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CLINICAL & BASIC RESEARCH

Molecular and Clinical Features of Heterogeneous Vancomycin-Intermediate *Staphylococcus aureus* in Tertiary Care Hospitals in South India

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abstract: ***Objectives:*** This study aimed to detect heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) among methicillin-resistant *S. aureus* (MRSA) isolated from healthcare-associated infections and identify staphylococcal cassette chromosome *mec* (SCC*mec*) types. ***Methods:*** This study was conducted from February 2019 to March 2020 and included patients admitted in 4 tertiary care hospitals in Karnataka, India. Isolation and identification of MRSA were done using standard bacteriological methods. Antimicrobial susceptibility testing was done using Kirby–Bauer disc diffusion; macrolide-lincosamide-streptogramin B phenotypes were identified using the D test. The minimum inhibitory concentration (MIC) of vancomycin was determined using agar dilution. hVISA were confirmed by the modified population analysis profile-area under the curve test. SCC*mec* types and the Panton-Valentine leukocidin (*pvl*) gene were detected using multiplex polymerase chain reaction. ***Results:*** Of 220 MRSA stains, 14 (6.4%) were hVISA. None of the MRSA isolates was vancomycin-intermediate or -resistant and all hVISA were susceptible to linezolid and teicoplanin. The macrolide-streptogramin B phenotype was present in 42.9% of hVISA; 92.9% of the hVISA strains had vancomycin MIC in the range of 1–2 μg/mL. Majority of the hVISA and vancomycin-susceptible MRSA were isolated from patients with skin and soft tissue infections. SCC*mec* III and IV were present in 50% and 35.7% of hVISA, respectively; 14.3% of the hVISA harboured SCC*mec* V. ***Conclusion:*** The prevalence rate of hVISA among MRSA was 6.4%. Therefore, MRSA strains should be tested for hVISA before starting vancomycin treatment. None of the isolates was vancomycin-intermediate or -resistant and all the hVISA strains were susceptible to linezolid and teicoplanin. The majority of the hVISA were isolated from patients with skin and soft tissue infections and harboured SCC*mec* III and IV.

Keywords: MRSA; Hospital Infection; Molecular Typing; Vancomycin; India.

Advances in Knowledge

- *This study showed a high frequency of staphylococcal cassette chromosome mec types III and IV among heterogeneous vancomycin-intermediate Staphylococcus aureus (hVISA).*
- *To the best of the authors' knowledge, this is the first report of hVISA infections in tertiary care hospitals of coastal Karnataka, South India.*

Applications to Patient Care

- *Methicillin-resistant S. aureus isolated from clinical specimens should be tested for the presence of hVISA before starting vancomycin treatment.*
- *All hVISA strains are susceptible to linezolid and teicoplanin.*

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be an important pathogen that causes a variety of healthcare- and community-associated infections.[1] Although, after its introduction, vancomycin became the drug of choice for severe MRSA infections, the emergence of organisms with reduced susceptibility or complete resistance to vancomycin has been a challenge in the treatment of such cases.[2] MRSA with reduced susceptibility to vancomycin include heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus* (VISA), both first reported in Japan in 1997.[3] The Clinical and Laboratory Standards Institute (CLSI) defines VISA as *S. aureus* with a vancomycin minimum inhibitory concentration (MIC) of 4–8 μg/mL.[4] hVISA shows a vancomycin MIC in the susceptible range (≤2 μg/mL) but contains a subpopulation at a rate of 10^{-5} to 10^{-6} with vancomycin MIC in the intermediate range (4–8 μg/mL).[5] The prevalence of hVISA and VISA has increased worldwide from 4.68% and 2.05%, respectively, in 2006 to 7.01% and 7.93%, respectively, in 2014.[6] A recent study in South India showed the prevalence of hVISA to be at 12.4%.[7]

Mutations of genes associated with cell wall thickening, slow growth and reduced autolysis are

