

# Detection of *mecA*, *vanA* and *vanB* Genes Among Methicillin- Resistant *Staphylococcus aureus* Isolates from Various Clinical Specimens in Moradabad, India

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## ABSTRACT

**Background and Aim:** The most effective antibiotic for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infections is vancomycin. However, vancomycin-intermediate *Staphylococcus (S.) aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) have been found to be more common in various parts of the world. This study aimed to determine the prevalence of *mecA*, *vanA* and *vanB* genes among MRSA isolates and to categorize vancomycin susceptibility profiles in clinical specimens from TMMC & RC, Moradabad.

**Materials and Methods:** In the central laboratory of TMMC and RC Moradabad, *S. aureus* was isolated from various clinical samples and MRSA was detected by Kirby-Bauer disk diffusion method. Minimum inhibitory concentration (MIC) of vancomycin was determined by broth microdilution (BMD) method and categorized it into VSSA, VISA, and VRSA isolates. The *mecA*, *vanA* and *vanB* genes were detected on extracted genomic DNA using polymerase chain reaction.

**Results:** Out of 314 MRSA strains, 296 (94.27%) were VSSA, 14 (4.46%) were VISA and 4 (1.27%) were VRSA, as found on the basis of MIC determination through BMD method. All MRSA strains possessed the *mecA* gene, 1 VRSA had *vanA* and 3 VRSA had *vanB*. Among VISA isolates no *vanA* and *vanB* genes were detected.

**Conclusion:** The high prevalence of MRSA (52.5%) and the emergence of VRSA (1.27%) carrying *vanA* and *vanB* genes in our setting highlight a serious public health threat and underscore the need for continuous surveillance and stringent infection control measures.

**Keywords:** Cefoxitin, Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, Vancomycin, Vancomycin-intermediate *Staphylococcus aureus*, Vancomycin-resistant *Staphylococcus aureus*

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## 1. Introduction

*Staphylococcus (S.) aureus* is catalase and coagulase positive bacteria and most commonly isolated from the nosocomial infections, accounting for almost 30 to 50% of bloodstream infections. Increased antibiotic resistance has been reported against this organism,

making it a major public health issue (1). Certain characteristics of *S. aureus* in the hospital setting, such as the horizontal transfer of the antibiotic resistance genes and the production of various virulence factors, have a major impact on the emergence and persistence of antibiotic resistance.

MRSA outbreaks are now a significant problem in healthcare settings around the world. Treatment options for MRSA infections are limited to ceftaroline, tigecycline, glycopeptides, and linezolid (2). The horizontal transfer of mobile genetic elements, known as staphylococcal cassette chromosome mec (SCCmec), leads to methicillin resistance, which is mediated by the *mecA* gene (3). The presence of the *mecA* gene in MRSA isolates causes resistance to methicillin. They are also resistant to a group of antibiotics, such as tetracycline, macrolides, lincosamides, and streptogramin B. The preferred antibiotic to treat and prevent the infections caused by MRSA is vancomycin.

However, vancomycin that is frequently prescribed in empirical therapy can lead to rapid emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) (4). Over the last ten years, *S. aureus* has been reported with decreased sensitivity to vancomycin. VRSA and VISA have been isolated from different areas of the world. These strains are associated with higher death rates and morbidity because after the vancomycin treatment failure, limited therapeutic options remain (5). The acquisition of the *vanA* operon from Enterococcus species is the reason of high level of vancomycin resistance in *S. aureus*. The *vanA* and *vanB* genes are used as markers for identification of VRSA and indicate the potential resistant to glycopeptide (6).

This study aimed to determine the prevalence of MRSA, VISA, and VRSA isolates from a hospital in Moradabad, India and to characterize their resistance by detecting the *mecA*, *vanA*, and *vanB* genes.

## 2. Materials and Methods

### 2.1 Study Design and Population

A cross-sectional study was carried out from November 2023 to February 2025, in the Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Moradabad after the approval of Institutional Ethics Committee.

### 2.2 Sample Size Calculation

Sample size was calculated using the formula:

$$n = Z^2 \times P \times Q / E^2$$

n=required sample size; Z= standard normal variant (1.96 at 95% confidence interval); P=prevalence of study (28.6%) (7); Q=(100-P); E= allowable error (5%).

$$n=313.78$$

Minimum 314 MRSA samples were taken in this study.

### 2.3 Inclusion and Exclusion Criteria

All the MRSA strains isolated from inpatient (IPD) and outpatient (OPD) wards were included in this study. Coagulase negative Staphylococci and methicillin-sensitive *Staphylococcus aureus* (MSSA) were excluded from the study.

### 2.4 Sample Collection, Processing and Identification of Bacteria

Different types of clinical specimens were collected aseptically from the patients and were labeled with date, time, collection method, and patient history. The clinical samples were inoculated on culture media (Blood and MacConkey) agar and incubated overnight (18 to 24 hours) at 37°C. Then, the characteristics of colonies were observed and bacteria were identified based on the Gram staining, positive catalase test, positive tube and slide coagulase test, golden yellow color colonies on mannitol salt agar, and beta hemolysis on blood agar (6). DNase test was carried out using DNase agar (Hi-Media). The bacteria were spot inoculated on DNase agar and incubated at 37°C for 24 hours. Then the plate was added with 1N HCl. After a few minutes, the plate was examined against dark background. Clearing zone around the spotted colony was considered as positive for DNase (8).

