Original Article

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Detection of Heterogeneous Vancomycin Intermediate Resistance in Methicillin- Resistant *Staphylococcus Aureus* Clinical Isolates

Shane Alam, Ph.D. Scholar[1], Umar Farooq, M.D.[2]

[1]Research Scholar, Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Teerthanker Mahaveer University, Moradabad (U.P.) 244001, India.

[2]Professor and Head, Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Teerthanker Mahaveer University, Moradabad (U.P.) 244001, India.

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Abstract:

Objective: To detect methicillin-resistant *Staphylococcus aureus* (MRSA) using the cefoxitin disk diffusion method. The study also determined the minimum inhibitory concentration (MIC) of vancomycin through the broth microdilution (BMD) method and categorized it into vancomycin sensitive-MRSA (VS-MRSA), vancomycin intermediate-MRSA (VI-MRSA), and vancomycin resistance-MRSA (VR-MRSA). All the VS-MRSA strains were screened for heterogeneous vancomycin intermediate-MRSA (hV-MRSA).

Material and Methods: In this cross-sectional study**,** 210 MRSA strains were isolated from the different types of clinical specimens. Vancomycin susceptibility in MRSA isolates was investigated by determining the MIC using the BMD method. We used brain heart infusion screen agar with 4 μg/ml of vancomycin (BHIV4) to screen VS-MRSA strains for hV-MRSA and performed the modified population analysis profile area under the curve (PAP-AUC) method to confirm hV-MRSA.

Results: On the basis of MIC determination of vancomycin, out of the 210 MRSA strains, we found 202 VS-MRSA, 7 VI-MRSA, and 1 VR-MRSA. BHIV4 applied and screened 202 VS-MRSA isolates, and these were confirmed with the PAP-AUC method, which revealed 34 hV-MRSA.

Conclusion: All MRSA strains identified from the different clinical samples had a prevalence of hV-MRSA, 16.83%. Most of the hV-MRSA detected from the VS-MRSA strains showing the MIC of vancomycin on the borderline (2 μg/ml) indicate that the hV-MRSA is a prior stage of development of VI-MRSA.

Keywords: Heterogeneous vancomycin-intermediate *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, Vancomycin-intermediate *Staphylococcus aureus*, Vancomycin-resistant *Staphylococcus aureus*, Vancomycin-susceptible *Staphylococcus aureus*

Contact: Shane Alam, Ph.D. Scholar
Research Scholar, Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Teerthanker Mahaveer University, Moradabad (U.P.) 244001, India.
E-mail: shanealam5361@gmail.com

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly reported in Indian healthcare centers and linked to both hospital-acquired and community-acquired infections[1]. MRSA causes a different types of clinical presentation, which ranges from superficial skin diseases to severe and life effective pervasive diseases like boils, abscesses, impetigo, cell destruction, infections in hair follicles, staphylococcal scalded skin syndrome, wound infections, respiratory tract infections (pneumonia), bloodstream infection (bacteremia), and sepsis; and, it is linked with the high rate of morbidity and mortality worldwide[2]. MRSA can arise due to a number of factors, including immunosuppression, advanced age, diabetes that requires insulin, long hospital stays, indiscriminate use of antibiotics, the existence of catheters and cannulas, and the presence of MRSA in anterior nares, nostrils, and the axilla of patients and healthcare workers[3].

The emergence of MRSA started in the UK. After 2 years of methicillin, it is well known that introduced, *S. aureus* develops resistance against the maximum types of antibiotics used in empirical therapy to target it; due to which, the treatment of MRSA infections can be difficult for clinicians[4]. Glycopeptide antibiotics (vancomycin) are considered the gold standard medication to treat MRSA infections. By forming a compound with the D-alanine-D-alanine terminus of the murein precursor, vancomycin prevents both transpeptidation and transglycosylation, which results in insufficient or ineffective peptidoglycan cross-linking during cell wall formation. Therefore, the cell wall of the organism is weak and incapable of changing osmotic pressures[5]. However, the emergence of decreased sensitivity of vancomycin had been reported in recent years, and vancomycin intermediate-MRSA (VI-MRSA) and heterogeneous vancomycin intermediate-MRSA (hV-MRSA) were first reported in 1997[6]. The strains of *S. aureus* with minimum inhibitory concentration (MIC) of vancomycin 4-8 µg/ml are called VISA, and may be ideally identified through broth and agar dilution techniques[7]. The expression of vancomycin intermediate can be of homogeneous or heterogeneous (hV-MRSA); hV-MRSA is a subpopulation of *S. aureus* strain that has one vancomycin intermediate cell per 10^5 to 10^6 vancomycin susceptible cells. hV-MRSA showing the vancomycin MIC is within the susceptible range (≤2 µg/ml)[1]. It is challenging to detect hV-MRSA using commonly utilized phenotypic laboratory techniques. The disk diffusion techniques and automated technologies often employed in clinical laboratories are not reliable for identifying these strains. These strains have become a cause of treatment failures and persistent infections[8].