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believed to be responsible for the reduced susceptibility of hVISA/VISA phenotypes to vancomycin.[8] Mutations in the *wal*KR (sensor protein kinase/regulator), *gra*SR (glycopeptide resistance–associated sensor/regulator) and *vra*SR (vancomycin resistance associated sensor/regulator) genes are considered important as well.[2,9,10] Prolonged exposure to vancomycin could induce these mutations.[11]

Vancomycin therapy has been shown to be ineffective in infections caused by hVISA.[2] Therefore, the detection of hVISA in clinical specimens before starting vancomycin treatment is essential. The detection of hVISA among MRSA is a challenge for clinical microbiologists because it exhibits vancomycin MIC within the susceptible range.[2,5] Antimicrobial susceptibility tests such as Kirby–Bauer disk diffusion, broth dilution, agar dilution and automated methods fail to detect hVISA, and screening tests such as the macro E-test (MET), vancomycin screen agar and glycopeptide resistance detection E-test vary in their sensitivity and specificity.[5,10,12] The population analysis profile-area under the curve (PAP-AUC) method, which is considered to be the reference method, is labour intensive, expensive and inappropriate for routine clinical microbiology laboratories.[12]

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is used to understand the epidemiology of MRSA infections. Healthcare-associated MRSA (HA-MRSA) normally harbours SCC*mec* I, II and III, whereas, community-associated MRSA (CA-MRSA) harbours SCC*mec* IV, V and the *Panton-Valentine leukocidin* (*pvl*) gene.[1,13] *pvl* is an important virulence factor in CA-MRSA.[13] Several recent studies have reported an overlapping of SCC*mec* types between HA-MRSA and CA-MRSA, and studies conducted in Europe, USA, Australia and Japan have shown the presence of SCC*mec* II, III and IV among hVISA.[6,14,15] However, reports from India have shown the predominance of SCC*mec* V among hVISA.[10,16] Therefore, there are differences in the SCC*mec* types harboured by MRSA in different parts of the world. The current study aimed to determine the prevalence rate of hVISA among MRSA isolated from healthcare-associated infections (HAIs) and identify the SCC*mec* types present in these strains.

Methods

This cross-sectional study was conducted on non-repetitive MRSA strains isolated from patients admitted in 4 tertiary care hospitals attached to a private medical college in coastal Karnataka, India; it was conducted from February 2019 to March 2020. HAIs were identified using the Center for Disease Control and Prevention's (CDC) guidelines.[17] The results were interpreted according to CLSI guidelines.[4]

Isolation and identification of *S. aureus* was done using standard bacteriological methods.[18] Methicillin resistance was detected using the cefoxitin (30 µg) disk diffusion method and confirmed by detecting the *mecA* gene using polymerase chain reaction (PCR).[4,19] *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively. Antimicrobial susceptibility testing was done using Kirby–Bauer disk diffusion. The following antibiotics (BD BBL™ Sensi-Disc™ antimicrobial susceptibility test disks, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) were used: ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), rifampicin (5 µg), teicoplanin (30 µg) tetracycline (30 µg) and trimethoprim-sulphamethoxazole (1.25µg/ 23.75 µg).

Macrolide-lincosamide-streptogramin B (MLSB) was identified using the D test.[4] Mueller-Hinton agar (MHA) plates (HiMedia Laboratories, Mumbai, India) were lawn-cultured with test bacterial inoculum having a turbidity matching the McFarland 0.5 standard (bacterial count 1.5×10^8 CFU/mL). Clindamycin (2 µg) and erythromycin (15 µg) disks were placed at a distance of 15 mm edge-to-edge on the inoculated plates, which were then incubated at 35°C for 16–18 hours.

