FREQUENCY OF VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS AMONG CLINICAL ISOLATES OF MRSA COLLECTED FROM TERTIARY CARE HOSPITALS OF LAHORE, PAKISTAN

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ABSTRACT

Objective: To determine the frequency of vancomycin resistant *Staphylococcus aureus* (VRSA) among clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA).

Study Design: Descriptive study.

Place and Duration of Study: Department of Microbiology, University of Health Sciences, Lahore from Jul 2014 to Dec 2014.

Material and Methods: A total of 240 (n=240) clinical isolates of MRSA were collected by consecutive sampling from different tertiary care hospitals of Lahore. Re-confirmation of MRSA was done by the standard microbiological methods using disc diffusion technique according to Clinical Laboratory Standards Institute (CLSI) guidelines 2014. Minimum inhibitory concentration (MIC) of the vancomycin was done by agar dilution method.

Results: It was found that vancomycin inhibited MRSA strains in the range of 1.0–2.0 µg/ml. Ninety percent (90%) of the strains inhibited at 1 µg/ml while 25 (10.41%) strains showed growth at 1 µg/ml which indicates that their MIC was 2 µg/ml. No vancomycin resistant (VRSA) or intermediate strains (VISA) of MRSA were found during the study but there were significant numbers of isolates having ≥1 µg/ml MIC of vancomycin.

Conclusion: Vancomycin has until now excellent activity against clinical isolates of Methicillin-resistant *Staphylococcus aureus*.

Keywords: Methicillin resistant staphylococcus aureus, Minimum inhibitory concentration, Staphylococcus aureus, Vancomycin resistant.

INTRODUCTION

Staphylococcus aureus is a gram positive coccus & normally causes skin and soft tissue infections such as cellulitis, abscesses and scalded skin syndrome. It can also cause life threatening conditions like pneumonia, endocarditis, meningitis, sepsis and causes both endemic and epidemic infections acquired in hospitals[1,2]. The problem of antibiotic resistance is increasing day by day due to extensive and unwarranted use of antibiotics and its impact is more in poor countries where infection rates are high due to unhygienic environment and poor health facilities[1]. Misuse and overuse of the antibiotics also helped in natural bacterial evolution which becomes resistant to drugs and fight against the infections. The problem is more exacerbated by those individuals who do not follow the proper guidelines of skilled professionals and self-prescribe the antibiotics[3].

Staphylococcus aureus has developed multiple ways to become resistant to various antibiotics which includes production of enzymes, changes in the cell wall structure and genetic mutations[4]. Methicillin Resistant *Staphylococus Aureus* (MRSA) is a strain of *Staphylococci* bacteria that become resistant to those antibiotics (methicillin, cephalosporins) which are normally used to treat these infections. Resistance to methicillin is accorded by the mecA gene which is a fragment of Staphylococcal cassette chromosome (SCCmec) that encodes low-affinity penicillin binding protein (PBP)[5]. The first case of MRSA was reported in 1960s and now it has increased at a dramatic rate and is associated with higher rates

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of morbidity and mortality in nosocomial infections[6].

Vancomycin is a glycopeptide and is very popular and competent antimicrobial drug for treating MRSA infections but unfortunately resistance to vancomycin have also been reported since 1997. This vancomycin resistance is actually due to the presence of Van resistance genes which are acquired from enterococci and are encoded on R-plasmid or chromosome. There are total six genes responsible for vancomycin resistance which includes vanA, vanB, vanC, vanD, vanE and vanG whereas VanA and VanB genes are the most important and prevalent genes[7,8]. The examples of resistant strains are vancomycin intermediate *S. aureus* (VISA), vancomycin resistant *S. aureus* (VRSA) and heterogeneous VISA (hVISA) strains.

The mechanism of resistance for both vanA and vanB genes encompass three sets of enzymes harbored by glycopeptide-producing bacteria. The enzymes are dehydrogenase that reduces pyruvate into D-Lactate, ligase that synthesizes D-Alanyl–D-Lactate and a dipeptidase that hydrolyzes D-Alanyl–D-Alanine. The collective action of these three enzymes incorporate D-Alanyl–D-Lactate instead of D-Alanyl–D-Alanine into peptidoglycan which prevents the attachment of vancomycin[9]. The vanA gene contributes higher resistance to vancomycin and teicoplanin both glycopeptides while VanB gene which is also an effective resistant gene encodes resistance to vancomycin only. The vanB gene clusters (vanB1, vanB2, and vanB3) are generally carried by large elements (90-250 kb) which are transferable by conjugation from one chromosome to another and often contain the transposon Tn1547[10]. Studies have also been conducted on detection of vanB gene in clinical isolates of Staphylococcus aureus strains but a few cases have been reported so far[11].