### 2.5 Antimicrobial Susceptibility Testing

Modified Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing using Mueller-Hinton agar (Hi-Media) following Clinical and Laboratory Standards Institute (CLSI M100-2024) guideline. Isolated pure colonies of test organism were inoculated in peptone water broth and incubated at 35-37°C for 4-6 hours. The density of the organism in broth was adjusted to approximately  $1.5 \times 10^8$  CFU/mL by comparing its turbidity with that of 0.5 McFarland opacity standard tube. The broth was inoculated on Mueller-Hinton agar by lawn culture using a swab stick (7).

### 2.9 Screening of MRSA Isolates

The Kirby-Bauer disk diffusion method was used to screen MRSA using cefoxitin disk and interpretation was based on the CLSI guideline (2024). If the zone of inhibition (ZOI) halo around the cefoxitin disk was  $\leq 21$  mm, then the *S. aureus* was considered MRSA, and if the ZOI was  $\geq 22$  mm, then the *S. aureus* was considered MSSA (9).

### 2.10 Vancomycin MIC Determination Among MRSA Isolates

The MIC of vancomycin among MRSA isolates was determined by BMD method and VISA and VRSA were

found out. BMD method followed the standard operating procedure for antimicrobial resistance surveillance national AMR surveillance network. Cation-adjusted Mueller Hinton Broth was prepared with various concentrations of vancomycin in Hi-Media (0.062 to 32 µg/mL) (10). *S. aureus* (ATCC 25923) of known MIC was also included in each test as a vancomycin susceptible control, and ATCC 51299 of *Enterococcus faecalis* was used as vancomycin resistant control (11). According to the MIC of vancomycin, *S. aureus* was categorized into three groups: MIC ≤2 µg/mL was considered susceptible; 4 to 8 µg/mL was considered intermediate; and ≥16 µg/mL was considered resistant (9).

### 2.11 PCR Amplification of *mecA*, *vanA* and *vanB* genes

Molecular assay was performed in the Molecular Laboratory of Pioneer Centre of Biosciences, Ghaziabad, by DNA extraction, PCR amplification, and gel electrophoresis.

The Qiagen-DNeasy blood and tissue kit was used to extract bacterial DNA according to the guideline provided by manufacturer (12). A commercially available Genei master mix was utilized to prepare the reaction mixture. The kit was used according to the guideline provided by manufacturer.

### 2.12 Detection of *mecA*, *vanA*, and *vanB* Gene Among MRSA Isolates

Amplification of the target genes was carried out by PCR (Bio-Rad) technique on *S. aureus* extracted DNA, and primers synthesized by Barcode Biosciences. The oligonucleotide sequences to amplify *mecA*, *vanA*, and *vanB* genes are listed in Table 1. The PCR programming cycles are mentioned in Table 2. A total volume of 25 µL, including 12.5 µL master mix, 5 µL of DNA template, 2 µL of both forward and reverse primers, and nuclease free water up to 25 µL were used for PCR reaction. The reaction mixture was then placed in the PCR machine. Programming of PCR was performed similar to that of Nashra et al (7). The PCR amplicons were analyzed using 2% agarose gel electrophoresis, and images were taken under UV transilluminator. Amplification of *mecA* gene produced a segment with 584 bp size. Amplification of the *vanA* and *vanB* genes produced segments with sizes of 717 and 433 bp, respectively. The ATCC strains used as positive and negative controls are mentioned in Table 3.

### 2.13 Statistical Analysis

Data are mentioned as mean±SD. The chi-square test and fisher's exact test were used to statistically assess the results. P-value≤0.05 was deemed statistically significant. SPSS version 20.0 (IBM Corp., Chicago, Illinois, USA) was used for this purpose.

**Table 1.** Oligonucleotides sequences to amplify *mecA*, *vanA* and *vanB* genes.

Target	Primer	Sequence (5'-3')	Amplified Product Size	Reference
<i>mecA</i>	Forward	AGAAGATGGTATGTGGAAGTTAG	584 bp	Azimian et al (13)
	Reverse	ATGTATGTGCGATTGTATTGC		
<i>vanA</i>	Forward	CTGGGAAAACGACAATTGCT	717 bp	Nashra et al (7)
	Reverse	TGTACAATGCGCCGTTACG		
<i>vanB</i>	Forward	GTGACAAACCGAGGCGAGGA	433 bp	Saadat et al (14)
	Reverse	CCGCCATCCTCTGCAAAAAA		

**Table 2.** PCR programming for the amplification of *mecA*, *vanA* and *vanB* genes.

Gene		<i>mecA</i>	<i>vanA</i>	<i>vanB</i>
Initial Denaturation		94° C for 5 min	94° C for 5 min	94° C for 5 min
Cycle*	Denaturation	94° C for 30 sec	94° C for 30 sec	94° C for 30 sec
	Annealing	50.5° C for 30 sec	52° C for 45 sec	56° C for 45 sec
	Extension	72° C for 30 sec	72° C for 45 sec	72° C for 45 sec
Final Extension		72° C for 7 min	72° C for 7 min	72° C for 7 min

\*The number of cycles for the genes *mecA*, *vanA* and *vanB* was fixed at 34.

**Table 3.** The ATCC strains served as the quality control strains.

Gene	Organism Name	Control	ATCC
<i>mecA</i>	<i>S. aureus</i>	Positive	43300
	<i>S. aureus</i>	Negative	25923
<i>vanA</i>	<i>E. faecium</i>	Positive	51559
	<i>S. aureus</i>	Negative	25923
<i>vanB</i>	<i>E. faecalis</i>	Positive	51299
	<i>S. aureus</i>	Negative	25923

The provided strains served as positive and negative controls for the *mecA*, *vanA* and *vanB* genes detection.

