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Research Article

Prevalence of Staphylococcal bloodstream infections and its antibiogram from a tertiary-care hospital in India: A descriptive cross-sectional study

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A B S T R A C T

Background: Bloodstream infections (BSIs) can cause self-limiting infections that recover within one to two days in healthy individuals and life-threatening sepsis in those with predisposing conditions.

Aim: The present study aimed to assess the frequency and antibiogram of *Staphylococcus spp.* isolated from blood culture and further detect methicillin resistance and vancomycin resistance among the isolates.

Methods: A total of 120 *Staphylococcus* species isolated over a period of six months from patients with BSIs were included in the study. In addition to the antibiogram, vancomycin resistance was also determined using the vancomycin screen agar test and the E-test for determination of minimum inhibitory concentration (MIC).

Results: Among the Staphylococcal isolates (n= 120), comprising *S. aureus* (66.7%, n= 80) and coagulase-negative Staphylococci (CoNS) (33.3%, n= 40), a total of 56 (70%) *S. aureus* and 24 (60%) CoNS isolates were detected as methicillin-resistant. Of the methicillin-resistant CoNS, 33.3% (n=6), 50% (n=6), and 40% (n=4) were methicillin-resistant *S. epidermidis*, *S. haemolyticus*, and *S. hominis*, respectively. All the Staphylococcal isolates were susceptible to linezolid and minocycline. Of the MRSA isolates, two strains were found to be resistant to vancomycin by Kirby Bauer's disc diffusion method. Additionally, a D-test was done for the MRSA strains (n = 56), of which 20 (35.7%) exhibited inducible clindamycin resistance.

Conclusion: This study highlights the increasing methicillin resistance in staphylococcal blood isolates. Resistance to the majority of the antibiotics, including vancomycin, the drug of choice for treatment of infections caused by MRSA strains, has reached alarming levels and continues to increase.

Keywords: Bloodstream infections, *Staphylococcus aureus*, CoNS, MRSA, antibiogram

Introduction

Bloodstream infections (BSIs) are potentially life-threatening, affecting individuals of all age groups and especially immunecompromised patients. The spectrum of this condition encompasses self-limiting infections that typically resolve within one to two days in healthy individuals, but in those with predisposing factors, it can progress to a severe and potentially life-threatening sepsis. [1,2] Thus, prompt and accurate diagnosis, including identification of pathogens by blood culture and determining their antibiogram, is crucial for minimizing morbidity and mortality. Automated blood culture systems offer continuous monitoring capabilities that enhance the detection of microbial growth and optimize the sensitivity and specificity of blood cultures. [3]

The pathogen profile of BSIs exhibits considerable variability, with *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci (CoNS), *Enterococcus spp.*, *Klebsiella spp.*, *Streptococcus spp.*, and *Pseudomonas aeruginosa* being the most frequent bacterial etiologies. [4] Globally, a notable rise in the prevalence of Staphylococcal isolates was documented, with prevalence ranging from 23.9% to 79.2% and the majority of the isolates were exhibiting methicillin resistance. [2] In an Indian study, the occurrence of Gram-positive organisms in BSIs within neonatal intensive care units was reported as 47%, comprising 15% *S. aureus* (15%) and CoNS (13%). [5]

Methicillin-resistant *S. aureus* (MRSA), initially documented in 1961, has become prevalent within Indian healthcare facilities and is widely recognized as a prominent nosocomial pathogen. Methicillin resistance is caused by the acquisition and expression of the Staphylococcal cassette chromosome (SCC mec) element, a 40–60 kb long foreign mobile DNA segment that contains the mec A gene. This gene encodes an altered form of penicillin -binding protein 2, which has little affinity for penicillin binding. [6] An increase in the number of MRSA isolates from BSIs, from 28% in 2017 to 35% in 2019, was reported from India. [7] Vancomycin, a glycopeptide antibiotic, remains the first-line agent and choice of drug for treatment of infections caused by MRSA. [8] However, vancomycin-resistant *S. aureus* strains have been identified globally since its first identification in Japan in 1997. [9-12] According to a recent study done on systemic review and meta-analysis, a gradual increase in the prevalence of VRSA was reported from <2% prior to 2006 to 7% in 2006-2014. [13]

Limited studies are available on the prevalence caused by *Staphylococcus spp.*, especially from rural India. Therefore, the present study was conducted to assess the frequency and antimicrobial resistance profile of Staphylococcus spp. isolated from blood culture and also the resistance pattern of MRSA isolates to vancomycin from a tertiary care hospital setup.

