



Received on 17 July 2026; received in revised form, 29 August 2026; accepted, 23 October 2026; published 01 November 2026

MINIMUM INHIBITORY CONCENTRATION OF LINEZOLID IN CLINICAL ISOLATES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AT A TERTIARY CARE HOSPITAL

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Keywords:

MRSA, Linezolid, Minimum inhibitory concentration, MIC50, MIC90, Antimicrobial resistance

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ABSTRACT: Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital and community-acquired infections, with substantial impact on infection rates, morbidity, mortality, and healthcare costs. Vancomycin, remained a longstanding cornerstone in MRSA treatment, but the emergence of vancomycin resistant MRSA (VRSA), necessitates alternative antimicrobial solutions. Linezolid, stands out as a promising candidate. Linezolid antibiotic is available as an oral formulation for resistant staphylococcal infections. It has unique advantages such as an absence of renal toxicity and improved lung parenchymal diffusion compared. However, emerging resistance to linezolid has become a concern worldwide. There is clonal dissemination of Linezolid Resistant *Staphylococcus aureus* (LRSA) within or across healthcare settings which demands continuous surveillance to determine the emergent risk of resistant strains and to establish guidelines for appropriate use. **Objectives:** To determine the minimum inhibitory concentration (MIC) of linezolid among MRSA isolates and to compare Kirby-Bauer disk diffusion method with MIC testing by agar dilution method for linezolid resistance detection. **Methods:** A hospital-based descriptive study was conducted over one year in the Department of Microbiology, Guru Gobind Singh Medical College & Hospital, Faridkot. Two hundred clinically significant MRSA isolates obtained from various clinical specimens were included. Identification and antimicrobial susceptibility testing were performed according to CLSI guidelines. Linezolid susceptibility was assessed using Kirby-Bauer disk diffusion and agar dilution MIC methods. **Results:** Of the 200 MRSA isolates, maximum MRSA were isolated from pus (67.5%) followed by blood (30.5%). All MRSA isolates were susceptible to linezolid by both disk diffusion and MIC methods. MIC distribution showed that 85.5% isolates had MIC ≤ 0.25 $\mu\text{g/mL}$, 8% had MIC 0.5 $\mu\text{g/mL}$, 3.5% had MIC 1 $\mu\text{g/mL}$, and 3% had MIC 2 $\mu\text{g/mL}$. No isolate demonstrated resistance (MIC ≥ 8 $\mu\text{g/mL}$). MIC50, MIC90, and geometric mean MIC were 0.25 $\mu\text{g/mL}$, 0.5 $\mu\text{g/mL}$, and 0.2952 $\mu\text{g/mL}$ respectively. Perfect agreement was observed between disk diffusion and agar dilution method (Kappa = 1). **Conclusion:** Linezolid demonstrated excellent in vitro activity against MRSA isolates in the present study. Routine surveillance of MIC trends remains essential for early detection of emerging resistance and prevention of linezolid MIC creep.

INTRODUCTION: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major multidrug-resistant pathogen responsible for a wide range of infections in both community and hospital settings¹.

MRSA has become a significant global public health problem². MRSA infections are associated with increased morbidity, mortality, prolonged hospital stay, and greater healthcare costs³.

MRSA strains are broadly classified into healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA)⁴. HA-MRSA commonly spreads in hospitals through direct contact with contaminated hands, wounds, or medical devices, while CA-MRSA is increasingly seen among healthy individuals and is often

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.17(11).3596-04</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(11).3596-04</p>
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associated with skin and soft tissue infections³. Several risk factors contribute to MRSA infection, including prolonged hospitalization, ICU admission, recent antibiotic use, invasive procedures, hemodialysis, HIV infection, open wounds, indwelling catheters, and residence in nursing homes⁵. Skin and soft tissue infections are the most common manifestations, but MRSA can also cause severe invasive diseases such as osteomyelitis, septic arthritis, infective endocarditis, bloodstream infections, ventilator-associated pneumonia, and necrotizing pneumonia. Community-associated MRSA is especially associated with cellulitis, diabetic foot ulcers, and necrotizing fasciitis^{6,7}.