### 3. Results

*Staphylococcus aureus* samples (n=598) were isolated from November 2023 to February 2025. Out of them 314 (52.5%) MRSA and 284 (47.5%) MSSA were isolated from TMU hospital and distributed on the basis of sources and specimens.

The number of 314 MRSA isolates were distributed on the basis of source and clinical specimens. Around 154 (49.04%) MRSA were isolated from the ward patients, 112 (35.67%) from ICU patients, and 48 (15.29%) MRSA were isolated from OPD patients. The highest MRSA strains were isolated from pus [130 (41.4%)], followed by blood [85 (27.07%)] and urine specimens [34 (10.83%)]. Association between specimens and sources was found statistically significant at  $P\text{-value} \leq 0.05$ . ( $\chi^2=128.027$ ,  $df=26$ ,  $P\text{-value} \leq 0.001$ ) (Table 4).

Majority of MRSA samples were isolated from the 16-30 years old age group 78 (24.84%), followed by the 46-60 years old age group 77 (24.52%) and from >60 years old age group 74 (23.57%), MRSA were isolated. In the present study, the patient ages varied from less than 1 to 87 years. Among 314 MRSA strains, 166 (52.87%) were isolated from male patients and 148 (47.13%) from female patients. Association between age groups and gender was found statistically significant at  $P\text{-value} \leq 0.05$ . ( $\chi^2=12.576$ ,  $df=4$ ,  $P\text{-value}=0.014$ ) (Table 5).

The vancomycin MIC values among MRSA isolates was determined through BMD method. Out of 314 MRSA strains, 4 strains were identified as VRSA, 14 as VISA and 296 as VSSA. Data are mentioned in Table 6 and Figure 1.

All 314 phenotypically identified MRSA strains were subjected to PCR. All isolates possessed the *mecA* gene shown in Figure 2. Phenotypically identified 14 VISA and 4 VRSA isolates were also subjected to PCR for detection of the *vanA* and *vanB* genes by PCR. The results showed only 1 *vanA* gene detected in VRSA isolates and among 14 VISA isolates *vanA* gene was not detected (Figure 3). The result showed 3 *vanB* gene detection in VRSA

isolates and no *vanB* gene was detected among 14 VISA isolates as shown in Figure 4.

The Kirby-Bauer disk diffusion method was used for the antibiotics susceptibility testing other than vancomycin, which was carried out by broth microdilution method. The VRSA, VISA, and VSSA isolates from non-urine samples showed variable antibiotic susceptibility patterns against antibiotic agents of different classes. Only 4 VRSA strains were isolated and 100% were sensitive to linezolid and rifampicin followed by doxycycline, tetracycline, minocycline, and daptomycin (75%), sensitive to co-trimoxazole (50%) and gentamycin (25%) and 100% resistant to vancomycin, azithromycin, ciprofloxacin, clindamycin, and erythromycin.

Among 12 VISA isolates, 83.33% were sensitive to linezolid, and daptomycin, followed by co-trimoxazole (75%), gentamycin (66.67%), clindamycin, doxycycline and rifampicin (33.33%), minocycline and tetracycline (25%), and azithromycin, ciprofloxacin, and erythromycin (8.33%). They were sensitive to vancomycin 100% intermediate in VISA cases.

Among 264 VSSA isolates, 100% were sensitive to vancomycin, followed by linezolid (99.24%), daptomycin (98.11%), rifampicin (84.85%), tetracycline, minocycline and doxycycline (82.20%), co-trimoxazole (50.76%), gentamycin (47.73%), clindamycin (46.97%), erythromycin and azithromycin (15.15%), and ciprofloxacin (4.27%). Association between VSSA, VISA and antibiotics was statistically significant only in cases of doxycycline, tetracycline, daptomycin, rifampicin, minocycline, linezolid, and vancomycin ( $P < 0.001$ ) as mentioned in Table 7.

From urine samples, 34 MRSA were isolated. Out of these, 32 VSSA, and 2 VISA but no VRSA were isolated. The VISA and VSSA isolates showed variable antibiotics susceptibility pattern against various antibiotics agent as shows in the Table 8. Norfloxacin and nitrofurantoin were only reported in urine isolates as these antibiotics are concentrated almost exclusively in urine, not in blood

or tissues. Association between VSSA, VISA, and antibiotics was statistically significant only in cases of

vancomycin, norfloxacin, linezolid, and ciprofloxacin with P-value<0.001, and daptomycin with P-value<0.006.

**Table 4.** MRSA distribution on the basis of sources and specimens.

Specimens	Sources			Total	X <sup>2</sup> , df, P-value
	ICU	Ward	OPD		
Ascitic fluid	0	1	0	1	128.027, 26, <0.001
BAL fluid	0	7	0	7	
Blood	65	20	0	85	
Bone	0	1	0	1	
CSF	2	2	0	4	
ET secretion	2	0	0	2	
Foley's tip	4	7	0	11	
HVS	0	8	2	10	
Pleural fluid	2	3	0	5	
Pus	21	72	37	130	
Bone screw	1	0	0	1	
Sputum	7	12	0	19	
Tissue	1	3	0	4	
Urine	7	18	9	34	
Total	112	154	48	314	

