International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN:0975-9174, Volume 4, Issue 5, 2012, pp.-223-226.
Available online at http://www.bioinfo.in/contents.php?id=27

LEVELS OF VANCOMYCIN SUSCEPTIBILITY AMONGST ISOLATED *Staphylococcus aureus* STRAINS IN A TERTIARY CARE HOSPITAL

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Received: April 20, 2012; Accepted: June 05, 2012

Abstract- Out of 260 strains isolated from the clinical specimens from different wards of hospital, 105 were identified as Methicillin resistant *Staphylococcus aureus* (MRSA). Minimum inhibitory concentrations (MIC) of vancomycin of these strains were determined by agar dilution method following CLSI guidelines. Sensitivity of different antibiotics was determined by Kirby Bauer disc diffusion method. Eight strains had MIC of vancomycin of 4 microgram /ml (µg/ml), 138 strains had MIC of 2 microgram /ml and 114 had MIC of 1 microgram/ml. All strains having MIC 4µg/ml were MRSA and were resistant to more than five antibiotics. Out of 138 strains having MIC of 2 µg/ml, 88 were MRSA and 50 were MSSA. Significant relation was found between increase in MIC of vancomycin and methicillin resistance. The most common risk factors associated with increased MIC of vancomycin were prior treatment of vancomycin and prolonged hospitalization .
Keywords- VISA, MRSA, MIC

Citation: Sachin Kishore, et al. (2012) Levels of Vancomycin Susceptibility Amongst Isolated *Staphylococcus aureus* Strains in a Tertiary Care Hospital. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN:0975-9174, Volume 4, Issue 5, pp.-223-226.

Introduction

Multidrug resistant *Staphylococcus aureus* is a common cause of nosocomial infection. Currently, measures to control *Staphylococcus aureus* infections are challenged by a large and continuing increase in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) worldwide, the spread of highly virulent community-associated MRSA and the emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin and other glycopeptides [1]. The condition has been further worsened by the emergence of Vancomycin intermediate sensitive *Staphylococcus aureus* (VISA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) [2]. Strains with upper level of Minimum inhibitory concentration (MIC) of vancomycin in the sensitive range (1-4 µg/ml), results in more morbidity and mortality of the patients, as compared to those which have lower range of vancomycin MIC (less than 1 µg/ml) [2-3]. As per latest clinical and laboratory standard institute guidelines staphylococci with MIC of vancomycin ≤ 2 µg/ml is susceptible, while for which MIC is 4-8 µg/ml are intermediate and those with MIC ≥ 16 µg/ml are resistant [4]. So the present study was aimed to determine the risk factors associated with higher range of minimum inhibitory concentration of vancomycin, to identify different clinical settings or wards where the resistant strains of *Staphylococcus aureus* are more prevalent and also to find out the different levels of minimum inhibitory concentration of vancomycin and oxacillin in a tertiary care hospital of a north Indian city.

Materials and Methods

This observational study was conducted after approval from institutional ethical committee in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow among indoor patients of Departments of Surgery, Gynecology and Obstetrics, Medicine, Pediatrics and Orthopedics from September 2008 to December 2009. Two hundred sixty *Staphylococcus aureus* strains were isolated from various clinical specimens collected from patients from respective wards including pus, urine, wound swab, catheters, blood, sputum, throat swab, cerebrospinal fluid, high vaginal swab and other body fluids. Past history of the patient was recorded for diabetes mellitus, chronic renal illness and any other chronic illness leading to prolonged hospitalization.

Specimen Processing was Done in Two Parts

Part I- Isolation and identification of Staphyloccos *aureus* by culture and biochemical tests.

Culture of Specimens

All the specimens received in the bacteriology laboratory were inoculated on Blood agar and McConkey agar plates & incubated at 37°C for 24-48 hours

Identification of *Staphylococcus aureus*

Presumptive identification was done on the basis of colony characteristics, Gram's staining, catalase and slide coagulase test. Confirmation was done by Tube coagulase test, Growth on mannitol salt agar, DNAse test, Modified Hugh and Leifson O/F test.