Vancomycin has traditionally been considered the drug of choice for serious MRSA infections because most strains remain susceptible to it³. However, extensive use of glycopeptide antibiotics has led to the emergence of strains with reduced susceptibility to vancomycin, including vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA)⁸. Linezolid, the first member of the oxazolidinone class of antibiotics, has emerged as an important therapeutic option for MRSA infections, especially VISA and VRSA strains⁹. It acts by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit and blocking initiation of translation¹⁰. Unlike many other protein synthesis inhibitors, linezolid acts at the initial stage of protein synthesis, giving it a unique mechanism of action¹¹. It has excellent activity against multidrug-resistant Gram-positive bacteria and does not show cross-resistance with other antibiotic classes¹².

One of the major advantages of linezolid is its excellent oral bioavailability, allowing easy transition from intravenous to oral therapy^{10,13}. It is widely used for skin and soft tissue infections, diabetic foot infections, pneumonia, infective endocarditis, meningitis, and eradication of MRSA colonization¹⁰. Despite its effectiveness, resistance to linezolid has started emerging worldwide. The first case of linezolid-resistant *Staphylococcus aureus* (LRSA) was reported within a year of its clinical introduction¹⁴. Several laboratory methods are available for detection of linezolid resistance, including Kirby-Bauer disk diffusion, E-test, broth dilution, agar dilution, VITEK 2, PCR, and pulsed-

field gel electrophoresis¹⁵. In routine laboratories, disk diffusion is commonly used because it is simple and economical. However, disk diffusion results can sometimes be variable. Therefore, resistant isolates should be confirmed using MIC determination methods, which provide a more precise and quantitative assessment of antibiotic susceptibility¹⁶. Considering the increasing emergence of linezolid-resistant MRSA strains and the limitations of routine susceptibility testing methods, accurate detection of resistance has become crucial for effective patient management and infection control. Hence, the present study was undertaken to compare the disk diffusion method with MIC determination method for the detection of linezolid resistance in MRSA isolates.

MATERIALS AND METHODS: A hospital-based descriptive study was conducted over a period of one year from September 2024 to August 2025, in the Department of Microbiology, Guru Gobind Singh Medical College & Hospital, Faridkot, Punjab. Convenient sampling method was used. According to the hospital statistics, availability and feasibility, the first consecutive 200 MRSA isolates from patients of all age groups and both sexes were included. Repeat samples from the same patient were excluded for this study. The study was conducted after taking ethical approval from the institution's ethical committee (No.BFUHS/2K26p-TH/1792).

All the clinical specimens received in the Microbiology department were inoculated on culture plates [blood agar and MacConkey's agar; cystine lactose electrolyte deficient (CLED) agar for urine specimens] and incubated at 37 °C for 24-48 hours and direct microscopy was performed¹⁶.

Control Strains used in the Study: *S. aureus* ATCC 25923 and MRSA 43300 Methicillin resistance was detected using cefoxitin disc (30µg), which is a surrogate marker and a potent inducer of MECA gene. The strains of *S. aureus* with a zone diameter of ≤ 21 mm were considered as MRSA.

***S. aureus* Isolation and Identification**¹⁷: Identification was done by colony morphology and gram staining. Bacterial colonies showing Gram positive cocci arranged in clusters were further subjected to catalase test, slide and tube coagulase

test. The bacteria that were catalase positive and both slide and tube coagulase test positive were identified as *S. aureus*.

Antibiotic Susceptibility Testing (Antibiogram)

¹⁷: In accordance with CLSI standards, Kirby-Bauer's disc diffusion method was used to evaluate the antibiotic sensitivity pattern of all *S. aureus* isolates to different antibiotics. The antibiotics (Hi-Media Ltd., Mumbai, India) used were Ampicillin, 10µg; Erythromycin, 15µg; Cefoxitin, 30µg; Ciprofloxacin, 5µg; Amikacin, 10µg; Clindamycin, 2µg; Cotrimoxazole, 1.25/23.75µg; Linezolid, 30µg; Nitrofurantoin, 300µg; Norfloxacin, 10µg; and Vancomycin was tested by MIC agar dilution method.

