

# Prevalence of Methicillin Resistance & Comparison of Vancomycin Minimum Inhibitory Concentration by E TEST & VITEK 2 in Staphylococcus Aureus Isolates

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## ABSTRACT

**Introduction:** Resistance to methicillin and other  $\beta$ - lactamase resistant penicillins was first observed in *Staphylococcus aureus* soon after methicillin was introduced into clinical use in Britain in 1961. Methicillin resistant *S. aureus* (MRSA) is responsible for around 30% or more of all *S. aureus* infections. MRSA is a multidrug resistant organism that threatens the continued effectiveness of antibiotics worldwide and causes a threat in hospitals and long-term care settings. Vancomycin has been the treatment of choice for serious infections caused by MRSA. But there has been uncertainty regarding the method for detection of minimum inhibitory concentration of vancomycin, clinical relevance of reduced vancomycin susceptibility in *S. aureus* & increasing concern regarding the efficacy of vancomycin for treatment of MRSA infections.

**Materials and Methods:** A study was planned to determine the prevalence of methicillin resistance in clinical isolates of *S. aureus* isolated from samples of patients attending OPDs & IPDs of Career Institute of Medical Sciences, Lucknow. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method. The isolates were tested for methicillin resistance by using cefoxitin disc (30 $\mu$ g) by disc diffusion method. The results were interpreted according to CLSI criteria. Vancomycin MICs were compared by two methods viz. E TEST & VITEK 2.

**Results:** A total of 163 isolates were studied and 66(40.4%) were found to be methicillin resistant. MRSA isolates showed greater resistance to multiple drugs as compared to methicillin sensitive *S. aureus* (MSSA) isolates. 48.48%, 86.36%, and 42.42% of MRSA were resistant to chloramphenicol, cotrimoxazole & doxycycline as compared to 4.12%, 15.46% & 12.37% of MSSA respectively. On determination of MICs for vancomycin for the MRSA isolates, all were identified as VSSA by E Test & Vitek 2 methods but the MIC values were variable on testing with both methods. Out of 66 isolates, 51 isolates had MIC=1.5 $\mu$ g/mL & 15 isolates had MIC = 2  $\mu$ g/mL by E Test method. When these isolates were tested with Vitek 2, only 20 had MIC=1.5 $\mu$ g/mL while 46 had MIC=2 $\mu$ g/mL.

**Conclusion:** An ever rising isolation of MRSA from various infections was observed. These isolates were also associated with high level of co-resistance to other group of antibiotics. There is a need to study the epidemiology of such infections. Robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce emergence of resistance.

**Keywords:** *Staphylococcus aureus*, Antibiotic susceptibility, MRSA, Vancomycin, MIC

## INTRODUCTION

*S. aureus* has been recognized as an important cause of human disease for more than 100 years. [1] *S. aureus* is recognized as

a cause of a wide range of infections, from minor skin infections and chronic bone infections to devastating septicemia and endocarditis. [2,3] Significant events in the

evolution of *S. aureus* have included the development of methicillin resistance, now a problem for many hospitals around the world. In addition, MRSA strains are important for their resistance to many other commonly used antibiotics and the emergence of resistance to vancomycin, the drug that has been used to treat MRSA infections for more than three decades. The glycopeptide antibiotic vancomycin was first released in 1958. Subsequently, vancomycin has been the treatment of choice for serious infections caused by methicillin resistant *S. aureus* (MRSA), which are becoming increasingly common globally. For many years there was no indication that vancomycin resistance in *S. aureus* was likely to be a problem. Therefore, initial reports of reduced vancomycin susceptibility in clinical isolates of *S. aureus* from Japan in 1997 generated significant concern in the medical community.<sup>[4,5]</sup> Since that time there has been uncertainty regarding optimal laboratory detection and the clinical relevance of reduced vancomycin susceptibility in *S. aureus*, changes in Clinical and Laboratory Standards Institute (CLSI) breakpoints for vancomycin against *S. aureus*, and increasing concern regarding the efficacy of vancomycin for the treatment of *S. aureus* infections. In view of this rising importance of methicillin resistance and associated resistance of other commonly used antibiotics for MRSA infections the present study proposes to assess the reduced susceptibility in *S. aureus* isolates in an Indian tertiary care facility & compare their vancomycin MICs by two methods viz. E TEST & VITEK 2.

