International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Mic Pattern of Vancomycin by E-Test in Methicillin- Resistant Staphylococcus Aureus from Various Clinical Samples in A Tertiary Care Hospital.

Ms. Farah Haseen[1], Dr. Sana Siddique[2], Dr. Vineeta Khare[3], Ms. Anjali Gupta[4]

[1,4]Department of Microbiology, Era University, Lucknow, Uttar Pradesh (Farahhaseen5@gmail.com)
[3]Professor & Head of Department, Department of Microbiology, Era University, Lucknow (U.P.)
[2]Asst. Professor, Assistant Professor, Department of Microbiology, Era University, Lucknow (U.P.)

Abstract

Introduction:

Staphylococcus aureus is one of the leading causes of both community-acquired and hospital-acquired infections. The emergence of Methicillin-resistant Staphylococcus aureus (MRSA) has become a major therapeutic challenge due to its resistance to multiple antibiotics. Vancomycin remains the drug of choice for the treatment of MRSA infections; however, increasing minimum inhibitory concentration (MIC) values within the susceptible range have raised concerns regarding reduced therapeutic efficacy, treatment failure, and the emergence of vancomycin resistance. Therefore, continuous monitoring of vancomycin MIC among MRSA isolates is essential for effective antimicrobial stewardship and patient management.

Objectives:

- To isolate methicillin-resistant Staphylococcus aureus from various clinical samples.
- To study the minimum inhibitory concentration (MIC) pattern of vancomycin by E-Test in methicillin-resistant Staphylococcus aureus strains.

Methods:

A cross-sectional study was conducted in the Department of Microbiology, Era's Lucknow Medical College & Hospital, Lucknow, from November 2024 to April 2025. A total of 95 non-duplicate MRSA isolates obtained from various clinical specimens were included in the study. MRSA isolates were identified using the cefoxitin (30 µg) disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. The minimum inhibitory concentration (MIC) of vancomycin was determined using the Epsilometer (E-test) method following standard laboratory procedures.

Results:

All 95 MRSA isolates were found to be susceptible to vancomycin, and no Vancomycin-resistant Staphylococcus aureus (VRSA) isolate was detected. Although majority of isolates demonstrated a vancomycin MIC of 1 µg/mL (66.3%) but higher MIC values within the susceptible range were also

observed in a subset of isolates, including MICs of 1.5 µg/mL (8.4%), 2 µg/mL (20%), and 3 µg/mL (5.3%). Even if all isolates remained susceptible according to CLSI criteria, the distribution of higher MIC values indicated a shift towards elevated vancomycin MICs among MRSA isolates.

Conclusion:
The present study demonstrated that all MRSA isolates remained susceptible to vancomycin, with no VRSA. However, the occurrence of higher MIC values within the susceptible range indicates a possible trend toward vancomycin MIC creep, which may adversely affect clinical outcomes if left unmonitored. Regular surveillance of vancomycin MIC patterns, along with judicious antibiotic use and antimicrobial stewardship practices, is recommended to prevent treatment failure and limit the emergence of vancomycin resistance. Further molecular studies are warranted to investigate the underlying mechanisms of reduced vancomycin susceptibility among MRSA isolates.

Keywords:
Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin, Minimum inhibitory concentration (MIC), E-test, Vancomycin-resistant Staphylococcus aureus (VRSA), Antimicrobial resistance.

1. Introduction

Background of the study:

Staphylococcus aureus is a common human flora present on the skin and mucous membranes, particularly the nasal cavity but also a major human pathogen. In healthy individuals, colonization is usually asymptomatic; however, when the organism breaches mucosal barriers, it can cause infections ranging from mild skin and soft tissue infections to life-threatening conditions such as pneumonia, meningitis, endocarditis, bacteraemia, toxic shock syndrome and sepsis.[1,2]