Inoculum Preparation ¹⁷: 0.5 McFarland turbidity standard inoculum of the isolated strain was applied to Mueller-Hinton agar (MHA) plates and the plates were incubated at 37°C for 18-24 hours. The zone of inhibition diameters were then measured with the help of caliper and interpreted as per CLSI criteria. In case of cefoxitin, the plates were incubated for 24 hours ²⁶. Linezolid zone diameter ≥ 21 mm with a 30µg disc was considered as sensitive and ≤ 20 mm as resistant.

Maintenance and Preservation of Culture Strains ¹⁶: Organisms grown in appropriate media for 18 hours were preserved in brain heart infusion (BHI) broth with 20% glycerol and stored without significant loss of viability at 20°C until MIC was done.

Minimum Inhibitory Concentration of Linezolid by Agar Dilution Method ^{16, 17}: The minimum inhibitory concentration (MIC) of linezolid for *S. aureus* isolates was determined by agar dilution method. Mueller-Hinton agar (MHA) was supplemented with different concentration of linezolid according to CLSI guidelines.

Stock Solution Preparation: The following formula was used to create a stock solution of linezolid:

$$W = V \times C \times 1000 / P$$

Where, W = Weight of antibiotic to be dissolved, V = Volume, C = final Concentration of solution, P = Potency.

Using the following formula, $C_1 V_1 = C_2 V_2$, where:

C_1 = Concentration of linezolid stock solution, V_1 = Unknown Volume of stock solution needed to make the dilution concentration, C_2 = Required concentration of linezolid for dilution, V_2 = Required volume of concentration of linezolid for dilution, serial two-fold dilutions of linezolid were prepared: (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25) µg/mL above known concentrations of linezolid were incorporated in plates of mueller-hinton agar and each plate (with different concentration of linezolid) thus obtained was spotinoculated (using 10µL bacterial culture) to find out MIC of all the isolates. The plates were incubated for 24 hours at 37°C. The lowest concentration of linezolid agar plate that entirely prevented the growth of isolate was the minimum inhibitory concentration (MIC) of linezolid for that particular isolate. MIC ≥ 8 µg/mL were considered resistant; MIC of ≤ 4 µg/mL as susceptible to linezolid for MRSA isolates as per CLSI ¹⁷.

RESULTS: A total of 13816 samples were received in the department of Microbiology over one year and processed. Of all the specimens received, 33.4% were of blood, 32.4% of urine, 17.17% of pus, 11.01% specimens were of sterile body fluids, 4% specimens were of sputum, 1% were of catheter tip (1%), 0.6% of bronchoalveolar lavage, 0.4% of stool and 0.02% of biopsy tissue (0.02%). Maximum number of 61.13% *Staphylococcus aureus* (*S. aureus*) isolates were obtained from pus samples, then followed by 35.37% from blood, 2.62% from sterile body fluids and 0.43% from catheter tip and biopsy tissue.

Methicillin resistance using cefoxitin disc (30µg) method showed 90.83% *S. aureus* were Methicillin resistant *Staphylococcus aureus* (MRSA) and 9.17% methicillin sensitive *Staphylococcus aureus* (MSSA). Of all the MRSA isolated, first 200 consecutive MRSA strains were considered for this study.

More number of MRSA isolates were obtained from patients with age group 21-40 years (47%) followed by 27% from age group 41-60 years. Of all the MRSA strains isolated, 70.5% isolates were

from males and 29.5% from females. Male to female ratio was 2.39:1.

MRSA isolates were obtained from specimens received from medicine department (23.5%), followed by plastic surgery (17%).

Maximum MRSA were isolated from pus (67.5%), as shown in **Table 1**. **Fig. 1** shows majority of

TABLE 1: DISTRIBUTION OF MRSA ISOLATES AMONG VARIOUS CLINICAL SPECIMENS (N=200) AND SPECIMEN-WISE LINEZOLID MIC ANALYSIS (µg/mL)

Type of Clinical specimen	Number of MRSA Isolates	Percentage	MIC 50 (µg/mL)	MIC 90 (µg/mL)	Geometric Mean (µg/mL)
PUS	135	67.5%	0.25	0.50	0.318
Blood	61	30.5%	0.25	0.25	0.25
Pleural Fluid	2	1%	0.25	0.25	0.25
Cerebrospinal Fluid	2	1%	0.25	0.25	0.25
Total	200	100%	0.25	0.50	0.2952

Pus isolates demonstrated significantly higher MIC90 and geometric mean MIC values of **Table 1**.

