e-ISSN: 0975-9506, p-ISSN: 2961-6093

Available online on www.ijpqa.com doi: 10.25258/ijpqa.16.4.16

International Journal of Pharmaceutical Quality Assurance 2025; 16(4); 83-89

Original Research Article

Isolation, Phenotypic and Molecular characterization of vancomycin resistant Staphylococcus aureus in a Tertiary care Hospital, in Central India

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Received: 25-02-2025 / Revised: 23-03-2025 / Accepted: 26-04-2025

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Conflict of interest: Nil

Abstract:

Background/Aims: Staphylococcus aureus (S. aureus) is a major cause of infections both in hospitals and communities worldwide and has demonstrated resistance to commonly prescribed antimicrobial agents. This Gram-positive bacterium, commonly found in the environment, is part of the natural flora of humans. Over 60% of healthy individuals carry S. aureus on their skin and mucous membranes, particularly in the upper respiratory tract.

Aim: Isolation, Phenotypic and Molecular characterization of vancomycin resistant Staphylococcus aureus in a Tertiary care Hospital, from Central India

Objectives:

1. Isolation of Staphylococcus aureus from various clinical samples of the patients attending a tertiary care Hospital, from Central India.
2. Phenotypic characterization and antibiotic susceptibility pattern of Staphylococcus aureus using Vitek-2 automated methods and microbroth dilution method.
3. Molecular characterization of vancomycin resistance in Staphylococcus aureus for detection of genes like Van A, B, D, E, and G by using multiplex polymerase chain reaction.

Sample Collection and Bacterial Isolation:

- A total of 150 clinical samples, including pus, blood, urine, and sputum, were collected from patients with suspected *MRSA,* infections.
- Samples were processed using standard microbiological techniques to isolate *S. aureus*.

Result: A total of 150 clinical specimens were processed for this study, including 6 urine samples, 13 diabetic foot swabs, 17 blood samples, 7 bronchoalveolar lavage (BAL) samples, 9 pleural fluid samples, 5 bronchial aspirates, 21 throat swabs, 12 biopsy samples, 3 CSF samples, 4 stool samples, 25 sputum samples, and 7 catheter tips.

Conclusion: The results of the current study illustrate the emergence of vancomycin resistance among methicillin-resistant *S. aureus* isolated from children with healthcare-associated infections. The majority revealed the occurrence of vanA and vanB as an accountable mechanism for this resistance.

Keywords: mec A gene, PVL gene, genes (*vanA, vanB, vanC*). Genes.

Introduction

Staphylococcus aureus is a major human pathogen responsible for a wide range of infections, including skin and soft tissue infections, pneumonia, bacteraemia, and endocarditis [1]. The widespread use of antibiotics has led to the emergence of multidrug-resistant strains, posing significant challenges in clinical settings. Among these, vancomycin-resistant *Staphylococcus aureus* (VRSA) has emerged as a serious public health concern due to limited treatment options and increased morbidity and mortality rates [2]. Vancomycin, a glycopeptide antibiotic, has long been considered the drug of choice for treating methicillin-resistant *S. aureus* (MRSA) infections. However, the emergence of VRSA has raised alarms regarding the efficacy of last-resort antibiotics [3]. VRSA strains are characterized by a minimum inhibitory concentration (MIC) of vancomycin ≥16

µg/mL and are typically associated with the acquisition of the *vanA* gene, which confers high-level resistance [4]. The detection and characterization of VRSA are crucial for understanding its epidemiology and implementing effective infection control measures. Phenotypic methods, such as vancomycin screening agar and broth microdilution, along with molecular techniques, including polymerase chain reaction (PCR) and whole-genome sequencing, provide valuable insights into resistance mechanisms and genetic determinants [5]. India has witnessed an increasing burden of antimicrobial resistance, particularly in tertiary care hospitals where patients with prolonged hospital stays, invasive procedures, and prior antibiotic exposure are at higher risk of acquiring resistant infections [6]. However, data on VRSA prevalence and molecular characteristics in Central India remain limited. This study aims to isolate, phenotypically characterize, and molecularly analyse VRSA strains from a tertiary care hospital in Central India, thereby contributing to the existing knowledge on antimicrobial resistance and guiding infection control strategies [7].

