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THE STUDY OF CHANGING TREND IN MINIMUM INHIBITORY CONCENTRATION (MIC) OF VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS (VRSA) BY AGAR DILUTION METHOD AND ITS COMPARISON WITH E-TEST

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ABSTRACT **Background:** The emergence of Vancomycin-Resistant Staphylococcus aureus (VRSA) among Methicillin-Resistant Staphylococcus aureus (MRSA) strains is a significant concern in the medical industry. Vancomycin and other glycopeptide antibiotics are frequently used to treat infections caused by methicillin-resistant Staphylococcus aureus. In recent years, the incidence of vancomycin intermediate S. aureus (VISA) and vancomycin resistant S. aureus (VRSA) has increased in many parts of the world. Accurate calculation of the Minimum Inhibitory Concentration (MIC) is critical for confirming VRSA and directing suitable treatment. This study examines the accuracy and utility of two commonly used MIC measurement techniques: agar dilution and the E-test strip method. **Aim And Objective:** A comparison of the agar dilution method and the e-test strip method for determining the minimum inhibitory concentration (MIC) to confirm VRSA among MRSA. **Material And Methods:** This was an observational cross-sectional study conducted in the Department of Microbiology at Government Medical College, Kota, Rajasthan. The samples were processed immediately on receiving the lab, if there was a delay, they were refrigerated at 40-70Celsius. A total of 185 clinical MRSA isolates were screened from 384 Staphylococcus aureus isolates, and vancomycin MICs were determined using both agar dilution and E-test strip procedures in accordance with CLSI guidelines. **Results:** In the current investigation, the Vancomycin screen agar method was used on 185 clinical isolates of MRSA, 6 (3.24%) of which were identified as VRSA. Out of the 185 MRSA isolates, two (1.08%) were VRSA and three (1.62%) were VISA. The incidence of VRSA was discovered to be 1.62%. Both the agar dilution and E-test strip methods identified 180 (97.30%) VSSAs. The MIC values of the 185 MRSA were determined using both the agar dilution method and the E-test strip method. In this work, the Agar dilution method was used as the gold standard for MIC determination. It produces exact and consistent results but is laborious and time-consuming. The e-test strip approach is a more convenient and faster option. It is simple to execute and analyse, however the findings may vary compared to the agar dilution approach. **Conclusion:** Both the agar dilution method and the E-test strip approach are suitable for determining MIC values. Both the agar dilution and E-test strip methods provide slightly different MIC values. The e-test strip method is easier to use than the agar dilution method. The agar dilution method is time consuming and labor-intensive, yet it is a reliable gold standard approach for MIC determination. The optimum clinical management of VRSA infections requires the use of an appropriate MIC determination method.

KEYWORDS : MRSA, VRSA, VISA, MIC determination, agar dilution, E-test strip

INTRODUCTION

One of the most pressing concerns in modern medicine is the growing prevalence of antibiotic resistance in bacterial infections. MRSA, which stands for methicillin-resistant Staphylococcus *aureus,* has been a significant challenge for a long time due to its resistance to a variety of treatments, including methicillin. The development of vancomycin-resistant *Staphylococcus aureus* (VRSA) in recent years has made treatment regimens even more problematic. Because vancomycin has been the cornerstone for treating MRSA infections, the development of resistance to this antibiotic severely limits the treatment options available and emphasises the importance of utilising precise diagnostic approaches to detect the existence of VRSA infection [1, 2].

The determination of the Minimum Inhibitory Concentration (MIC) is an important step in classifying bacterial isolates as antibiotic-resistant or antibiotic-susceptible. Agar dilution and the E-test strip technique are two popular methods for determining the minimum inhibitory concentration (MIC). The agar dilution technique, commonly considered as the gold standard, involves adding different amounts of an antibiotic to an agar medium and then assessing the growth of bacteria. Despite its reliability, the proposed approach is labor-intensive and time-consuming. The E-test strip technique, on the other hand, is a more convenient and quick alternative. This method includes applying a gradient of antibiotic concentrations to a strip, which is then put on an infected agar plate. The result is a minimum inhibitory concentration (MIC) measurement at the intersection of the bacterial growth inhibition ellipse[1].

In spite of the fact that both approaches are designed to precisely identify the MIC, differences between them might have an effect on therapeutic judgments. The incorrect categorization of VRSA may result in the selection of improper treatments, the subsequent spread of resistant strains, and unfavorable consequences for patients. For this reason, it is very necessary to analyze and contrast the performance of different approaches in order to guarantee accurate MIC determination.

An Overview of the Problem of Antibiotic Resistance

Antibiotic resistance has become a global hazard to people's health, jeopardising the efficacy of treatments that were once capable of curing bacterial illnesses with amazing regularity. This growing threat complicates a wide range of medical therapies that rely on effective antibiotic prophylaxis, undermining contemporary medicine's fundamental triumphs. Infections that were once manageable are now potentially lethal. Antibiotic resistance is caused by a number of methods that bacteria can use either alone or in combination. Bacteria produce enzymes that degrade or alter antibiotics, rendering them ineffective. Bacteria do this mostly through enzymatic degradation. Beta-lactamase enzymes are an excellent example since they have the ability to degrade beta-lactam medicines like penicillin. Alterations to target sites are another approach. bacterium have the ability to change certain places inside their cells that antibiotics target, which may result in a reduction in the drug's ability to attach to the bacterium. The mecA gene, which produces an altered penicillin-binding protein (PBP2a) with a decreased affinity for beta-lactam antibiotics, is a good example of this. MRSA is a particularly good example of this [3].