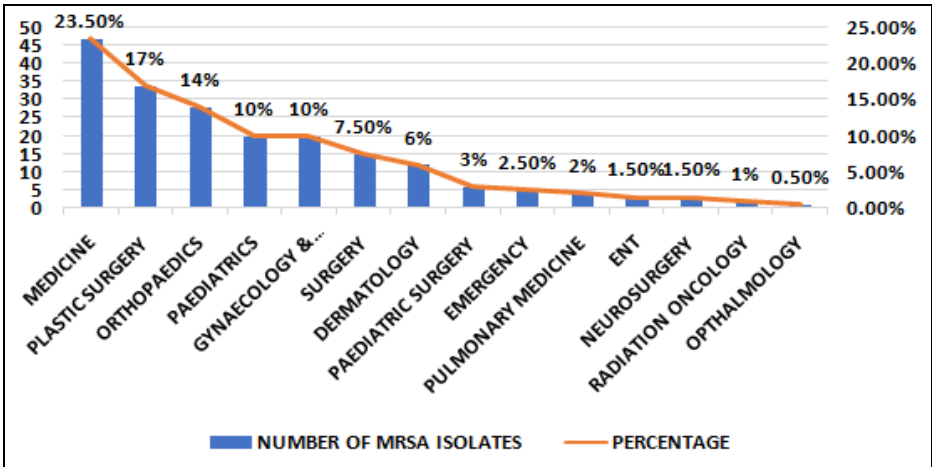


FIG. 1: DISTRIBUTION OF MRSA (n=200) IN VARIOUS CLINICAL DEPARTMENTS

The Antimicrobial Susceptibility Testing (AST) of 200 MRSA isolates showed hundred percent susceptibility to linezolid. The maximum resistance among MRSA isolates was observed against ampicillin (100%), followed by 92% resistance to ciprofloxacin **Fig. 2** and vancomycin was found to be susceptible by using MIC agar dilution method (MIC<2µg/ml). Inducible clindamycin resistance detected by D test was found to be positive for 25.2% of MRSA isolates **Table 2**.

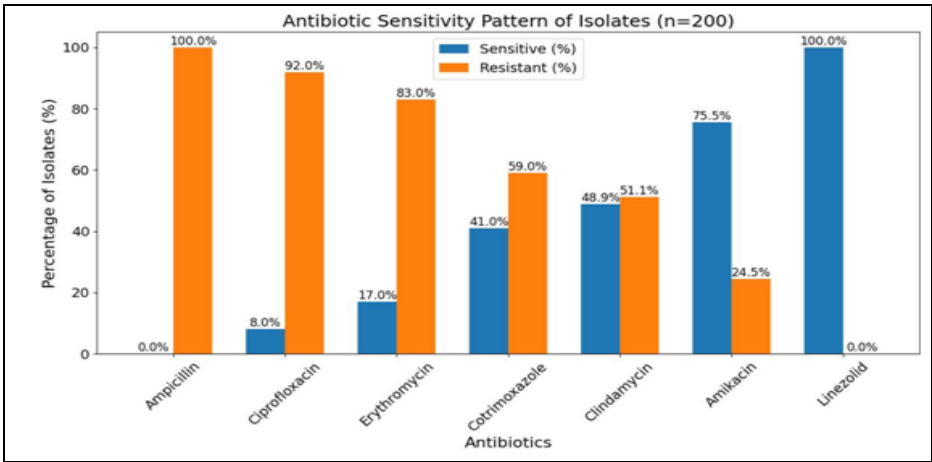


FIG. 2: ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF VARIOUS ANTIBIOTICS OF MRSA ISOLATES BY KIRBY BAUER DISK DIFFUSION (n=200)

TABLE 2: RESISTANCE PATTERN OF CLINDAMYCIN AMONG MRSA ISOLATES FROM PUS SAMPLES (n=135)

Resistance pattern of clindamycin	Number of MRSA isolates	Percentage
E-R; CD-R (MLSBc) Phenotype	69	51.11%
E-R; CD-R MLSBi Phenotype (D-Test Positive)	34	25.2%
E-R, CD-S MS Phenotype (D-Test Negative)	43	31.85%
E-S, CD-S	23	17.03%

E-R – Erythromycin resistant, CD-R – Clindamycin resistant, CD-S – Clindamycin sensitive. MLSBc – Macrolide-lincosamide-streptogramin resistant, (Constitutive resistance). MLSBi – Macrolide-lincosamide-streptogramin resistant, (Inducible resistance; D-test positive). MS phenotype – Macrolide, (Erythromycin) resistant; lincosamide (clindamycin) sensitive (D-test negative).

