

**Original article:**

## **Comparison of Vancomycin MIC against methicillin resistant *Staphylococcus aureus* and methicillin sensitive *Staphylococcus aureus* isolated at a tertiary care centre in Jaipur, India**

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### **ABSTRACT:**

**Introduction:** Vancomycin, a glycopeptide antibiotic had been considered to be the drug of choice for methicillin resistance *Staphylococcus aureus*. In MSSA infection, Injudicious and infrequent use of vancomycin has resulted in emergence of the strains with higher vancomycin MIC. Vancomycin MIC is a gold standard test to determine the sensitive ( $\leq 2$   $\mu\text{g/ml}$ ), intermediate (4-8  $\mu\text{g/ml}$ ) or resistant ( $\geq 16$   $\mu\text{g/ml}$ ) *S. aureus* strains, according to the CLSI guidelines.

**Material and methods:** The current study was a comparative study to determine Vancomycin MIC value in MRSA and MSSA. Total 53 *Staphylococcus aureus* (*S. aureus*) strains were screened for methicillin resistance using a 30  $\mu\text{g}$  cefoxitin disc (HiMedia, India) on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) guideline. Vancomycin MIC (minimum inhibitory concentration) was determined by agar dilution method.

**Observation:** Out of 53 *S. aureus* isolates, 42 (79%) were methicillin resistant *S. aureus* (MRSA) and 11(21%) methicillin sensitive *S. aureus* (MSSA). Majority of the isolates (14% of MRSA and 9% of MSSA) showed vancomycin MIC  $\leq 0.5$   $\mu\text{g/ml}$ . Isolates with higher vancomycin MIC values (0.75-1  $\mu\text{g/ml}$ ) were MRSA (n=36 of 42 isolates) as compared to MSSA (n=6 of 11 isolates).

**Conclusion:** The results showed that higher vancomycin MIC 0.75-1  $\mu\text{g/ml}$  was observed in 55% of MRSA compared to 45% MSSA strains) with higher vancomycin MIC (mean 0.840) in MSSA was compared to the mean vancomycin MIC (mean 0.833) in MRSA with significant difference ( $p < 0.0001$ ), which may explain the inappropriate use of vancomycin in prophylaxis and treatment.

**Key words:** Vancomycin MIC (minimum inhibitory concentration), *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA), methicillin sensitive *Staphylococcus aureus* (MSSA).

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### **INTRODUCTION**

*Staphylococcus aureus* is a dangerous threat to human health. Its commonly associated with both hospital and community acquired infection<sup>1</sup>. *S. aureus* colonise the anterior nares which is the most frequent site, followed by extra-nasal sites such as GI tract, axilla,

vagina etc. Being a commensal it is easily transmitted to other sites and causes localized as well as disseminated infection<sup>2</sup>. These infections range from superficial skin lesions to deep seated infections. It is the most common cause of localized suppurative lesions as pustules, boils, carbuncles,

abscesses, styles, impetigo and wound infection. The bacterium also causes toxin-mediated diseases such as food poisoning, toxic shock and staphylococcal scalded skin syndrome. A wide range of antibiotics are used to treat the staphylococcal infection including penicillin, cephalosporin, macrolide, fluroquinolone and glycopeptide group of antibiotics<sup>3</sup>. *S. aureus* has undergone genetic modification that has resulted in all strains of *S. aureus* are resistant to natural penicillins because of the genes acquisition that encode drug-inactivating enzymes, initially known as penicillinase and now called  $\beta$ -lactamase that hydrolyze the penicillin, which is treated by methicillin and oxacillin. Most of the strains show resistant to methicillin which is primarily mediated by the *mecA* gene, which are also resistant to other higher  $\beta$ -Lactam group of antibiotic including cephalosporins. Cotrimoxazole, aminoglycosides, erythromycin, clindamycin etc are used to treat MRSA infection. Vancomycin, a glycopeptide antibiotic had been considered to be the drug of choice. Apart from vancomycin, other effective drugs as linezolid and teicoplanin are also widely used. In MSSA infection, Injudicious and infrequent use of vancomycin has resulted in emergence of the strains with higher vancomycin MIC<sup>4</sup>. In early twenties, only VISA (Vancomycin intermediate *S. aureus*) strains were reported but now in India, there is emergence of *S. aureus* strain with higher vancomycin MIC<sup>5</sup>. Currently Vancomycin resistant *S. aureus* (VRSA) strains have been reported. This higher vancomycin MIC has been attributed

by increased thickness of the cell wall as in case of VRSA and also by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan<sup>6</sup>.