Methods

Study design and setting

A hospital-based cross-sectional study was carried out for a period of six months (July 2021 to December 2021) in the Department of Microbiology at a tertiary care hospital in Haryana, India. Ethical approval was obtained from the Institutional Ethics Committee (SEC/FMHS/02/06/21-02) before starting the study.

Laboratory analysis

Blood samples for culture and sensitivity received in the laboratory during the study period were included in the study. The samples were inoculated on aerobic blood culture bottles and incubated in the BacT/Alert blood culture system (BioMérieux, France). Once the culture bottle flagged positive, subculture was done on blood agar, MacConkey agar, and chocolate agar. After incubation, Gram-positive bacterial isolates were further identified by standard bacteriological techniques. [13] Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Muller Hinton agar, and results were interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines. [14] The following antibiotic discs were used: azithromycin (15 µg), cefoxitin (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), co-trimoxazole (1.25/23.752 µg), doxycycline (30 µg), erythromycin (15 µg), gentamicin (30 µg), levofloxacin (5 µg), linezolid (30 µg), minocycline (30 µg), nitrofurantoin (300 µg), penicillin (10 µg) and tetracycline (30 µg). *S. aureus* ATCC 25923 was used as the control strain.

Vancomycin screen agar test: Staphylococcal isolates showing resistance to cefoxitin in the AST were considered as MRSA strains. These strains were further subjected to vancomycin screen agar for determination of vancomycin resistance. Briefly, 10 µl of bacterial inoculum matched with 0.5 McFarland standard was inoculated on Brain Heart Infusion agar supplemented with 6 µg/ml of vancomycin and further incubated at 35°C for 24 hours. Any visible growth was interpreted as reduced susceptibility to vancomycin. *S. aureus* ATCC 29213 and *E. faecalis* ATCC 51299 were used as vancomycin-susceptible and -resistant control strains, respectively. [11]

E-test for vancomycin: Bacterial inoculum was prepared from a 24 h bacterial culture and matched with 0.5 McFarland standard before swabbing on MHA using a

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sterile cotton swab, and vancomycin E-test strips were placed. Plates were incubated at 37°C for 18 to 24 h. After incubation, the readings were taken by observing the strips from the top of the plate, and results were interpreted as per CLSI guidelines. Vancomycin MIC ≤2 µg/ml indicates susceptibility while MIC 4-8 µg/ml indicates intermediate, and MIC ≥16 µg/ml indicates resistance to vancomycin.[15]

Statistical analysis: Data obtained were entered and recorded in Microsoft Excel 2020, and results were expressed in terms of frequency (number) and percentage. Categorical variables were compared using the Chi-square test, and a P-value < 0.05 was considered statistically significant.

Results

A total of 120 consecutive, non-duplicate *Staphylococcus spp.* isolated from blood cultures of OPD and IPD patients were included in the study. Of the total Staphylococcal isolates, 66.7% (n=80) were S. aureus, and the remaining isolates were CoNS (n=40, 33.3%), comprising of *S. epidermidis* (n=18, 15%), *S. haemolyticus* (n=12, 10%), and *S. hominis* (n=10, 8.3%). The majority of the isolates were obtained from adults (45%), followed by pediatric patients (33.3%) and neonates (21.7%), a with male preponderance of 58.3% and a male:female ratio of 1:0.7. Among the Staphylococcal isolates, 53.3% were from patients attending the Pediatrics department, followed by those attending General Medicine (28.4%), Surgery (10%), Obstetrics & Gynecology (5%), and two patients (3.3%) were from the ENT department. A total of 66 isolates (55%) were patients attending IPD, while 45% (n=54) were from OPD of various departments.

Out of the total *S. aureus* isolates, 70% (n=56) were found to be MRSA, and the remaining 30% (n = 24) were methicillin-sensitive S. aureus (MSSA). Among the CoNS isolates, 60% (n=24) were methicillin sensitive, and the remaining 16 (40%) isolates were methicillin resistant, of which 33.3% (n=6), 50% (n=6), and 40% (n=4) were methicillin-resistant *S. epidermidis, S. haemolyticus, and S. hominis,* respectively (Fig. 1).