Linezolid minimum inhibitory concentration (MIC) determined using agar dilution method is shown in Table 2. No MRSA isolates showed MIC ≥ 4 µg/ml. Both MIC and disk diffusion method, showed 100% susceptibility to Linezolid in MRSA isolates Table 3.

MIC50, MIC90 and Geometric mean MIC of linezolid for all MRSA isolates were calculated to

be 0.25 µg/ml, 0.5 µg/ml and 0.2952 µg/ml respectively Fig. 3. MIC90 and geometric mean MIC of linezolid for pus isolates were significantly higher than other specimens Table 1.

Although statistically not significant, linezolid geometric mean MIC reduced from 0.3 µg/ml in the first six months to 0.29 µg/ml in the next six months Fig. 4.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION (MIC) OF LINEZOLID BY AGAR DILUTION METHOD (in µg/mL)

MIC value (in µg/mL)	NO. of MRSA Isolates	Percentage of MRSA Isolates
≤ 0.25	171	85.5%
0.5	16	8%
1	7	3.5%
2	6	3%
4	0	0%
Total	200	100%

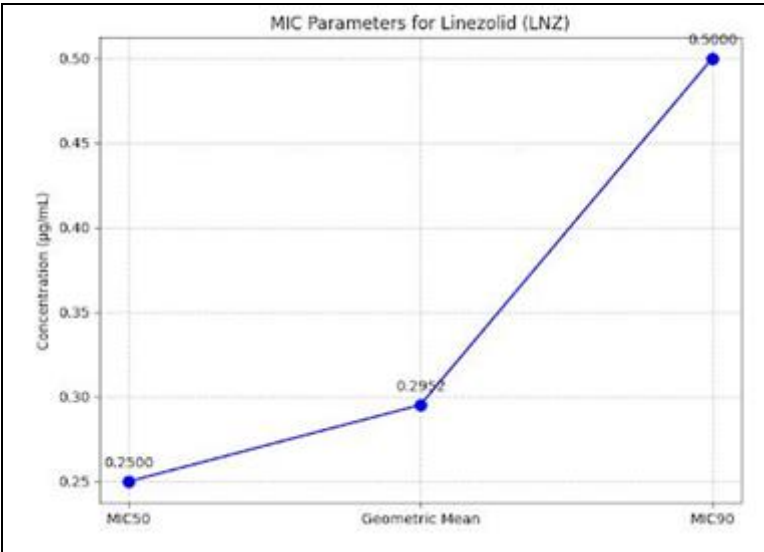


FIG. 3: MIC50, MIC90 AND GEOMETRIC MEAN MIC OF MRSA ISOLATES (n=200)

TABLE 3: COMPARISON OF DISC DIFFUSION AND MIC METHOD (AGAR DILUTION) FOR DETECTION OF LINEZOLID RESISTANCE IN MRSA ISOLATES