The development and spread of antibiotic resistance in bacterial pathogens constitute a substantial human health hazard worldwide, making antimicrobial resistance a serious public health problem. Staphylococcus aureus proves to be a prime example of this quandary because of its capacity to acquire resistance to a variety of antimicrobial drugs.[3] Sir Alexander Fleming's discovery of penicillin marked the beginning of the modern antibiotic era, and it was widely used for the treatment of Staphylococcus aureus infections.[4] However, the unsystematic use of penicillin during and after the Second World War led to the emergence of penicillin-resistant Staphylococcus aureus strains.[5] Subsequently, methicillin-resistant Staphylococcus aureus (MRSA) emerged during the late 1960s.[6] MRSA strains possess the mecA gene, which confers resistance to methicillin and other β-lactam antibiotics, making them multidrug resistant.[1,7,8] MRSA has long been recognized as an important nosocomial pathogen causing hospital-acquired MRSA (HA-MRSA), while in recent years it has also emerged as a significant cause of community-acquired MRSA (CA-MRSA) infections.[9]

The prevalence of MRSA varies considerably across different geographical regions, ranging from approximately 1% to more than 70% globally. In India, the prevalence of MRSA in healthcare settings ranges from 30% to 60%, highlighting its growing clinical importance.[7]

Thus, in order to treat MRSA infections effectively, Vancomycin has emerged as an essential therapeutic

option.[1,8] Although its clinical use was initially limited because of the availability of β-lactam antibiotics, the increasing prevalence of β-lactam resistance has restored its importance in clinical practice.[9] Vancomycin exerts its antibacterial activity by inhibiting late-stage peptidoglycan synthesis in Gram-positive bacteria through binding to the terminal D-Ala-D-Ala residues of cell wall precursors.[10]

Staphylococcus aureus develops vancomycin resistance through acquisition of van gene clusters, particularly the VanA gene, resulting in alterations of cell wall precursors and reduced binding affinity of vancomycin to its target site.[9] Strains with reduced susceptibility to vancomycin were first reported from Japan, followed by the first report of vancomycin-resistant Staphylococcus aureus (VRSA) from the United States in 2002. Since then, vancomycin-intermediate Staphylococcus aureus (VISA) and VRSA have been increasingly reported worldwide.[11] Because vancomycin is considered the drug of last resort for MRSA infections, the emergence of reduced susceptibility or resistance is of major clinical concern.[1]

The laboratory identification of MRSA and assessment of vancomycin susceptibility are essential for appropriate patient management. The Clinical and Laboratory Standards Institute (CLSI) recommends the cefoxitin disc diffusion method for the detection of MRSA.[12] Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent capable of inhibiting visible bacterial growth, and determination of vancomycin MIC by methods such as the E-test is recommended for all Staphylococcus aureus isolates.[13] According to CLSI criteria, isolates are classified as vancomycin-susceptible Staphylococcus aureus (VSSA) with MIC ≤2 µg/mL, vancomycin-intermediate Staphylococcus aureus (VISA) with MIC 4–8 µg/mL, and vancomycin-resistant Staphylococcus aureus (VRSA) with MIC ≥16 µg/mL.[12]

Need of the Study: Although most MRSA isolates remain classified as vancomycin susceptible, increasing clinical failures have been reported among isolates exhibiting elevated minimum inhibitory concentration (MIC) values within the susceptible range, particularly those with MICs greater than 1 µg/mL[11,13]. Considering that vancomycin MIC patterns vary between hospitals and geographical regions, continuous monitoring of MIC trends is essential for the early detection of reduced susceptibility, prevention of treatment failure, and optimization of antimicrobial therapy. Therefore, the present study was undertaken to determine the vancomycin MIC pattern among MRSA isolates using the E-test method in a tertiary care hospital.

Inclusion Criteria: -
All clinical samples from patients attending the IPD and OPD of all age group were included in this study.

Exclusion Criteria;-
- Methicillin sensitive Staphylococcus aureus strains were not included.
- Duplicate isolates from the same patient received within a week were not included in the study.