X<sup>2</sup>= Chi-square, df= Degree of freedom, P-value= Probability-value

**Table 5.** Distribution of MRSA according to age and gender.

Age Group	Gender		Total (%)	X <sup>2</sup> , df, P-value
	Male (%)	Female (%)		
0-15	18 (10.84)	7 (4.73)	25 (7.96)	12.576, 4, 0.014
16-30	29 (17.47)	49 (33.12)	78 (24.84)	
31-45	33 (19.88)	27 (18.24)	60 (19.11)	
46-60	43 (25.9)	34 (22.97)	77 (24.52)	
>60	43 (25.9)	31 (20.95)	74 (23.57)	
Total	166 (52.87)	148 (47.13)	314 (100)	

**Table 6.** Distribution of VRSA, VISA, and VSSA among MRSA isolates on the basis of MIC values.

S. No.	MRSA Strains N=314 (%)	MIC in µg/mL	Categories
1	3 (0.96)	0.125	
2	8 (2.55)	0.25	
3	47 (14.97)	0.5	VSSA = 296 (94.27%)
4	127 (40.45)	1	
5	111 (35.35)	2	
6	12 (3.82)	4	VISA = 14 (4.46%)
7	2 (0.64)	8	
8	3 (0.96)	16	VRSA = 4 (1.27%)
9	1 (0.32)	32	

**Table 7.** Antibiotics susceptibility pattern among VRSA, VISA and VSSA isolated from non-urine samples.

Antibiotics	Strains	S (%)	I (%)	R (%)	df	χ <sup>2</sup> Test	P-value
<b>Gentamycin</b>	VSSA (264)	126 (47.73)	49 (18.56)	89 (33.71)	2	3.076	0.215
	VISA (12)	8 (66.67)	0 (0.00)	4 (33.33)			
	VRSA (4)	1 (25)	0 (0.00)	3 (75)			
	Total (280)	135 (48.21)	49 (17.5)	96 (34.29)			
<b>Azithromycin</b>	VSSA (264)	40 (15.15)	15 (5.68)	209 (79.17)	2	1.266	0.531
	VISA (12)	1 (8.33)	0 (0.00)	11 (91.67)			
	VRSA (4)	0 (0.00)	0 (0.00)	4 (100)			
	Total (280)	41 (14.64)	15 (5.36)	224 (80.00)			
<b>Ciprofloxacin</b>	VSSA (264)	11 (4.17)	0 (0.00)	253 (95.83)	1	0.479	0.489
	VISA (12)	1 (8.33)	0 (0.00)	11 (91.67)			
	VRSA (4)	0 (0.00)	0 (0.00)	4 (100)			
	Total (280)	12 (4.29)	0 (0.00)	268 (95.71)			
<b>Clindamycin</b>	VSSA (264)	124 (46.97)	0 (0.00)	140 (53.03)	1	0.858	0.354
	VISA (12)	4 (33.33)	0 (0.00)	8 (66.67)			
	VRSA (4)	0 (0.00)	0 (0.00)	4 (100)			
	Total (280)	128 (45.71)	0 (0.00)	152 (54.29)			
<b>Co-Trimoxazole</b>	VSSA (264)	134 (50.76)	0 (0.00)	130 (49.24)	1	2.702	0.100
	VISA (12)	9 (75)	0 (0.00)	3 (25)			
	VRSA (4)	2 (50)	0 (0.00)	2 (50)			
	Total (280)	145 (51.79)	0 (0.00)	135 (48.21)			
<b>Doxycycline</b>	VSSA (264)	217 (82.20)	0 (0.00)	47 (17.8)	1	17.176	<0.001*
	VISA (12)	4 (33.33)	0 (0.00)	8 (66.67)			
	VRSA (4)	3 (75)	0 (0.00)	1 (25)			
	Total (280)	224 (80.00)	0 (0.00)	56 (20)			
<b>Erythromycin</b>	VSSA (264)	40 (15.15)	26 (9.85)	198 (75.00)	2	1.962	0.375
	VISA (12)	1 (8.33)	0 (0.00)	11 (91.67)			

Antibiotics	Strains	S (%)	I (%)	R (%)	df	X <sup>2</sup> Test	P-value
	VRSA (4)	0 (0.00)	0 (0.00)	4 (100)			
	Total (280)	41 (14.64)	26 (9.29)	213 (76.07)			
Tetracycline	VSSA (264)	217 (82.20)	0 (0.00)	47 (17.80)	1	23.218	<0.001*
	VISA (12)	3 (25)	0 (0.00)	9 (75)			
	VRSA (4)	3 (75)	0 (0.00)	1 (25)			
	Total (280)	223 (79.64)	0 (0.00)	57 (20.36)			
Daptomycin	VSSA (264)	259 (98.11)	0 (0.00)	5 (1.89)	1	10.134	0.001*
	VISA (12)	10 (83.33)	0 (0.00)	2 (16.67)			
	VRSA (4)	3 (75)	0 (0.00)	1 (25)			
	Total (280)	272 (97.14)	0 (0.00)	8 (2.86)			
Rifampicin	VSSA (264)	224 (84.85)	11 (4.17)	29 (10.98)	2	23.874	<0.001*
	VISA (12)	4 (33.33)	1 (8.33)	7 (58.33)			
	VRSA (4)	4 (100)	0 (0.00)	0 (0.00)			
	Total (280)	232 (82.86)	12 (4.29)	36 (12.86)			
Linezolid	VSSA (264)	262 (99.24)	0 (0.00)	2 (0.76)	1	20.340	<0.001*
	VISA (12)	10 (83.33)	0 (0.00)	2 (16.67)			
	VRSA (4)	4(100)	0 (0.00)	0 (0.00)			
	Total (280)	276 (98.57)	0 (0.00)	4 (1.43)			
Minocycline	VSSA (264)	217 (82.20)	0 (0.00)	47 (17.80)	1	23.218	<0.001*
	VISA (12)	3 (25)	0 (0.00)	9 (75)			
	VRSA (4)	3(75)	0 (0.00)	1 (25)			
	Total (280)	223 (79.64)	0 (0.00)	57 (20.36)			
Vancomycin	VSSA (264)	264 (100)	0 (0.00)	0 (0.00)	1	276.00	<0.001*
	VISA (12)	0 (0.00)	12	0 (0.00)			
	VRSA (4)	0 (0.00)	0 (0.00)	4			
	Total (280)	264 (94.29)	12(4.29)	4(1.43)			