The MIC of vancomycin for MRSA was determined using the agar dilution method.[4] MHA agar plates with different vancomycin (Sigma-Aldrich Corporation, St. Louis, USA) concentrations (32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/mL) were prepared. A total of 2–3 colonies of the test organism grown on blood agar plates were inoculated into the Mueller-Hinton broth (HiMedia laboratories) and incubated at 37°C for 4 to 6 hours until the turbidity matched that of the McFarland 0.5 standard. The broth culture was diluted 10^{-1} to prepare the working inoculum (1.5×10^7 CFU/mL), 2 µL of which was spot-inoculated on each plate, and the plates were incubated at 35°C for 24 hours and observed for growth. The minimum concentration of vancomycin required to inhibit bacterial growth was referred to as the MIC. MRSA isolates with a vancomycin MIC of ≤2 µg/mL, 4–8 µg/mL and ≥16 µg/mL were considered to be vancomycin-susceptible *S. aureus*, VISA and vancomycin-resistant *S. aureus* (VRSA), respectively.[4] *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were used as vancomycin-susceptible controls. *E. faecalis* ATCC 51299 was used as the vancomycin-resistant control.

Screening the MRSA for hVISA was done using the brain-heart infusion agar (BHIA) (HiMedia Laboratories), which contains 16 g/L of pancreatic

digestion of casein and 4 µg/mL of vancomycin.[12] The test organisms were grown in the brain-heart infusion broth till the turbidity matched with the McFarland 0.5 and 2.0 standards. Four 10 µL-drops of each suspension were spot-inoculated on BHI screen agar plates and allowed to dry for 10 minutes. The plates were then incubated at 35°C for 48 hours and observed for bacterial growth. An isolate was considered hVISA if at least one drop had 2 or more colonies.[12] *S. aureus* ATCC 700698 (Mu3 strain of hVISA) and *S. aureus* ATCC 29213 were used as the positive and negative controls, respectively.

Confirmation of hVISA was done using the PAP-AUC method.[20] Briefly, the test and control (Mu3) were grown at 35°C for 4–6 hours in the brain-heart infusion broth, and the turbidity matched with the McFarland 0.5 standard (1.5 × 10^8 CFU/mL). The broth culture was further diluted by 10^{-4} to achieve a viable bacterial count of 10^4 CFU/mL and then used for inoculation.[5] A 10 µL-bacterial inoculum was spread on BHIA plates with vancomycin at various concentrations (16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/mL). The inoculated plates were incubated at 35°C for 48 hours and the colonies were counted. The log10 number of colonies was plotted against the vancomycin concentrations and the area under the curve (AUC) was determined using the GraphPad Prism software Version 9.0 (Graphpad Software USA).[20] AUC_{test}/AUC_{Mu3} ratio was calculated and used for the confirmation of hVISA. MRSA strains with an AUC_{test}/AUC_{Mu3} ratio of 0.9–1.3 were considered to be hVISA and strains with an AUC ratio >1.3 were considered to be VISA [Figure 1].[5] Mu3 strain of hVISA (*S. aureus* ATCC 700698) and *S. aureus* ATCC 29213 (VSSA) were used as positive and negative controls, respectively.

SCC*mec* types I–V and *pvl* in the test organisms were identified using multiplex PCR with specific primers and controls.[19,21] DNA was extracted using the Qiagen DNA extraction kit (QIAGEN, Hilden, Germany) as per manufacturer's instructions. The principle of the multiplex PCR performed in this study was based on a previous study by Zhang *et al.*[19] A Multiplex PCR kit (QIAGEN) was purchased. The primers used for the molecular detection and characterisation of HA-MRSA isolates are listed in Supplementary Table 1.

A 50 µL PCR mixture containing 25 µL of multiplex master mix (which contains Taq DNA polymerase, dNTPs and 1X Qiagen Multiplex PCR buffer with 6 mM $MgCl_2$), 5 µL of 10X primer mix, 15 µL of water and 5 µL of DNA extract was prepared in 0.2 mL PCR tubes. Multiplex PCR was performed for one cycle of initial denaturation at 97°C for 5 minutes, followed by 30 cycles lasting 30 seconds at 94°C, 30 seconds at 54°C and 90 seconds at 72°C, with a final extension of 10 minutes at 72°C. The amplicons were analysed using 2% agarose gel electrophoresis in 1X Tris-Acetate EDTA buffer. The electrophoresis was performed at 120 V for 90 minutes, and the gel was stained with ethidium bromide staining solution for