Efflux pumps, another type of resistance mechanism, allow antibiotics to be expelled from the cell before reaching their targets. This enables bacteria to transmit resistance to multiple drugs at the same time. Bacteria may also have lower permeability by modifying their cell membranes to prevent antibiotics from entering their cells. Porin proteins, which control the passage of substances into and out of the bacterial cell, are frequently modified to achieve this purpose. Biofilms are complex communities that exist within a protective extracellular matrix. These biofilms prevent antibiotics from reaching the bacteria and protect them from both the host's immune response and antimicrobial medications. Certain bacteria create biofilms [4].

Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Staphylococcus aureus* (VRSA) are two varieties of resistant bacteria that are among the most worrying. A substantial contributor to morbidity and death on a global scale, methicillin-resistant *Staphylococcus aureus* (MRSA) was primarily a hospital-acquired illness but has now expanded to community settings. Because methicillin is not effective against MRSA, which is resistant to all beta-lactam antibiotics, it is necessary to use other therapies such

as vancomycin. On the other hand, the appearance of vancomycin-resistant strains of the bacterium VRSA has caused the medical community to express widespread concern. The vanA gene, which modifies the terminal amino acid residues of peptidoglycan precursors and thereby reduces the binding affinity of vancomycin, is one of the resistance mechanisms that VRSA strains have developed.

The global spread of antibiotic-resistant bacteria has resulted in increased death rates, longer hospital stays, and higher medical costs, all of which pose a substantial risk to public health. This puts further load on healthcare systems since resistant infections complicate the treatment of common infectious diseases. Further complicating matters is the fact that the pipeline for new antibiotics is insufficient to keep up with the rapid development of resistance. The development of effective treatment options and the mitigation of the effects of these resistant strains necessitates a full understanding of their mechanisms and prevalence. The promotion of antibiotic stewardship, which assures the appropriate use of antibiotics in both healthcare and agriculture, and the improvement of global surveillance systems to monitor the spread of resistant strains, the investment in the research and development of new antibiotics and alternative therapies such as bacteriophages and immunotherapies, the improvement of infection control practices in healthcare settings, and the education of the general public on the significance of responsible antibiotic use and the problems that are associated with antibiotic resistance are all important strategies [5,6].

In order to guarantee the continuous effectiveness of antimicrobial drugs and to protect the health of people all over the world, it is necessary to take a coordinated and multidisciplinary approach to the issue of antibiotic resistance. It is vital to have a comprehensive understanding of the complicated processes and the prevalence of resistance infections across the world in order to successfully address this catastrophe.

Considering the Importance of Vancomycin in the Treatment of MRSA

Vancomycin has long been considered the best antibiotic for treating MRSA infections. This is especially true in circumstances where other antibiotics are rendered ineffective due to resistance. Because of its ability to suppress Staphylococcus aureus's synthesis of cell walls, it has become a significant component in the treatment of severe MRSA infection. However, the development of vancomycin-resistant Staphylococcus aureus strains (VRSA) poses a significant risk to this last protection barrier. The acquisition of resistance mechanisms by VRSA strains reduces both the binding affinity and efficiency of vancomycin.

One such method is the vanA gene, which modifies the peptidoglycan precursors found in bacterial cell walls. Not only does this resistance make treatment regimens more difficult to follow, often necessitating the employment of less effective or more dangerous alternatives, but it also emphasises the crucial importance of correct diagnostic techniques. When it comes to minimising treatment failures and restricting the emergence of highly resistant strains, precise VRSA identification and control are critical [7]. As a result, it is critical in clinical settings to develop and implement reliable diagnostic procedures for determining vancomycin's Minimum Inhibitory Concentration (MIC). This is necessary in order to guarantee that appropriate treatment interventions are carried out and to reduce the negative effect that VRSA has on public health [8-10].

The Importance of Finding an Accurate MIC

The determination of the Minimum Inhibitory Concentration (MIC) is an important step in clinical microbiology. It is required to classify bacterial isolates as antibiotic-resistant or antibiotic-susceptible. Accurate MIC values are critical because they immediately influence therapeutic decisions on the selection of appropriate antimicrobial drugs. This ensures that patients receive the most effective therapy possible. Because drugs like vancomycin are critical for the treatment of MRSA infections, finding the minimum inhibitory concentration (MIC) with precision is even more vital. Given that vancomycin is the primary treatment for methicillin-resistant Staphylococcus aureus (MRSA), it is critical to correctly assess when the antibiotic is no longer able to prevent the formation of germs, which indicates resistance. Detecting strains of vancomycin-resistant *Staphylococcus aureus* (VRSA) requires a level of accuracy that is very difficult to achieve. Incorrect resistance categorization due to wrong MIC values can lead to poor therapeutic decisions, resulting in treatment failures, protracted infections, and an increased risk of transmission. Furthermore, accurate MIC calculation allows doctors to adapt therapeutic measures accordingly, optimising doses and selecting alternative therapies as needed. As a result, an accurate minimum inhibitory concentration (MIC) test is a necessary component of effective antimicrobial management. This test helps to ensure that existing antibiotics remain effective while also reducing the spread of resistant strains [11]. Therefore the present study was undertaken to study the changing trend in minimum inhibitory concentration (mic) of vancomycin resistant *staphylococcus aureus* (VRSA) by agar dilution method and its comparison with e-test

MATERIAL AND METHODS

Study Design:

This study's design was observational and descriptive, with a cross-sectional approach. This approach permits data to be collected at a single point in time, with no intervention or variable modification required. It is appropriate for determining the prevalence and features of Staphylococcus aureus, particularly methicillin-resistant strains, in a specific population.