S: Sensitive, I: Intermediate, R: Resistant, x<sup>2</sup>: Chi-square test, df: Degree of freedom, \*: Significant ( $P < 0.05$ )

**Table 8.** Antibiotics susceptibility pattern among VRSA, VISA and VSSA isolated from urine samples.

Antibiotics	Strains	S (%)	I (%)	R (%)	df	X <sup>2</sup> Test	P-value
Gentamycin	VSSA (32)	17 (53.12)	5 (15.62)	10 (31.25)	2	1.889	0.389
	VISA (2)	1 (50)	1 (50)	0 (0.00)			
	Total (34)	18 (52.94)	6(17.65)	10 (29.41)			
Ciprofloxacin	VSSA (32)	1 (3.13)	0 (0.00)	31 (96.87)	1	16.485	0.001*
	VISA (2)	0 (0.00)	0 (0.00)	2 (100)			
	Total (34)	1 (2.94)	0 (0.00)	33 (97.06)			
Co-Trimoxazole	VSSA (32)	18 (56.25)	0 (0.00)	14 (43.25)	1	0.030	0.863
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	19 (55.88)	0 (0.00)	15 (44.12)			

Antibiotics	Strains	S (%)	I (%)	R (%)	df	X <sup>2</sup> Test	P-value
Doxycycline	VSSA (32)	22 (68.75)	0 (0.00)	10 (31.25)	1	0.302	0.582
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	23 (67.65)	0 (0.00)	11 (32.35)			
Minocycline	VSSA (32)	22 (68.75)	0 (0.00)	10 (31.25)	1	0.302	0.582
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	23 (67.65)	0 (0.00)	11 (32.35)			
Tetracycline	VSSA (32)	22 (68.75)	0 (0.00)	10 (31.25)	1	0.302	0.582
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	23 (67.65)	0 (0.00)	11 (32.35)			
Linezolid	VSSA (32)	32 (100)	0 (0.00)	0 (0.00)	1	16.485	0.001*
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	34 (100)	0 (0.00)	0 (0.00)			
Daptomycin	VSSA (32)	31 (96.87)	0 (0.00)	1 (3.13)	1	7.471	0.006*
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	32 (94.12)	0 (0.00)	2 (5.88)			
Norfloxacin	VSSA (32)	1 (3.13)	0 (0.00)	31 (96.87)	1	16.485	0.001*
	VISA (2)	0 (0.00)	0 (0.00)	2 (100)			
	Total (34)	1 (2.94)	0 (0.00)	33 (97.06)			
Nitrofurantoin	VSSA (32)	26 (81.25)	3 (9.36)	3 (9.36)	2	3.060	0.217
	VISA (2)	1 (50)	0 (0.00)	1 (50)			
	Total (34)	27 (79.41)	3 (8.82)	4 (11.76)			
Rifampicin	VSSA (32)	21 (65.63)	2 (6.25)	9 (28.13)	2	4.443	0.108
	VISA (2)	0 (0.00)	0 (0.00)	2 (100)			
	Total (34)	21 (61.76)	2 (5.88)	11 (32.35)			
Vancomycin	VSSA (32)	32 (100)	0 (0.00)	0 (0.00)	1	34.000	0.001*
	VISA (2)	0 (0.00)	2 (100)	0 (0.00)			
	Total (34)	32 (94.12)	2 (5.88)	0 (0.00)			

S: Sensitive, I: Intermediate, R: Resistant, x<sup>2</sup>: Chi-square test, df: Degree of freedom, \*: Significant ( $P < 0.05$ )