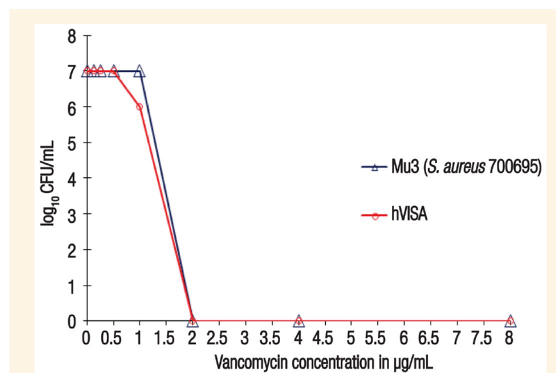

Figure 1: Confirmation of heterogeneous vancomycin-intermediate *Staphylococcus aureus* using the modified population analysis profile-area under the curve method.

CFU = colony forming unit; hVISA = heterogeneous vancomycin-intermediate Staphylococcus aureus.

Mu3-hVISA reference strain (S. aureus ATCC 700698).

AUCtest = 9.750; AUCMu3 = 10.50; AUCtest/AUCMu3 ratio = 0.93 (hVISA).

Table 1: Isolation of heterogeneous vancomycin-intermediate *Staphylococcus aureus* and vancomycin-susceptible methicillin-resistant *Staphylococcus aureus*

Type of infection (n)	n (%)		*P* value
	hVISA (n =14)	Vancomycin-susceptible MRSA (n =206)	
Surgical site infection (87)	4 (28.6)	83 (40.3)	0.385
Wound infection (63)	3 (21.4)	60 (29.1)	0.762
Bacteraemia (25)	3 (21.4)	22 (10.7)	0.220
Abscess (18)	1 (7.1)	17 (8.3)	0.883
Cellulitis (6)	1 (7.1)	5 (2.4)	0.295
Osteomyelitis (6)	0 (0.0)	6 (2.9)	0.517
Carbuncle (5)	0 (0.0)	5 (2.4)	0.555
Gangrene (3)	1 (7.1)	2 (1.0)	0.054
Septic arthritis (2)	0 (0.0)	2 (1.0)	0.711
Umbilical site infection (2)	0 (0.0)	2 (1.0)	0.711
Necrotising fascitis (2)	0 (0.0)	2 (1.0)	0.711
Sepsis (1)	1 (7.1)	0 (0.0)	0.064

hVISA = heterogeneous vancomycin-intermediate Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus.

Table 2: Antimicrobial resistance profile of heterogeneous vancomycin-intermediate *Staphylococcus aureus* and vancomycin-susceptible methicillin-resistant *Staphylococcus aureus*

Antimicrobial agents	Resistant n (%)		*P* value
	hVISA (n =14)	Vancomycin-susceptible MRSA (n = 206)	
Ciprofloxacin	14 (100.0)	179 (86.9)	0.227
Clindamycin	3 (21.4)	32 (15.5)	0.472
Erythromycin	13 (92.9)	173 (84.0)	0.701
Gentamicin	8 (57.1)	102 (49.5)	0.784
Linezolid	0 (0.0)	0 (0.0)	-
Rifampicin	6 (42.9)	11 (5.3)	<0.001
Teicoplanin	0 (0.0)	0 (0.0)	-
Tetracycline	5 (35.7)	63 (30.6)	0.767
Trimethoprim-sulphamethoxazole	4 (28.6)	101 (49.0)	0.172
MLS_B phenotypes			
$iMLS_B$	4 (28.6)	59 (28.6)	1.000
$cMLS_B$	3 (21.4)	32 (15.5)	0.472
MS_B	6 (42.9)	82 (39.8)	1.000

hVISA = heterogeneous vancomycin intermediate Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus; MLS_B = macrolide lincosamide streptogramins B; $iMLS_B$ = inducible clindamycin resistance; $cMLS_B$ = constitutive clindamycin resistance; MS_B = macrolide streptogramins B.