The purpose of this study was to identify MRSA causing various infections, focusing on the detection of hV-MRSA isolated from various clinical samples.

Material and Methods

Study setting and design

This cross-sectional study was conducted after receiving approval from the Institutional Ethics Committee from November 2023 to October 2024 in the Department of Microbiology, Teerthanker Mahaveer Medical College and Research Centre, Moradabad. A total of 505 staphylococci were isolated from various clinical specimens: urine, blood, body-fluid respiratory samples (sputum, endotracheal aspirate, and bronchoalveolar lavage (BAL)), and swabs from wounds, ears, and throats received during the study period for investigation. *S. aureus* strains showing zone of inhibition around cefoxitin disk ≤21 mm (Resistant Cefoxitin) were included in the study (screening test for MRSA)[7]. Coagulase-negative *Staphylococcus* was excluded from the study.

Identification and isolation of bacteria

Gram stain, colony characteristics, β-hemolysis, pigment production on nutrient agar, enzymatic tests, such as catalase and coagulase, and fermentation of mannitol are the standard bacteriological procedures used to identify *S. aureus*. MRSA was detected through the Kirby-Bauer disk diffusion method using a cefoxitin disk (30µg) recommended by the clinical laboratory standard institute (CLSI) guidelines (2024). The zone size surrounding the cefoxitin disk ≤21 mm was considered MRSA[7].

Vancomycin MIC determination

The broth microdilution (BMD) method was used for determining the MIC of vancomycin among MRSA isolates. Cation-adjusted Mueller Hinton broth (MHB) with various concentrations (32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.062 µg/ml) of vancomycin (Hi-Media) was prepared. After that, 3-5 well-isolated colonies with the same characteristics were picked from 18-24 hours old culture plates, then inoculated into normal saline and matched its turbidity with 0.5 McFarland standard (1.5×10^8 CFU/ml).

For further dilution, 0.5 of McFarland inoculum into 1:75 times was prepared by mixing 10 µl of inoculum with 740 µl of sterilized MHB medium. To achieve a bacterial concentration of approximately 5×10^4 CFU/well, 25 µl of diluted solution was taken and added to each well in the columns from 1 to 11, which already contained 75 µl solution (50 µl MHB+25 µl vancomycin solution). Within 15 minutes of adding the inoculum to the microtitre plate, it was incubated at 35 ±2 °C for 24 hours in the incubator. Next day, it was observed that the minimum concentration of vancomycin in the microdilution wells, which totally inhibited the growth of *S. aureus* as seen by the naked eye, was called the MIC of vancomycin[9]. Based on the MIC of vancomycin, MRSA was categorized into 3 groups: if the MIC ≤2 µg/ml was considered vancomycin sensitive-MRSA (VS-MRSA), 4 to 8 µg/ml was considered VI-MRSA, and ≥16 µg/ml was considered vancomycin resistance-MRSA (VR-MRSA), according to CLSI guidelines (2024)[7]. *Enterococcus faecalis* ATCC 51299 was used as the vancomycin-resistant control, and *S. aureus* ATCC 25923 was used as the vancomycin-susceptible control[1].

hV-MRSA identification

hV-MRSA screening through the brain heart infusion agar with vancomycin 4 µg/ml BHIV4 screen agar

Screening of hV-MRSA was completed using the BHIV4 screen agar. BHIV4 screen agar was prepared according to the Figure 1.

