

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Abstract

Objectives: To assess the current situation of vancomycin MIC values and to find out the possible presence of vancomycin intermediate or resistant *Staphylococcus aureus* strains in a tertiary care hospital of Lahore, Pakistan.

Study Design: Non-interventional, descriptive study.

Place and Duration of Study: Pathology Department, Post Graduate Medical Institute, Lahore from February 2013 to October 2013.

Methodology: A total of 50 consecutive isolates of methicillin resistant *Staphylococcus aureus* (MRSA) were identified by using cefoxitin disc diffusion test. The confirmation of methicillin resistance was done by PBP2a latex agglutination test. E-test strips (AB Biodisk, Biomeurix) were used for the determination of Vancomycin MIC values for MRSA strains and results were interpreted according to clinical and laboratory standards institute (CLSI) guidelines.

Results: Result of our study showed that the range of MIC values of vancomycin for MRSA was 1.5-4 ug/ml. Among a total of 50 MRSA isolates, four strains (8%) were identified as vancomycin intermediate *Staphylococcus aureus* (VISA) with MIC value of 4µg/ml. Mean vancomycin MIC value was 2.5ug/ml whereas MIC50 and MIC90 values of vancomycin were 2ug/ml and 3ug/ml respectively.

Conclusion: The emergence of vancomycin Intermediate *Staphylococcus aureus* in our setup is an alarming situation. The widespread use of vancomycin for the treatment of MRSA might have induced a selection pressure, thus resulted in the emergence of these notorious resistance strains. Our study accentuates the need for continuous monitoring of vancomycin MIC levels in MRSA infections. A strict regulation on irrational antibiotic usages might be an effective approach in this direction. Proper infection control policies must be adopted to prevent the transmission of resistance strains.

Key words: Methicillin Resistant Staphylococcus aureus, Minimum Inhibitory concentration, Epsilometer Test, heteroresistant Vancomycin Intermediate Staphylococcus aureus,Vancomycin Intermediate Staphylococcus aureus, Vancomycin Resistant Staphylococcus aureus.

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Introduction

Methicillin Resistant *Staphylococcus aureus* is a major human pathogen responsible for a broad range of hospital as well as community acquired infections. [1] MRSA is currently endemic in many hospitals around the world with approximately 1.5 million cases per year.[2] In Pakistan, the prevalence of MRSA ranges from 38% to 62% .[3] The rise in the incidence of MRSA infections has resulted in the overuse of glycopeptides. [4]

Vancomycin although being an effective parenteral therapy for the treatment of MRSA infections for a long time [5] but its use has increased noticeably all over the world. Clinicians started using vancomycin as an empirical treatment for MRSA infections as there was absence of any alternate options.[6, 7] Moreover, vancomycin treatment failures with increased vancomycin MICs are a major concern, especially when these MICs are within the susceptible range. This increase in MICs over time has been referred to as "MIC Creep". [8, 9] Several studies indicate that "MIC Creep" phenomenon is associated with a significant risk of vancomycin treatment failure and a higher mortality rate.[10] An increase in vancomycin MIC values needs continuous surveillance to allow timely detection and identification of the emergence of vancomycin resistant strains, since the increasing vancomycin MIC may indicate a first step towards vancomycin intermediate strains. [11]

Reduced vancomycin susceptibility was first reported in Japan in 1996 and was designated as vancomycin intermediate susceptible *staphylococcus aureus* (VISA). [12] Another population of *S.aureus,* known as heteroresistant VISA which is defined as the presence of subpopulation of VISA within the population of MRSA at the rate of one organism per 10^5 to 10^6 organisms. [13] hVISA is presumably a stage that precedes the development of VISA. [14]

The strains of hVISA and VISA strains possess a thicker cell wall i.e. increased numbers of D-Ala-D-Ala residues and expresses resistance to Vancomycin. [15] This results in vancomycin 'trapping' in the outer layers, making it inaccessible for target molecules. This phenomenon is called "Clogging Phenomenon". [16, 17] Now the medical community must be aware of the consequences of another superbug called vancomycin-resistant *Staphylococcus aureus* (VRSA) strains. These strains are still very rare but steps should be taken for the prevention of their spread. [18, 19] Since 2002, several isolates of VRSA have been reported from patients in the United States. [20] The underlying mechanism for vancomycin resistance is acquisition of *vanA* gene from vancomycin resistant enterococci. [21]

Emergence of hVISA, VISA and VRSA strains are related with increasing Vancomycin treatment failures. [13, 22-24] The present study was planned to access the current status of vancomycin MIC values and to find out the presence of any VISA or VRSA strain in our setup.

Methodology

The study was non interventional, descriptive study. It was conducted at Microbiology Department PGMI, Lahore from February 2013 to October 2013. Fifty consecutive isolates of MRSA from various clinical specimens of pus, blood, wound swab, urine, sputum and aspirates of patients admitted in Lahore General Hospital sent for culture and sensitivity to the Department of Microbiology PGMI, were included in this study.