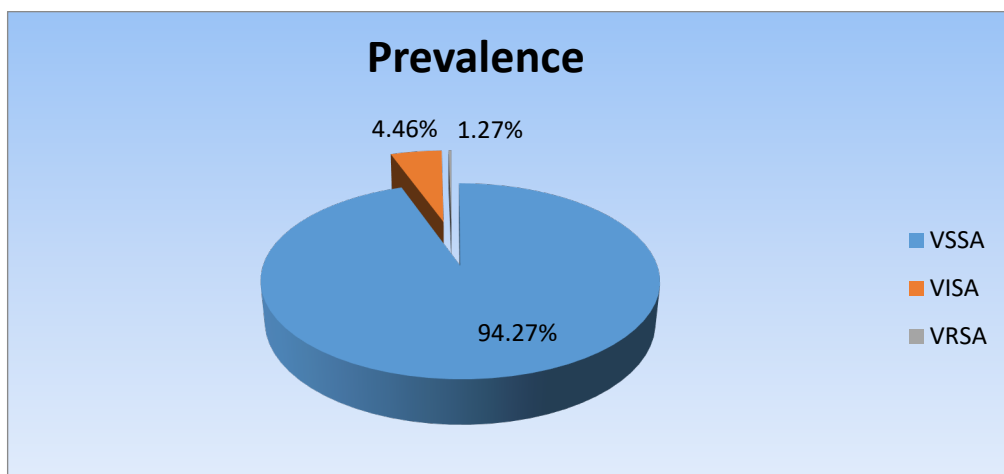
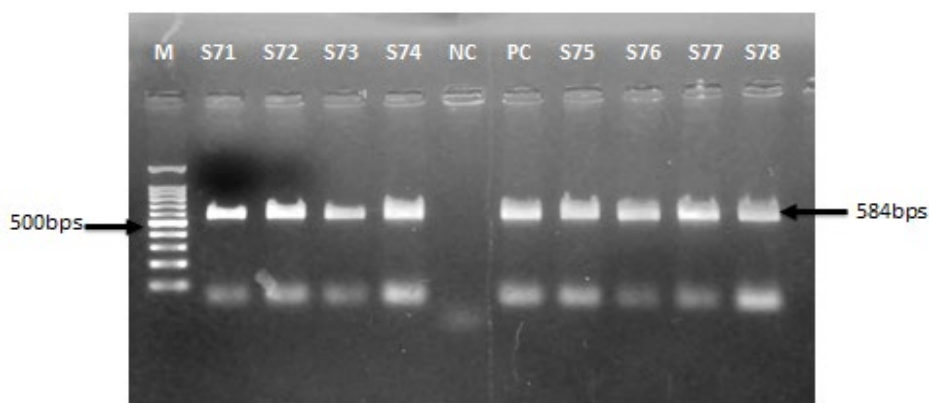
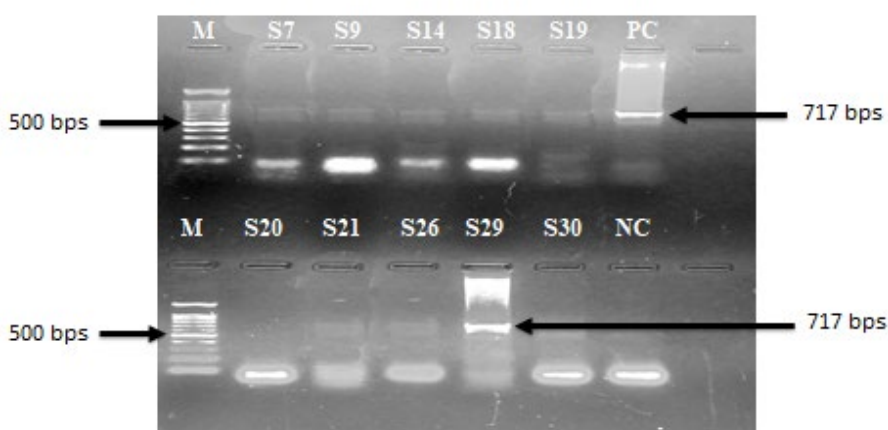


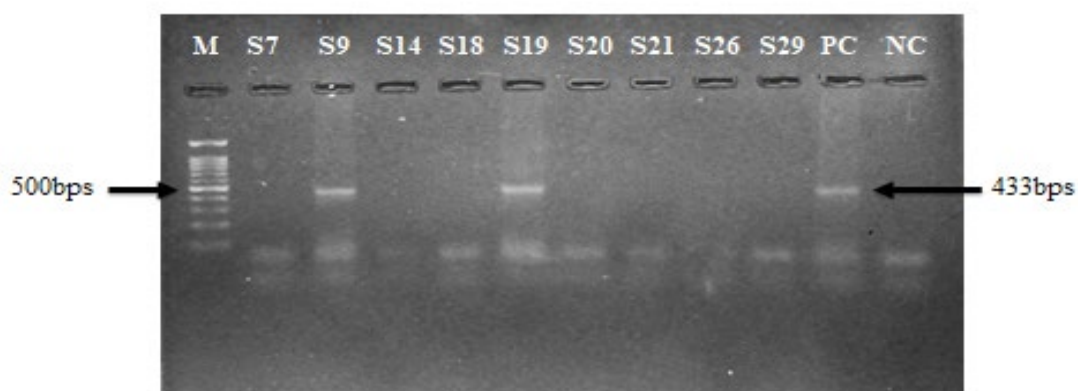
Figure 1. The chart shows the prevalence of VSSA, VISA and VRSA (Prepared by Authors, 2025).



**Figure 2. Representative gel image of the *mecA* gene PCR (584 bp).** Lanes: M, molecular weight marker; PC, positive control (*S. aureus* ATCC 43300); NC, negative control (*S. aureus* ATCC 25923); S71, S72, S73, S74, S75, S76, S77 and S78, MRSA isolates positive for *mecA*. (Prepared by Authors, 2025).



**Figure 3. Representative gel image of the *vanA* gene PCR (717 bp).** Lanes: M, molecular weight marker; PC, positive control (*E. faecium* ATCC 51559); NC, negative control (*S. aureus* ATCC 25923); S29, a VRSA isolate positive for *vanA*; S7, S9, S14, S18, S19, S20, S21, S26 and S30, VISA and VRSA isolates negative for *vanA*. (Prepared by Authors, 2025).