The turbidity of the prepared bacterial inoculum was compared with the 0.5 McFarland standard. Ten microliters of bacterial inoculum were placed onto the BHIV4 screen agar and allowed to absorb for 10 minutes. After that, the plate was incubated at 35±2 degree Celsius for 48 hours, and the growth of the bacteria was observed. On the BHIV4 screen agar, if at least 2-3 colonies were observed, then the strain was considered hV-MRSA[10]. *S. aureus* ATCC 25923 and *S. aureus* ATCC 700698 were used as negative and positive controls, respectively[1].

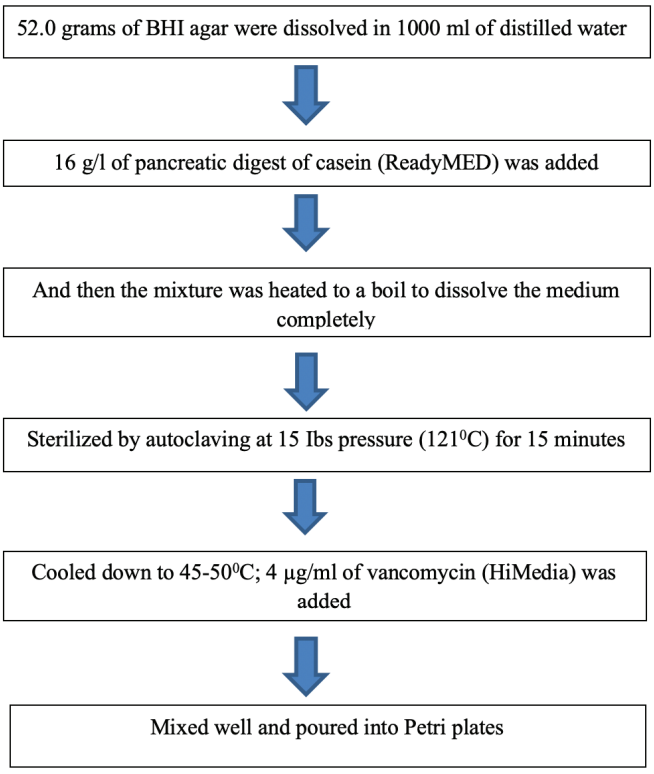
Using the modified population analysis profile-area under the curve (PAP-AUC) method to confirm hV-MRSA

All the VS-MRSA strains were subjected to the confirmation of hV-MRSA using the PAP-AUC technique. Wootton et al.[11] previously described this method in their study. Briefly, in this method, the clinical isolates and ATCC 700698 (Mu3 standard) were subcultured on the nutrient agar and incubated overnight. Two to 3 well-isolated colonies were inoculated into the Brain Heart Infusion (BHI) broth and incubated at 35 °C for 6 hours. The turbidity of the

resulting inoculum was then compared with a 0.5 McFarland standard (1.5×10^8 CFU/ml). Inoculum was again diluted from 10^{-2} to 10^{-6}. Now, the turbidity (bacterial count) of the inoculum was 10^4 CFU/ml used for the inoculation. BHI agar was prepared with different concentrations of vancomycin (16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 µg/ml). The bacterial inoculum was then inoculated onto the surface of the agar and allowed to dry for 10 minutes. The culture plate was incubated at 35 °C for 48 hours. The colonies grew on the culture media and were counted on each vancomycin concentration culture plate; then, the proper dilution factor was used to convert the colony count into the number of CFU/ml. To plot the *S. aureus* colony counts (Log10 CFU/ml) versus the drug concentration (0.125 to 16µg/ml), GraphPad Prism software version 10.Ink (GraphPad Software, USA) was utilized. Area under the curve (AUC) was calculated with the help of the plotted data. The calculated AUC for each strain was divided by the AUC value of the positive control (*S. aureus* ATCC 700698). An isolate with an AUC ratio of ≥0.9 to 1.3 was considered hV–MRSA, while isolates with an AUC ratio <0.9 and >1.3 were considered VS–MRSA and VI–MRSA, respectively[12].

Statistical analysis

The prevalence of MRSA among *S. aureus* and hV–MRSA among VS–MRSA were expressed as a percentage. Results were statistically analyzed using the chi-square test. P-value ≤0.05 was considered statistically significant. Statistical package for the social sciences version 20.0 (IBM Corp., Chicago, Illinois, USA) was used for this purpose.



