Original Research Article Iran J Med Microbiol. 2025; 19(5): 345-359

Category: Medical Bacteriology Peer Reviewed | Open Access

10.30699/ijmm.19.5.345

Iranian Journal of Medical Microbiology | ISSN:2345-4342

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

Shane Alam*, Umar Farooq

Department of Microbiology, Teerthanker Mahaveer University, Moradabad, India

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*

Received: 2025/07/27; **Accepted**: 2025/10/28; **Published Online:** 2025/11/11;

Corresponding Information: **Shane Alam**, *Department of Microbiology, Teerthanker Mahaveer University, Moradabad, India* & Email: shanealam5361@gmail.com

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 meticillin-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 1.5×10^8 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 ≤21 mm, then the *S. aureus* was considered MRSA, and if the ZOI was ≥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
mecA	Forward	AGAAGATGGTATGTGGAAGTTAG	584 bp	Azimian et al **(13)**
	Reverse	ATGTATGTGCGATTGTATTGC		
vanA	Forward	CTGGGAAAACGACAATTGCT	717 bp	Nashra et al **(7)**
	Reverse	TGTACAATGCGGCCGTTACG		
vanB	Forward	GTGACAAACCGGAGGCGAGGA	433 bp	Saadat et al **(14)**
	Reverse	CCGCCATCCTCCTGCAAAAAA		

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

Gene		*mecA*	*vanA*	*vanB*
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
mecA	*S. aureus*	Positive	43300
	S. aureus	Negative	25923
vanA	*E. faecium*	Positive	51559
	S. aureus	Negative	25923
vanB	*E. faecalis*	Positive	51299
	S. aureus	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-value≤0.05. (χ2=128.027, df=26, *P*-value≤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-value≤0.05. (χ2=12.576, df=4, *P*-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^2, 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^2= 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^2, 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	VSSA = 296 (94.27%)
2	8 (2.55)	0.25	
3	47 (14.97)	0.5	
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	X^2 Test	*P*-value
Gentamycin	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)			
Azithromycin	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)			
Ciprofloxacin	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)			
Clindamycin	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)			
Co-Trimoxazole	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)			
Doxycycline	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)			
Erythromycin	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^2 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^2: 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^2 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^2 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^2: Chi-square test, df: Degree of freedom, *: Significant (*P*<0.05)

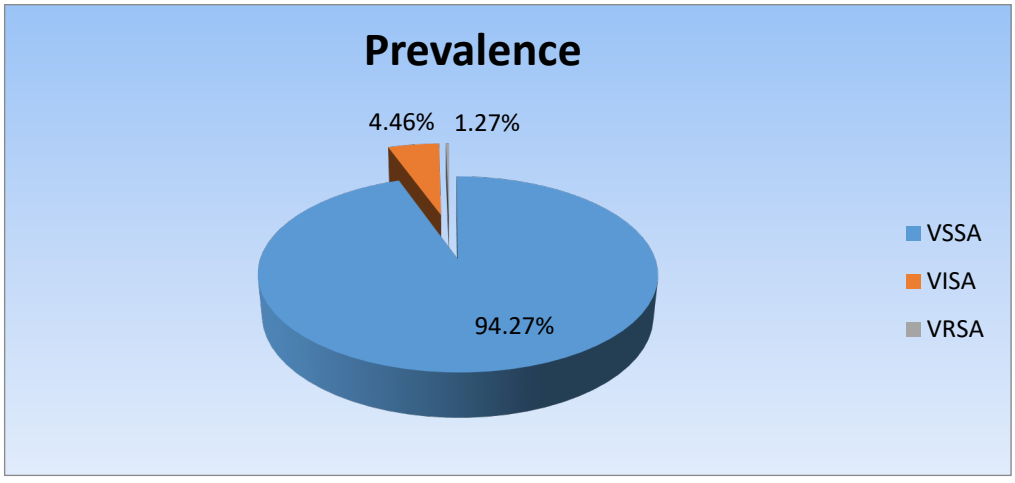

Figure 1. The chart shows the prevalence of VSSA, VISA and VRSA **(Prepared by Authors, 2025)**.