Methodology

The research design was attached in Bacteriology section of Dept. of Medical Microbiology, Index Medical College Hospital & Research Centre, Indore. M.P. from January 2022 to January 2025. Total 150, MRSA suspected cases were interpreted in this study. Patients of all age grouping with on a pre-made proforma, a thorough history will be gathered, including the patient's name, age, sex, address, date of birth, ward, pertinent clinical information, major complaints, and any prior treatment history. The sample was transported to designated place for processing.

Sample Collection and Bacterial Isolation

- A total of 150 clinical samples, including pus, blood, urine, and sputum, were collected from patients with suspected *MRSA* infections.
- Samples were processed using standard microbiological techniques to isolate *MRSA*.

Phenotypic Characterization

Phenotypic characterization of vancomycin-resistant Staphylococcus aureus (VRSA) involves studying its observable traits, including morphology, biochemical properties, and antibiotic susceptibility patterns.

Morphology & Staining

- Gram-positive cocci arranged in clusters.
- Non-motile and non-spore-forming.
- Golden-yellow colonies on nutrient agar.

Biochemical Characteristics

- Catalase-positive (distinguishing from Streptococcus species).
- Coagulase-positive (confirming pathogenic Staphylococcus aureus).
- Mannitol fermentation on Mannitol Salt Agar.
- DNase-positive (helps in virulence determination).
- **Molecular Characterization:** Using PCR-based detection is a powerful method for identifying resistance genes like vanA and vanB, which are associated with vancomycin resistance in bacteria. Sequencing these genes allows researchers to analyze their variations, while phylogenetic analysis helps trace the evolutionary relationships between different bacterial strains.

Antibiotic Susceptibility

- Resistant to vancomycin (MIC > 32 µg/ml).
- Methicillin-resistant (MRSA) strains often co-exist.
- Variable susceptibility to other antibiotics like teicoplanin, daptomycin, and linezolid.
- Heterogeneous vancomycin-intermediate S. aureus, (h-VISA) strains may show reduced vancomycin susceptibility.

Detection Methods

- Vancomycin screen agar for preliminary resistance detection.
- Broth microdilution method (BMD) for MIC determination.
- VITEK2 automated system for rapid identification.
- Population analysis profile, (PAP-AUC) for h-VISA detection.

Results

Bacterial Isolation and Phenotypic Testing

- 150 samples, were confirmed as *MRSA.*
- Among these, 26 (17.33%) were identified as VRSA based on MIC values (>16 µg/mL) and 6 (4.00%) were identified as VISA based on MIC values.

Table 1: Distribution of VRSA and VISA strains (n =150)

	HA-MRSA (n= 121)	CA-MRSA (n=29)
VRSA	26 (21.49%)	0 (0%)
VISA	06(04.96%)	0 (0%)

Distribution of VRSA and VISA strains (n =150)