Data Collection and Procedure

Place of Study: The study was conducted in the Department of Microbiology, Government Medical College, Kota, Rajasthan, India.

Duration of Study: Data collection took place over a period of three years, starting from September 7, 2019, to September 6, 2022, following approval from the Departmental Research Committee (DRC).

Sample Size Determination- The sample size was calculated using a statistical formula based on the prevalence rate of vancomycin-resistant *Staphylococcus aureus* (VRSA) among various clinical samples. An average prevalence rate of 20% was used, resulting in a calculated sample size of 384.

The Ethical Letter: The Ethical clearance was duly obtained from the Institutional Medical College of GMC, Kota.

Specimen Collection and Processing- *Staphylococcus aureus* isolated from various clinical samples, including pus, urine, sputum, blood, throat swab, and pleural fluid, were processed according to standard protocols in the bacteriology lab of the Department of Microbiology.

Identification of the Organism: Isolated *Staphylococcus aureus* underwent microscopic examination, subculture, and manual identification tests including Gram staining, nutrient agar, blood agar, and mannitol salt agar. Biochemical tests such as the catalase and coagulase tests were also conducted for the identification.

Detection of MRSA and VRSA: Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected using the cefoxitin disk diffusion method, while the presence of vancomycin-resistant strains (VRSA) was determined by using vancomycin agar screen plates. MIC determination to confirmVRSA was performed by using the agar dilution method and E-test strip test.

Statistical Analysis

Statistical analysis may include calculations of prevalence rates, descriptive statistics of sample characteristics, and comparisons of antimicrobial susceptibility patterns among different clinical samples. Additionally, the correlation between demographic variables and antibiotic resistance profiles may be analyzed using appropriate statistical tests. The significance level for statistical tests may be set at $p < 0.05$.

Vancomycin Screen Agar Method [12]:

Vancomycin Agar Screen test was utilized to screen *Staphylococcus aureus* strainsfor resistance to vancomycin. For the isolates, which gave positive result on vancomycin agar screen test, were tested for MIC determination by using agar dilution method and E-test.

Preparation of Vancomycin Agar Screen plates:

i.) Medium used for vancomycin agar screen plates was Brain Heart Infusion (BHI) agar supplemented with 6 µg/ml vancomycin.
ii.) For the preparation of 100 ml of BHI agar plates containing 6

µg/ml vancomycin, 1 vial of aliquoted 600 µl of the 1 mg/ml vancomycin stock was taken out from deep freezer and add in 100 ml of autoclaved warm BHI media.

iii.) Immediately after adding antibiotic stock solution, it was mix slowly and pour approx. 5 plates.

"The following formula was used for making 6 µg/ml vancomycin BHI agar plate from 1 mg/ml stock solution (1000 µg/ml)".

C1 V1 = C2 V2

C1 = Concentration of vancomycin stock solution.

V1= Volume taken from stock solution for making 6 µg/ml vancomycin BHI agar.

C2= Concentration of vancomycin BHI agar.

V2= Volume of the BHI agar.

C1 V1 = C2 V2

1000 µg/ml V1 = 6 µg/ml x 100 ml

V1 = 600 µl

Inoculum preparation:

i.) Fresh culture of the strain was used to be prepared a suspension of tested strain equivalent to 0.5 Mc Farland standard.

ii.) Standardized inoculum was prepared of 0.5 McFarland turbidity standard (approx. $1.5x10^8$ CFU/ml) by using the direct colony suspension method. 3-5 well isolated colonies of the same morphological type were taken from 18–24-hour culture plate and prepare saline suspensions in tubes containing sterile saline.

Plate Inoculation:

i.) The suspension was inoculated by using a micropipette to spot a 10 µl drop (final conc. 10^6cfu/ml) on the surface of the BHI agar plate containing 6 µg/ml vancomycin.

ii.) Square grid template was preparedto spot approx. 13 – 14 test strains and 2 QC strains in one plate.

iii.) Incubation conditions: The plates were incubated at 35±2°C for 18-24 hrs in an inverted position.

Interpretation and Reporting:

Presence of more than one colony of the strain or light film of growth was interpreted as reduced susceptibility to vancomycin.

MIC determination of Vancomycin:

MIC determination methods recommended by CLSI and CDC for VRSA are agar dilution method and E-test.

A. Agar Dilution Method [13]: **-**

I. Introduction:

i.) The agar dilution method was utilized to determine MIC.

ii.) Determination of minimum inhibitory concentration (MIC) of vancomycin for *S. aureus* isolates which grew on vancomycin agar screen plates were identified by agar dilution method.

iii.) CLSI recommends agar dilution method for the detection of MIC for VRSA.