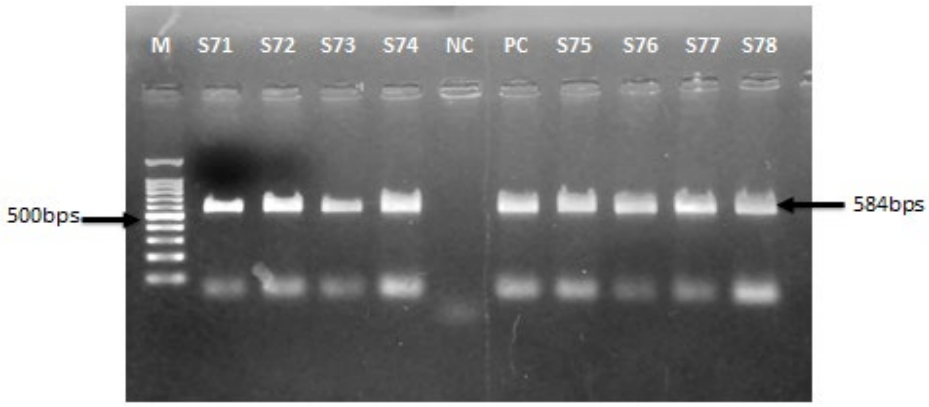

Figure 2. Representative gel image of the *mecA* gene PCR (584 bp). Lanes: M, molecular weight marker; PC, positive control (*S. aureus* ATCC 43300); NC, negative control (*S. aureus* ATCC 25923); S71, S72, S73, S74, S75, S76, S77 and S78, MRSA isolates positive for *mecA*. **(Prepared by Authors, 2025)**.

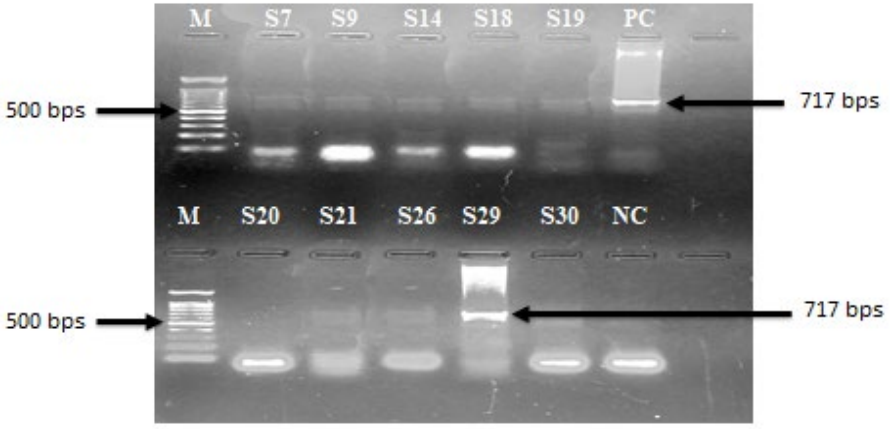

Figure 3. Representative gel image of the *vanA* gene PCR (717 bp). Lanes: M, molecular weight marker; PC, positive control (*E. faecium* ATCC 51559); NC, negative control (*S. aureus ATCC* 25923); S29, a VRSA isolate positive for *vanA*; S7, S9, S14, S18, S19, S20, S21, S26 and S30, VISA and VRSA isolates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