Vancomycin MIC is a gold standard test to determine the sensitive, intermediate or resistant strains. According to the CLSI<sup>7</sup> guidelines, *S. aureus* is considered as susceptible to vancomycin if MIC is  $\leq 2$   $\mu\text{g/ml}$ , resistant strains shows MIC  $\geq 16$   $\mu\text{g/ml}$  whereas VISA strain shows MIC of 4-8  $\mu\text{g/ml}$ <sup>3</sup>.

#### AIMS & OBJECTIVES

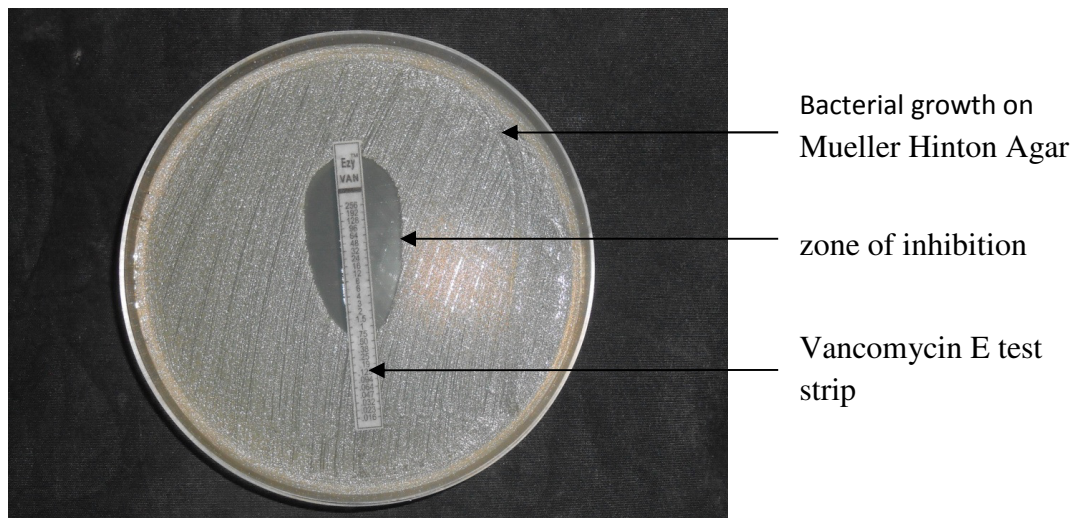
1. To determine vancomycin MIC against isolated *Staphylococcus aureus* from different clinical sample.
2. Comparison of vancomycin MIC in MRSA & MSSA strains.

#### MATERIALS AND METHODS

The study was carried out in the Department of Medical Microbiology, NIMS medical College and Hospital, Jaipur, Rajasthan during the period of January 2015 to June 2015. A total of 100 clinical samples were collected and inoculated on blood agar and incubated overnight at 37°C for *S. aureus* screening. Strains were identified by colony characteristics on blood agar media, gram's staining and free and bound coagulase enzyme production. Total 53 *S. aureus* strains were screened for methicillin resistance using a 30  $\mu\text{g}$  Cefoxitin disc (HiMedia, India) on Mueller Hinton agar according to CLSI guideline. Plates were incubated at 37°C overnight. Strain with zone inhibition  $\leq 22$  mm on MHA around Cefoxitin 30  $\mu\text{g}$  (HiMedia) considered as MRSA and  $\geq 22$  mm as MSSA (Wayne,

2014). Vancomycin MIC was determined by E-test method. On Mueller Hinton Agar media under specific incubation condition strains were inoculated, a paper strip (Vancomycin E-

strip, Hi-media) comprises of predefined antibiotic gradient was used and incubated for overnight at 35°C to determine the MIC, in µg/ml (Figure 1).