BHI=brain heart infusion

Figure 1 Preparation of BHIV4 screen agar

Results

A total of 505 staphylococci were isolated from November 2023 to October 2024. Out of them, 392 *S. aureus* were isolated from various clinical samples. These *S. aureus* were further divided into MRSA 210 (53.6%) and MSSA 182 (46.4%). MRSA (210) isolates were distributed according to the source department and clinical specimens; 164 (78.1%) MRSA were isolated from in-patient department patients, and 46 (21.9%) were isolated from out-patient department patients. It was observed that the maximum MRSA strains were isolated from MICU 29 (13.8%), followed by the medicine ward 28 (13.3%), and from the surgery ward 21 (10%). The maximum MRSA strains that were isolated from pus 93 (44.3%), followed by blood, 48 (22.9%), and from the urine specimen, 29 (13.8%) Table 1.

Table 1 Distribution of MRSA strains according to source and clinical specimens

Sources	Clinical specimens										
IPD 164 OPD 46 Department	Pus	Blood	BAL fluid	Urine	Sputum	Tissue	Semen	Foley's tip	Other fluid	High vaginal swab	Total
Medicine	12	3	0	12	1	0	0	0	0	0	28
Surgery	19	1	0	1	0	0	0	0	0	0	21
Paediatrics	2	0	0	2	0	0	0	0	0	0	4
Orthopedics	7	0	0	0	0	0	0	0	0	0	7
Gynecology	4	0	0	3	0	0	0	0	0	4	11
Casualty	0	0	0	0	0	0	0	0	1	0	1
TB & Chest	0	8	4	0	6	0	0	0	1	0	19
Emergency	0	0	0	1	0	0	0	0	0	0	1
Dermatology	1	0	0	0	0	0	0	0	0	0	1
ENT	8	0	0	0	0	0	0	0	0	0	8
FMW	4	1	1	1	0	0	0	0	0	0	7
FOW	2	0	0	0	0	0	0	0	0	0	2
FSW	4	0	0	1	1	0	0	0	0	0	6
HDU	1	0	0	0	0	0	0	4	1	0	6
IVF	0	0	0	0	0	0	2	0	0	0	2
MMW	2	0	0	0	0	0	0	2	1	0	5
MOW	7	0	0	0	0	2	0	0	0	0	9
MSW	4	0	0	0	0	1	0	0	0	0	5
Private	1	0	0	2	0	0	0	0	0	0	3
PSW	1	0	0	0	0	0	0	0	0	0	1
Urology	0	0	0	1	0	0	0	0	0	0	1
MICU	7	16	0	4	1	0	0	1	0	0	29
NICU	1	2	0	0	0	0	0	1	0	0	4
PICU	1	5	0	1	0	0	0	0	2	0	9
RICU	2	5	0	0	2	0	0	1	0	0	10
SICU	3	7	0	0	0	0	0	0	0	0	10
Total	93	48	5	29	11	3	2	9	6	4	210

IPD=in-patient department, OPD=out-patient department, TB=Tuberculosis, ENT=ear, nose and throat, FMW=female medical ward, FOW=female observation ward, FSW=female surgical ward, HDU=high dependency unit, IVF=in vitro fertilization, MMW=male medical ward, MOW=male observation ward, MSW=male surgical ward, PSW=pediatric surgical ward, MICU=medical intensive care unit, NICU=neonatal intensive care unit, PICU=pediatric intensive care unit, RICU=respiratory intensive care unit, SICU=surgical intensive care unit

The maximum MRSA strains isolated from the 41–50 years of age group were 36 (17.1%), followed by 61–70 years, 35 (16.7%), and 21–30 years, 33 (15.7%). In this study, the age of the patients varied from <1 to 86 years of age with a median of 42 years of age. Among all the 210 MRSA strains isolated, 129 (61.4%) were from male patients and 81 (38.6%) were from female patients Table 2.