## MATERIALS & METHODS

The study included 163 clinical isolates of *S. aureus* that were received over a period of 1 year from OPDs & IPDs in the Clinical Microbiology Laboratory of the Department of Microbiology, Career Institute of Medical Sciences, Lucknow from 02 December 2015 to 30 November 2016. These isolates were obtained from

various clinical samples like pus, urine, endotracheal secretions and body fluids. The clinical isolates of *S. aureus* were identified as per standard bacteriological techniques.<sup>[6]</sup> Test for methicillin resistance was performed by Kirby Bauer disc diffusion method using cefoxitin (30µg) disc on Mueller Hinton agar with 16-18 hours incubation at 35<sup>0</sup>C. Antibiotic sensitivity testing was performed by Kirby-Bauer disc diffusion method for the following antibiotics: gentamicin (10µg), tetracycline (30µg), ciprofloxacin (5µg), cotrimoxazole (1.25/23.75µg), levofloxacin (5µg), moxifloxacin (5µg), penicillin (10units), doxycycline (30µg), erythromycin (15 µg), clindamycin (2 µg), chloramphenicol (30µg) & linezolid (30µg) according to CLSI guidelines.<sup>[7]</sup> The reagents & antibiotics were obtained from HiMedia, Mumbai, India. *Staphylococcus aureus* ATCC 43300-mecA positive (zone ≤21 mm), *Staphylococcus aureus* ATCC 25923-mecA negative (zone 23-29mm), were used for quality control. Vancomycin susceptibility testing was performed on the MRSA isolates by two methods viz. Vitek 2 & E test.

### CLSI MIC interpretative criteria for Vancomycin in S.aureus:

Vancomycin susceptible *S.aureus* (VSSA) ≤2µG/mL

Vancomycin intermediate *S.aureus* (VISA) 4-8µG/mL

Vancomycin resistant *S.aureus* (VRSA) ≥16µG/mL

**E Test:** The E test strips were brought to room temperature. The inoculum was prepared by making a direct broth suspension of isolated colonies selected from an overnight growth on blood agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland. Lawn culture from this suspension was made using a swab according to standard protocol. After being dried for approximately 10 minutes, E Test strips (HiMedia) for vancomycin (0.016 to 256 µg/ml) were applied. All plates were

incubated at 35<sup>0</sup>C for 24 hours. MIC was read where a clearly defined zone of inhibition intersected the strip. [8]

**Vitek 2:** A pair of plastic tubes was used for each isolate. 3 ml of 0.45% NS (normal saline) was taken in each tube. Colonies from an overnight growth were picked up with an inoculating wire and emulsified in the first tube. 280µl ml from first tube was pipetted into the second tube. The turbidity equivalent to 0.5 Mc Farland was measured using densicheck. Then GP 67 card was put in the second tube. The card with the test tubes was fed into the Vitek 2 machine for antibiotic sensitivity testing where bacterial suspension got vacuum filled in the antimicrobial susceptibility testing card. The card was then inserted in the incubator-reader of the Vitek 2 system and the results were expressed as MIC values in µg/mL. (Vitek 2 Compact Systems Version:06.01)

Statistical analysis was performed using the SPSS version 10.0. Chi-square test ( $\chi^2$ ) was used to determine the significance between proportions. Karl Pearson's

correlation coefficient was applied to determine the correlation between normally distributed values. Arithmetic mean & p-value were the other statistical tools applied.

## RESULTS

A total of 163 isolates of *S. aureus* were obtained from different clinical samples of patients attending various OPDs & IPDs of the hospital. Out of the 163 *S. aureus* isolates, 110(67.5%) isolates were obtained from various IPDs which were clearly in excess of 53(32.5%) isolates obtained from various OPDs. Eighty two (74.5%) out of 110 isolates obtained from various IPDs, were obtained from pus samples which formed the largest group of samples followed by 20(18.2%) from endotracheal secretions & 4(3.6%) from urine samples. Other samples like sputum, CSF & endocervical swab were less in number. Fifty three isolates were obtained from various OPDs of which pus again formed the larger group. (Table 1)

TABLE 1: Type of samples from IPDs and OPDs (n=163)

SAMPLE	Total	%	IPD	%	OPD	%
Pus	128	78.53	82	74.55	46	86.79
E.T SECR	20	12.27	20	18.18	0	0.00
Urine	11	6.75	4	3.64	7	13.21
Sputum	2	1.23	2	1.82	0	0.00
Endocervical swab	1	0.61	1	0.91	0	0.00
CSF	1	0.61	1	0.91	0	0.00
Total	163	100.00	110	100.00	53	100.00

The *S.aureus* isolates were tested for methicillin susceptibility; 40.4% were MRSA and 59.5% were MSSA (Table 2).