**Figure 1 : Vancomycin E test strip on Mueller Hinton Agar showing elliptical zone of inhibition (both diffusion as well as dilution)**

#### OBSERVATION AND RESULTS

A total of 53 clinical samples of *S. aureus* were isolated and Vancomycin E-test was performed on the isolates. Among 53 *S. aureus* isolates, 42 (79%) were MRSA and 11(21%) MSSA.

**Table 1 : Distribution of samples in percentage (%-age)**

TYPE OF SAMPLES	NO. OF PATIENT S(n=)	MRSA (n=)	MSSA (n=)
Pus	22(41%)	15(28%)	7(13%)
Blood	2(4%)	1(2%)	1(2%)
Nasal Swab	18(34%)	16(30%)	2(4%)
Gastric Aspirates	1(2%)	1(2%)	0
Sputum	3(6%)	3(6%)	0
Urine	3(6%)	3(6%)	0
Other Swabs	4(7%)	3(6%)	1(2%)
Total	53(100%)	42(79%)	11(21%)

MRSA were mostly isolated in our study (Table 1) from clinical samples as (41%; 22/53) pus samples, followed by (4%; 2/53) blood samples, (34%; 18/53) nasal swabs,

(2%; 1/53) gastric aspirates, (6%; 3/53) sputum, (6%; 3/53) urine, and (8%; 4/53) other swabs.

**Table 2: MIC values of MRSA and MSSA isolates against vancomycin**

MIC Values (µg/ml)	MRSA(n=)	MSSA(n=)	TOTAL (n=)
< 0.25	3(7%)	0	3(6%)
0.25- 0.5	3(7%)	1(9%)	4(8%)
0.5- 0.75	13(31%)	5(45%)	18(34%)
0.75 - 1	23(55%)	5(45%)	28(53%)
Total	42(79%)	11(21%)	53(100%)

All the isolated staphylococci are sensitive to Vancomycin <4 µg/ml (Table 2). Highest vancomycin MIC was 1 µg/ml. Vancomycin MIC value 0.25 µg/ml was determined for 3 strains (MRSA), 0.5 µg/ml determined for 4 strains (MRSA:MSSA;3:1), 0.75 µg/ml

determined for 18 (MRSA:MSSA;13:5), and 1 µg/ml determined for 28 (MRSA:MSSA;23:5). But the higher vancomycin MIC 0.75-1 µg/ml was observed in 55% of MRSA compared to 45% MSSA strains (Table 2).

**Table 3 : Mean and standard deviation of Vancomycin MIC in MRSA and MSSA**

MIC Value	MRSA	MSSA
Mean Value	0.833	0.840
Standard deviation	1.0587	0.1685
Total	42	11

( F= 39.4772, P <0.0001)

In our study, mean value and standard deviation of vancomycin MIC in MRSA is 0.833 and 1.0587 and MSSA is 0.840 and 0.1685 (Table 3). There is significant difference in mean value

of vancomycin MIC in MRSA and MSSA (p < 0.0001). The higher vancomycin MIC (mean 0.840) in MSSA was compared to the mean vancomycin MIC (mean 0.833) in MRSA with

significant difference ( $p < 0.0001$ ) obtained. All isolated MSSA strains have higher vancomycin MIC in compared to MRSA. This variation of vancomycin MIC in developed and developing countries may be due to selective use of antibiotics in MRSA and MSSA strains.