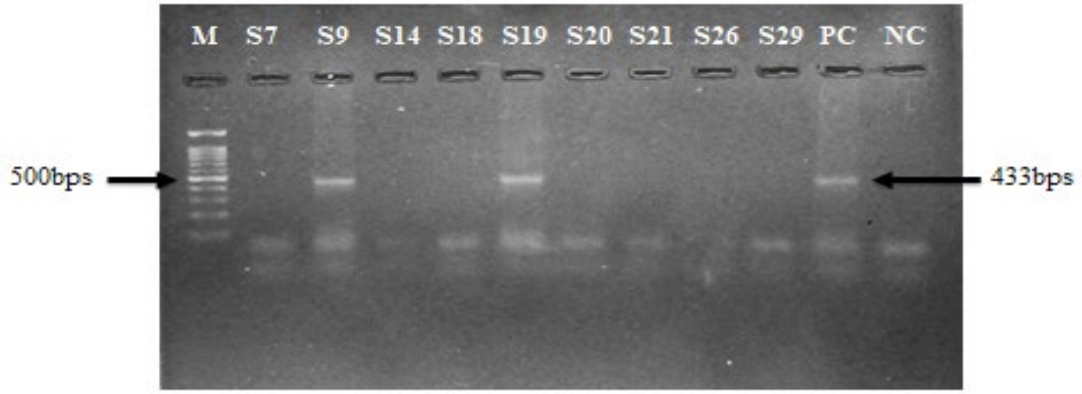

Figure 4. Representative gel image of the *vanB* gene PCR (433 bp). Lanes: M, molecular weight marker; PC, positive control (*E. faecalis* ATCC 51299); NC, negative control *(S. aureus* ATCC 25923); S9, S19, VRSA isolates positive for vanB; S7, S14, S18, S20, S21, S26 and S29, VISA and VRSA isolates 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 β-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 mecA gene	MRSA isolates negative for mecA 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. Antibiotics susceptibility pattern among VRSA, VISA and VSSA isolated from urine samples.

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 β-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

We would like to express our heartfelt thanks to the academic and laboratory staff of the Department of Microbiology, Teerthanker Mahaveer Medical College 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.

References

1. Maharjan M, Sah A K, Pyakurel S, Thapa S, Maharjan S, Adhikari N, et al. Molecular Confirmation of Vancomycin-Resistant Staphylococcus aureus with vanA Gene from a Hospital in Kathmandu. Int J Microbiol. 2021; 2021(1):3847347. [DOI:10.1155/2021/3847347] [PMID] [PMCID]
2. Aubaid AH, Mahdi ZH, Abd-Alraoof TS, Jabbar NM. Detection of mecA, vanA and vanB genes of Staphylococcus aureus Isolated from Patients in Al Muthanna Province Hospitals. Indian J Forensic Med Toxicol. 2020;14(2):1002-8. [DOI:10.37506/ijfmt.v14i2.3037]
3. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, Staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. Antimicrob Agents Chemother. 2000;44(6): 1549-55. [PMID] [PMCID] [DOI:10.1128/AAC.44.6.1549-1555.2000]
4. Nepal N, Mahara P, Subedi S, Rijal KR, Ghimire P, Banjara MR, et al. Genotypically confirmed vancomycin-resistant Staphylococcus aureus with vanB gene among clinical isolates in Kathmandu. Microbiol Insights. 2023;16:

11786361231183675. [PMID] [PMCID] [DOI:10.1177/11786361231183675]

5. Kim JW, Kang GS, Yoo JI, Kim HS, Lee YS, Yu JY, et al. Molecular typing and resistance profiles of vancomycin-intermediate Staphylococcus aureus in Korea: results from a National Surveillance Study, 2007-2013. Ann Clin Microbiol. 2016;19:88-96. [DOI:10.5145/ACM.2016.19.4.88]

6. Dhungel S, Rijal KR, Yadav B, Dhungel B, Adhikari N, Shrestha UT, et al. Methicillin-Resistant Staphylococcus aureus (MRSA): prevalence, antimicrobial susceptibility pattern, and detection of mec a gene among cardiac patients from a tertiary care heart center in Kathmandu, Nepal. Infect Dis Res Treat. 2021;14:11786337211037355. [PMID] [DOI:10.1177/11786337211037355] [PMCID]

7. Nashra A, Sujatha R, Sameer D. Screening and molecular characterization of VISA and VRSA among the MRSA isolates at a tertiary care centre, Kanpur. Int J Health Sci Res. 2019;9(5): 46-54.

8. Vedavati BI, Halesh LH, Koppad M, Premalatha DE, Akshatha YJ. Study of the virulence factors and antimicrobial susceptibility profile of Staphylococcus aureus isolated from clinical samples at tertiary care centre. Trop J Path Microbiol. 2019;5(3):120-5. [DOI:10.17511/jopm.2019.i03.02]

9. Wayne PA. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 34th informational supplement M100, antimicrobial testing standards, M02, M07 and M 11. 2024.