II. Preparation of Vancomycin Hydrochloride stock solution:

i.) First step was to prepare the 1 mg/mL stock solution of vancomycin hydrochloride.

ii.) Use of the following formula to determine the amount of powder needed for a standard solution:

Weight (mg) = Volume (mL) × Conc. (µg/mL)
Potency (µg/mg)

Example: To prepare 100 mL of a stock solution containing 5120 µg/mL concentration with antimicrobial powder that has a potency of 950 µg/mg.

The amount of vancomycin hydrochloride powder was calculated as follows:

Weight (mg) = Volume (mL) × Conc.(µg/mL)
Potency (µg/mg)
Weight (mg) = 100 (mL) × 5120 (µg/mL)
950 (µg/mg)
Weight (mg) = 538.95 mg

Therefore, dissolve 538.95 mg of antimicrobial powder in 100 mL of diluent.

iii.) Prepare each aliquots containing 1-2 ml of 1 mg/ml stock solution of vancomycin hydrochloride.

iv.) Label each vial of aliquoted 1 mg/ml stock solution as **"Working Stock of Vancomycin".**

III. Preparation of vancomycin MIC plates:

For making vancomycin MIC plates of different concentrations (0.5-256 µg/ml) dilution of the stock solution was made according to the given table 1.

Table 1: Dilution of Vancomycin for MIC plates.

Step	Concentration (µg/mL)	Source	Vol. (mL)	Diluent (mL)	Intermediate Concentration (µg/mL)	Final Conc. At 1:10 dilution in Agar (µg/mL)
1	5120	Stock	2	2	2560	256
2	5120	Stock	1	3	1280	128
3	5120	Stock	1	7	640	64
4	640	Step 3	2	2	320	32
5	640	Step 3	1	3	160	16
6	640	Step 3	1	7	80	8
7	80	Step 6	2	2	40	4
8	80	Step 6	1	3	20	2
9	80	Step 6	1	7	10	1
10	10	Step 9	2	2	5	0.5
11	10	Step 9	1	3	2.5	0.25
12	10	Step 9	1	7	1.25	0.125

Readings were interpreted according to recent CLSI guideline: -

- MIC ≤2 mg/l for vancomycin-susceptible *S. aureus* (VSSA).
- MIC of 4-8 mg/l for vancomycin-intermediate *S. aureus* (VISA).
- MIC ≥16 mg/l for vancomycin-resistant *S. aureus* (VRSA).

B. E-test Strip Method:

I. Introduction:

i.) Another method for the Determination of minimum inhibitory concentration (MIC) of vancomycin for *S. aureus* isolates which grew on vancomycin agar screen plates was done by E-test.

ii.) CDC recommends E-test method for the detection of MIC for VRSA.

II. Procedure:

i.) In this test inoculum suspensions of 0.5 McFarland standard were prepared.

ii.) Dip a sterile cotton swab to the inoculum suspension and carefully streak the entire surface Muller-Hinton agar evenly in three directions.

iii.) Allow excess moisture to be fully absorbed and ensured that the surface of Muller-Hinton agar was completely dry before applying E-test strips.

iv.) An E-test strip containing a concentration gradient of Vancomycin ranging from 0.016 - 256 µg/ml was used to check the susceptibility.

v.) E-test strip was applied on to the agar surface with the MIC scale facing upwards. This was done by using forceps.

vi.) It was ensured that the whole strips were in complete contact with the agar surface.

vii.) Plates were incubated in an inverted position at 37°C for overnight incubation.

Fig.1 E-test strip method.

Interpretation of MIC values: -

Readings were interpreted according to recent CDC guideline:

- MIC≤2 µg/ml for vancomycin-susceptible *S. aureus* (VSSA).
- MIC of 4-8 µg/ml for vancomycin-intermediate *S. aureus* (VISA).
- MIC≥16 µg/ml for vancomycin-resistant *S. aureus* (VRSA)[14].

Advantages and Disadvantages:

Agar Dilution Method: Highly accurate, suitable for research

settings, but requires extensive resources and trained staff.

E-test Strip Method: Practical for clinical use, faster results, but may be less precise than the agar dilution method.

RESULT

In the present study a total of 384 *Staphylococcus aureus* isolates were collected from different clinical samples. These isolates were from patients admitted to Maharao Bhim Singh Hospital (MBSH) and New Medical College Hospital (NMCH), as well as from out-patients at both hospitals of Government Medical College in Kota, Rajasthan, India.

Table 2:*Staphylococcus aureus* segregation by age and sex (n = 384).