All the Staphylococcal isolates were susceptible to antibiotics viz., linezolid and minocycline. Two isolates showed resistance to vancomycin; thus, the resistance rate was noted as 1.7%. Members of the tetracycline group showed low resistance rates, i.e., 16.7% for tetracycline and 11.7% for doxycycline. Additionally, the Staphylococcal isolates showed low resistance rates to antibiotics such as co-trimoxazole (8.3%), and 18.3% to both gentamicin and chloramphenicol. A moderate degree of resistance was noted for antibiotics such as levofloxacin (31.7%), clindamycin (51.7%), azithromycin (65%), and erythromycin (66.7%). Among the antibiotics tested, the highest resistance rate was shown by penicillin (96.7%) (Fig. 2). Additionally, a D-test was done for the MRSA strains (n = 56), of which 20 (35.7%) exhibited inducible clindamycin resistance (Fig. 3).

The comparison of antibiotic resistance patterns exhibited by *S. aureus* and CoNS isolates showed significant differences for antibiotics such as levofloxacin and cefoxitin (P<0.05). While the higher resistance rates were observed for antibiotics viz. the tetracycline group, azithromycin, gentamicin, clindamycin, erythromycin, co-trimoxazole, chloramphenicol, and penicillin in CoNS compared to *S. aureus*, though statistically insignificant (P>0.05) (Table 1).

All the *S. aureus* and CoNS isolates were susceptible to linezolid and minocycline. Comparative analysis of antibiotic resistance patterns exhibited by MRSA and MSSA isolates showed significant differences for antibiotics such as penicillin, azithromycin, and erythromycin (P<0.05). Although the resistance rates for most antibiotics in MSSA were lower compared to MRSA, this difference was found to be statistically insignificant (P>0.05) (Table 2). Complete susceptibility to linezolid and minocycline was seen, irrespective of the methicillin-sensitive or -resistant *S. aureus* strains.

In addition to the vancomycin disc diffusion test for detection of vancomycin resistance, all MRSA isolates were subjected to the vancomycin screen agar test, and further confirmation was done by E-test, which is the gold standard method. It was observed that two isolates of VRSA were identified by the vancomycin disc diffusion test, although none of the isolates exhibited resistance to vancomycin when tested on vancomycin screen agar. The E-test method also indicated that all isolates were sensitive to vancomycin. All the MRSA isolates were sensitive to vancomycin with an MIC of less than 2 µg/ml. The majority of MRSA isolates (53.6%, 30/56) had a vancomycin MIC value of 1.5 µg/ml and 42.9% (24/56) of isolates had a vancomycin MIC value of 1.0. Only two (3.5%) isolates had an MIC value of 2 µg/ml; no isolates were found in the range of 0-0.5. Therefore, vancomycin intermediate resistant (VISA) and vancomycin resistant (VRSA) were not detected when the vancomycin MIC was analyzed by E-test.

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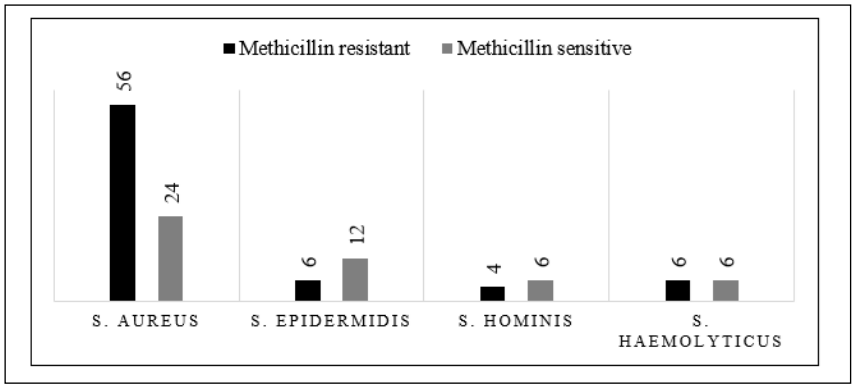

Figure 1.Distribution of MRSA and MSSA among the Staphylococcal blood culture isolates