TABLE 2: Occurrence of MRSA versus MSSA among *S. aureus* isolates (n=163)

Methicillin susceptibility	No.	%
Sensitive(MSSA)	97	59.5%
Resistant(MRSA)	66	40.4%
Total	163	100%

Amongst 163 *S.aureus* isolates, 110 were obtained from patients admitted in the hospital, 53 (48.18%) of which were MRSA. The isolates obtained from various OPDs were 53, out of which only 13(24.53%) were MRSA (Table3). A significant association was observed between MRSA in various in patient & outpatient departments

(Chi-square=8.3054, p=0.0042) at 5% level of significance. MRSA were significant higher in IPD (48.18%) than OPDs (24.53%). (Table 3)

A total of 128 *S. aureus* isolates were obtained from pus samples, out of which 45(35.2%) were MRSA and 83(64.8%) were MSSA. 20 isolates were obtained from endotracheal secretions, out of which 16(80%) were MRSA and 4(20%) isolates were MSSA. (Table 4)

A total of 45 isolates were MRSA from the pus samples. Out of 45 MRSA isolates, higher numbers of isolates were obtained from IPDs (33) as compared to OPDs (12). Sixteen isolates out of 20 endotracheal secretion samples were MRSA

and all were obtained from IPDs. From the 11 urine samples, 4 isolates were MRSA, 3 out of which were obtained from various IPDs as compared to only 1 isolates from OPD. Single isolate of endocervical swab which was an MRSA, was obtained from

IPD. These findings suggest a higher association of MRSA isolates in IPD patients as compared to OPDs.(Table5). The resistance data of MRSA and MSSA is shown in Table 6.

**Table 3: Distribution of MRSA and MSSA amongst In Patient & Outdoor Patients (n=163)**

IPDs/OPDs	Total	%	MRSA	%	MSSA	%
IPDs	110	67.48	53	48.18	57	51.82
OPDs	53	32.52	13	24.53	40	75.47
Total	163	100.00	66	40.49	97	59.51

Chi-square=8.3054 P = 0.0042 \*

\*p<0.05

**Table 4: Distribution of S. aureus isolates according to the type of sample(n=163)**

Type of sample	Total	%	MRSA	%	MSSA	%
Pus	128	78.53	45	35.16	83	64.84
E.T SECR.	20	12.27	16	80.00	4	20.00
Urine	11	6.75	4	36.36	7	63.64
Others*	4	2.45	1	25.00	3	75.00
Total	163	100.00	66	40.49	97	59.51

Chi-square=4.9449, P = 0.0020\*

(Others\*: 2 sputum samples, 1 endocervical swab & 1 CSF)

\*p<0.05

**Table 5: Samplewise distribution of MRSA & MSSA isolates in IPDs and OPDs (n=163)**

IPDs/OPDs	Pus (128)		Endotracheal secretion (20)		Urine (11)		Others* (4)	
	MRSA- (45)	MSSA (83)	MRSA (16)	MSSA (4)	MRSA (4)	MSSA (7)	MRSA (1)	MSSA (3)
IPDs	33	49	16	4	3	1	1	3
OPDs	12	34	0	0	1	6	0	0
	Chi-square= 2.5951 P = 0.1070		-		With Yates's correction, chi-square = 1.8526 P = 0.1732		-	

(Others\* include 2 sputum samples, 1 endocervical swab & 1 CSF)

**Table 6: Antibiogram Showing Resistance Pattern Of MRSA & MSSA Isolates**

Antibiotic	MRSA	%	MSSA	%	Total	Chi-square	p-value
DO	28	42.42	12	12.37	40	19.1565	0.00001*
COT	57	86.36	15	15.46	72	80.0605	0.00001*
CD	45	68.18	15	15.46	60	46.9279	0.00001*
C	32	48.48	4	4.12	36	44.9161	0.00001*
GEN	51	77.27	18	18.56	69	55.4676	0.00001*
E	53	80.30	37	38.14	90	28.2301	0.00001*
P	NA	NA	82	84.54	NA	NA	NA
MO	38	57.58	13	13.40	51	35.6486	0.00001*
TE	37	56.06	16	16.49	53	28.0204	0.00001*
CIP	56	84.85	65	67.01	121	6.5339	0.0106*
LZ	0	0.00	0	0.00	0	0.0000	1.0000
Total	66	100.00	97	100.00	163		

\*p<0.01

(DO: Doxycycline, COT: Cotrimoxazole, CD: Clindamycin, C: Chloramphenicol, GEN: Gentamicin, E: Erythromycin, P: Penicillin, MO: Moxifloxacin, TE: Tetracycline, CIP: Ciprofloxacin, LZ: Linezolid)