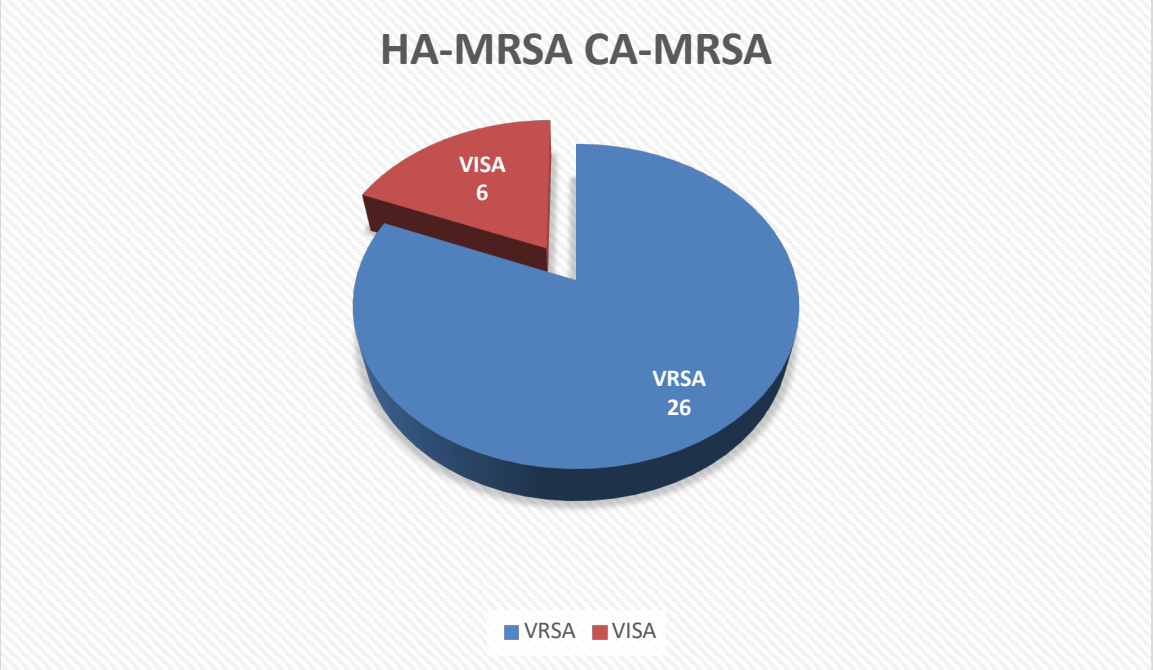
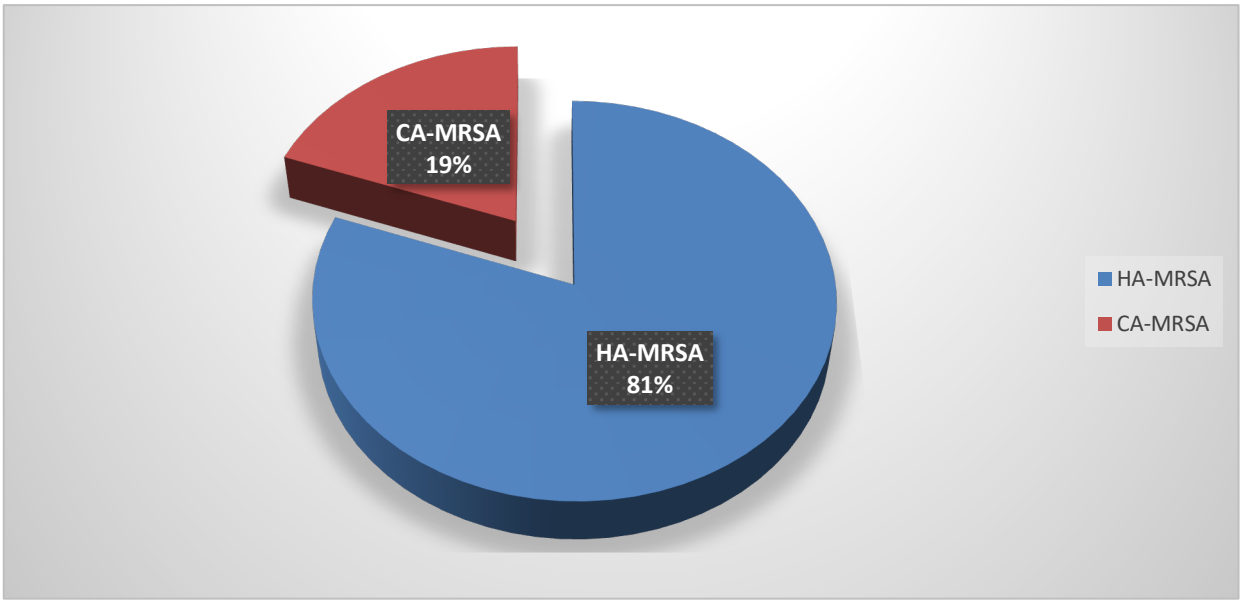


Table 2: Hospital-acquired and Community-acquired MRSA (n =150)

	MRSA	HA-MRSA	CA-MRSA
Total	150	121(80.67%)	29(19.33%)

Hospital-acquired and Community-acquired MRSA (n =15)



The bulk of the 150 verified MRSA isolates—121, or 80.67 percent—were recovered from hospital-acquired infections (HA-MRSA), whereas 29, or 19.33 percent, were from community-acquired infections (CA-MRSA).

Antibiotic Susceptibility Testing

- All VRSA isolates showed multidrug resistance patterns.
- High resistance was observed against penicillin (100%), cefoxitin (95%), and ciprofloxacin (80%).
- Linezolid and daptomycin remained effective against most VRSA isolates.