Table 3: Minimum inhibitory concentration of vancomycin to heterogeneous vancomycin-intermediate *Staphylococcus aureus* and vancomycin-susceptible methicillin-resistant *Staphylococcus aureus*

Vancomycin MIC in µg/mL	n (%)	
	hVISA (n = 14)	Vancomycin-susceptible MRSA (n = 206)
0.125	0 (0.0)	0 (0.0)
0.25	0 (0.0)	5 (2.4)
0.5	1 (7.1)	55 (26.7)
1	8 (57.1)	93 (45.1)
2	5 (35.7)	53 (25.7)
4	0 (0.0)	0 (0.0)
8	0 (0.0)	0 (0.0)
16	0 (0.0)	0 (0.0)
32	0 (0.0)	0 (0.0)
aMIC50 in µg/mL	1	1
bMIC90 in µg/mL	2	2

MIC = minimum inhibitory concentration; hVISA = heterogeneous vancomycin intermediate Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus; aMIC50 = MIC value at which growth was inhibited in 50% of isolates; bMIC90 = MIC value at which growth was inhibited in 90% of isolates.

Table 4: Staphylococcal cassette chromosome *mec* types of vancomycin to heterogeneous vancomycin-intermediate *Staphylococcus aureus* and vancomycin-susceptible methicillin-resistant *Staphylococcus aureus*

SCC*mec* types	n (%)		*P* value
	hVISA (n = 14)	Vancomycin-susceptible MRSA (n = 206)	
SCC*mec* I	0 (0.0)	0 (0.0)	-
SCC*mec* II	0 (0.0)	3 (1.5)	0.649
SCC*mec* III	7 (50.0)	73 (35.4)	0.389
SCC*mec* IVa	4 (28.6)	47 (22.8)	0.621
SCC*mec* IVb	0 (0.0)	0 (0.0)	-
SCC*mec* IVc	0 (0.0)	12 (5.8)	0.353
SCC*mec* IVd	1 (7.1)	20 (9.7)	0.752
SCC*mec* V	2 (14.3)	37 (18.0)	0.727

SCCmec = staphylococcal cassette chromosome mec; hVISA = heterogeneous vancomycin intermediate Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus.

30 minutes and then visualised under an ultraviolet illuminator. The size of the bands was compared with the 100 base pair DNA ladder (Bangalore Genei Private Limited, Bengaluru, India).

Sensitivity and specificity analyses were performed to evaluate the performance of the vancomycin agar screen. The data were analysed using the Statistical Package for the Social Sciences (SPSS) Version 29.0 (IBM Corp., Armonk, New York, USA). The prevalence rate of hVISA among MRSA is expressed in percentage, and the results were analysed using Fisher's exact test. A *P* value of ≤0.05 was considered statistically significant.

This study was approved by the institutional ethics committee of Kasturba Medical College, Mangalore (IEC KMC MLR 03-19/75). The isolates for the current study were obtained from clinical specimens brought to the laboratory for investigation. The samples were anonymised and the patients' details were not disclosed. Therefore, informed consent was not required for the present study.

Results

Of the 220 non-repetitive strains of MRSA isolated form HAIs, 14 (6.4%) were confirmed to be hVISA by PAP-AUC, and the remaining 206 (93.6%) were vancomycin susceptible. Vancomycin screen agar, using both McFarland 0.5 and 2.0 standard inoculum densities, detected hVISA in 21 (9.5%) MRSA isolates, including the 14 isolates confirmed by PAP-AUC. The sensitivity and specificity of the screening method were 100% and 96.6%, respectively. However, the