There are very few studies available on VRSA detection particularly on vanA and vanB genes in Pakistan also. Therefore we planned this

Table-I: Details of MIC of vancomycin against MRSA isolates.

Isolates	Anti-microbial Agent	Percentage of isolates susceptible at MIC µg/ml											Concentration µg/ml		
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	Range	MIC 90	MIC 100
MRSA	Vancomycin	0	0	0	0	0	89.59	100	100	100	100	100	1-2	1	2

Table-II: Distribution of MRSA (n=240) isolates in clinical specimens.

Clinical specimen	MRSA isolates	Percentage (%)
Pus	193	80.41
Wound Swab	17	7.08
Blood	6	2.5
Tracheal Secretion	5	2.08
Tips	4	1.66
Tissue Biopsy	4	1.66
Urine	3	1.25
HVS	3	1.25
Sputum	2	0.83
Pleural Fluid	2	0.83
Throat Swab	1	0.41
Total	240	100

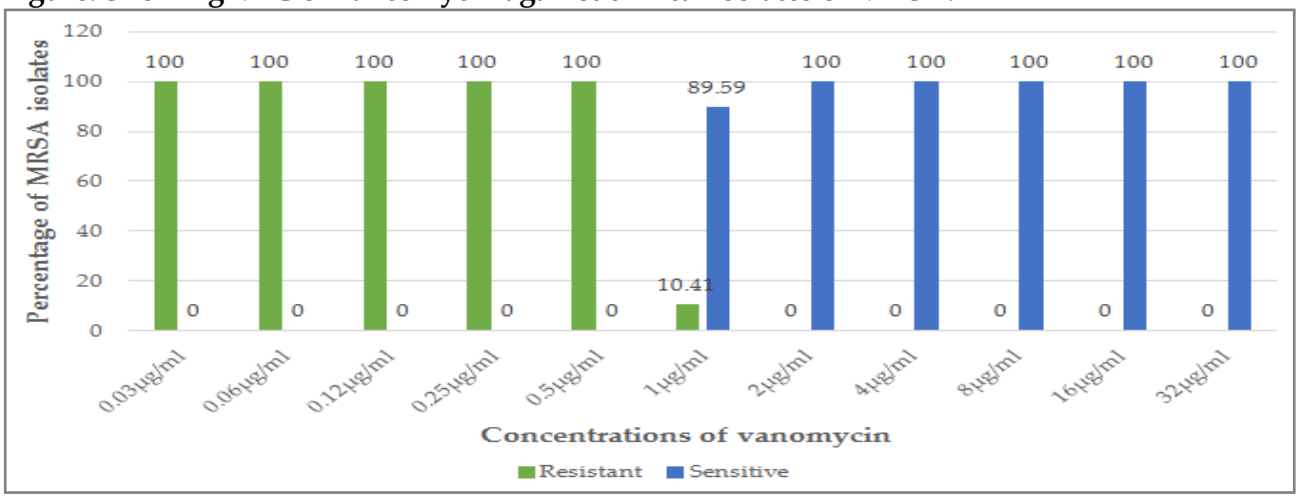
study to determine the frequency of VRSA among clinical isolates of MRSA and molecular detection of van resistant genes in these isolates if found resistant to vancomycin phenotypically.

MATERIAL AND METHODS

This cross-sectional descriptive study was conducted at the department of Microbiology, University of Health Sciences Lahore, Pakistan from July 2014 to December 2014. A total of 240 (n=240) MRSA were collected by consecutive sampling from different tertiary care hospitals; Combined Military Hospital, Shalamar Hospital, Jinnah Hospital and Chughtai Labs Lahore. All coagulase negative *Staphylococci* were excluded from the study while coagulase positive *Staphylococci* were included in the study. The *Staphylococci* inoculated on Mueller Hinton Agar (MHA) were transported from different hospitals to the microbiology Lab. These isolates were then re-identified by gram staining, Catalase test, Coagulase test, DNAse test and preserved in 20% v/v glycerol in brain heart infusion (BHI) at -70˚C till further use. 30µg Cefoxitin disc was used to confirm MRSA by Kirby-Bauer Disc Diffusion method. In this method a lawn of the bacterial suspension adjusted to 0.5 Mc Farland standard was made on MHA and the cefoxitin disc was placed on the agar surface and incubated overnight at 37ºC. After overnight incubation reading was taken by measuring the zone of inhibition and results were interpreted according to CLSI guidelines 2014.