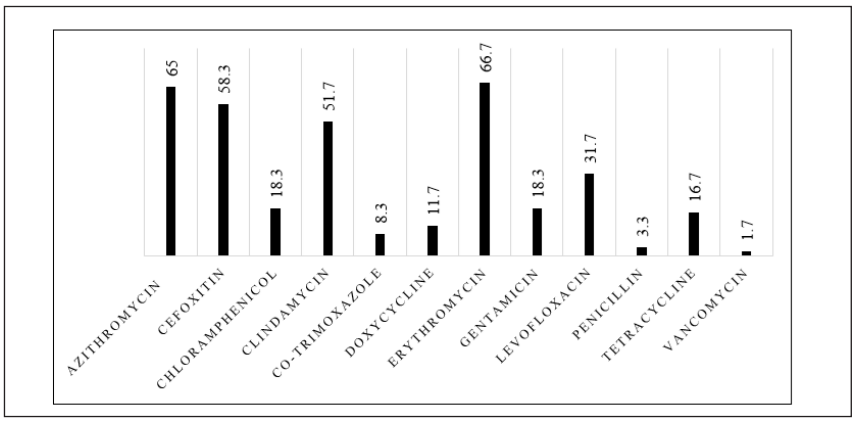

Figure 2.Antibiogram of Staphylococcal blood culture isolates

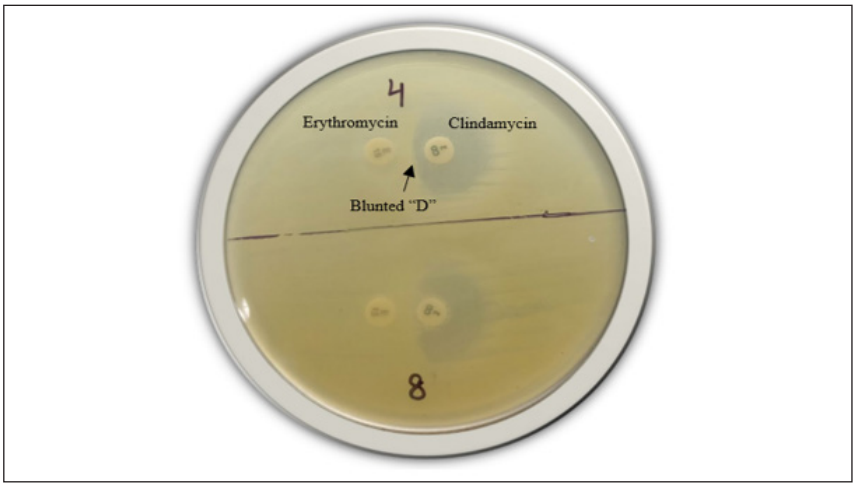

Figure 3.Positive D-test showing blunted "D" zone

Table 1.Comparative analysis of antibiotic resistance pattern of *S. aureus* and CoNS isolates

Antibiotic	Antibiotic resistance pattern, No. (%)		P- value
	S. aureus (n= 80)	CoNS (n=40)	
Azithromycin	52 (65)	26 (65)	1
Cefoxitin	56 (70)	16 (40)	0.02*
Chloramphenicol	10 (12.5)	12 (30)	0.1
Clindamycin	38 (47.5)	24 (60)	0.4
Co-trimoxazole	4 (5)	06 (15)	0.2

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Doxycycline	10 (12.5)	04 (10)	0.8
Erythromycin	54 (67.5)	26 (65)	0.8
Gentamicin	12 (15)	10 (25)	0.3
Levofloxacin	30 (37.5)	08 (20)	<0.001*
Penicillin	76 (95)	40 (100)	0.5
Tetracycline	12 (15)	08 (20)	0.6
Vancomycin	2 (2.5)	00 (00)	0.7

*P<0.05 was considered as statistically significant

Table 2.Comparative analysis of antibiotic resistance pattern of methicillin-resistant and methicillin-sensitive *S. aureus* isolates

Antibiotic	Antibiotic resistance pattern, No. (%)		P- value
	Methicillin-resistant *S. aureus* (n=56)	Methicillin-sensitive *S. aureus* (n=24)	
Azithromycin	48 (85.7)	04 (16.7)	<0.001*
Chloramphenicol	10 (17.9)	00 (00)	0.3
Clindamycin	32 (57.1)	06 (25)	0.06
Co-trimoxazole	4 (7.1)	00 (00)	0.6
Doxycycline	10 (17.9)	00 (00)	0.3
Erythromycin	46 (82.1)	08 (33.3)	0.03*
Gentamicin	12 (21.4)	00 (00)	0.3
Levofloxacin	26 (46.4)	04 (16.7)	0.08
Penicillin	56 (100)	04 (16.7)	<0.001*
Tetracycline	12 (21.4)	00 (00)	0.3
Vancomycin	2 (3.6)	00 (00)	0.7