10. Directorate General of Health Services, Ministry of Health and Family Welfare Government of India. Standard Operating Procedure for Antimicrobial Resistance Surveillance National AMR Surveillance Network (NARS-Net). National Programme on Antimicrobial Resistance Containment National Centre for Disease Control, India. 2nd Edition. January 2023. [https://ncdc.mohfw.gov.in/wp-content/uploads/2024/03/5849549352168188 0873.pdf]

11. Sreejisha M, Mulki S S, Shenoy S, Dhanashree B, Chakrapani M, Bhat G. Heterogeneous vancomycin intermediate Staphylococcus aureus infections in diabetic and non-diabetic patients-A hospital-based comparative study. Infect Drug Resist. 2023;16:9-17. [DOI:10.2147/IDR.S393415] [PMID] [PMCID]

12. Qiagen D.N. easy Blood and tissue handbook Available from: [https://www.qiagen.com/mx/resources/ resourcedetail?id=6b09dfb8-6319-464d-996c 79e8c7045a50&lang=en]

13. Azimian A, Havaei S A, Fazeli H, Naderi M, Ghazvini K, Samiee S, et al. Genetic characterization of a vancomycin-resistant Staphylococcus aureus isolate from the respiratory tract of a patient in a university hospital in northeastern Iran J Clin Microbiol. 2012;50(11):3581-5. [PMID] [PMCID] [DOI:10.1128/JCM.01727-12]

14. Saadat S, Solhjoo K, Norooz-Nejad M J, Kazemi A. VanA and vanB positive vancomycin-resistant Staphylococcus aureus among clinical isolates in Shiraz, South of Iran. Oman Med J. 2014;29(5):335-39. [DOI:10.5001/omj.2014.90] [PMID] [PMCID]

15. Aslanimehr M, Attar A, Barikani A. Phenotypic and Molecular Characterization of Vancomycin and Linezolid Resistance in Clinical Isolates of Staphylococcus aureus. Arch Clin Infect Dis. 2024;19(3):1-9. [DOI:10.5812/archid-132652]

16. Ghaderi H, Shiri Malekabad E, Vahidi M, Dadashi A. Evaluation of genotypic and phenotypic biofilm formation by Staphylococcus aureus isolated from clinical samples and their association with antimicrobial resistance. Iran J Med Microbiol. 2020;14(5):441-59. [DOI:10.30699/ijmm.14.5.441]

17. Anupurba S, Sen M R, Nath G, Sharma B M, Gulati A K, Mohapatra T M. Prevalence of methicillin resistant Staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol. 2003;21(1): 49-51.

18. Pradhan P, Rajbhandari P, Nagaraja SB, Shrestha P, Grigoryan R, Satyanarayana S, et al. Prevalence of methicillin-resistant Staphylococcus aureus in a tertiary hospital in Nepal. Public Health Action. 2021;11(1):46-51. [DOI:10.5588/pha.21.0042] [PMID] [PMCID]

19. Arora S, Devi P, Arora U, Devi B. Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care hospital in Northern India. J Lab Physicians. 2010;2(02):78-81. [DOI:10.4103/0974-2727.72154] [PMID] [PMCID]

20. Dhar E, Urs TA, Ramesh PS, Devananda D, Karthik MVSK. Beyond antimicrobial resistance: mec-genes detection as a factor of

epidemiological characterization among methicillin resistant Staphylococcus aureus (MRSA) isolates in a Tertiary Care Hospital, South India. Online J Health Allied Sci. 2024; 23(1):1-6.