Part II- In Vitro Antibiotic Susceptibility Testing by Disc Diffusion Method of Kirby Bauer and MIC by Agar Diilution Method.

Screening of MRSA was done by cefoxitine (30µg) disc diffusion test. Confirmation of methicillin resistance was done by determination of oxacillin MIC by agar dilution method. Screening of vancomycin resistance was done by disc diffusion method with vancomycin (30 µg) disc .Screening of VISA was done by inoculating isolated *Staphylococcus aureus* strains over Brain Heart Infusion (BHI) screen agar containing 6 µg/ml vancomycin. Levels of susceptibility was determined by MIC's of vancomycin by agar dilution method.

Detection of MRSA was done by the use of cefoxitin disc (30 micro grams) diffusion test. The strains of *Staphylococcus aureus* with a zone diameter of < 19 mm were taken as MRSA and those with zone diameters >20 mm were considered sensitive [5]. All strains of *Staphylococcus aureus* were confirmed as methicillin resistant by oxacillin agar dilution using Muller Hinton agar (MHA) supplemented with 2% NaCl. The concentrations tested ranged from 1 µg/ml to 16 µg/ml of oxacillin. Those strains having minimum inhibitory concentration (MIC) ≤2 µg/ml were taken as sensitive and those with MIC ≥ 4 µg/ml were taken as resistant [4].

Disk Diffusion Sensitivity by Vancomycin

30 microgram disc was carried out using Kirby Bauer method. Muller-Hinton agar (MHA) plates were overlaid with the inoculum turbidity equivalent to that of a 0.5 McFarland Standard of the *Staphylococcus aureus* following CLSI criteria. Zone diameters were measured at 24 hrs. following CLSI criteria i.e. zone of inhibition more than or equal to 15 mm reported as sensitive and less then 15mm were further confirmed by MIC testing and growth on BHI screen agar. *Staphylococcus aureus* ATCC 29213 was used as reference strain. This strain had been also used in other previous studies [6].

Determination of Vancomycin MIC was done by Agar Dilution Method

All strains were tested for MIC by vancomycin agar dilution method using MHA. The concentrations tested ranged from 1 µg/ml to 32 µg/ml of vancomycin. Strain of *Staphylococcus aureus* ATCC 29213 (susceptible) and Enterococcus feacalis 51299 (resistant) were taken as control strains. Further detection of VISA was done by BHI Vancomycin Screen Agar. As per CDC guidelines, in-house prepared BHI agar (Hi-Media, India) screen plates containing 6 microgram/ml Vancomycin (Lilly Pharma, Giessen, Germany) was prepared. Inoculum suspensions were prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies were transferred to sterile saline to produce a suspension that matched the turbidity of 0.5 McFarland standard. The final inoculum concentration of 10^5 to 10^6 CFU per spot was prepared by adding the sterile saline to the bacterial suspension [7]. These suspensions were spot inoculated on BHI screen agar plates and plates were incubated for 24 hrs. at 35°C aerobically. Any visible growth was indicated for Vancomycin resistance. Statistical analysis was performed using SPSS 14.0 version software. Chi-square test and Z test were applied. A p-value of <0.05 was taken as statistically significant.