**Table 7: Agreement between E Test & Vitek 2 methods for MRSA isolates (n=66)**

Vitek 2 method	E test method					
	0.5	0.75	1.0	1.5	2.0	Total
0.5	0	0	0	0	0	0
1.0	0	0	0	0	0	0
1.5	0	0	0	20	0	20
2.0	0	0	0	31	15	46
Total	0	0	0	51	15	66
Agreement	Expected Agreement	Kappa statistic	Std. Err.	Z-value	p-value	
53.03%	39.26%	0.2268	0.0781	2.9100	0.0018*	

\*p<0.05

Out of 66 MRSA isolates, 51 isolates had MIC=1.5µg/mL & 15 isolates had MIC = 2 µg/mL by E Test method. When these isolates were tested with Vitek 2, only 20 had MIC=1.5µg/mL while 46 had MIC=2µg/mL. Agreement between the two methods is 53.03% as against the expected agreement of 39.26%. Kappa statistics is 0.2268 which shows that it is statistically significant (p<0.05). (Table 7)

## DISCUSSION

S.aureus is one of the most important pathogens, causing severe morbidity and fatal infections. The rapid evolution of antibiotic resistance in S.aureus is of considerable concern. Methicillin was indicated for treatment of staphylococcal infections due to penicillinase producing staphylococci. Methicillin resistant strains gradually evolved during last three decades which accounted for less than 0.1% of S.aureus in 1960s. Since then MRSA have become well established as hospital acquired pathogen. [9]

Resistance to vancomycin in MRSA (MIC of  $\geq 2$  mg/liter) remains infrequent, but there is growing evidence in the literature that vancomycin may be ineffective against an increasing proportion of isolates with MICs between 1 and 2 mg/liter. [10] A shift in MIC population distributions may have important implications for the incidence of treatment failure beyond individual risk assessment.

In the present cross sectional, comparative study, out of 163 isolates of S.aureus, 128 (78.53%) isolates were obtained from pus samples which formed the largest group of samples followed by endotracheal aspirates, urine and other body fluids. Similar type of pattern was also observed by Shanthi et al. in their studies who have also reported that maximum number of Staphylococcus aureus (67.5%) were obtained from samples like pus & wound swabs. [11] The study conducted in Mangalore by Pai et al. also showed that pus & wound swabs accounted for majority (76.3%) of isolates followed by urine,

respiratory specimens, blood and body fluids. [12] Similarly, Perwaiz et al. have also reported that majority of S.aureus isolates were from pus (59.47%) followed by endotracheal aspirates, blood, urine & body fluids. [13]

On the contrary, Ahmad et al. in their study isolated the maximum number of S. aureus from blood (40%) followed by wound swabs (12%), respiratory specimens (6%) & urine(2.5%). [14]

Various studies show that the epidemiology of MRSA over different parts of India and worldwide is not uniform. In our study, out of 163 S.aureus isolates, 66(40.5%) isolates were recognized to be MRSA. Similarly, Shanthi et al. have reported 45.5% MRSA isolates in their study. [11] Likewise, Tiwari et al. in their study conducted in BHU, Varanasi have also reported 38.44% MRSA isolates. [15] Perwaiz et al. have also reported that 43% isolates of S.aureus were MRSA. [13] Parveen et al. have observed isolation of 48% MRSA in their study from Andhra Pradesh. [16] Still higher isolation of MRSA was observed in a tertiary referral hospital in eastern Uttar Pradesh by Anupurba et al. who have reported isolation of 54.85% of MRSA which is much higher than our study. [17] Likewise, Behera et al. have reported a higher isolation of MRSA(75%) in their study from All India Institute Of Medical Sciences, New Delhi. [18] On the contrary, Pai et al. in their study have observed that only 69/ 237 (29.1%) of S.aureus isolates were found to be MRSA which is much lower than our study. [12] Similarly, Mehta et al. from Chandigarh have reported a low (24%) isolation of MRSA in their study. [19]

The MRSA isolates were associated with a high degree of co- resistance to other groups of antimicrobial agents. In our study, 42.42% of MRSA isolates were resistant to doxycycline, 86.36% were resistant to cotrimoxazole, 80.30% were resistant to erythromycin, 68.18% were resistant to clindamycin, 48.48% were resistant to chloramphenicol, 77.27% were resistant to