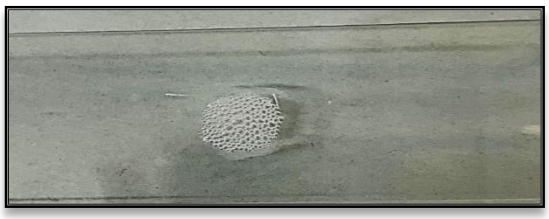
Data Collection & Sample Collection: -
Data was collected and the following details were noted-- Patient name, age, sex and brief clinical history. All clinical samples (pus, sputum, wound and soft tissue, urine, blood) were collected using aseptic

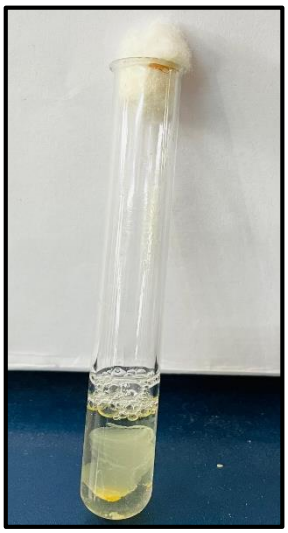
technique as per standard microbiology laboratory protocol with the informed written consent of the patient.

Sample Processing: Clinical specimens were processed under aseptic conditions. A direct smear was prepared from each sample, followed by inoculation onto appropriate culture media, including Blood agar, MacConkey agar, and Cysteine Lactose Electrolyte Deficient (CLED) agar for urine samples. The inoculated media were incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified based on colony morphology and standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.[20] Methicillin-resistant Staphylococcus aureus (MRSA) isolates were identified using the cefoxitin disk diffusion method, and the minimum inhibitory concentration (MIC) of vancomycin was determined by the E-test method following CLSI 2024 guidelines.[12]

Isolation and Identification of Staphylococcus aureus

Clinical specimens were processed under aseptic conditions. Direct Gram staining was performed on the specimens, and samples were cultured on Blood agar, MacConkey agar, and Cysteine Lactose Electrolyte Deficient (CLED) agar for urine specimens. The inoculated plates were incubated aerobically at 37°C for 24–48 hours. Presumptive Staphylococcus aureus isolates were identified based on colony morphology, Gram staining, Catalase test, and Coagulase test (slide and tube coagulase) using standard microbiological procedures and following standard laboratory guidelines.[2,12]





[Figure 1. Catalase positive test;
Figure 2. Tube Coagulase positive test]

Detection of MRSA

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines.[12] Methicillin resistance was determined using the cefoxitin (30 µg) disc, and isolates were categorized as methicillin-resistant Staphylococcus aureus (MRSA) based on CLSI interpretative criteria.[12]

Determination of Vancomycin MIC by E-test

The minimum inhibitory concentration (MIC) of vancomycin for all MRSA isolates was determined using the Epsilometer test (E-test). A 0.5 McFarland bacterial suspension was prepared, inoculated onto Mueller–Hinton agar plates, and the vancomycin E-test strip was applied to the agar surface. After that it was incubated at 37°C for 18–24 hours & the MIC was recorded at the point where the elliptical zone of inhibition intersected the scale on the strip. Interpretation of MIC values was performed according to CLSI 2024 guidelines.[12]

[Figure 3. MHA plate of MRSA showing 1 µg /ml MIC of Vancomycin

Figure 4. MHA plate of MRSA showing 3 µg /ml MIC of Vancomycin]

Results:

A total of 300 Gram-positive cocci (GPC) isolates were received in the Department of Microbiology during the study period. Of these, 200 isolates were identified as S. aureus based on standard clinical methods. Among the 200 S. aureus isolates, 95 were confirmed as methicillin-resistant S. aureus (MRSA) using the cefoxitin (30 µg) disk diffusion method according to CLSI 2024 guidelines.

The prevalence of MRSA was higher among female patients as compared to male patients. The maximum number of MRSA isolates was observed in the 20–29 years age group. Among the various clinical specimens, pus was the most common source of MRSA isolates. Of the 95 MRSA isolates, the majority were obtained from admitted (IPD) patients, with the highest number originating from the Surgery ward. [Table -1]

Table 1. Distribution of MRSA based on different parameters.

