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DETERMINATION OF VANCOMYCIN MIC IN *STAPHYLOCOCCUS AUREUS* FOR ISOLATES SHOWING REDUCED ZONE OF INHIBITION BY DISC DIFFUSION METHOD

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ABSTRACT

Introduction: Methicillin Resistant *Staphylococcus aureus* (MRSA) is resistant to the majority of antimicrobial agents available for clinical use, the glycopeptides vancomycin has been proposed as the drug of choice for treating such infections. Indiscriminate use of vancomycin leads to the emergence and spread of vancomycin resistance in multidrug resistant strains is of growing concern in the recent years. The minimum inhibitory concentration (MIC) represents the concentration of antimicrobial at which there is complete inhibition of growth of organism. Measurement of minimum inhibitory concentration (MIC) of antibiotics is an important aid to determine antibiotic resistance to the bacteria.

Materials and Methods: The present study aims to determine the MIC of 240 *Staphylococcus aureus* which showed reduced zone of inhibition by Vancomycin disk diffusion test. Minimal inhibitory concentration (MIC) of vancomycin was determined by agar dilution method in Muller Hinton agar

Results: Out of 240 *S.aureus* 225 isolates were VSSA (MIC≤ 2 µg/mL), 12 were VISA (MIC 4-8 µg/mL) and 3 strains were VRSA (MIC>16 µg /mL).

Conclusion: Wide spread usage of vancomycin to treat infections caused by MRSA has been reported to result in the emergence of low level resistance. Continuous efforts should be made to prevent the spread and the emergence of glycopeptide resistance by early detection of the resistant strains and using the proper infection control measures in the hospital setting.

KEYWORDS : MIC,MRSA,VRSA, VISA,

Introduction:

Staphylococcus aureus is a major pathogen causing a diversity of infections including bacteraemia, pneumonia, skin, soft tissue and osteo-articular infections (1). *Staphylococcus aureus* infections used to respond to ß-lactam and related group of antibiotics but, the emergence of *Methicillin-resistant S. aureus* (MRSA) has posed a serious therapeutic challenge (2). The first case of MRSA was reported in 1961, these MRSA isolates are usually resistant to multiple classes of antimicrobial agents including macrolides, lincosamides, tetracyclines, fluroquinolones and aminoglycosides and it has made the therapy of staphylococcal disease a global challenge. Recommended therapeutic for treatment of MRSA infections are glycopeptides in particular vancomycin (3, 4, 5).

Since the emergence of vancomycin resistance in enterococci in 1988 and its *in vitro* demonstration that its resistance genes (*Van A and Van B*) are transmissible to other bacterial species including *S.aureus*, emergence of vancomycin resistance in clinical staphylococci has become a great concern (6). The incidence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) has been increasing in various parts of the world (7). The first case of diminished vancomycin susceptibility (VISA) (MIC 8-16mg/l) in a clinical isolate of *S. aureus* was reported from Japan in 1997 and subsequently in United States (3). It was due to decreased availability of the vancomycin for intracellular target molecules. This was in turn caused by unusually thickened cell wall having dipeptides used for binding vancomycin (6).

Tiwari and Sen have reported a VRSA which is *van A* gene negative. Subsequently, VISA and VRSA strains were reported from France, United Kingdom, Brazil, Germany and other countries of the world (7). Other strains, named hetero-VISA, appear to be borderline susceptible to vancomycin (MIC 2–4 mg/L) but exhibit low-level subpopulations (10–6 cells) able to grow at vancomycin concentrations of 4–8 mg/L. Such strains have been described in Europe, Asia and Brazil. Hetero-VISA strains could represent first-step mutants that are precursors of VISA strains in patients receiving prolonged courses of vancomycin (1).

The aim of the present study was to determine the MIC of vancomycin to *S. aureus* isolates from different clinical specimens showing reduced zone of inhibition to vancomycin by disc diffusion test and to determine the sensitivity of these isolates to different antimicrobial agents.

Materials and Methods:

***Isolation of Staphylococci from clinical samples*:** A total of 240 consecutive isolates of *S.aureus* from clinical samples (Urine, Blood, Sputum, C.S.F, Catheter tip, Pus and Body fluids) were collected between January 2015 to April 2017 in the Department of Microbiology, SVIMS, Tirupati, were included in the study. All the isolates were identified as *S. aureus* by culture and biochemical tests which included test for clumping factor, free and bound coagulase and mannitol fermentation.

Antibiotic susceptibility testing: All *S. aureus* isolates were then subjected to antibiotic susceptibility testing by the Kirby–Bauer disc diffusion method using different antimicrobial agents e.g., Penicillin (10 units), Ampicillin (10µg), Cloxacillin (5µg), Oxacillin (1µg), Cefoxitin (30µg), Cephotaxime (30µg), Gentamicin (50 µg), Coprofloxacin (10 µg), Vancomycin (30µg), Amikacin (30µg) (Hi-media).

All the *S.aureus* strains resistant to vancomycin (30µg) were subjected to MIC determination. The diameter of zone of inhibition was compared with CLSI zone size interpretative chart.

Interpretation: The strain was considered as sensitive if zone size was ≥ 15mm.

The strain was considered as resistant if zone size was <15 mm.

Determination of Vancomycin MIC: Minimal inhibitory concentration (MIC) of vancomycin was determined by agar dilution method in Muller Hinton agar according to the protocol of CLSI (9). Briefly, gradient plates of Mueller-Hinton agar (Hi-media) were prepared with vancomycin (0.5-256 mg/l). 0.5 McFarland equivalent inoculum prepared using 18-24 h old culture. 0.01 ml of inoculum was spotted using a calibrated loop on to gradient plates. Plates were incubated at 37°C for 48 h. before assessing the visible growth, *S. aureus* ATCC 25923 was used as control. Before reading and interpreting the results, growth control and results with quality controls mains were checked. The minimum inhibitory concentration (MIC) was checked by examining the plates for the lowest

concentration of vancomycin that inhibited visible growth.