Observation

Table 1- Distribution of Staphylococcus aureus strains isolated from the various clinical specimens

TYPE OF SPECIMEN	NUMBER OF SPECIMENS	S. AUREUS ISOLATED (%)
PUS	395	128 (32.41%)
BLOOD	149	25 (16.78%)
URINE	956	45 (4.7%)
VAGINAL SWAB	134	10 (7.46%)
SPUTUM, TRACHEAL ASPIRATE	149	36 (24.1%)
THROAT SWAB, PLEURAL FLUID, PERITONEAL FLUID, CSF, TISSUE ASPIRATE	245	16 (6.53%)

Table 2- Staphylococcus aureus resistance pattern among the patients

VAN MIC µg/ml	OXA MIC µg/ml	Cn 30 µg	T 15 µg	Cp 5 µg	G 10 µg	E 15 µg	Cd 2 µg	P 10 U	Lz 30 µg	Pm 15 µg	Clo 30 µg
4	8	R	R	R	S	R	R	R	S	S	S
4	16	R	R	R	R	R	R	R	S	R	R
4	8	R	R	R	R	R	R	R	S	R	R
4	16	R	R	S	R	R	R	R	S	R	S
4	8	R	R	R	R	-	R	R	S	S	S
4	16	R	I	R	R	R	R	R	R	R	S
4	16	R	R	R	S	R	S	R	S	S	R
4	8	R	R	R	R	-	S	R	S	S	R

(Cn-Cefoxitine, Van-Vancomycin, T-Tetracycline, Cp-Ciprofloxacin, E-Erythromycin, G-Gentamycin, Cd-Clindamycin, Lz -Linezolid, Pm-Pristinomycin, Clo-Cloremphenicol, Oxa-Oxacillin)

Table 3- Association of different levels of MICs of vancomycin with methicilin resistance

MIC of Vancomycin	NUMBER of MRSA	NUMBER of MSSA	Total
MIC = 2-4 µg/ml.	8	0	8
MIC = 1-2 µg/ml.	88	50	138
MIC = < 1 µg/ml.	9	105	114
Chi-square (df), P-value		82.6 (2), 0	

Two hundred and sixty *Staphylococcus aureus* strains after identification by standard biochemical tests were tested for methicillin resistance using 30 µg cefoxitin disc on MHA by Kirby-Bauer disc diffusion method, 105 isolates showed resistance after 18-24 hours incubation at 35°C. Remaining 155 isolates were sensitive.

Confirmation of Methicillin resistance was done by agar dilution method following standard protocols of Clinical and Laboratory Standards Institute (CLSI). Out of 260 strains, 105 were grown on agar plate having 4 µg/ml concentration of oxacillin. Seventy five *Staphylococcus aureus* strains had MICs of 8 µg/ml. Thirty *Staphylococcus aureus* strains had MIC of 16 µg/ml. All 260 isolates were found sensitive to vancomycin (30 µg) disc by Kirby Bauer disc diffusion method. In our study no isolate was grown over the BHI screen agar containing vancomycin 6 µg/ml after 24-48 hours of incubation. By agar dilution method, out of 260 *Staphylococcus aureus* strains, 8 strains had MIC of < 4 microgram/ml after 24hours of incubation at 37°C, 138 had MIC of < 2 microgram/ml and 114 strains had MIC 1 µg/ml. (Table 1 and 2)

Vancomycin has been the most reliable therapeutic agent against MRSA. However increased use of vancomycin has set a basis of selection for vancomycin resistance in MRSA [8]. In our study we have noted that majority of pus specimens received in bacteriology lab were from surgery and orthopedics wards. Our study is similar to other workers who have also reported that *Staphylococcus aureus* was the commonest pathogen which was isolated from localized pyogenic and surgical wound infections from surgery ward. In another study *Staphylococcus aureus* was the commonest pathogen isolated from surgical site infections [9].

During our study we observed that all strains isolated from various clinical specimens had MIC of vancomycin between 0.5-4 µg/ml. This is similar to other studies which have reported that all clinical isolates were vancomycin sensitive [10,11]. Previously in another study it was reported that MRSA isolates having 2 µg/ml vancomycin MIC were 40% [12]. A single case of vancomycin resistant *Staphylococcus aureus* was isolated from Kolkata [13].