	Disc Diffusion	MIC
Sensitive	200	200
Resistant	0	0
Total	200	200

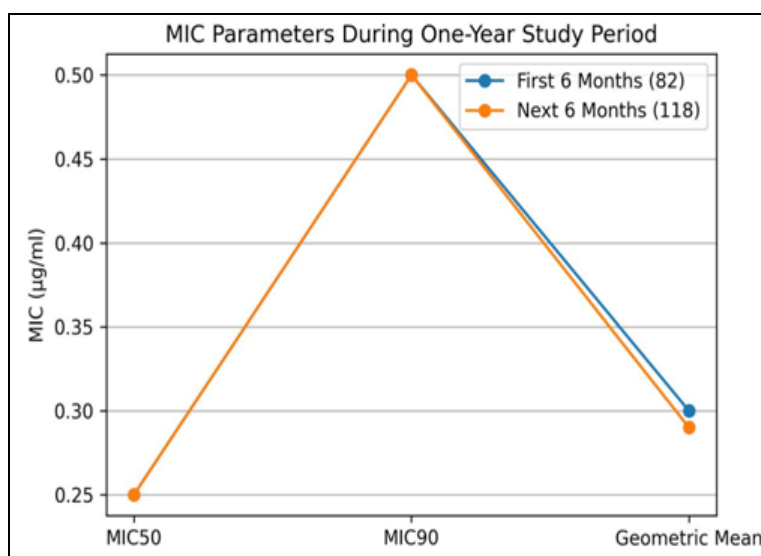


FIG. 4: MINIMUM INHIBITORY CONCENTRATION (MIC) ($\mu\text{g/mL}$) STATISTICS FOR LINEZOLID FROM 2024 TO 2025

DISCUSSION: Due to the emergence of VISA, hVISA, and VRSA strains, linezolid has become an important alternative for treating severe MRSA infections including hospital-acquired pneumonia, community-acquired pneumonia, and skin and soft tissue infections¹⁸. However, reports of reduced susceptibility to linezolid among gram-positive cocci have gradually emerged¹⁹. Resistance mechanisms of linezolid include mutations in the domain V region of the 23S rRNA gene, acquisition of the plasmid-mediated *cfr* gene, and alterations in ribosomal proteins L3 and L4. The most common mechanism is the G2576T point mutation in the 23S rRNA gene²⁰.

Minimum Inhibitory Concentration (MIC) is considered the best indicator for selecting appropriate antimicrobial therapy in serious infections²¹.

In the present study of the total 13,816 clinical specimens processed for culture and sensitivity testing, blood samples constituted the majority, followed by urine. Similar distributions have been reported in studies by Kansal R *et al.* and Regmi S *et al.*^{22, 23}. Of all the samples processed, 229 isolates (1.65%) were identified as *S. aureus*. The majority of these isolates were recovered from pus samples (61.13%) as similar to studies conducted by Sharlee R *et al.*, and Moses VK *et al.* where pus samples yielded the highest number of *S. aureus* isolates^{9, 24}. In the present study, 90.83% of isolates were identified as MRSA, while only

9.17% were methicillin-sensitive *Staphylococcus aureus* (MSSA). The prevalence of MRSA in this study was significantly higher than that reported in many previous studies, although similar high prevalence was observed by Kansal R *et al.*²³. Variations in MRSA prevalence across studies may be attributed to environmental factors, irrational antibiotic use, differences in infection control practices, and varying inclusion criteria. First 200 consecutive MRSA isolates were included in this study.

Most MRSA isolates were obtained from patients aged 21–40 years, followed by those aged 41–60 years, which correlated with the findings of Kansal R *et al.*²³. Male patients accounted for 70.5% of MRSA isolates, consistent with studies conducted by Arumugam A *et al.* and Sharlee R *et al.*^{8, 9}. Among clinical specimens, pus samples contributed the majority of MRSA isolates followed by blood, pleural fluid, and cerebrospinal fluid. This is in accordance with studies conducted by Kansal R *et al.*, Moses VK *et al.*, and Husain A *et al.*^{23, 24, 25}. Surgical departments such as plastic surgery, general surgery, pediatric surgery, and neurosurgery accounted for the highest proportion of MRSA isolates, followed by medicine and orthopedics departments. Similar trends were observed in studies conducted by Kansal R *et al.*, Moses VK *et al.*, and Srinivasan S *et al.*^{23, 24, 26}.

Antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method demonstrated

extensive multidrug resistance among MRSA isolates. All isolates were resistant to ampicillin, while high resistance rates were observed for ciprofloxacin (92%), erythromycin (83%), cotrimoxazole (59%) and clindamycin (51.11%). For amikacin resistance rate was 24.5%. Similar resistance patterns have been reported by Chitnis S *et al.* and Bawankar NS *et al.*^{27, 28}. Importantly, no resistance to linezolid was detected in the present study. Studies conducted by Niveditha N *et al.*, Xu Yanlei *et al.*, Arumugam A *et al.*, Husain A *et al.*, and Chitnis S *et al.*, also reported 100% susceptibility of MRSA isolates to linezolid^{2, 5, 8, 25, 27}. In the current investigation, 25.2% of MRSA isolates showed inducible clindamycin resistance, which is higher than 14% inducible resistance observed by Kansal R *et al.*, from the same institution in 2024 indicating the declining susceptibility to clindamycin, and erythromycin²³. While Sharma V *et al.*, Sharma NK *et al.*, and Kumar R *et al.* reported similar percentages of 33.7%, 21.38%, and 27%^{29, 30, 31}.

All MRSA isolates were evaluated for vancomycin susceptibility using the agar dilution MIC method. Every isolate showed vancomycin MIC values below 2 µg/mL, indicating 100% susceptibility. Similar findings have been documented by Anitha TK *et al.*, Husain A *et al.*, Xu Yanlei *et al.*, Niveditha N *et al.*, and Hortiwakul R *et al.*^{21, 25, 32, 33, 34}.

The major focus of the study was the evaluation of linezolid susceptibility using both Kirby–Bauer disk diffusion and agar dilution MIC methods. All MRSA isolates showed inhibition zone diameters greater than 21 mm with linezolid discs, confirming susceptibility by disk diffusion testing. MIC values ranged from ≤0.25 µg/mL to 2 µg/mL. The majority of isolates (85.5%) demonstrated MIC values ≤0.25 µg/mL, while smaller proportions showed MICs of 0.5 µg/mL, 1 µg/mL, and 2 µg/mL. No isolate demonstrated MIC values greater than 4 µg/mL. These findings were comparable to those reported by Arumugam A *et al.*⁸, although Bawankar NS *et al.* reported higher MIC values and even linezolid-resistant isolates²⁸.

A perfect concordance was observed between disk diffusion and MIC methods with 100% percentage agreement. Similar findings were reported in

several Indian and international studies^{8, 9, 10, 25, 27, 32, 33, 34, 35}. Although disk diffusion is more practical for routine laboratory use, MIC determination remains important for detecting early shifts in susceptibility and possible emergence of resistance²⁸. While no linezolid-resistant MRSA isolates were identified in this study, some isolates had MIC values at the upper end of the susceptible range, which is concerning because previous studies by Bawankar NS *et al.*, Husain A *et al.*, and Wali M *et al.* have documented reduced susceptibility and frank resistance to linezolid^{25, 28, 36}.

The study calculated linezolid MIC₅₀, MIC₉₀, and geometric mean MIC values as 0.25 µg/mL, 0.5 µg/mL, and 0.2952 µg/mL respectively. Pus isolates showed significantly higher MIC₉₀ and geometric mean MIC values compared to blood and fluid isolates, similar to observations made by Xu Y *et al.*³². Over the study period, MIC₅₀ and MIC₉₀ remained stable while geometric mean MIC showed a slight decrease, unlike the “MIC creep” reported by Husain A *et al.*, and Miyazaki M *et al.*^{25, 37}.

CONCLUSION: The study concludes that linezolid remain highly effective against MRSA isolates in this institution. However, MIC values on higher side of the susceptible range highlight the need for continuous surveillance, antibiotic stewardship, and strict infection control practices. Linezolid should be reserved for severe MRSA infections. The study also acknowledges limitations including its single-center design, predominance of pus samples, lack of molecular typing methods, and use of agar dilution instead of more precise gradient methods like E-test. Further multicentric studies with molecular analysis are recommended to better understand resistance patterns and clonal dissemination of MRSA strains.

ACKNOWLEDGEMENT: None

Ethical Approval: The study was performed after taking approval from the institution’s Ethical committee.

Funding Source: NIL

A.I used: None

CONFLICTS OF INTEREST: None**REFERENCES:**

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How to cite this article:

Gogoi R, Sharma V and Arora S: Minimum inhibitory concentration of linezolid in clinical isolates of methicillin-resistant *Staphylococcus aureus* at a tertiary care hospital. Int J Pharm Sci & Res 2026; 17(11): 3596-04. doi: 10.13040/IJPSR.0975-8232.17(11).3596-04.

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