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Vancomycin Resistance Screening in Methicillin-Resistant Staphylococci: Comparison of Vancomycin Screen Agar, E-strip and Broth Microdilution

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Abstract: ***Background: Detection of vancomycin resistance in methicillin-resistant staphylococci is essential because resistance may affect therapeutic choices and infection-control decisions. Vancomycin screen agar is a screening method, whereas E-strip and broth microdilution (BMD) provide MIC-based results. Objective: To describe and compare vancomycin screen agar, E-strip and BMD findings among methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS) reported in this study. Methods: This cross-sectional laboratory study was conducted at Government Medical College and associated hospitals, Kota, Rajasthan, from March 2025 to February 2026. Two hundred clinical staphylococcal isolates were identified by standard microbiological methods. Methicillin resistance was assessed using cefoxitin disk diffusion method. Vancomycin susceptibility was screened using vancomycin screen agar and MICs were assessed by E-strip and BMD method. The study included 116 MRSA and 21 MRCoNS isolates. Screen-agar positivity and MIC-based vancomycin susceptibility was detected among methicillin resistant isolates. Wilson 95% confidence intervals (CIs) were calculated for proportions, and two-sided Fisher exact tests compared MRSA and MRCoNS proportions. Results: Vancomycin Screen agar was positive in 1/116 MRSA and 1/21 MRCoNS. E-strip and BMD both reported one vancomycin-resistant MRSA (1/116) and no resistant MRCoNS (0/21). The MRCoNS screen-positive result was not confirmed by either MIC method. Conclusion: One MRSA isolate was reported as vancomycin-resistant by VSA method confirmed by both E-strip and BMD, whereas no MRCoNS isolate was resistant to vancomycin by either of these methods. One MRCoNS screen-agar-positive (Vancomycin Resistant) result was discordant with MIC testing (susceptible by both E-strip and BMD).***

Keywords: vancomycin resistance; MRSA; MRCoNS; vancomycin screen agar; E-strip; broth microdilution; MIC; antimicrobial susceptibility testing

1. Introduction

Methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative staphylococci are important causes of healthcare-associated and community-onset infections [(1)]. CoNS may be clinically significant in bloodstream and device-associated infections, particularly in patients with intravascular catheters or implanted prosthetic material. Reliable antimicrobial susceptibility testing is therefore important for guiding treatment and infection-control measures.

Vancomycin susceptibility should be interpreted using validated methods and the applicable organism-specific standard. Vancomycin Screen agar may flag isolates requiring further evaluation, but screening positivity is not equivalent to a confirmed resistant phenotype. E-strip estimates the MIC by gradient diffusion, while broth microdilution is a quantitative dilution method commonly used as a reference approach [(2)].

2. Materials and Methods

Study Design and Setting

A cross-sectional laboratory study was conducted in the Department of Microbiology, Government Medical College and associated Hospitals, Kota, Rajasthan, India, from March 2025 to February 2026. Study population was 200.

Study Isolates

A total of 200 clinical staphylococcal isolates from patients of different age groups were included. Organisms were identified using standard microbiological procedures. All procedures were preformed using CLSI [(3)] and EUCAST [(4)] guidelines. Analysis of sample-wise distribution revealed that pus samples were the most common source of Staphylococcus aureus isolates, accounting for 51.7% of cases. Other important sources included sputum (20.35%), urine (11.6%), bone and joint aspirates (3.5%), and CSF (2.9%). Smaller numbers of isolates were also obtained from blood, pleural fluid, suction tips, throat swabs, and other body fluids. Similarly, among CoNS isolates, pus (35.7%) and urine (28.6%) were the most common sources, followed by CSF (17.9%) and blood (10.8%).

Clinical sample	S. aureus n (%)	CoNS n (%)
Pus	89 (51.7%)	10 (35.7%)
Sputum	35 (20.35%)	0 (0%)
Urine	20 (11.6%)	8 (28.6%)
Blood	3 (1.7%)	3 (10.8%)
CSF	5 (2.9%)	5 (17.9%)
Central line	1 (0.6%)	1 (3.6%)
Bone / joint aspirates	6 (3.5%)	0 (0%)
Pleural fluid	3 (1.7%)	0 (0%)
Peritoneal fluid	1 (0.6%)	0 (0%)
Suction tip	3 (1.7%)	1 (3.6%)
Synovial fluid	1 (0.6%)	0 (0%)
Throat swab	3 (1.7%)	0 (0%)
Vaginal swab	1 (0.6%)	0 (0%)
Liver abscess	1 (0.6%)	0 (0%)
Total	172 (100%)	28 (100%)