Sr. No.	Age group	S. aureus No. (%)	Male No. (%)	Female No. (%)
1	0-10	15 (3.91)	8 (53.33)	7 (46.67)
2	11-20	21 (5.47)	11 (52.38)	10 (47.62)
3	21-30	70 (18.23)	38 (54.29)	32 (45.71)
4	31-40	67 (17.45)	33 (49.25)	34 (50.75)
5	41-50	57 (14.84)	29 (50.88)	28 (49.12)
6	51-60	48 (12.50)	26 (54.17)	22 (45.83)
7	61-70	63 (16.41)	36 (57.14)	27 (42.86)
8	71-80	43 (11.20)	14 (32.56)	29 (67.44)
	Total	384 (100)	195 (50.78)	189 (49.22)

In the current study out of the 384 *S. aureus* isolates, 70 (18.23%) belonged to (21-30) age group followed by 67 (17.45%) from (31-40) age group, 63 (16.41%) from (61-70) age group, 57 (14.84%) from (41-50) age group, 48 (12.50%) from (51-60) age group, 43 (11.20%) from (71-80) age group, 21 (5.47%) from (11-20) age group and least in the age group 15 (3.91%) from (0-10) age group.

Among 195 *S. aureus* isolated the ratio of male patients observed that 38 belonged to (21-30) age group followed by 36 from (61-70) age group, 33 from (31-40) age group, 29 from (41-50) age group, 26 from (51-60) age group, 14 from (71-80) age group, 11 from (11-20) age group, 8 from (0-10) age group.

Among 189 *S. aureus* isolated from female patients, 34 belonged to (31-40) age group followed by 32 from (21-30) age group, 29 from (71-80) age group, 28 from (41-50) age group, 27 from (61-70) age group, 22 from (51-60) age group, 10 from (11-20) age group, 7 from (0-10) age group.

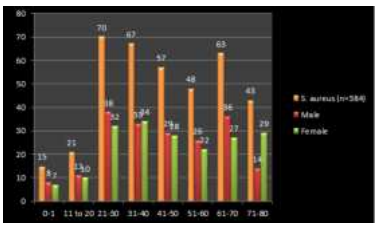

Fig. 2: *Staphylococcus aureus age and sex distribution (n = 384).*

Table 3: Distribution of *S. aureus* isolated from different clinical samples.

Sr. No.	Clinical Specimens	Number of Staphylococcus aureus	Percentage (%)
1	Pus	215	56
2	Urine	77	20
3	Sputum	50	13
4	Blood	27	7
5	Throat swab	8	2
6	Pleural fluid	7	2
	Total	384	100

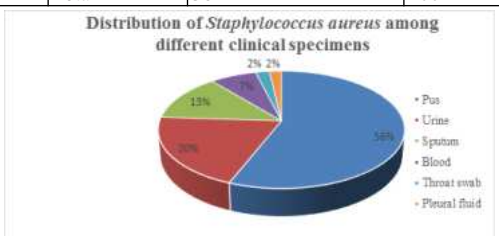

Fig 4: Distribution of *Staphylococcal aureus* among different clinical specimens.

Table 4: Distribution of *S. aureus* among MRSA and MSSA.

Staphylococcus aureus	384	100%
MRSA	185	48.18%
MSSA	199	51.82%

Among 384 *staphylococcus aureus*, 185 (48.18%) were methicillin resistant and 199 (51.82%) were methicillin sensitive.

Fig 4: Distribution of *Staphylococcal aureus* among MRSA and MSSA.

Table 5: The vancomycin screen agar technique of 6 µg/ml* MIC, *was used to identify VRSA among MRSA isolates.

MRSA No, %	VRSA No. %	VSSA No. %
185 (100%)	06 (3.24%)	179 (96.76%)

The agar dilution method and the E-test strip method were used to confirm the presence of 6 (3.24%) VRSA isolates out of 185 MRSA discovered by the vancomycin screen agar method.

Figure 5: *Distribution of MRSA among VRSA and VSSA.*

Table 6: MIC determination by agar dilution method and E-test strip method, a comparative study.

MIC (µg/ml)	Agar dilution	%	E-test	%
0.5	8	4.32	2	1.08
0.75	-	-	3	1.62
1	93	50.27	68	36.76
1.5	-	-	22	11.89
2	79	42.70	85	45.95
3	-	-	0	0
4	2	1.08	2	1.08
6	-	-	0	0
8	1	0.54	1	0.54
12	-	-	0	0
16	2	1.08	2	1.08
32	00	0	00	0
Total	185	100	185	100

Agar dilution method:

Out of 185 MRSA 8 (4.32%) MRSA shows 0.5 µg/ml MIC by agar dilution method followed by 93 (50.27%) MRSA shows 1 µg/ml MIC, 79 (42.70%) MRSA shows 2 µg/ml MIC, 2 (1.08%) MRSA shows 4 µg/ml MIC, 1 (0.54%) MRSA shows 8 µg/ml MIC, 2 (1.08%) MRSA shows 16 µg/ml MIC and 00 (0%) MRSA shows 32 µg/ml MIC.

E-test strip Method:

Of the 185 MRSA isolates 2 (1.08%) MRSA shows 0.5 µg/ml MIC by E-test strip method followed by 3 (1.62%) MRSA shows 0.75 µg/ml MIC, 68 (36.76%) MRSA shows 1 µg/ml MIC, 22 (11.89%) MRSA shows 1.5 µg/ml MIC, 85 (45.95%) MRSA shows 2 µg/ml MIC, 2 (1.08%) MRSA shows 4 µg/ml MIC, 1 (0.54%) MRSA shows 8 µg/ml MIC, 2 (1.08%) MRSA shows 16 µg/ml MIC and 0 (0%) MRSA shows 3, 6, 12, 32 µg/ml MIC.