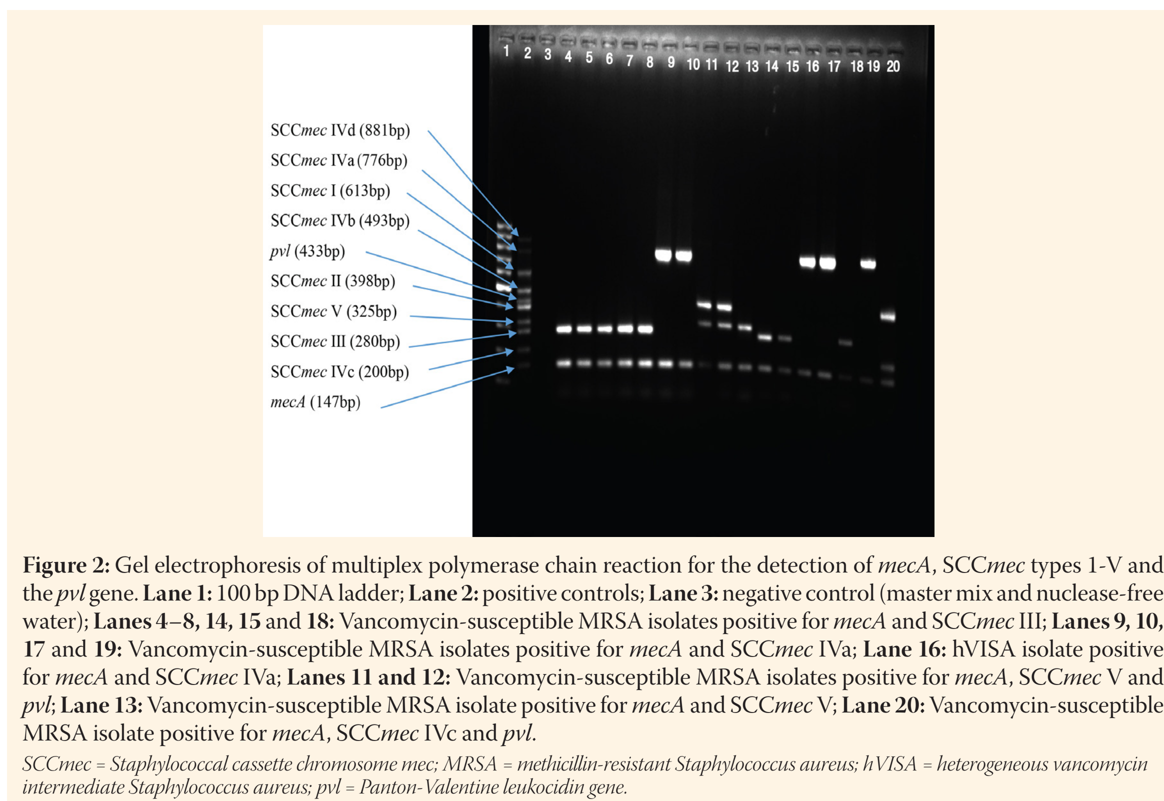

Figure 2: Gel electrophoresis of multiplex polymerase chain reaction for the detection of *mecA*, SCC*mec* types 1-V and the *pvl* gene. **Lane 1:** 100 bp DNA ladder; **Lane 2:** positive controls; **Lane 3:** negative control (master mix and nuclease-free water); **Lanes 4–8, 14, 15** and **18:** Vancomycin-susceptible MRSA isolates positive for *mecA* and SCC*mec* III; **Lanes 9, 10, 17** and **19:** Vancomycin-susceptible MRSA isolates positive for *mecA* and SCC*mec* IVa; **Lane 16:** hVISA isolate positive for *mecA* and SCC*mec* IVa; **Lanes 11 and 12:** Vancomycin-susceptible MRSA isolates positive for *mecA*, SCC*mec* V and *pvl*; **Lane 13:** Vancomycin-susceptible MRSA isolate positive for *mecA* and SCC*mec* V; **Lane 20:** Vancomycin-susceptible MRSA isolate positive for *mecA*, SCC*mec* IVc and *pvl*.