**Figure 4. Representative gel image of the *vanB* gene PCR (433 bp).** Lanes: M, molecular weight marker; PC, positive control (*E. faecalis* ATCC 51299); NC, negative control (*S. aureus* ATCC 25923); S9, S19, VRSA isolates positive for *vanB*; S7, S14, S18, S20, S21, S26 and S29, VISA and VRSA isolates negative for *vanB* (Prepared by Authors, 2025).

## 4. Discussion

The prevalence of MRSA is highly reported and the rise in VISA and VRSA strains are significantly affecting the healthcare setting nowadays. Vancomycin has emerged as the preferred medication for the treatment of MRSA infections due to the growing resistance to methicillin and inefficiency of  $\beta$ -lactam antibiotics (15). This study found that 314 (52.5%) *S. aureus* were classified as MRSA. All the MRSA strains possessed the *mecA* gene. Four (1.27%) VRSA and 14 (4.46%) VISA strains were detected by BMD method.

The indiscriminate use of the antibiotics is considered a significant contributing factor to the development of antibiotics resistance as supported by the study conducted by Ghaderi et al (16). The medical community in India faces a challenge to treat the bacterial infections due to arise and spread of antibiotic resistance in pathogenic bacteria. Although vancomycin resistance has been reported in many parts of the world, the worldwide spread of MRSA strains has increased the significance of managing and treating MRSA infections. Since the genes responsible for the development of vancomycin resistance can spread horizontally among different strains and species, determining how common they are in various populations can yield important therapeutic insights (15).

Thus, this investigation examined resistance to methicillin and vancomycin antibiotics and the related genes (*mecA*, *vanA*, and *vanB*) in *S. aureus* isolated from various clinical samples collected from the TMU hospital.

During the study period, 598 *S. aureus* strains were isolated from different types of clinical specimens in our hospital. The prevalence of MRSA was found to be 52.5% in this study, which is comparable to the findings of Vedavati et al (8) (53%), Anupurba et al (17) (54.85%), and Pradhan et al (18) (57%). However, another research group like Arora et al (19) reported the prevalence of MRSA at 46%.

In the present study, all phenotypically identified MRSA strains possessed the *mecA* gene detected through conventional PCR using a specific primer pair. Several studies agree with this finding and indicate that the *mecA* gene is found in the maximum number of MRSA isolates. Several previous studies listed in Table 9, have reported that the MRSA strains possess the *mecA* gene.

In 1997, Hiramatsu et al (27) reported the first case of VISA from Japan and in June 2002, Sievert et al (28) reported the first case of VRSA from Michigan, USA. Vancomycin resistance in *S. aureus* has grown to be a serious issue and a public health risk. The infections caused by VRSA have been reported few in number

globally, but the infections caused by VISA have been continuously reported from all over the world. Because of this, the study on VISA and VRSA is a topic of intensive research. The current study was created to look into the molecular basis of the phenotypic identified MRSA and vancomycin in our environment.

For determination of vancomycin MIC the BMD method is recommended by CLSI guideline (2024). We found 4 (1.27%) VRSA on the basis of MIC of vancomycin showed in Table 6. Our result was comparable to the findings of previous studies like Ahmed and Chand (29) from Kota, Rajasthan, Kaur et al (30) from Amritsar, Punjab, and Tawfeek et al (26) from Egypt who reported the prevalence of VRSA at 0.52%, 0.5% and 1%, respectively. Other researchers, such as Nashra et al (7) from Kanpur, Uttar Pradesh, India; Kaur et al (31) from Punjab, India; and Moses et al (32) from Hyderabad, India; reported the prevalence of 1.42%, 2.46% and 6.08%, respectively.

However, 14 VISA strains were isolated and showed different MIC of vancomycin as shown in Table 6. In the present study, prevalence of VISA strain was found to be 4.46%, which is comparable to the findings of Nashra et al (7) from Kanpur, Uttar Pradesh, India. Tawfeek et al (26) from Egypt reported the prevalence of VISA at 7%. In other studies, such as Kaur et al (30) from Punjab, India; Ahmed and Chand (29) from Kota, Rajasthan, India; Dhungel et al (6), Nepal et al (4) and Bamigboye et al (34), the prevalence of VISA was reported 1.3%, 1.62%, 8.8%, 10.5% and 15.1%, respectively and some similar studies also mentioned in Table 10. Infections caused by VISA strains are reported to be associated with prolonged infections and vancomycin treatment failure, which leads to inadequate and poor clinical outcomes (26).

In this study VISA and VRSA isolates were tested for *vanA* and *vanB* genes. From four VRSA isolates, 1 (25%) possessed *vanA* and 3 (75%) possessed *vanB* genes and these isolates were resistant to methicillin but 100% sensitive to linezolid and rifampicin and 75% sensitive to daptomycin, minocycline, tetracycline, and doxycycline. Thus, these antibiotics could be used as alternative treatments for MRSA infections. The finding of this study was agreed with Saadat et al (14) who reported the prevalence of *vanA* and *vanB* genes at 34% and 37%, respectively. The supported study conducted by Maharjan et al (1) detected the *vanA* gene in 2 (40%) VRSA isolates and *vanB* gene was not detected in his study. A comparable study conducted by Abdulrazzaq et al (35) reported the prevalence of *vanA* gene at 100% (12/12), and *vanB* gene at 66.66% (8/12) among VRSA isolates.