All specimens were inoculated on blood agar and MacConkey agar. Incubation was done at 37°C for 24 hours. Preliminary identification of *Staphylococcus aureus* was done by noting the colony morphology on blood agar plates, Gram stain and catalase tests. Confirmation of isolates was done by Coagulase and DNase tests.

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 30 µgcefoxitin disk (Oxoid, UK). For each strain, a bacterial suspension adjusted to 0.5 McFarland turbidity standards was used. Zone of inhibition was determined after 24 hours of incubation at 35°C and interpreted according to CLSI criteria. [25] All isolates that showed cefoxitin resistance were tested for *mecA* product (PBP2a) using latex agglutination kit (Slidex, Biomeurix) for confirmation of MRSA. The procedure was done by following the manufacturer's instructions provided with the kit.

MICs of vancomycin for MRSA isolates were determined using the E-test method according to the guidelines provided by the CLSI. [25] For the preparation of direct colony suspension, well isolated colonies from an overnight agar culture were suspended directly into physiological saline and were mixed to achieve 0.5 McFarland turbidity (corresponds to approximately 1.5 x 10^8 CFU/ml). The turbidity was visually compared with MF standards against a card with a white background and contrasting black lines. The suspension was used within 15 minutes of preparation. A sterile cotton swab was dipped into the adjusted suspension. Then it was pressed firmly against the inside wall of the bottle, above the suspension level to remove the excess inoculum. Entire surface of dried Mueller Hinton agar plates was uniformly inoculated with the swab. This procedure was repeated three times by rotating the plate at approximately 60°C, taking care to ensure no gaps were left in the deposited inoculum. The strip was gripped with a pair of sterile forceps were applied by placing the end with the lowest concentration onto the plate first. The strip was rolled carefully onto the agar surface to ensure good contact along the entire length of the strip. Using sterile forceps, the strip was gently smoothened onto the agar. Care was taken not to move the position of the strip. The plates were incubated at 37°C for 16 to 18 h. MIC values were read where the respective inhibition ellipses intersected the strip according to CLSI 2012 .MRSA ATCC 33591 and MSSA ATCC 25923 were used as positive and negative control respectively.

Results

Most of the MRSA isolates were recovered from pus samples as shown in Table 1. The range of MIC values of vancomycin for MRSA isolates was 1.5ug/ml to 4ug/ml. The majority of the isolates were handled at MIC value of ≥ 2µg/ml of vancomycin. The most frequently detected MIC value for Methicillin Resistant *Staphylococcus aureus* was 3µg/ml (42%) for vancomycin. The frequency distribution of MIC values of vancomycin for 50 isolates of methicillin resistant *Staphylococcus aureus* is shown in Figure 2. MIC 1.5 µg/ml of vancomycin was observed for 7 (14%) isolates of methicillin resistant *Staphylococcus aureus*. 18 (36%) isolates of MRSA were inhibited at MIC 2 µg/ml. While MIC 3 µg/ml and MIC 4 µg/ml of vancomycin was observed for 21 (42%) and 4 (8%) isolates of MRSA respectively. These 4 (8%) isolates were in the range of vancomycin intermediate *Staphylococcus aureus* (VISA) with MIC value of 4µg/ml. MIC50 and MIC90 values of vancomycin were 2ug/ml and 3 ug/ml respectively as shown in figure 3.

Table 1: Distribution of Methicillin Resistant Staphylococcus aureus isolates from patients according to clinical specimen (n = 50)

Specimen	Frequency	Percentage
Pus	24	48
Blood	10	20
Urine	2	4
Wound Swab	3	6
Bronchial washings	1	2
CSF	5	10
CV Tip	1	2
Pleural Fluid	1	2
Sputum	1	2
HVS	2	4

Figure 1: Vancomycin E-Test showing MIC (4µg/ml) in the Range of Vancomycin Intermediate Resistant *Staphylococcus aureus* (VISA)

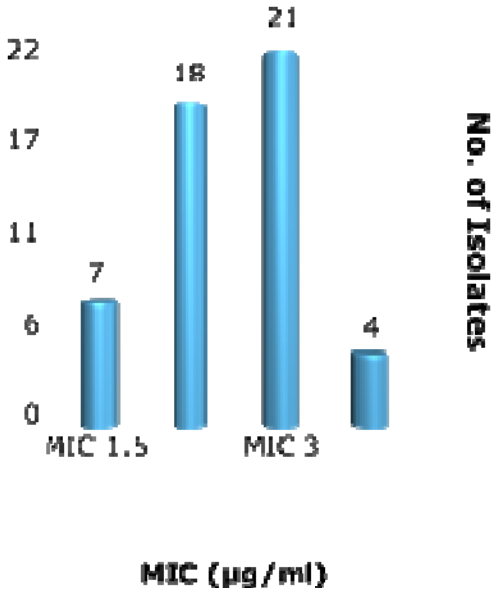

Figure 2: Graphical Presentation of Vancomycin MICs Values for Methicillin Resistant Staphylococcus aureus Isolates