In our study out of 260 *Staphylococcus aureus* strains isolated from various clinical specimens, 8 of them showed MIC value of 4 µg/ml. This is of concern as this level is at the higher margin of susceptible level. This may be prelude to developing tolerance or frank resistance. These 8 strains which had MIC of 4 µg/ml, were isolated from 5 pus specimens (4 from orthopedics and 1 from surgery ward), 1 blood culture (from ICU) and two urine specimens (one each from ICU ward and surgery ward). These 8 strains were also found to be MRSA and multiple drug resistant. In our study all of the strains were isolated from hospitalized patients. Prolonged hospitalization and exposure to glycopeptides or vancomycin were the important risk factors found for high MIC of vancomycin as all the 8 strains having MIC of vancomycin 4 µg/ml were isolated from patients who had prolonged hospital stay (>21 days) and underwent prior treatment with vancomycin.

In our study 138 strains had MIC of 2 µg/ml (53%). Out of 138 strains which had MIC of 2 µg/ml, were isolated from 10 blood specimens, 20 urine specimens, 79 pus specimens, 20 sputum specimens, 4 pleural fluid and 5 high vaginal swab specimens. These strains were found to be more frequently MRSA as compared to those which had a lower MIC of Vancomycin. Significant association of *Staphylococcus aureus* strains was found with increase concentration of vancomycin and methicilin resistance as shown in Table 3.

Out of 138 strains having vancomycin MIC of 2 µg/ml, 79 were isolated from pus specimens from patients admitted in orthopaedics, surgery and ICU ward. All these patients had longer duration of hospital stay as compared to *Staphylococcus aureus* strains having vancomycin MIC of <1 µg/ml. Almost similar findings were observed with other strains having vancomycin MIC 2 µg/ml isolated from blood, sputum and urine specimens. Also 27 patients out of 138 were also taking either insulin or oral hypoglycemic for diabetes mellitus. 19 patients out of 138 were suffering from chronic renal diseases. This is similar to another study which has also reported that long term hospitalization and prior treatment with vancomycin are significant risk factors for increased level of vancomycin MIC in *Staphylococcus aureus* [2]. Peritoneal dialysis, renal failure and diabetes mellitus were found to be significant risk factors in another study [14].

Some retrospective studies have independently found that MRSA strains with vancomycin minimum inhibitory concentrations (MICs) at the upper limits of microbiological susceptibility (vancomycin MIC 1-2 µg/ml) are associated with inferior vancomycin treatment outcomes in pneumonia and bacteremia [15]. In our study we isolated 8 *Staphylococcus aureus* strains having vancomycin MIC of 4 µg/ml, from patients who were chronically ill and had a prolonged hospital stay. We also observed that the clinical improvement in them was slow as compared to those patients who had vancomycin MIC 1 µg/ml. Another study has suggested a paradoxically inverse relationship between vancomycin MIC and clinical outcome [3].

In our study linezolid showed good activity against a variety of multiple resistant *Staphylococcus aureus.* This drug is rapidly and completely absorbed after oral administration with a mean bioavailability of approximately 100% [16]. We observed that this drug is effective against multiple drug resistant strains of *Staphylococcus aureus* .This was also observed in other studies [17].

So we suggest that the indiscriminate use of antibiotics without antibiotic susceptibility testing should not be prescribed by clinicians which can lead to emergence of resistance worldwide. A good antibiotic policy should be laid down between the clinician and microbiologist in all tertiary care hospitals and a strict antibiotic regimen should be applied by clinicians. As there are only a limited drugs available for the treatment of VISA, irrational use of antibiotics should be avoided and a rational antibiotic policy must be adopted. We have also come to this conclusion in our study that there are several risk factors associated with reduced susceptibility to vancomycin like prolonged hospitalization, prior exposure to glycopeptides and chronic renal failure. A comprehensive history should also be taken regarding antibiotic therapy from all patients being admitted for surgery. Those patients identified with history of chronic illness like diabetes mellitus, renal failure, peritoneal dialysis should be dealt with utmost care.

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