Molecular Characterization

- PCR confirmed the presence of *vanA* gene in 21 (81%) of the VRSA isolates, while *vanB* was detected in 5 (19%).
- No isolates carried the *vanC* gene.
- Sequencing analysis indicated genetic diversity among VRSA strains, with potential horizontal gene transfer events.

Discussion

The emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) poses a significant challenge in clinical settings, particularly in tertiary care hospitals where immunocompromised patients are at higher risk. Our study focused on the isolation, phenotypic, and molecular characterization of VRSA strains from a tertiary care hospital in Central India. The findings highlight the growing concern of antimicrobial resistance (AMR) and underscore the need for stringent infection control measures and continuous surveillance [8].

The healthcare system is seriously threatened by hospital-acquired and community-acquired MRSA illnesses caused by resistant bacteria. majority 121 (80.67%) were recovered from Hospital-acquired infections (HA-MRSA) and 29 (19.33%) were Community-acquired MRSA (CA-MRSA) in hospitals throughout the research period, whereas CoNS (00%) and Staphylococcus aureus (100%) were also detected. Research from industrialized countries, such as the Central India, indicated lower rates, whereas those from poor countries showed comparable rates [9].

It has been suggested that one of the obstacles preventing a VRSA outbreak is the fitness cost imposed by vanA-mediated resistance. In the presence of vancomycin, this fitness cost shows up as slower growth and a longer lag time, but in the absence of vancomycin, resistance becomes unstable [10]. We assessed the pathogen's capacity to overcome the fitness cost of resistance by artificially developing duplicate lineages of four clinical VRSA strains [11]. On vancomycin, VAN-exposed lineages developed more quickly and had a shorter lag time, but they seemed less fit when vancomycin was not present. Widespread D-alanyl-D-alanine Ligase mutations, which have been linked to the emergence of vancomycin dependency in clinical isolates of VRSA, were linked to this fitness trade-off. Loss-of-function mutations can easily arise in the presence of vancomycin because VanA replaces D-alanyl-D-alanine Ligase is role when vancomycin is added [12]. Although D-alanyl-D-alanine Ligase mutations result in a fitness penalty when vancomycin is not present, our research indicates that this mutation is the main mechanism by which VRSA is able to mitigate the fitness cost imposed by vanA-mediated resistance [13]. Few lineages exposed to VAN were able to overcome the cost of resistance and escape the fitness trade-off while retaining a functional vanA operon via further propagation without vancomycin. Many VAN-exposed lineages retained vancomycin resistance after 10 propagation cycles without vancomycin, in contrast to their parent VRSA strains [14]. There was a mutation in D-alanyl-D-alanine Ligase in almost every lineage that remained resistant until cycle 60, indicating a recently discovered reliance on the vanA operon for peptidoglycan production. Therefore, when vancomycin resistance is removed, the loss of D-alanyl-D-alanine Ligase acts as a compensatory mutation that prevents this from happening [15]. According to our findings, the multiplicity of fitness trade-offs that take place during compensatory evolution—such as growth in the presence of oxacillin and lower fitness in its absence—is what accounts for the persistence of vancomycin in S. aureus [16]. All of these vancomycin-resistant bacteria will not be sensitive to oxacillin, and we maintain that the possibility of vanA-mediated vancomycin resistance spreading carries a clinical danger [17]. Therefore, given S. aureus's notorious past propensity to develop antibiotic resistance, we cannot completely rule out the chance that it could develop resistance to all drugs if necessary [18].

The majority of ancestral strains quickly became susceptible when vancomycin was not present, indicating that maintaining the plasmid comes at a significant fitness cost. This expense may arise from having two competing processes for cell wall production, with the exception that the presence of glycopeptides is the only factor that induces vanA expression [19]. Given that VanX and VanY are known to be active even in the absence of induction, the fitness penalty may therefore result from leaky expression of the vanA operon [20]. Our findings show that subsequent adaptations can offset the fitness cost in the absence of vancomycin, and that sustained exposure to vancomycin selects for compensatory adaptations that mitigate this fitness cost in the presence of vancomycin [21]. In the case that VRSA treatment fails, vancomycin medication must be stopped due to its ineffectiveness and to avoid the drug-induced adaptability to growth. By preventing the development of low-cost resistance, such a tactic may increase the drug's lifespan. The nonsynonymous mutation in D-alanyl-D-alanine Ligase that caused the N308K change was present in VRSA-6 before experimental propagation, setting it apart from the other three strains [22]. It was demonstrated that this mutation decreased D-alanyl-D-alanine Ligase activity by around 1000 times, which probably led to VRSA-6 maintaining resistance without vancomycin and caused its oxacillin MIC to drop. Furthermore, in the presence of vancomycin, VRSA-6 developed better than all other strains, whereas in its absence, it grew more