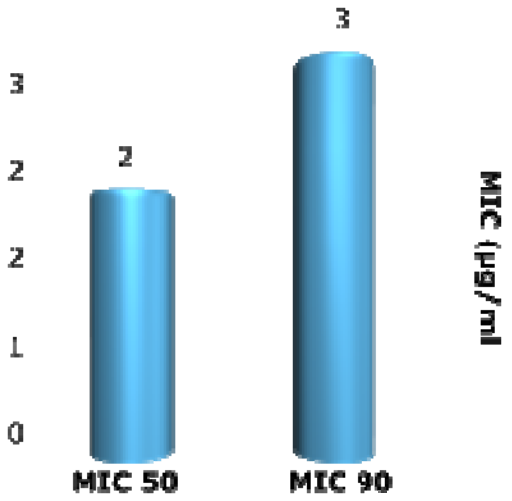

Figure 3: Graph Showing Comparison of MIC 50 & MIC 90 Values of Vancomycin for Methicillin Resistant *Staphylococcus aureus* isolates

Discussion

Vancomycin has been the main therapeutic agent for the treatment of MRSA infections but the high rate of use and exposure pressure of vancomycin have resulted in increasing levels of resistance strains i.e. heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA), vancomycin intermediate *Staphylococcus aureus* (VISA) and vancomycin resistant *Staphylococcus aureus* (VRSA). [26]

Results of our study indicate an alarming situation about the emergence of increased vancomycin resistance among MRSA strains in our setup. Moreover, a trend of elevated vancomycin MIC values all over the world have resulted in increased rate of treatment failure and these failures have led to utilization of higher doses of vancomycin than those approved by FDA. These higher doses results in nephrotoxicity especially in those who already have underlying renal insufficiency, while inadequate dosing of vancomycin increases the chances of selecting heteroresistant strains. [27]

Results of our study are comparable with a study conducted by Hakim et al at Karachi [28] and found that 13% of the strains were intermediates (VISA). A study done by Mahmood et al [29] showed that all the MRSA isolates had vancomycin MIC values ranged from 1 to 3µg/ml. However, no vancomycin-intermediate *Staphylococcus aureus*(VISA) isolates was found in their study. Similarly, Kaleem et al [30] conducted a study at Islamabad from December 2009 to June 2011. Although, there was no VISA or VRSA strain detected in their study but a large number of isolates had vancomycin MIC > 1 ug/ ml.On the contrary, several researchers from Pakistan have reported complete susceptibility of MRSA to vancomycin. [31-34]

Other studies beside Pakistan also show the increasing trend of vancomycin MIC values and emergence of VISA and VRSA isolates. Assadullah et al [35] conducted a study in Srinagar, Kashmir in 2003and 3.3% VISA isolates were identified in their study. Mehdinejad et al in Ahwaz, Iran (2008) [36] reported 7.5% VISA while only one VRSA was isolated by Bataineh (2006) [37] in Zarqa Jordan. Similarly, Menezes et al [38] identified one VISA isolate at Jawaharlal India while Tiwari et al [39] identified 6 VISA and 2 VRSA isolates from Banaras India in 2006. A study conducted in Pennsylvania 2008 by Shick et al [40] identified three vancomycin-intermediate *Staphylococcus aureus*(VISA) (0.3%) and two heterogeneous VISA (0.2%) isolates.

Several studies have shown that higher vancomycin MICs are more frequently associated with treatment failure and poor outcome even when these MICs are below the breakpoint. [41] So these higher vancomycin MICs are a matter of serious concern and an alarming

situation in a developing country like Pakistan when there are already limited alternative therapeutic options for the treatment of serious MRSA infections. Care should be taken to prescribe drug after proper diagnosis of MRSA, in serious clinical situations. Serum levels of the drug should be checked and maintained. Factors like poor tissue penetration, chiefly in the lung and the potential for nephrotoxicity should also be kept in consideration while using vancomycin. This will help avoid the consequences of the development resistant strains in our setup.

Conclusion

Our study has exposed the emergence of Vancomycin intermediate *Staphylococcus aureus* in a tertiary care hospital of Lahore Pakistan. However, no vancomycin resistant strain was found in our setup. The emergence of vancomycin Intermediate *Staphylococcus aureus* is a real threat because there are hardly any treatment options available in Pakistan.

Recommendations: Steps should be taken for implementation of improved infection control measures for preventing patient to patient transmission of resistant organisms as there is a rapid rise in the development of resistance all over the world. The use of vancomycin as an empirical treatment must be avoided. Factors regarding increased vancomycin prescription and consumption must be identified and addressed as otherwise response to one emerging resistance infection will breed another resistance infection.

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