According to CLSI MIC revelatory criteria, *S. aureus* having MIC of 2µg/ml are defined as vancomycin susceptible and 16 µg/ml are known as vancomycin resistant. The isolates having MIC in the range of 4 to 8 µg/ml are categorized as vancomycin intermediate[1i]. The protocol for MIC was followed as previously reported in the literature[12]. According to method, isolates were sub cultured on blood agar and after overnight incubation at 35°C; three to five colonies were emulsified in sterile isotonic saline. The organism's suspension was adjusted to 0.5 McFarland standard which gave us the concentration of (108 CFU/ml). It was further diluted 1:10 in sterile saline to achieve the final concentration of (107 CFU/ml). This suspension was then spot inoculated onto MHA plates containing serial (two-fold) dilutions of vancomycin in duplicates from 0.03 to 32 mg/L via multipoint inoculator (MPI). This instrument grasps 35 pins each having a diameter of approximately 3 mm; which convey the inoculum approximately 3µl/spot. After overnight incubation, MIC was interpreted against dark non-reflecting surface as the first antibiotic concentration that inhibits the growth of the organism completely. The presence of hazy growth caused by the inoculum or colony was

Figure: Showing MIC of vancomycin against clinical isolates of MRSA.



not considered as growth. Results of MIC were interpreted according to the cutoff points given by CLSI 2014. ATCC 29213 *S. aureus* was used as reference strain. .

RESULTS

All 240 (n=240) of the strains were gram positive cocci and were catalase, coagulase and DNAse positive. Most of the strains were from pus followed by wound swab, blood and tracheal secretions etc. as shown in table-II. All *Staphylococci* showed the zone size of <22 mm which indicates that these strains were resistant to Cefoxitin and were declared as MRSA. Vancomycin inhibited the strains of MRSA in the range of 1.0-2.0 µg/ml. Out of 240 MRSA 215 (89.59%) strains inhibited at 1 µg/ml while 25 (10.41%) strains showed growth at 1 µg/ml which indicates that their MIC was 2 µg/ml as shown in table-I & figure. No vancomycin resistant (VRSA) or intermediate strains (VISA) of MRSA were isolated. As there were no VRSA detected in clinical isolates of MRSA so we were unable to perform the molecular detection of van resistant genes in these isolates.

DISCUSSION

Vancomycin has an important role in the treatment of MRSA infections and still it is a best therapeutic agent for the treatment of highly resistant nosocomial infections but it should be used with proper prescription and laboratory evaluation. In this study we intended to find out the frequency of VRSA from clinically isolated MRSA strains (n=240). If we look at the results of MIC we have found that no VISA or VRSA were detected among MRSA isolates. Though there were no VISA or VRSA strains detected but a significant number of isolates are found having vancomycin MIC ≥1 µg/ml. Ninety percent of the strains showed MIC of 1 µg/ml, which is a very good sign for vancomycin efficacy while 25 (10.41%) strains had showed growth at 1µg/ml which indicates that their MIC was 2µg/ml which needs to be observed frequently.

These results are in accordance with the results of a study already conducted in Pakistan who also reported MRSA strains with higher MIC against the vancomycin[13]. Another study conducted in Pakistan reported one isolate having MIC of 32µg/ml (VRSA) along with four isolates had intermediate level resistance (VISA) with two strains having MIC of 16µg/ml and two having MIC of 8µg/ml[11]. The similar studies also conducted in different parts of the world and reported VISA, hVISA and VRSA strains[11-13]. The reason for this sneaking of MIC is not known, but lesser vulnerability to vancomycin is likely due to overuse as well as to sub-optimal dosing of the vancomycin.

This global problem of antimicrobial resistance particularly requires immediate attention more in the developing countries, where the infectious diseases burden is already high and cost restriction prevents the widespread application of newer and more expensive agents. This gradual increase in MIC of vancomycin against MRSA infections is an alarming situation for us, soon it could be difficult to treat these infections if not controlled properly specially, when newer antimicrobials are not available. As concluded in previous studies conducted in Pakistan that a continuous monitoring is mandatory so this study is a follow up study for vancomycin MIC. For future, we recommend that to cope with the problem of increasing antimicrobial resistance, misuse and overuse of the drugs should be stopped immediately and continue the monitoring of resistance patterns of MRSA carefully. This will surely help in avoiding the development of resistant strains in our setup.

CONCLUSION

It is concluded from our study that vancomycin has until now excellent activity against clinical isolates of Methicillin-resistant *Staphylococcus aureus*.

CONFLICT OF INTEREST

There is no conflict of interest between the authors for this article.

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