CLSI MIC interpretative criteria for vancomycin in *S.aureus* (8):
Vancomycin susceptible *S.aureus* (VSSA): ≤2µg/mL
Vancomycin intermediate *S.aureus* (VISA) : 4-8µg/mL
Vancomycin resistant *S.aureus* (VRSA) : ≥16µg/mL

Results:
Out of 240 subjects, 162 (67.5%) patients were male and 78 (17.5%) were female patients. The male to female ratio in the present study was 2:1. Majority of the patients were of age 41-50 years. Out of the total 240 samples 129 samples were pus, 27 blood samples, 9 sputum samples, 15 catheter tip samples, body fluids 3 sample, CSF sample 3 and 54 urine samples, as shown in (***Table: 1***).

Among the 240 clinical isolates of *S. aureus*, the MIC for 225 (93.75%) for vancomycin was ≤2 mg/l indicating that all were sensitive to vancomycin (VSSA). 12 isolates showed an MIC range between 4-8 mg/l, indicating vancomycin intermediate resistance (VISA). Out of 12 VISA isolates 9 were from urine, and 3 from blood sample. For the remaining 3 isolates, the MIC was in the range of >16mg/l indicating that these three isolates were vancomycin resistant (VRSA)(***Table: 2***). VRSA strains was isolated from blood, urine and pus samples. All these 3 isolates were sensitive to Linezolid and Gentamicin in common.

Antibiotic Susceptibility Pattern: *S.aureus* isolates Antibiogram of S.aureus showed highest resistance to Ampicillin 96%, followed by Penicillin 92%, VRSA isolates were susceptible to Tetracyclin, Erythromycin, Gentamicin, Cotrimoxazole and Linezolid. All the three Vancomycin Resistant *Staphylococcus aureus* were sensitive to Linezolid in common.

Discussion:
The emergence and spread of resistance to vancomycin is a threat to the already challenging therapy of MRSA and raise an alarming situation to the clinicians in hospital as well as in community (10). The spread of MRSA from the hospital to the community, coupled with the emergence of VISA and VRSA, have become a major concern among healthcare providers (11).

Out of 240 subjects, 162 (67.5%) patients were male and 78 (32.5%) were female patients. Male to female ratio was 2:1. The increased rate of infection among males could be due to their outdoor occupation, more prone for injuries, smoking and due to exposure to contaminated environment. A similar observation has been made by Siddique et al (12) who has reported a male to female ratio of 2.6:1.

Out of 80 cases, most of the cases were seen after 4th decade, majority being between 41-50 years. This may be because of waning immunity as age advances and underlying hormonal abnormalities. The MIC value of 240 isolates varied from 0.5-32 µg/mL. 225 strains had MIC between 0.5-2 µg/mL (VSSA), 12 strains had MIC between 4-8 µg/ mL (VISA) and 3 straisn had MIC f 32 µg/mL (VRSA).

Horieh Saderi et al (2008) in Tehran investigated for vancomycin MIC for 164 *S.aureus* isolates and reported 97.5% isolates were shown MIC ≤ 2µg/ml and one strain as VRSA (NIIC -256 µg/ml) . In our study 93.75% of strains showed MIC ≤2 µg/ml and one strain as VRSA (MIC -32 µg/ml) (13).

A study by Venubabu Thati et al (4) showed similar findings with our study The MIC for 335 of 358 isolates (93.57%) for vancomycin was ≤2 mg/l indicating that all were sensitive to vancomycin . Sixteen (4.4%) isolates were identified as VISA (MIC = 4-8 mg/L). All VRSA isolates (n = 7) were MRSA and had a vancomycin MIC in the range of 16-64 mg/L.

Conclusion:
There are only limited drugs available for the treatment of VRSA. Linezolid, Quinupristin-dalfopristin, Daptomycin and Tigecycline are the newer antimicrobial agents currently available with activity against drug-resistant staphylococci (including most VISA and VRSA strains *in vitro*), clinicians and patients still need options for treatment of MRSA infection. Though cross-resistance has not been noted for Linezolid, isolates have known to develop resistance during therapy.

There is a need to reduce the global burden of infections caused by Gram-positive pathogens and its resistant strains (mainly MRSA). Continuous efforts should be made to prevent the spread and the emergence of glycopeptide resistance by early detection of the resistant strains and using the proper infection control measures in the hospital setting.

Table 1: Age, Sex and Sample distribution

Table - 1: Patient Characters			
S.No	**Characters**	**No.**	**Percentage**
1.	**Age (yrs) :**		
	1-10	6	2.5%
	11-20	6	2.5%
	21-30	21	8.75%
	31-40	27	11.25%
	41-50	66	27.5%
	51-60	36	15%
	61-70	51	21.25%
	71-80	27	11.25%
2.	**Sex:**		
	Male	162	67.5%
	Female	78	32.5%
3.	**Distribution of samples**		
	Blood	27	11.25%
	Urine	54	22.5%
	Sputum	9	3.75%
	Pus	129	53.7%
	Body fluids	3	1.2%
	CSF	3	1.25%
	Catheter tip	15	6.25%
	Total	**240**	

Table - 2: MIC values among samples

MIC value	No. of samples	Percentage
≤ 2 µg/mL	**225**	**93.75%**
4-8µg/mL	**12**	**5%**
>16 µg /mL	**3**	**1.25%**

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