21. Ramesh R, Sinha D, Ambica R, Shantala GB. Screening for mecA and mecC Gene Carriage among Clinical Isolates of Methicillin Resistant Staphylococcus aureus at a Tertiary Care Hospital: A Cross-sectional Study. J Clin Diagn Res. 2023;17(5):1-4. [DOI:10.7860/JCDR/2023/62215.17812]
22. Jauhari S, Pal S, Prakash R, Juyal D. Absence of mecA gene in methicillin resistant Staphylococcus aureus: a tertiary care center's perspective. Ann Int Med Dent Res. 2020;6(4): 1-5. [DOI:10.21276/aimdr.2020.6.4.MB1]
23. Sony S, Haneefa S, Suresh M. Comparative evaluation of phenotypic methods to detect methicillin resistance with mecA gene detection by PCR in Staphylococcus aureus. Indian J Microbiol Res. 2024;11(4):316-22. [DOI:10.18231/j.ijmr.2024.054]
24. Elhassan MM, Ozbak HA, Hemeg HA, Elmekki MA, Ahmed LM. Absence of the mecA gene in methicillin resistant Staphylococcus aureus isolated from different clinical specimens in Shendi City, Sudan. Biomed Res Int. 2015; 2015(1):1-5. [DOI:10.1155/2015/895860] [PMID] [PMCID]
25. Makgotlho PE, Kock MM, Hoosen A, Lekalakala R, Omar S, Dove M, et al. Molecular identification and genotyping of MRSA isolates. FEMS Immunol Med Microbiol. 2009;57(2):104-15. [DOI:10.1111/j.1574-695X.2009.00585.x] [PMID]
26. Tawfeek CE, Khattab S, Elmaraghy N, Heiba A A, Nageeb WM. Reduced vancomycin susceptibility in Staphylococcus aureus clinical isolates: a spectrum of less investigated uncertainties. BMC Infect Dis. 2024;24(1):1218. [DOI:10.1186/s12879-024-10047-2] [PMID] [PMCID]
27. Hiramatsu K, Kayayama Y, Matsuo M, Aiba Y, Saito M, Hishinuma T, et al. Vancomycin-intermediate resistance in Staphylococcus aureus. J Glob Antimicrob Resist. 2014;2(4): 213-24. [DOI:10.1016/j.jgar.2014.04.006] [PMID]
28. Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC. Vancomycin-resistant Staphylococcus aureus in the United States, 2002-2006. Clin Infect Dis. 2008;46(5):668-74. [DOI:10.1086/527392] [PMID]
29. Ahmed R, Chand A E. The Study of Antimicrobial Sensitivity Patterns of VRSA, Visa and VSSA Isolates Obtained from Various Clinical Specimens Among MRSA. Eur J Cardiovasc Med. 2024;14:1223-34.
30. Kaur G, Singh K, Oberoi L, Sidhu SK. Prevalence and in vitro susceptibility pattern of MRSA, VRSA, VISA isolates from various clinical samples in tertiary care hospital. Ann Int Med Dent Res. 2022;8(4):1-8. [DOI:10.53339/aimdr.2022.8.4.1]
31. Kaur K, Gill A K, Kaur M. Methicillin resistance, vancomycin intermediate and vancomycin resistance Staphylococcus aureus prevalence in a tertiary care hospital of Punjab, India. NJLM. 2019;8(3):1-3.
32. Moses VK, Kandi V, Rao SKD. Minimum Inhibitory Concentrations of Vancomycin and Daptomycin Against Methicillin-resistant Staphylococcus Aureus Isolated from Various Clinical Specimens: A Study from South India. Cureus. 2020;12(1):e6749. [DOI:10.7759/cureus.6749] [PMID] [PMCID]
33. Thati V, Shivannavar CT, Gaddad SM. Vancomycin resistance among methicillin resistant Staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res. 2011;134(5):704-8. [DOI:10.4103/0971-5916.91001] [PMID] [PMCID]
34. Bamigboye BT, Olowe OA, Taiwo SS. Phenotypic and molecular identification of vancomycin resistance in clinical Staphylococcus aureus isolates in Osogbo, Nigeria. Eur J Microbiol Immunol. 2018;8(1):25-30. [PMID] [PMCID] [DOI:10.1556/1886.2018.00003]
35. AbdulRazzaq AB, Shami AM, Ghaima KK. Detection of vanA and vanB genes Among Vancomycin Resistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. I.J.B. 2022;21(1):19-25.
36. Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, et al. Cell wall thickening is a common feature of vancomycin resistance in Staphylococcus aureus. J Clin Microbiol. 2003; 41(1):5-14. [DOI:10.1128/JCM.41.1.5-14.2003] [PMID] [PMCID]
37. Meehl M, Herbert S, Götz F, Cheung A. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in

Staphylococcus aureus. Antimicrob Agents Chemother. 2007;51(8):2679-89. [DOI:10.1128/AAC.00209-07] [PMID] [PMCID]

38. Matsuo M, Hishinuma T, Katayama Y, Cui L, Kapi M, Hiramatsu K. Mutation of RNA polymerase β subunit (rpoB) promotes hVISA-to-VISA phenotypic conversion of strain Mu3. Antimicrob Agents Chemother. 2011;55(9): 4188-95. [DOI:10.1128/AAC.00398-11] [PMID] [PMCID]

39. McGuinness WA, Malachowa N, DeLeo FR. Vancomycin Resistance in Staphylococcus aureus. Yale J Biol Med. 2017;90(2):269-81. [PMID] [PMCID]