gentamicin, 57.58% to moxifloxacin, 56.06% to tetracycline, 84.85% to ciprofloxacin. All the isolates however were sensitive to vancomycin and linezolid. Similar high level of co resistance to other group of antibiotics has been observed by other workers too. Pai et al. observed in their study that about 40-50% of MRSA isolates were resistant to erythromycin, gentamicin & chloramphenicol. [12] Similarly, Anupurba et al. have also reported that MRSA isolates were found to have multi drug resistance. More than 80% of MRSA were resistant to cotrimoxazole, ciprofloxacin, gentamicin, erythromycin, tetracycline. [17] Likewise, Perwaiz et al. have reported 90- 95% resistance to erythromycin & clindamycin, 93% resistance to gentamicin & 54% resistance to amikacin in MRSA isolates. [12] In their study, Tiwari et al observed that almost all MRSA strains were resistant to penicillin, 95.68% were resistant to cotrimoxazole, 92.36% were resistant to chloramphenicol, 90.7% were resistant to norfloxacin, 76.1% were resistant to tetracycline, and 75.75% were resistant to ciprofloxacin. [15]

Persistently high & increasing rates of MRSA among S.aureus isolates have been observed for healthcare settings in many regions of the world. But prevalence of MRSA is also increasing in the community and has become an emerging public health problem. In our study, 80.3% MRSA were healthcare associated while only 19.7% MRSA were community isolates. Similarly, in a study by Albur et al. 76% MRSA were healthcare acquired while only 24% were community associated. [20] On the contrary, in their study Shrijana et al. have reported that 48.1% MRSA were isolated from the community. [21]

Subtle but potentially important variability in vancomycin MIC results is obtained with different methods. Broth Micro Dilution is considered to be the gold standard for measuring vancomycin MIC. However BMD is a cumbersome test and is not used routinely in clinical laboratories.

Currently most clinical laboratories use E Test and automated susceptibility tests for measuring the vancomycin MIC. Various automated systems like MicroScan WalkAway, Vitek 2, Phoenix, Sensititre, Vitek Legacy are available having variable sensitivities and specificities.

In our study we compared the vancomycin MICs obtained by E Test & Vitek 2 for same isolates. All the isolates were equally identified as VSSA by E Test & Vitek 2 (agreement of 53.3% as against the expected agreement of 39.26%). But out of 66 MRSA isolates, 51 isolates had MIC=1.5µg/mL & 15 isolates had MIC = 2 µg/mL by E Test method. When these isolates were tested with Vitek 2, only 20 had MIC=1.5µg/mL while 46 had MIC=2µg/mL. Although all the MRSA isolates were sensitive to vancomycin according to the MIC criteria, there was a difference in minimum inhibitory concentration values with the two methods. This difference is significant because it has been observed that the vancomycin MICs towards the higher side of sensitivity criteria are associated with treatment failures.

Hsu et al. in their study conducted in California, USA have reported highly variable results of vancomycin MICs using different methods like E test, Vitek1, Micro Scan and comparing them with BMD. They also reported that E Test method appeared to be relatively more reliable in predicting treatment response (PPV=89%) as compared to Vitek 1 (PPV=81%). [22]

In their study, Rybak et al. observed that on comparison of Vitek 2 and E Test methods with BMD, the E Test & BMD method had 36.7% agreement while Vitek 2 system & BMD method had 54.3% agreement. [23]

Behera et al. from All India Institute Of Medical Sciences, New Delhi, in their study observed that out of 49 MRSA isolates, 2 had MIC in the intermediate range (8µg/mL) & 1 isolate had vancomycin MIC of 16 µg/mL by Vitek 2 method, but all the isolates were susceptible (<2µg/mL)

when tested by broth dilution & E Test methods. [13]

In the study by Kruzel et al., all isolates were susceptible by all testing methods. The vancomycin MICs determined by E Test method were consistently elevated than those determined by BMD. Using frozen Trek panels as the reference method, the essential agreement for in-house broth microdilution was 99.4%, while it was 76.4% for the E Test method, 96.3% for Vitek 2. [24]

Even though in this study we did not correlate the clinical outcome of MRSA infections, the possibility of vancomycin treatment failures in our hospital settings could not be ruled out and is an area of concern.

## CONCLUSION

Over the decades isolation of MRSA has been on the rise, more so from patients admitted in hospital than from patients coming to the OPD. MRSA isolates have also been observed to be associated with high level of co resistance to other commonly prescribed antibiotics for gram positive bacteria. Vancomycin has till now been the treatment of choice for MRSA. But MRSA isolates with higher MICs, even within the susceptibility range, are being observed more frequently which result in treatment failures with vancomycin. Several investigators have observed that the MICs of vancomycin differ with different methods. Therefore due to the variability in vancomycin MIC results obtained with the different methods, the use of the vancomycin MIC to predict the outcome of serious S. aureus infections needs to take into account the method used and the results of studies using that particular method.

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