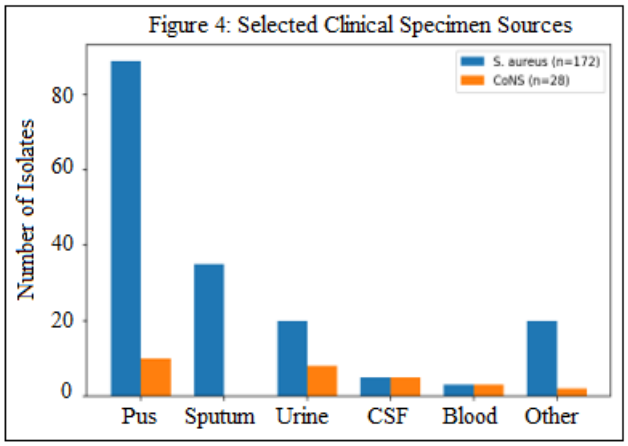
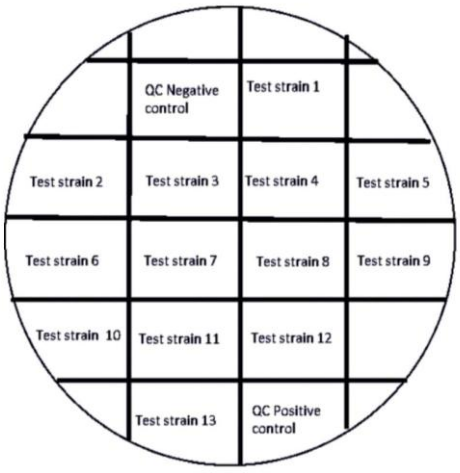


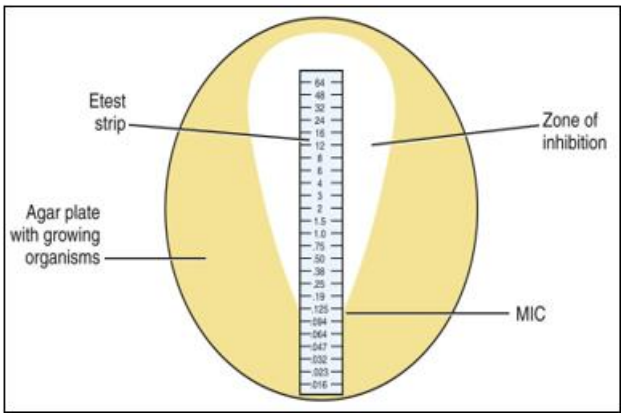
Figure: Analysis of sample-wise distribution

Vancomycin susceptibility methods

Vancomycin screen agar used (brain–heart infusion agar) containing vancomycin at 6 µg/mL[5]. Vancomycin MICs were assessed using **E-strip and BMD**. The staphylococcal isolates were categorized as susceptible at MIC ≤2 µg/mL, intermediate at 4–8 µg/mL, and resistant at ≥16 µg/mL[4].

3. Vancomycin Agar Screen Square Grid Template





MIC For Vancomycin Using E- Strip Test

Ethical Clearance

Ethical approval given by Institutional Ethical Committee Government Medical College, Kota, Rajasthan on 24/02/2025, Order No. F.3() Acad/Ethical Clearance/Batch2023/2025/05.

4. Results

The study included 200 clinical staphylococcal isolates: 172 S. aureus and 28 coagulase-negative staphylococci (CoNS). Methicillin resistance was assessed using cefoxitin disk diffusion. Among 200 isolates, 137 (68.5%) were methicillin-resistant: 116 MRSA and 21 MRCoNS. The study groups relevant to this analysis were 116 MRSA and 21 MRCoNS.

Isolates	Number of Isolates (n)	Percentage (%)
MRSA	116	58
MSSA	56	28
MRCoNS	21	10.5
MSCoNS	7	3.5
Total	200	100

There was female predominance with 108 (54%) female and 92 (46%) male patients with male to female ratio of 1: 1.17.

Gender	Number of Patients (n)	Percentage (%)
Male	92	46
Female	108	54
Total	200	100

Vancomycin susceptibility Comparison using VSA, E-strip and BMD

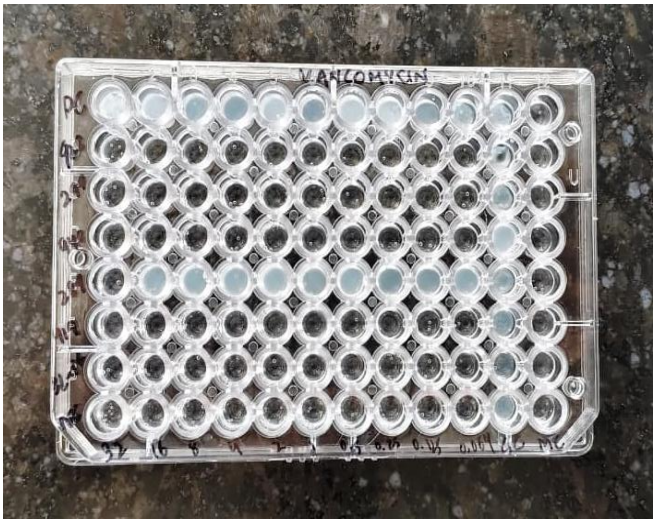
E-strip and BMD gave concordant aggregate resistance classifications in the study: one resistant MRSA isolate and no resistant MRCoNS isolates were resistant to Vancomycin. The MRCoNS isolate positive on Screen Agar was reported susceptible by both MIC methods.