*P<0.05 was considered as statistically significant

Discussion

Bloodstream infections are a global health concern that result in substantial morbidity and mortality. In the United States, hospital-acquired BSI has been regarded as the 10th leading cause of death. [17] *S. aureus*, CoNS, and Enterococci are frequently encountered Gram-positive bacteria responsible for BSIs, while *Escherichia coli* and *Salmonella Typhi* are the predominant Gram-negative bacteria associated with such infections. Staphylococcal BSIs have the potential to be life-threatening and are characterized by a significant global death incidence of up to 25%. In a study conducted by Tak et al., it was shown that patients with Staphylococcal BSIs in an Indian trauma care center had a mortality rate of 31%. [17]

In the present study, the majority of the *S. aureus* isolates were obtained from blood cultures of adult patients (45%), followed by pediatric patients (33.3%) and neonates (21.7%). The findings of our study correspond with the study conducted by Vasudeva et al. and Kulshrestha et al., which revealed that the majority of blood culture-positive cases were observed in adult patients aged above 21 years. [18,19] Since BSIs are serious illnesses requiring 24-hours medical supervision, [18] more than three-quarters of the Staphylococcal isolates were isolated from hospitalized patients in the study. Lohan et al. also reported comparable results, indicating that 75.3% of the positive blood cultures were obtained from hospitalized patients. [20]

Out of the Staphylococcal isolates from blood culture, *S. aureus* accounted for 67% of the cases, while CoNS were isolated from 33.33% of cases. An epidemiological study by Vasudeva et al. from India reported *S. aureus* as the dominant gram-positive pathogen (52%), followed by CoNS (32%), which is similar to our findings. [21] Tak et al. reported predominant isolation of *S. aureus* (53%) compared to CoNS (47%) from blood samples of critically ill trauma patients. [17] In the past, CoNS isolates were not considered clinically significant pathogens and were instead considered as contaminants that could potentially be introduced during blood collection. However, studies have shown that CoNS has recently emerged as a substantial etiological agent in bacteremia cases among immunocompromised patients.

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[22] In the present study, one-third of the Staphylococcal isolates were CoNS, comprising *S. epidermidis* (45%), *S. haemolyticus* (30%), and *S. hominis.* [25] Similar findings have been reported by other studies in which *S. epidermidis* was the most frequently isolated CoNS in BSIs (53.4%-62.4%), followed by *S. haemolyticus* (12.4%-25.8%) and S. hominis, comprising 6.5% -14%. [7,23]

The emergence of antimicrobial resistance in staphylococcal isolates, including high-priority pathogens such as MRSA and VRSA, provides a significant healthcare challenge due to their propensity for multidrug resistance, which consequently contributes to poor clinical outcomes. [24] In our study, more than two-thirds of the *S. aureus* isolates were found to be methicillin resistant. Findings from various studies from India and other countries were also in accordance with our study, indicating a prevalence rate of MRSA as 74.9 -76%. [18,25,26] Among the CoNS isolated during the study period, 40% were found to be methicillin resistant. Similar findings were reported by Khadri et al. and Mir et al., in which two-fifths of the CoNS isolates were methicillin resistant. [27,28]