Table 2 Age and gender-wise MRSA distribution

Age group (Year)	Male (%)	Female (%)	Total
<10	11 (8.5)	01 (1.2)	12
11–20	20 (15.5)	08 (9.9)	28
21–30	18 (13.9)	15 (18.5)	33
31–40	15 (11.6)	14 (17.3)	29
41–50	20 (15.5)	16 (19.8)	36
51–60	17 (13.2)	10 (12.4)	27
61–70	22 (17.1)	13 (16.0)	35
>70	06 (4.7)	04 (4.9)	10
Total	129	81	210

MRSA=methicillin-resistant Staphylococcus aureus

Amongst a total of 210 MRSA isolates, 95 (45.2%) isolates showed MIC 1 µg/ml, which was the maximum isolates, followed by 64 (30.5%) isolates showing MIC of vancomycin 2 µg/ml, and 34 (16.2%) isolates showing MIC of vancomycin 0.5 µg/ml, which was determined using the broth microdilution method. Out of 202 VS-MRSA strains, 34 (16.8%) hV-MRSA were detected through the PAP-AUC method Table 3.

On the basis of MIC of vancomycin determined through the BMD method, 202 VS-MRSA strains were detected and further tested for hV-MRSA status. The PAP-AUC test detected 34 (16.8%) hV-MRSA, while 168 (83.2%) were classified as non-hV-MRSA, whereas BHIV4 tests revealed that 38 (18.8%) VS-MRSA strains were able to grow on the BHIV4 screen agar and 164 (81.2%) strains could not grow, as mentioned in Table 4.

PAP-AUC test was considered the gold standard test for hV-MRSA detection, and the results were compared with the BHIV4 method, and the performance of the BHIV4 test was evaluated in terms of sensitivity, specificity, positive predictive value, and negative predictive value, as shown in Table 5.

The highest number of hV-MRSA isolates were detected in blood samples (17/46), followed by pus (5/86), urine (6 isolates), and Foley's catheter tips (3 isolates). The association between the specimens and hV-MRSA was found to be statistically significant at p-value<0.05 (χ^2=30.681, df=12, p-value=0.002).

VS-MRSA strains were subjected to hV-MRSA detection through the PAP-AUC method. Out of the 34 hV-MRSA detected, 19 hV-MRSA were isolated, which showed an MIC of vancomycin of 2µg/ml, 1 hV-MRSA showed MIC of vancomycin 0.5 µg/ml, and 1hV-MRSA showed MIC of vancomycin 0.25 µg/ml.

Table 3 Distribution of VS-MRSA, VI-MRSA, and VR-MRSA on the basis of MIC and detection of hV-MRSA through the PAP-AUC method among MRSA isolates

S. no.	MRSA strains n=210 (%)	MIC in µg/ml	hV-MRSA detected n=34 (%)	hV-MRSA not detected n=168 (%)	Categories
1	2 (0.9)	0.125	0 (0)	2 (1.2)	VS-MRSA
2	7 (3.3)	0.25	1 (2.9)	6 (3.6)	
3	34 (16.2)	0.5	1 (2.9)	33 (19.6)	
4	95 (45.2)	1	13 (38.2)	82 (48.8)	
5	64 (30.5)	2	19 (55.9)	45 (26.8)	

Table 3 (continued)

S. no.	MRSA strains n=210 (%)	MIC in µg/ml	hV-MRSA detected n=34 (%)	hV-MRSA not detected n=168 (%)	Categories
6	6 (2.9)	4	NA	NA	VI-MRSA
7	1 (0.5)	8	NA	NA	
8	1 (0.5)	32	NA	NA	VR-MRSA

S. no.=serial number, MRSA=methicillin-resistant *Staphylococcus aureus*, MIC=minimum inhibitory concentration, hV-MRSA=heterogeneous vancomycin-intermediate MRSA, VS-MRSA=vancomycin-susceptible MRSA, VI-MRSA=vancomycin-intermediate MRSA, VR-MRSA=vancomycin-resistant MRSA

Table 4 hV-MRSA detection in VS-MRSA strains by brain heart infusion agar with 4 µg/ml of vancomycin test and population analysis profile-area under the curve test

Test	PAP-AUC test (positive n=34)	PAP-AUC test (negative n=168)
BHIV4 (positive)=38	30	8
BHIV4 (negative)=164	4	160

BHIV4=brain heart infusion agar with vancomycin 4 µg/ml, PAP-AUC=population analysis profile-area under the curve

Table 5 Performance of hV-MRSA by BHIV4 screening method

Screening method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BHIV4	88.2	95.2	78.9	97.6

BHIV4=brain heart infusion agar with vancomycin 4 µg/ml, PPV=positive predictive value, NPV=negative predictive value