Vancomycin Resistance using Vanomycin Screen AGAR



MIC for Vancomycin using E-Strip Method



MIC for Vancomycin using Broth Micro-Dilution Method

Organism	Method	Resistant (VRSA / VRCoNS) n (%)	Intermediate (VISA / VICoNS) n (%)	Susceptible (VSSA / VSCoNS) n (%)
MRSA	VSA	1 (0.9%)	0 (0%)	115 (99.1%)
MRSA	E-strip	1 (0.9%)	0 (0%)	115 (99.1%)
MRSA	BMD	1 (0.9%)	0 (0%)	115 (99.1%)
MRCoNS	VSA	1 (4.8%)	0 (0%)	20 (95.2%)
MRCoNS	E-strip	0 (0%)	0 (0%)	21 (100%)
MRCoNS	BMD	0 (0%)	0 (0%)	21 (100%)

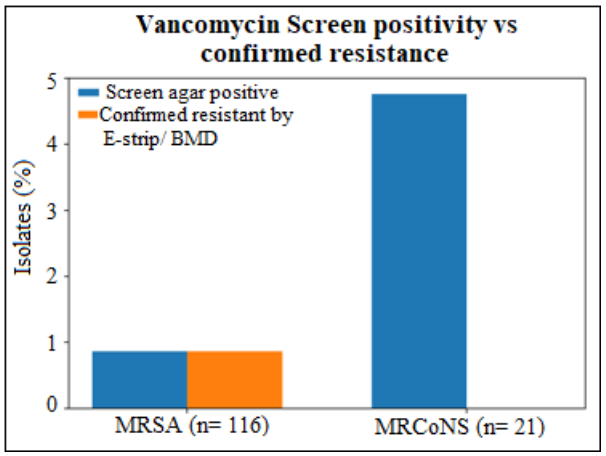


Figure 1: Bar diagram: screen agar positivity and MIC-confirmed resistance

Figure 1: Percentages using group-specific denominators. Screen positivity and confirmed resistance are distinct outcomes; the MRCoNS screen-positive isolate was not confirmed by C

5. Discussion

Age-wise distribution showed that young and middle-aged adults were most commonly affected by Staphylococcal infections. The highest number of isolates was observed in the 21–40 years age group (29%), followed closely by the 41–60 years group (28.5%). Patients aged 61–80 years accounted for 22.5% of cases, whereas 17% of isolates were obtained from patients aged 0–20 years. The least number of cases (3%) were observed in patients older than 80 years. Gender distribution demonstrated a slight female predominance, with 108 (54%) female patients compared to 92 (46%) male patients, resulting in a male-to-female ratio of approximately 1:1.17.

Vancomycin resistance was assessed using Vancomycin Screen Agar (VSA), E-strip MIC testing, and Broth Microdilution (BMD) methods. Using the VSA method, 1% of isolates showed vancomycin resistance, including one isolate each of VRSA and VRCoNS. However, confirmatory MIC testing using E-strip and BMD methods revealed only one VRSA isolate (0.5%), while all CoNS isolates remained susceptible. No VISA or VICoNS isolates were detected in the study.

These findings are consistent with the earlier published literature reporting strong vancomycin in-vitro activity and need for confirmation by either E-strip MIC and Broth Microdilution (BMD) methods against methicillin-resistant staphylococci, including reports by and colleagues, Hope[6] and colleagues, and Oksuz[7] and colleagues. The magnitude of susceptibility varies by population, study period, methods, breakpoints, and local antimicrobial exposure

6. Conclusion

In the present study, one MRSA isolate was reported resistant by both E-strip and BMD; One MRCoNS isolate was positive on vancomycin screen agar but susceptible by both MIC methods. The one screen-positive MRCoNS isolate is

important because it illustrates that a screening result may be discordant with quantitative MIC testing. One VSA resistant isolate should not be labelled vancomycin-resistant unless confirmed with either E-strip MIC or Broth Microdilution (BMD) methods as both methods showed same Susceptibility in present study.

7. Strengths and Limitations

Strengths include a direct comparison of three laboratory approaches within a defined clinical isolate collection and explicit reporting of the discordant screen-positive MRCoNS result. Limitations include a small MRCoNS sample and very few positive events.

Funding: None

Conflict-of-interest: None

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