Antibiograms of the Staphylococcal isolates in the present study revealed 100% susceptibility to linezolid and minocycline. Other antibiotics such as vancomycin, co-trimoxazole, doxycycline, tetracycline, and gentamicin were found to be effective, with susceptibility rates ranging between 81.7% and 98.3%. Moderate to high degrees of resistance were noted for antibiotics such as levofloxacin, clindamycin, azithromycin, and penicillin. Studies from India and the Maldives reported staphylococcal isolates exhibiting 100% susceptibility to vancomycin and linezolid. [21,29] An Indian study by Singh et al. reported Staphylococcal isolates showing 70.6 % susceptibility to levofloxacin and 63.8% susceptibility to gentamicin. [30] studies from Ethiopia reported staphylococcal isolates with a moderate degree of susceptibility to tetracycline, clindamycin, and erythromycin from patients with suspected BSIs. [31,32] Furthermore, we observed CoNS showing higher resistance to antibiotics viz., chloramphenicol, clindamycin, co-trimoxazole, gentamicin, and tetracycline, as compared to *S. aureus* isolates. Maharath et al. also observed higher antibiotic resistance rates to co-trimoxazole and gentamicin in CoNS isolates compared to *S. aureus* strains. [29] MRSA isolates were more resistant to antibiotics as compared to MSSA in the present study, which is consistent with the reports from other studies from India and China. [30,33,34] In the present study, 20 out of the total isolates of MRSA, accounting for 35.7%, demonstrated inducible clindamycin resistance as determined by the D-test. A study conducted by Maheshwari et al. at the same hospital observed that there was a 27.2% prevalence of inducible clindamycin resistance among MRSA strains in 2017. This finding indicates a rise in the identification of inducible clindamycin resistance among MRSA isolates. [35] Clindamycin is a preferred long-term oral antibiotic for MRSA-infected patients; however, the rise in erythromycin resistance, indicating the potential for transmission of cross-resistance, is of significant concern.

With the development of resistance in MRSA to all β-lactam antibiotics, the glycopeptide antibiotic vancomycin became the cornerstone of treatment against life-threatening MRSA infections. However, the emergence of strains that are resistant to this antibiotic was reported globally. Various factors contributing to the emergence of VISA and VRSA include inappropriate use in agriculture, hospitals, and husbandry and easy availability in medical stores without prescriptions. Therefore, it is imperative to prioritize the regulation of these practices, in conjunction with the implementation of precise laboratory techniques for assessing vancomycin susceptibility. We have evaluated vancomycin resistance among the MRSA strains by three phenotypic methods: the vancomycin disc diffusion test, the vancomycin screen agar test, and the E-test. Various studies reported the discrepancies in the results of vancomycin susceptibility through disc diffusion methods; thus, further confirmation was done by estimation of MIC by E-test. In our study, two MRSA strains were found to be resistant to vancomycin, as detected by the vancomycin disc diffusion method. Similar results were observed in a study carried out by Solanki et al., which detected one MRSA isolate that was resistant to vancomycin by the disc diffusion method. [26] In terms of vancomycin MIC, all the MRSA isolates were sensitive to vancomycin with a MIC range of <2 µg/ml. Similar findings were observed by Mehta et al., and Yadav et al. reporting 100% sensitivity to vancomycin in MRSA isolates (MIC <2 µg/ml). [34,36] The Infectious Disease Society of America MRSA guidelines 2011 recommended that in critically ill patients having vancomycin MIC >2 µg/l, an alternative to vancomycin should be used for management of significant risk of treatment failure. [19] It has also been concluded in a meta-analysis report that MRSA isolates having vancomycin MIC values of 1.5 or 2 µg/l were related to greater risk of treatment failure. Isolates identified as heterogenous VISA infections were also reported to be prevalent when the vancomycin MIC reaches 2 µg/l.[37]

Limitations

The present study included a small number of staphylococcal blood isolates, which may account for the few vancomycin-resistant bacteria detected.

Conclusion

The escalating incidence of MRSA isolates is a significant issue of utmost importance in the realm of public health. MRSA strains showed higher antibiotic resistance, especially multidrug resistance, thereby limiting treatment options. Additionally, the emergence of vancomycin resistance

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exhibited by MRSA isolates is worrisome, since vancomycin is the last resort for treatment of MRSA infections. Thus, novel antibiotics targeting MDR-Gram-positive bacteria should be prioritized. Most laboratories perform the antibiotic disc diffusion method, which cannot distinguish VISA from VRSA; hence, it should not be used to assess vancomycin susceptibility. All laboratories should evaluate vancomycin MIC values to help clinicians prescribe antibiotics. Hand hygiene, proper antibiotic usage, mask use, and regular intensive care unit disinfection can reduce MRSA transmission in hospitals.

Authors' contributions

The conceptualisation of the study and methodology was developed by MS, MK, MSh, and PB; RK was involved in data collection, and LSD conducted the data analysis. MS, MK, and MSh reviewed the findings and contributed to the final article. PB, LSD, and RK wrote the first draft, and M.S. edited it.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: None

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