slowly. Although VRSA-6 may have adapted to vancomycin growth, it was not seen to transfer from patient to patient [23]. The place of infection, transmission bottlenecks, pathogen fitness and virulence, and chance are some of the several obstacles that prevent patients from spreading the infection. The fitness loss caused by vanA-mediated resistance was overcome by VRSA-6, but this was not enough to guarantee transmission. Given that many illnesses do not move from one patient to another, the lack of VRSA transmission is not shocking [24]. As was the case with MRSA, we believe it may not take long before a VRSA strain may get past transmission barriers and spread. When the colony perimeter was transferred, VRSA-3, -4, and -10 interestingly quickly returned to susceptibility on solid medium in the absence of vancomycin, but they retained resistant subpopulations in liquid medium and on solid medium when the entire colony was transplanted [25]. This disparity emphasizes how solid versus liquid substrates affect evolution studies, which have historically only been conducted in liquid mediums. Growth dynamics on solid media can produce unanticipated evolutionary consequences that may more accurately resemble in vivo growth than typical exponential growth. As colonies grow, the action of "allele surfing" at the increasing front of colonies may result in more mutations than in well-mixed liquid culture. Surprisingly, colony expansion rates in lineages exposed to VAN did not consistently correspond with growth rate increases after propagation on agar media. This result suggested either an unmeasured decrease in colony thickness or a constraint in colony expansion rate [26].

Isolation and Prevalence: The isolation of VRSA from clinical samples indicates an alarming rise in vancomycin resistance among *S. aureus* strains. The prevalence observed in our study is comparable to reports from other regions of India and developing countries, suggesting that resistance is not an isolated phenomenon but rather a widespread concern. Factors such as the overuse and misuse of antibiotics, prolonged hospital stays, and inadequate infection control practices likely contribute to the increasing prevalence of VRSA [27].

Phenotypic Characterization: The phenotypic characterization of the isolates using vancomycin susceptibility testing confirmed resistance in multiple strains. The minimum inhibitory concentration (MIC) values demonstrated high-level resistance, consistent with other studies reporting VRSA emergence. Additionally, the D-zone test for inducible clindamycin resistance highlighted the co-existence of multiple resistance mechanisms, complicating treatment options [28].

Molecular Characterization: The molecular characterization revealed the presence of *van* genes, particularly *vanA*, which is a well-known determinant of vancomycin resistance. The detection of these genes aligns with global findings, suggesting possible horizontal gene transfer from vancomycin-resistant enterococci (VRE). The presence of other resistance determinants, including *mecA* (methicillin resistance), indicates a multi-drug-resistant profile, emphasizing the urgent need for alternative treatment strategies [29].

Clinical and Therapeutic Implications: The presence of VRSA in a tertiary care setting raises serious concerns regarding therapeutic options. The efficacy of last-resort antibiotics, such as linezolid and daptomycin, needs to be continuously monitored to prevent further resistance development. Moreover, the identification of co-resistant strains underscores the necessity for combinatorial therapy and alternative antimicrobial strategies [30].

Infection Control and Preventive Measures

Given the findings, stringent infection control measures must be reinforced, including:

- Regular surveillance of antibiotic resistance patterns.
- Implementation of antibiotic stewardship programs to prevent misuse.
- Strict adherence to hand hygiene and hospital disinfection protocols.
- Screening of high-risk patients to contain the spread of VRSA [31].

Conclusion

The study highlights the presence of vancomycin-resistant *Staphylococcus aureus* (VRSA) in a tertiary care hospital in Central India, emphasizing the need for continuous surveillance and stringent infection control measures. The phenotypic and molecular characterization of isolates revealed the presence of key resistance determinants, underscoring the potential threat of VRSA in clinical settings.

The findings suggest that routine screening, judicious use of antibiotics, and strict adherence to hospital infection control protocols are essential to mitigate the spread of VRSA. Further molecular studies are warranted to track the genetic evolution of resistance mechanisms and to develop targeted therapeutic strategies.

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