## DISCUSSION

*S. aureus* is the most common cause of nosocomial infections. Most of these infections are transmitted directly from the health care personnel to the patient by fingers and sneezing<sup>8</sup>, thereby producing clinical manifestations. Highest prevalence of MRSA (31%) compared to MSSA (13%) has also been reported by Agnihotri et al.<sup>8</sup>. Higher percentage of MRSA rate (79%) have been found in our study denotes the prior use of broad spectrum antibiotics in the treatment of both *S. aureus* infection whether MRSA or MSSA. Similar studies was found by Goyal et al. where highest prevalence was observed in pus samples (250; 66.03%) followed by urine (43; 11.45%), Blood (35; 9.16%), sputum (30; 8.02%), swab (9; 2.29%), CSF (9; 2.29%) and other body fluids (3; 0.76%)<sup>9</sup>.

Vancomycin is considered as drug of choice. But in present scenario, *S. aureus* strains with higher vancomycin MIC have been reported that influence the treatment options to combat *S. aureus* mediated infections. This higher vancomycin MIC has been attributed by increased thickness of the cell wall as in case of Vancomycin Intermediate *S. aureus* Pootoolal et al.,<sup>10</sup>.

Variation with higher vancomycin MIC in MRSA as well as MSSA may be due to inappropriate and injudicious use of

vancomycin in prophylaxis and treatment. Similar type of result was observed in the study performed by Gupta et al. (2014) reported vancomycin MIC  $\leq 0.5$   $\mu\text{g/ml}$  showed by majority of the isolates (73.2% of MRSA and 67.3% of MSSA)<sup>11</sup>, which is comparable with MIC 0.25  $\mu\text{g/ml}$  for 7% MRSA in our present study and MIC 1  $\mu\text{g/ml}$  for 9% MRSA and 25% MSSA. In another study by Dhawan et al. (2010) determined vancomycin MICs ranged from 0.5–2  $\mu\text{g/ml}$  (MIC 1  $\mu\text{g/ml}$ ) for both MSSA and MRSA strains<sup>3</sup>. Vancomycin MIC 0.5  $\mu\text{g/ml}$  for 26 *S. aureus* strains, MIC 1  $\mu\text{g/ml}$  for 4 isolates out of 160 *S. aureus* determined by P Bhateja et al.<sup>12</sup>. Highest prevalence of MRSA determined with MIC  $\leq 0.5$   $\mu\text{g/ml}$  in our study. In contrast higher vancomycin MIC in 16.3% MRSA and 6.5% MSSA was also reported by Lodise et al.<sup>13</sup>. Vancomycin MIC of 1  $\mu\text{g/ml}$  for 109 (26.4%), 1.5  $\mu\text{g/ml}$  for 213 strains (51.4%) and 2  $\mu\text{g/ml}$  for 92 (22.2%) *S. aureus* has been reported by Alex Soriano et al.<sup>14</sup>.

Although there was no strains of the VISA and VRSA was observed but different strains of the staphylococcus have the higher MIC  $\leq 1$   $\mu\text{g/ml}$  were reported, which is comparable to the study of Sharma et al. where 82.6% MRSA strains were observed to multidrug resistant (MDR) but all were sensitive to vancomycin<sup>15</sup>. Inappropriate vancomycin use in patients eliminate the susceptible strain and only strains higher MIC values remains that in future creates infections to the patients.

## CONCLUSION

The study shows a continuous increase in prevalence of MRSA in hospitals, is of great concern for our healthcare system. Good

hospital associate infection control policies may be a good barrier for these infections. Because the potential reservoirs of MRSA include infectious patients, hospital personnel and hospital environment. Regular monitoring of antimicrobial sensitivity pattern of all clinical isolates and continuous infection control measures like hand washing and other aseptic techniques will definitely control and decrease the incidence of MRSA as well as other multidrug resistant pathogens.

Emergence of higher vancomycin MIC strain warns the clinician for the selective use of vancomycin only in MRSA cases as MSSA strains are easily susceptible to penicillin and cephalosporin. Non-judicious use of vancomycin in treatment as well as prophylaxis will result in vancomycin intermediate as well as vancomycin resistant *S. aureus* strains that will leave only limited drugs options.

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