Table 7: The Agar dilution method and the E-test strip method were used to determine the VRSA, VISA, and VSSA isolates based on their MIC values.

MIC By	VRSA No. (%)	VISA No. (%)	VSSA No. (%)
Agar dilution method	02 (1.08)	03 (1.62)	180 (97.30)
E-test strip method	02 (1.08)	03 (1.62)	180 (97.30)

MRSA isolates were subjected to MIC determination by the agar dilution method and E-test strip method. Of the 185 MRSA isolates 02 isolates were identified as VRSA and 03 isolates were identified as VISA by both methods.

DISCUSSION

Staphylococci infections are a global health hazard because this bacterium has evolved increased antibiotic resistance [15]. It accounts for 30% of hospital-acquired infections, with *Staphylococcus* being the most often isolated organism on culture in around 50% of bloodstream infections [16, 17]. *Staphylococcus aureus* poses a public health problem due to its rising virulence and resistance patterns, which concerns WHO.

The rise in *S.aureus* isolates resistant to methicillin and less susceptible to vancomycin has raised concerns about the development of new antistaphylococcal medicines capable of killing resistant variants. The emergence of VRSA/VISA may be attributed to vancomycin-induced selective pressure.

In a recent scenario vancomycin is the treatment choice for MRSA (Methicillin Resistant *Staphylococcus aureus*) infections. However, it has resulted in the evolution of vancomycin intermediate *Staphylococcus aureus* (VISA) and (vancomycin resistant *Staphylococcus aureus* (VRSA).

For the treatment of MRSA; vancomycin is used as drug of choice. However, there are increasing numbers of reports on emergence of vancomycin intermediate-sensitive *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) . In addition, many researchers have reported the higher rates of likelihood of treatment failure leading to higher rates of mortality, due to the infection caused by MRSA having MIC of vancomycin at the upper end of susceptible range . The higher MIC of vancomycin to MRSA even in the susceptible range correlates with the drug resistance to many different classes of the antibiotics [12].

In this present study, there were total 384 isolates of *Staphylococcus aureus*,out of which maximum isolates were observed in the age group of 21-30 years (18.23%) followed by 31-40 years (17.45%). Our results were comparable to the study by Mandal M *et al*and Goyal A *et al* which showed high prevalence in the age groups of 21-30 years (36.11%) and 20-40 years (51%) respectively. However, in the study by Kaur K *et al* the prevalence was high in the age group ranging from 16-40 years (43.2%) which was in constrast to the present study. This could be due to wider margin of age range selected in their study (Table-8).

Table 8: Comparison of age-based prevalence of *Staphylococcus aureus* isolates with other studies

Present study (N=384)	Kaur K et al (N=162) [15]	Goyal A et al (N=379) [16]	Mandal M et al (N=108) [17]
21-30 years (70/18.23%)	16-40 years (70/43.2%)	20-40 years (193.29/51%)	21-30 years (39/36.11%)
31-40 years (67/17.45%)	-	0-20 years (98.54/26%)	11-20 years (23/21.29%)

In the study of Kaur K *et al*, there were 162 isolates of *Staphylococcus aureus*, of which 85 (52.4%) were obtained from females while 44 (47.5%) were obtained from male patients. The higher number of isolates was obtained from male patients which was similar to our study. In contrast, a study from Bihar conducted by Mandal M *etal*and a study conducted in Nepal by Adhikari R *et al* reported high prevalence of *Staphylococcus aureus* isolates in females compared to males (Table-9).

Table 9: Comparison of gender based (Male: M, Female F) prevalence of *Staphylococcus aureus* isolate with other studies

Present Study (N=384)		Kaur K et al (N=162) [15]		Mandal M et al (N=108) [17]		Sarrafzadeh F et al (N=250) [18]		Adhikari R et al (N=95) [19]	
M	F	M	F	M	F	M	F	M	F
195 (50.78%)	185 (49.22 %)	85 (52.4%)	44 (47.5 %)	37 (34.2 6%)	71 (65.7 4%)	188 (75. 2%)	62 (24. 8%)	41 (43.1 6%)	54 (56.8 4%)
Ratio	1.03:1	1.93:1		0.52:1		3.03:1		0.76:1	

In the present study most of the *Staphylococcus aureus* isolates i.e.215 were obtained from pus samples that accounted for 56% followed by urine samples, the incidence of which were 77 (20%). Like the present study, the number of *Staphylococcus aureus* isolates was high is pus sample in the studies conducted by Usha MG *et al* (126/66.31%), Kaur K et al (96/59.2%) and Nepal N *et al* (42/52.5%) followed by blood (16/8.42%), urine (30/18.5%) and pus (16/41%) respectively. In contrast to our study, the study of Kandel SN *et al* showed higher incidence in urine sample (9/23.1%) followed by pus (8/20.5%). Another, study of Maharjan M *et al* reported higher incidence in wound swab (27/47.4%) followed by pus sample (16/41%) (Table-10).