Table 6 Distribution of hV-MRSA according to the specimen

Specimen	hV-MRSA		Total	χ2, df, p-value
	Detected	Not detected		
Ascitic fluid	0	1	1	30.681, 12, <0.002
BAL fluid	0	5	5	
Blood	17	29	46	
Bone	0	1	1	
CSF	1	1	2	
Foley's tip	3	4	7	
HVS	0	3	3	
Pleural fluid	1	2	3	
Pus	5	86	91	
Semen	0	2	2	

Table 6 (continued)

Specimen	hV-MRSA		Total	$_{x}2$, df, p-value
	Detected	Not detected		
Sputum	1	9	10	30.681, 12, <0.002
Tissue	0	2	2	
Urine	6	23	29	
Total	34	168	202	

df=degree of freedom, hV-MRSA= Heterogeneous Vancomycin-Intermediate MRSA

Table 7 VS-MRSA strains showing MIC and PAP-AUC ratios ≥0.90 to ≤1.3 were defined as hV-MRSA

Patients ID	MIC of Vancomycin	PAP-AUC ratio	Patients ID	MIC of vancomycin	PAP-AUC ratio
3	2	0.99	107	2	1.03
4	2	0.95	109	0.5	0.91
18	2	0.93	132	2	0.92
22	2	1.14	134	1	1.05
24	1	0.91	143	1	1.04
32	2	1.14	146	2	0.92
40	2	0.91	151	1	0.93
41	2	0.96	152	1	1.09
46	1	1.06	168	2	0.91
47	1	0.94	175	2	1.08
62	2	1.1	182	2	0.96
69	1	0.98	187	2	1.04
82	2	1.02	196	1	0.99
84	0.25	1.09	198	1	0.94
91	2	0.91	200	1	0.92
95	2	0.94	204	1	0.96
102	1	0.96	206	2	1.04

MIC=minimum inhibitory concentration, PAP-AUC=population analysis profile-area under the curve

Discussion

There are serious concerns over the future of antimicrobial therapy for *S. aureus* infections due to the bacteria developing resistance to antibiotics. MRSA strains were first introduced just 2 years after methicillin was used for the treatment of *S. aureus* infections. Vancomycin was introduced around 20 years later in the 1980s, as an alternative and potent anti-staphylococcal antibiotic. Vancomycin was considered the gold standard antibiotic for treating MRSA infections until the first case of VI-MRSA was reported in Japan in 1997[13].

In the present study, 392 *S. aureus* were isolated from various clinical samples collected from TMU Hospital during the study period. Out of 392 *S. aureus,* 182 *S. aureus* were MSSA and 210 were MRSA, with a prevalence of 53.6%. Our results were similar to the findings of Rana-

Khara R et al.[14], who reported an MRSA prevalence of 52% from Vadodara District, Gujarat, India. Similarly, Raina D et al.[15] found an MRSA prevalence of 58% from Dehradun, Uttarakhand. However, Kaur K, et al.[16] found an MRSA prevalence of 51.2% from Punjab, India, and Mandal M et al.[17] reported the prevalence of MRSA to be 29.6% from Bihar, India.

S. aureus causes a variety of clinical manifestations, and the sample collection depends on the site of infection. Therefore, various clinical samples were collected and processed in the laboratory in order to isolate *S. aureus.* In this study, the highest number of MRSA was isolated from the pus samples, 93 (44.3%) out of the 210 total MRSA strains. Similar findings were also reported by other researchers, including Sapkota J et al.[18], Kaur K et al.[16], and Bhatt MP et al.[19], who isolated MRSA strains of 55.6%, 59.2%, and 68%, respectively, from the pus samples. The second most frequent MRSA strains were isolated from blood samples, 48 (22.9%), followed by urine, 29 (13.8%), and sputum, 11 (5.2%).

hV-MRSA is a subpopulation of *S. aureus* that becomes a major problem to the clinician due to the susceptible cells that are killed after vancomycin therapy, but a few cells (vancomycin intermediate) don't respond to vancomycin therapy. This type of cell flourishes and increases in number, which causes treatment failure and prolonged hospital stay of the patient, and increases the cost of the treatment. The prevalence of hV-MRSA isolates was 16.8 % in this study. Our results were similar to the findings of Amberpet R et al.[20], who reported that the prevalence of hV-MRSA was 13.2% in Pondicherry, India. Other researchers, such as Singh A et al.[21] found hV-MRSA prevalence was 12.6% in Lucknow, India. However, in another study by Chaudhari CN et al.[22] and Sreejisha M et al.[1], they reported that the prevalence of hV-MRSA was 8.6% and 6.4%, respectively.