SCCmec = Staphylococcal cassette chromosome mec; MRSA = methicillin-resistant Staphylococcus aureus; hVISA = heterogeneous vancomycin intermediate Staphylococcus aureus; pvl = Panton-Valentine leukocidin gene.

end-point (minimum of 2 colonies) was clear in the screening method using the McFarland 2.0 standard inoculum. None of the isolates was VISA or VRSA. Out of the 14 hVISA, 10 (71.4%) and 4 (28.6%) were isolated from male and female patients, respectively. Regarding the 206 vancomycin-susceptible MRSA, 133 (64.6%) and 73 (35.4%) were isolated from male and female patients, respectively. The majority of the hVISA (n = 6/14, 42.9%) were isolated from patients aged between 61 and 70 years, whereas the majority of the vancomycin-susceptible MRSA (n = 48/206, 23.3%) were isolated from patients aged between 41 and 50 years.

Of the 14 patients infected with hVISA, 11 (78.6%) were diabetic, 13 (92.9%) had been previously hospitalised, 8 (57.1%) had received previous vancomycin treatment and 8 (57.1%) had undergone a previous surgery. Majority of the hVISA and vancomycin-susceptible MRSA were isolated from patients with skin and soft tissue infections; 21.4% of hVISA and 10.7% of vancomycin-susceptible MRSA were isolated from patients with bacteraemia [Table 1].

Compared with vancomycin-susceptible MRSA, hVISA were more resistant to all the antimicrobial agents except trimethoprim-sulphamethoxazole. All the test organisms were susceptible to linezolid and teicoplanin [Table 2].

More than 80.0% of the isolates were resistant to ciprofloxacin and erythromycin. The MSB phenotype was more common in both hVISA (6/14; 42.9%) and vancomycin-susceptible MRSA (n = 82/206, 39.8%); 92.9% of the hVISA had a vancomycin MIC ranging from 1 to 2 µg/mL. For both hVISA and vancomycin-susceptible MRSA, MIC50 and MIC90 were 1 µg/mL and 2 µg/mL, respectively [Table 3].

Majority of the hVISA and vancomycin-susceptible MRSA carried SCC*mec* III and IV, and there was no significant difference between hVISA and vancomycin-susceptible MRSA regarding SCC*mec* type; 6.8% of vancomycin-susceptible MRSA were non-typeable. The *pvl* gene was detected in 2/14 (14.3%) of the hVISA and 57/206 (27.7%) of the vancomycin-susceptible MRSA isolates. The results of SCC*mec* typing are presented in Table 4 and Figure 2.

Discussion

The prevalence and molecular features of hVISA in 4 tertiary care hospitals in coastal Karnataka, India are presented. The hVISA phenotype was detected among 6.4% of the MRSA strains isolated from HAIs. A recent systematic review and meta-analysis has reported the prevalence rate of hVISA worldwide.[22] The hVISA phenotype was detected, in 82 studies, on a total of 47,721 strains, with an average prevalence of 4.6%. This study showed that the prevalence of hVISA has increased over the last few years in different parts of the world.[22] Three previous studies in India reported

the prevalence of hVISA as ranging from 2–12.4%.[7,23,24] The differences in the prevalence of hVISA could be due to the geographical area in which the study was conducted, the sample size, patient population or testing methods used. Nevertheless, the increase in the prevalence rate of hVISA is a matter of concern. Furthermore, since hVISA is considered to be the precursor stage of VISA, an increase in the prevalence rate of VISA may be expected in the future.[2,3]

In the current study, there was no association between hVISA and the type of infection. Factors such as age, extended hospital stay, previous vancomycin treatment, diabetes mellitus, instrumentation and surgery may increase the risk of hVISA infections.[2] This study showed that more than 50% of the patients infected with hVISA had risk factors such as diabetes mellitus, previous hospitalisation and previous vancomycin treatment. The clinical profile of *pvl* positive cases was not different from that of *pvl* negative ones.

Treatment of hVISA infections with vancomycin may result in the persistence of the infection, a greater risk of complications and treatment failure.[2,25] Some researchers believe that hVISA arises as a consequence of prolonged vancomycin treatment.[25] Studies have demonstrated that a vancomycin AUC/MIC of >400 μg/mL can bring about effective treatment.[26] This can be achieved if vancomycin's MIC is ≤1 μg/mL. The European Committee on Antimicrobial Susceptibility Testing classifies *S. aureus* with a vancomycin MIC of >2 μg/mL as vancomycin resistant.[27] A previous study reported a higher mortality rate among patients with hVISA infection admitted in the intensive care unit.[28] In the current study, patients with deep hVISA infections responded to vancomycin treatment. However, in cases where vancomycin toxicity developed, vancomycin was replaced with teicoplanin.