Table 10: Comparison of prevalence of *Staphylococcus aureus* isolates with other studies according to various clinical specimen

Present study (N=384)	Usha MG et al N=190 [20]	Kandel SN et al (N=39) [21]	Kaur K (N=162) [15]	Nepal N et al (N=80) [22]	Maharjan M et al (N=74) [23]
Pus 215 (56%)	Pus 126 (66.31%)	Urine 9 (23.1%)	Pus and Wound 96 (59.2%)	Pus 42 (52.5%)	Wound swab 27 (47.4%)
Urine 77 (20%)	Blood 16 (8.42%)	Pus 8 (20.5%)	Urine 30 (18.5%)	Wound swab 21 (26.3)	Pus 16 (41%)

The present study reported that, of 384 *Staphylococcus aureus* isolates, 185 (48.18%) were methicillin resistant (i.e. MRSA) while 199 (51.82%) were methicillin sensitive (MSSA). In the study of Osman MM *et al*, 25 (41%) of *Staphylococcus aureus* isolates were MRSA while 36 (59%) were MSSA which was similar to our study. Likewise, in the study of Jayshree N et al, the prevalence of MRSA was 41.38% (12) while the study of Thati V *et al* showed a very high prevalence of MRSA i.e. 79.6% (285) which was higher than that observed in our study (table-11).

Table 11: Comparison of prevalence of MRSA among *Staphylococcus aureus* isolates with other studies

Present Study (N=384)		Osman MM et al N=61 [24]		Arora S et al N=250 [25]		Jayshree et al N=29 [26]		Thati V et al N=358 [27]	
MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA
199 (51.82%)	185 (48.18%)	36 (59%)	25 (41%)	135 (58.7 %)	115 (41.3 %)	17 (58.6 2%)	12 (41.3 8%)	73 (20. 4%)	285 (79.6 %)

In the present study, VRSA and VISA strains among the MRSA isolates were determined using vancomycin screen agar method and was further confirmed by agar dilution method and E-test strip method. Of 185 MRSA isolates vancomycin screen agar method detected 3.24% (3) of VRSA isolates and 96.76% (179) VSSA. The incidence of VRSA strains was documented in this study was lower than that observed in the previous studies of Thati V *et al* (23/6.42%), Kaur K *et al* (23/27.71%) and Olufunmiso O *et al* (89/33.5%) (Table-12).

Table 12: Comparative determination of VRSA and VSSA among MRSA isolates by using vancomycin screen agar method (MIC = 6 µg/ml) with other studies

Present Study (N=185)		Kaur K et al (N=83) [15]		Thati V et al (N=358) [27]		Olufunmiso O et al (N=266) [28]	
VRSA	VSSA	VRSA	VSSA	VRSA	VSSA	VRSA	VSSA
6 (3.24%)	179 (96.76 %	23 (27.71 %)	60 (72.28%)	23 (6.42 %)	335 (93.57 %)	89 (33.5%)	147 (55.26%)

On agar dilution method, it was found that for most of the MRSA isolates (50.27%) were represent the MIC 1 µg/mL while for 0.54% of cases MIC was 8 µg/mL. On E-test strip method, it was found that for most of the MRSA isolates (57.84%), the MIC was 1.5-2 µg/mL while for 0.54% of cases MIC was 8 µg/mL. None of the isolates showed MIC of 32 µg/mL. Previous studies have also determined the MIC of vancomycin as per CLSI guidelines, the comparative analysis of which with the present study is shown in the given table-13.

In the study of Mongy MA *et al*, 96% of MRSA isolates had MIC of 2 µg/mL, while in the study of Mandal M *et al*, 50% of the isolates showed MIC of 2 µg/mL. Mohanty *et al* determined the MIC of vancomycin using E test method. They found that for 40.16% of the MRSA isolates, the MIC was 2 µg/mL. Likewise, Kumari J *et al* and Kaur K *et al* also documented comparable findings to our study. In their study too, the MIC for most of the isolates was ≤2 µg/mL which was in support to the present study. In the study by other investigator Kaur K *et al*recorded 11.7% of VRSA strains with the MIC between 4-8 ug/ml (VISA) and 2.46% had MIC > 16 ug/mL showing complete resistance to vancomycin.

Table 13: MIC determination for vancomycin by agar dilution method and E-test strip method, a comparative study.