Numerous studies have found that the isolation rate of hV-MRSA is greater in VS-MRSA strains, which had an MIC of vancomycin closer to the breakpoint of 2 µg/ml[20,23]. This supports our study, as 94.2% of hV-MRSA had an MIC within the range of 1-2 µg/ml compared to 5.8%, where the MIC was <1 µg/ml, as mentioned in Table 3.

In the present study, it was found that 35.29% of hV-MRSA-infected patients were on vancomycin therapy and had an average hospital stay of 10 days before hV-MRSA isolation. It was not required that all the hV-MRSA-infected patients should have been on prolonged vancomycin exposure. Successful horizontal transmission of hV-MRSA among hospitalized patients was demonstrated. In this study, all the hV-MRSA-infected patients, for whom treatment and outcome details were available, survived. The majority of bacteraemic patients were treated with linezolid, in addition to vancomycin, which could have contributed to a better outcome. Vancomycin treatment failure was substantially higher in hV-MRSA-infected patients than in non-hV-MRSA-infected patients. However, in some other studies, no increase in mortality was observed in the hV-MRSA-infected patients who were treated with linezolid[20,24].

The result of this investigation demonstrated that hV-MRSA prevalence has increased globally in recent years. According to 4 investigations conducted in India, the prevalence of hV-MRSA ranged from 6.4% to 13.2%[1,20-22]. The variation in the prevalence of hV-MRSA could be due to the geographical area where the study was carried out, the patient population, and the sample size. However, the rise in the hV-MRSA prevalence rate is cause for concern. Moreover, since hV-MRSA is considered to be the prior stage of VI-MRSA, an increase in the prevalence rate of VI-MRSA may be expected in the future.

The first case of VISA was reported in 1997 by Hiramatsu K, et al.[6] in a 4-month-old infant from Japan, whereas in 2002, the first case of VRSA was found in

Michigan, USA. In India, the VI-MRSA isolates were found frequently, while VR-MRSA isolates were reported to be few in number. BMD using the agar dilution method is the standard method for detection of MIC of vancomycin, recommended by CLSI guidelines (2024). In this study, the MIC of vancomycin was determined through the BMD method, and the prevalence of VI-MRSA was reported to be 3.3%. A similar result of 2.7% was reported by Huang SH et al.[25] from Taiwan. The study conducted by Tawfeek CE et al.[12] from Suez Canal University, Islamia, Egypt, and Song KH et al.[26] from South Korea reported prevalence of VI-MRSA 7.3% and 8.5%, respectively, which is in concordance with our study.

In this study, only 1 isolate was found from VR-MRSA out of the 210 MRSA isolates, and the prevalence of VR-MRSA was found to be 0.5%. Tawfeek CE et al.[12] found one VR-MRSA isolate from 96 isolates of MRSA, and the prevalence was 1.0%, which is similar to our result. Other researchers, like as Kejela et al.[27], reported a VR-MRSA prevalence of 7.9%.

Conclusion

In this study, we observed that the reduced susceptibility of vancomycin may emerge due to the hV-MRSA phenotype, which is not routinely tested in the clinical laboratory for vancomycin susceptibility. It is unfortunately said that hV-MRSA is responsible for vancomycin treatment failure, prudent prescription of vancomycin can help prevent treatment failures in the future. Although the prevalence of hV-MRSA among MRSA isolated from various clinical samples was 16.8%, the maximum isolation rate of hV-MRSA from the blood samples indicates that the *S. aureus* strains causing bacteremia were not responding to vancomycin therapy, which may lead to a prolonged hospital stay for patients. Most of the hV-MRSA detected from the *S. aureus* strains showing the MIC of vancomycin on the borderline (2 µg/ml) indicates that the hV-MRSA is a prior stage of development of VI-MRSA.

Limitation

The limitation of this study is the absence of molecular confirmation for hV-MRSA, which would have strengthened the validity of the findings.

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Conflict of interest

The author declared that there are no conflicts of interest.

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