Identifying hVISA phenotype among MRSA is difficult.[2,12] The screening methods vary in sensitivity, specificity and validity. The vancomycin screen agar method used in the present study had a sensitivity and specificity of 100% and 96.6%, respectively. The PAP-AUC, which is the reference method for the confirmation of hVISA, is laborious, and as such, it may be difficult to test all MRSA strains for hVISA.[12] In the current study, 92.9% of hVISA had a vancomycin MIC ranging from 1–2 μg/mL; similar observations were made by other researchers.[10,29] Therefore, the authors suggest that MRSA strains with an MIC range of 1–2 μg/mL could be chosen for the detection of hVISA phenotype. In critically ill patients with MRSA infections, hVISA identification may have to be done upfront. In non-critical conditions, hVISA identification may be carried out if clinical response is sub-optimal.

Moreover, in this study, none of the MRSA was vancomycin-intermediate or -resistant. All hVISA and vancomycin-susceptible MRSA were susceptible to linezolid and teicoplanin. MSB was the most common phenotype detected, followed by iMLSB (inducible clindamycin resistance). In routine disk diffusion test, MRSA exhibiting inducible clindamycin appeared resistant to erythromycin but susceptible to clindamycin. If clindamycin is wrongly used for the treatment of infections caused by such organisms, treatment failure occurs. Therefore, hVISA strains resistant to erythromycin and susceptible to clindamycin should be subjected to D test to detect the possibility of inducible clindamycin resistance.

Furthermore, the majority of the hVISA harboured SCC*mec* III and IV. This in contrast to some previous Indian studies which reported a high frequency of SCC*mec* V among hVISA.[7,10,16] hVISA harbouring SCC*mec* IV, V and *pvl* in the current study is suggestive of the entry of CA-MRSA into hospitals. This also shows that the molecular differences between HA-MRSA and CA-MRSA are not distinct. Although all hVISA strains in the present study were typeable, 6.8% of the vancomycin-susceptible MRSA were non-typeable. It is possible that these strains harbour SCC*mec* types not included in the present study. A recent study in South India also reported non-typeable strains among clinical isolates of MRSA.[30]

The present study was subject to some limitations. It was difficult to draw general conclusions based on investigations conducted on a few hVISA. A larger sample size should be used to gain a better understanding of hVISA infections. Multiplex PCR was designed for the detection of SCC*mec* types I-V only. Additional genetic and molecular tests could have given a better understanding of the epidemiology of hVISA.

Conclusion

The prevalence rate of hVISA among MRSA was 6.4%. MRSA strains should be tested for hVISA phenotypes before commencing vancomycin treatment. Vancomycin agar screen with 4 μg/mL vancomycin and McFarland 2.0 inoculum could be used for screening MRSA for hVISA. However, confirmation requires the use of PAP-AUC. None of the isolates were vancomycin-intermediate or -resistant and all hVISA strains were susceptible to linezolid and teicoplanin. The majority of the hVISA were isolated from patients with skin and soft tissue infections and

SCC*mec* III and IV were predominant among hVISA and vancomycin-susceptible MRSA.

Authors' Contribution

SM collected and organised the data, performed the experiments, carried out statistical analysis of the results and wrote the initial draft of the article. GBK conceived and designed the study, reviewed the results, analysed and interpreted the data, wrote the initial and final drafts of the article and supervised the study. SM and GBK acquired financial support for the project and participated in writing the literature review, methods and discussion. ShSM, SuSM and GBK participated in planning and executing the research activity. SuSM provided logistic support and provided research materials. ShSM, SuSM, DB and CM designed the study, analysed and interpreted the data, participated in the literature review, methods and discussion as well as in the final writing and provided supervision. All authors approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interests.

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