Present Study (N=185)		Mongy MA et al N=50 [29]	Mandal M et al N=32 [17]	Mohanty et al N=127 [30]	Kumari J et al N=98 [31]		Kaur K et al N=162 [15]	Raina D et al N=50 [32]	
Agar dilution	E-strip	Agar dilution	Agar dilution	E-strip	Agar dilution	E-strip	Agar dilution	Agar dilution	E-strip
0.5 µg/mL: 8 (4.32%)	0.5-0.75 µg/mL: 5 (2.70%)	-	-	0.5-0.75 µg/mL: 0 (0%)	0.5 µg/mL: 11 (11.2%)	0.5-0.75 µg/mL: 1 (1%)	-	-	-
1 µg/mL: 93 (50.27%)	1 µg/mL: 68 (36.76%)	-	1 µg/mL: 1 (3.12%)	1 µg/mL: 23 (18.11%)	1 µg/mL: 37 (37.8%)	1 µg/mL: 11 (11.2%)	-	-	-
2 µg/mL: 79 (42.70%)	1.5-2 µg/mL: 107 (57.84%)	2 µg/mL: 48 (96%)	2 µg/mL: 16 (50%)	1.5-2 µg/mL: 95 (74.80%)	2 µg/mL: 46 (46.9)	1.5-2 µg/mL: 41 (40.82)	<2 µg/mL: 139 (85.80%)	<2 µg/mL: 25 (86.2%)	<2 µg/mL: (68.9%)
4 µg/mL: 2 (1.08%)	4 µg/mL: 2 (1.08%)	4-8 µg/mL: 49 (98%)	4 µg/mL: 3 (9.37%)	3-4 µg/mL: 9 (7.09%)	4 µg/mL: 4 (4.1%)	3-4 µg/mL: 45 (45.92%)	4-8 µg/mL: 19 (11.7%)	4-8 µg/mL: (13.8%)	4-8 µg/mL: (13.8%)
8 µg/mL: 1 (0.54%)	8 µg/mL: 1 (0.54%)	8 µg /mL: 49 (98%)	8 µg/mL: 8 (25%)	-	-	-		-	-
16 µg/mL: 2 (1.08%)	16 µg/mL: 2 (1.08%)	16 µg/mL: 50 (100%)	16 µg/mL: 1 (3.12%)	-	-	-	16 µg/mL: 3 (1.8%)	-	-
32 µg/mL: 0	32 µg/mL: 0	32 µg/mL: 50 (100%)	32 µg/mL: 2 (6.25%)	-	-	-	32 µg/mL: 1 (0.6%)	-	-
-	-	-	64 µg/mL: 1 (3.12%)	-	-	-	-	-	-

By agar dilution method and E-test strip method 2 (1.08%) VRSA and 3 (1.62%) of VISA isolates were detected(Table-7). This result was comparable to the study of Thati V *et al* who documented 1.9% of VRSA strains and 4.46% of VISA strains. In the study of Osman MM *et al*, there was no isolation of any VRSA stains and the overall prevalence of VISA was 12%.

They were mostly observed in the patients having underlying conditions such as long-term hospitalization, serious disease and immune-suppressive therapy (Table-14).

Table-14: Comparative determination of VRSA, VISA and VSSA isolates by Agar dilution method and E-test strip method on the basis of MIC values.

Present study N=185			Thati V et al (N=358) [27]			Osman MM et al (N=25) [24]			Maharjan M et al (N=45) [23]		
VRSA	VISA	VSSA	VRSA	VISA	VSSA	VRSA	VISA	VSSA	VRSA	VISA	VSSA
2 (1.08%)	3 (1.62%)	180 (97.29%)	7 (1.95%)	16 (4.46%)	335 (93.57%)	0	3 (12%)	22 (88%)	5 (11.11%)	15 (33.33%)	25 (55.55%)

Agar dilution technique and E-test strip method both are used to determine the minimum inhibitory concentration (MIC) of vancomycin among MRSA isolates. The comparison research offers useful insights into the efficiency of both of these methods. In each case, there are benefits and disadvantages associated with the different techniques. In clinical settings, the decision of which approach to use may be contingent on the particular needs of accuracy, the availability of resources, and the demand for speedy findings.

The use of these strategies in a variety of situations, as well as the influence that they have on the management and treatment of infections caused by MRSA and VRSA, might be the subject of exploration in further study. For the purpose of effective antibiotic stewardship and infection control, the results highlight the need of accurately determining the minimum inhibitory concentration (MIC).

When this antibiotic was first released in 1858, it was assumed that no resistance would develop because resistance was difficult to produce. This has triggered off alarms in the medical community as *S. aureus*causes life-threatening infections in hospitalized and non-hospitalized patients [33,34] as Vancomycin is the main antimicrobial agent available to treat serious infections with MRSA but unfortunately, many nations have lately reported a decline in vancomycin susceptibility of *S. aureus* as well as the isolation of vancomycin-intermediate and resistant *S. aureus* [35,36].

Implementing infection control practices and controlling the risk factors will help in management of MRSA infections. Drug resistance to glycopeptides can be avoided by regular screening of vancomycin creeps by different susceptibility methods in order to avoid treatment failures [37,38].

CONCLUSION

In this work, the Agar dilution method was used as the gold standard for MIC determination. It produces exact and consistent results but is laborious and time-consuming. The E-test strip approach is a more convenient and faster option. It is simple to execute and analyse, however the findings may vary compared to the agar dilution approach.

The prevalence of antibiotic-resistant pathogenic microorganisms, notably *Staphylococcus aureus* resistant to vancomycin, is on the rise, which is concerning. Vancomycin will continue to dominate until resistance to vancomycin is controlled or a new antibiotic with a greater efficacy becomes available. However, controlling VRSA has been difficult because VRSA isolates have demonstrated resistance to multiple current antibiotics (a condition known as multi drug resistant VRSA).

It has resulted in a scarcity of therapeutic alternatives, resulting in ineffective antibiotic therapy and increased mortality or morbidity in affected people. Furthermore, once MRSA or VRSA have established a permanent presence in the environment or in medical settings, they are nearly tough to remove. Given the prevalence of antibiotic abuse, authorities must take immediate action to prevent the spread of VRSA and VISA strains. Strict limits on irrational antibiotic use may be a useful strategy. Furthermore, a statewide surveillance system is required to map the vancomycin susceptibility pattern in the country.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

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