Parameter	Category	Number of MRSA isolates, n (%)
Gender	Male	37 (38.9)
	Female	58 (61.1)

Age Group (Years)	0–15	18 (18.9)
	16–30	37 (38.9)
	31–50	24 (25.3)
	>50	16 (16.8)
Clinical Specimen	Pus	53 (55.8)
	Urine	29 (30.5)
	Sputum	4 (4.2)
	Blood	2 (2.1)
	Catheter Tip	1 (1.1)
	Others	6 (6.3)
Hospital Department	Surgery	23 (24.2)
	Medicine	14 (14.7)
	ICU	6 (6.3)
	Orthopaedics	3 (3.2)
	OPD	37 (38.9)
	Others	12 (12.6)

The antimicrobial resistance pattern of the 95 methicillin-resistant Staphylococcus aureus (MRSA) isolates against the tested antimicrobial agents is presented in **Table 2**. All isolates were resistant to cefoxitin, confirming their methicillin-resistant phenotype. The highest resistance was observed against amoxicillin–clavulanate, followed by levofloxacin and ciprofloxacin, whereas the lowest resistance was observed against doxycycline and amikacin. No resistance was observed against linezolid and teicoplanin among the MRSA isolates.

Note 1: Norfloxacin susceptibility testing was performed only for urinary MRSA isolates (n = 29). All 95 MRSA isolates were susceptible to Linezolid and Teicoplanin; therefore, no resistant isolate was observed against these antimicrobial agents.

Table 2. Antimicrobial resistance pattern of Methicillin-resistant S. aureus (MRSA)

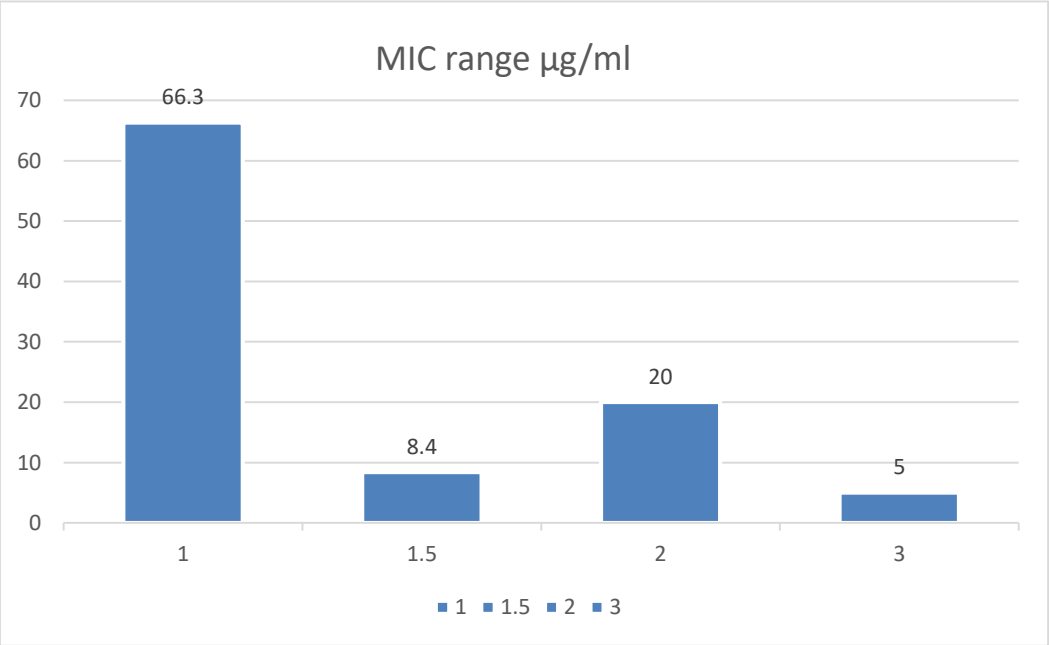
Antibiotic Disc	**MRSA, n (%)**
Cefoxitin	95 (100)
Amikacin	2 (2.1)
Doxycycline	1 (1.1)
Ciprofloxacin	48 (50.5)
Levofloxacin	54 (56.8)
Gentamicin	6 (6.3)
Amoxicillin–Clavulanate	62 (65.3)
Clindamycin	5 (5.3)
Norfloxacin	25 (86.2)

The distribution of vancomycin minimum inhibitory concentration (MIC) values among the 95 MRSA isolates is presented in **Table 3**. The majority of MRSA isolates demonstrated a vancomycin MIC of 1 µg/mL, followed by 2 µg/mL and 1.5 µg/mL. A small proportion of isolates exhibited an MIC of 3 µg/mL. No isolate showed an MIC within the VISA (4–8 µg/mL) or VRSA (≥16 µg/mL) range, indicating that all MRSA isolates were susceptible to vancomycin according to CLSI 2024 criteria.