In this study, 14 MRSA isolates were confirmed as VISA on the basis of MIC determination. The *vanA* and

*vanB* genes were not detected in VISA isolates that indicate *van* genes are not responsible for VISA. The mechanism behind VISA isolates is the fundamental characteristics of the VISA phenotype including increased cell wall thickness, caused by differentially regulated cell wall biosynthesis and stimulatory

pathway, reduced cross-linking of peptidoglycan, decreased autolytic activity of the enzymes responsible to cell-wall turnover, altered surface protein profile, dysfunction of the accessory gene regulator (*agr*) system, and changes to growth characteristics (36).

**Table 9.** Comparative studies of MRSA isolates prevalence.

Studies	MRSA isolates detected through cefoxitin disc diffusion method	MRSA isolates positive for <i>mecA</i> gene	MRSA isolates negative for <i>mecA</i> gene
Dhar et al (20)	246	162(65.85%)	84 (34.14%)
Ramesh et al (21)	41	33 (80.49%)	18 (19.51%)
Jauhari et al (22)	196	164 (83.7%)	32 (16.3%)
Sony et al (23)	112	101 (90.2%)	11 (9.8%)
Elhassan et al (24)	123	111 (90.2%)	12 (9.8%)
Aslanimehr et al (15)	152	144 (94.7%)	8 (5.3%)
Makgotlho et al (25)	97	96 (98.99%)	1 (1.01%)
Tawfeek et al (26)	96	96 (100%)	0 (0%)
Aubaid et al (2)	72	72 (100%)	0 (0%)
Present study	314	314 (100%)	0 (0%)

**Table 10.** Similar studies of VSSA, VISA and VRSA isolates prevalence.

Present study N=314			Thati et al (33) N= 358			Ahmed and Chand (29) N=185		
VRSA	VISA	VSSA	VRSA	VISA	VSSA	VRSA	VISA	VSSA
4 (1.27%)	14 (4.46%)	296 (94.27%)	7 (1.96%)	16 (4.45%)	335 (93.56%)	2 (1.08%)	3 (1.62%)	180 (97.3%)

There are several genes/mutations known to contribute to the development of VISA. Two-component regulatory system such as *graRS* and *walkR*, has been linked to glycopeptide resistance (37). A gene encoding DNA-dependent RNA polymerase  $\beta$ -subunit (*rpoB*) is also commonly associated with increased resistance to vancomycin, prolonged propagation time, and increased cell wall thickness (38). *GraRS* differentially regulates transcription of cell wall biosynthesis genes and has been associated with a broad array of genes and regulators that play a role in the intermediate resistance phenotype. Moreover, *graRS* mutations are linked to modified expression of global regulators, repressor of toxins (*rot*) and *agr*. Altered expression of global gene regulators has a tremendous downstream effect, and thus could play a role in a VISA phenotype. *WalkR* is another two-component gene regulatory system associated with the VISA phenotype (39). A comparable study was conducted by Aslanimehr et al (15) who reported that the *vanA* and *vanB* genes were not detected in four VISA isolates.

Our research validates the presence of *mecA* in MRSA isolates, aligning with findings from other regional studies; however, in Moradabad, U.P. region the presence of *vanA* and *vanB* genes among VRSA strains was significant, as it decreased the sensitivity to vancomycin and similar drugs. The integration of molecular detection for resistance genes facilitates the early identification of high risk isolates and promotes a more informed, gene-guided choice of alternative antibiotics (linezolid and daptomycin), highlighting the clinical importance of regional surveillance.

#### Limitations

In the present study, the authors were unable to detect the *van* operons other than the *vanA* and *vanB* genes among VISA and VRSA. The *mecC* gene detection among MRSA was also a limitation of this study due to time and resource constraints.

Furthermore, the use of *E. faecalis* ATCC 51299 (*vanA* genotype) as a positive control for the *vanB*

PCR, while supported by some literature, may not be ideal, and future studies should employ a confirmed *vanB*-positive control strain.

### Future implications

The presence of *vanA* and *vanB* genes among MRSA strains may signal the emergence of vancomycin-resistant *S. aureus* isolates in this region, which could significantly complicate treatment options. These findings emphasize the need for implementing molecular diagnostics in routine surveillance to enable timely detection and control of resistant strains. Future research could focus on understanding the genetic transfer mechanisms, resistance evaluation, and regional epidemiology to inform targeted antimicrobial stewardship and public health strategies.

## 5. Conclusion

This study revealed the existence of different types of antibiotic resistant strains like MRSA, VISA and the emergence of VRSA by molecular detection of the *mecA*, *vanA*, and *vanB* genes in clinical specimens from a healthcare setting in Moradabad, India. Detection of these genes alarms the spread of vancomycin-resistant genes among clinical *S. aureus* isolates and other bacterial pathogens. Moreover, MRSA infections are treated by vancomycin, but the emergence of vancomycin resistance poses a serious concern for the healthcare system.

## 6. Declarations

### 6.1 Acknowledgment

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and Research Centre, and Dr. Manish Sharma, director of the Pioneer Centre of Biosciences, for their support during the period of this study.

### 6.2 Ethical Considerations

This study was approved by Institutional Ethics Committee of Teerthanker Mahaveer Medical College and Research Center with reference number "TMU/IEC Nov. 23/60".

### 6.3 Authors' Contributions

SA wrote the manuscript. UF supervised the project. All authors read and approved the final manuscript for publication.

### 6.4 Conflict of Interests

The authors declare that they have no conflicts of interest.

### 6.5 Financial Support and Sponsorship

This research was not funded by any public, commercial, or not-for-profit funding agency.

### 6.6 Using Artificial Intelligence Tools (AI Tools)

All authors declare that there is no use of AI Tools in this study, including the writing of this manuscript.

### 6.7 Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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