According to CLSI 2024 guidelines, Staphylococcus aureus isolates with vancomycin MIC ≤2 µg/mL are classified as vancomycin-susceptible Staphylococcus aureus (VSSA)**,** MIC 4–8 µg/mL as vancomycin-intermediate Staphylococcus aureus **(**VISA**)**, and MIC ≥16 µg/mL as vancomycin-resistant Staphylococcus aureus (VRSA). Although no VRSA was detected yet the increased range of MIC among VSSA was observed which is the initial turning point towards development of VRSA.

Table 3. Observation of Vancomycin MIC in MRSA

Vancomycin MIC (µg/mL)	MRSA isolates, n (%)
1.0	63 (66.3)
1.5	8 (8.4)
2.0	19 (20.0)
3.0	5 (5.3)



[Figure 5. MIC range for Vancomycin]

2. Discussion:

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as one of the most important causes of both hospital- and community-acquired infections, contributing significantly to increased morbidity and mortality worldwide. The CLSI recommends detection of the mecA gene by PCR as the gold standard for the diagnosis of MRSA; however, its routine use is limited in resource-constrained settings due to financial and technical constraints. Consequently, phenotypic methods such as cefoxitin disc diffusion and E-test continue to be widely employed for the identification of MRSA and determination of vancomycin susceptibility. [14,15]

Vancomycin remains the cornerstone for the treatment of severe MRSA infections. Nevertheless, treatment failures have increasingly been reported despite isolates remaining susceptible according to current CLSI breakpoints, emphasizing the importance of monitoring minimum inhibitory concentration (MIC) values. Among the available phenotypic methods, the E-test provides a reliable and practical approach for MIC determination, thereby assisting clinicians in selecting appropriate antimicrobial therapy and improving patient outcomes, particularly in critically ill patients. [16,17]
The present study was conducted in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow, to evaluate the vancomycin MIC pattern among MRSA isolates using the E-test method. During the six-month study period, a total of 95 MRSA isolates were recovered. The prevalence of MRSA was higher among female patients (61%) than male patients (39%). Similar findings were reported by Sharma S et al. (2012), who also observed a higher proportion of MRSA isolates among female patients (60.86%) compared to males (39.13%). [18]

The present study demonstrated the highest prevalence of MRSA among the 20–29 years age group (31.6%), followed by 10–19 years (14.7%), 30–39 years (13.7%) and patients aged >60 years (12.6%), whereas the lowest prevalence was observed in the 50–59 years age group (4.2%). Comparable findings were reported by Sarrafzadeh et al. (2016), who observed the highest prevalence among individuals aged 19–40 years, followed by those aged >55 years. [18] Similarly, Moses VK et al. (2020) reported the highest isolation of MRSA among patients aged 20–50 years, followed by the 0–19 years age group. [12]

The predominance of MRSA among females and young adults observed in the present study may be attributed to increased exposure to skin trauma and micro-abrasions associated with personal care practices, shaving, tattooing, and body piercing, which facilitate colonization and subsequent infection by Staphylococcus aureus.

3. Recommendation:

To gain a more comprehensive understanding of these findings and their broader implications, future research should involve long-term surveillance and incorporate molecular diagnostic methods for more precise evaluation of vancomycin MIC trends.

4. Conclusion:

Although no vancomycin-resistant Staphylococcus aureus (VRSA) isolates were detected in this study, variations in minimum inhibitory concentration (MIC) were observed within the susceptible range. An increase in MIC values above 1 µg/mL may be indicative of vancomycin overuse in clinical settings, while values exceeding 2 µg/mL could suggest the early stages of MIC creep.

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