E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.01.181

**Research Article**

# Identification of Methicillin-Resistant Staphylococcus aureus (MRSA) Isolates by Conventional Methods and Determination of Their Minimum Inhibitory Concentrations Using VITEK 2: A Prospective Observational Study

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Received: 08.04.25, Revised: 24.05.25, Accepted: 03.06.25

**ABSTRACT**
**Background:** MRSA represents a central global contributor to hospital- and community-acquired infections because it produces severe healthcare complications that result in numerous deaths. The main component creating resistance in MRSA is the mecA gene that generates penicillin-binding protein PBP2a/PBP2 through its coding function. Effectual infection control requires immediate identification of MRSA with accurate results to determine proper therapeutic approaches plus prevent spread of multidrug-resistance.
**Methods:** A six-month prospective observational research determined its findings. Biochemical tests identified 314 S. aureus isolates that researchers obtained from different clinical specimens. The testing laboratory performed cefoxitin (30 µg) disc diffusion assays using Mueller–Hinton agar with 2% NaCl enhancement to evaluate all obtained isolates. Additionally they used mannitol salt agar-Ceinasefoxitin (MSA-CFOX) containing 8 mg/L of the antibiotic. VITEK 2 automated system tested the minimum inhibitory concentrations (MIC) values for the identified MRSA isolates after the confirmation step. The lab designation of MRSA required either cefoxitin inhibition zones equal to or less than 21 mm and MIC values at or above 4 µg/mL.
**Results:** Among 314 S. aureus isolates, cefoxitin disc diffusion found 90 cases of MRSA corresponding to 29% of these strains. MSA-CFOX detected 96 potential MRSA isolates based on its results (31%) yet six of these cases turned out to be incorrect due to MIC values which failed to meet the MRSA threshold. The laboratory confirmed 29% of MRSA cases through VITEK 2 testing. The results from cefoxitin disc diffusion tests match closely with VITEK 2 readings which show the validity of disc diffusion testing for MRSA identification but users need to recognize that MSA-CFOX may produce false-positive results in select cases.
**Conclusion:**Definitive MRSA isolate identification uses Cefoxitin disc diffusion together with the VITEK 2 system for accurate MIC value determination in a cost-efficient manner. Therapeutic decision support along with suitable infection control protocols depend on proper identification timing and accurate MIC measurement.

**Keywords**: MRSA, cefoxitin disc diffusion, mannitol salt agar with cefoxitin, minimum inhibitory concentration, VITEK 2, *Staphylococcus aureus*, antibiotic resistance

## INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) functions as a major worldwide healthcare problem because it causes substantial mortality and morbidity as well as increased healthcare expenses [1]. As an opportunist pathogen Staphylococcus aureus affects patients

through various severe infections starting from uncomplicated skin infections to fatal bloodstream infections and pneumonia [2]. MRSA strains have made therapeutic management more difficult because these pathogens show antibiotic resistance across all β-lactam agents except for one type of drug [3].

The main cause of methicillin resistance in S. aureus exists through the mecA gene that leads to penicillin-binding protein 2a (PBP2a/PBP2) enzyme production. The modified protein has a weak connection to β-lactam antibiotics which makes standard pharmaceutical approaches inadequate [4]. Treatment limitations emerge because doctors must resort to second-line drug options which include vancomycin and newer generation antibiotics that tend to be toxic or costly [5].

The immediate identification of MRSA represents an essential need in both patient care and disease monitoring operations. The early discovery of MRSA enables doctors to begin treating patients with the right antimicrobial drugs that can enhance treatment results. Urgent identification of infections plays a crucial role in preventive transmission by enabling strict control procedures such as patient seclusion and contact protection and personnel monitoring [6]. The detection of MRSA utilizes cefoxitin disc diffusion as traditional laboratory methods because this phenotypic test demonstrates strong reliability in detecting methicillin resistance. Tests determine resistance in isolates when cefoxitin discs create a zone of inhibition smaller than 21 mm as per Clinical and Laboratory Standards Institute [CLSI] requirements [7].

The detection of resistant colonies uses mannitol salt agar (MSA) containing cefoxitin (MSA-CFOX) as a culture-based alternative. Selective media proves useful by providing an effective method to screen possible MRSA strains. The inconsistency in cefoxitin concentration together with different incubation requirements leads to unreliable tests that might produce minor wrong readings [8].

The popularity of VITEK 2 automated systems has increased for MIC determination because this system provides accurate and efficient resistance confirmation. The medical value of MIC assessment enables healthcare providers to select appropriate antibiotics while tracking antibiotic resistance changes across healthcare facilities.

This work assesses the reliability of cefoxitin disc diffusion and MSA-CFOX tests along with their ability to detect MRSA isolates and matches the results to VITEK 2 system MIC assessments. The adopted approach enables deep understanding of phenotypic detection and establishes resistance levels to assist evidence-based decisions in infectious disease prevention and control measures.

## MATERIALS AND METHODS

### Study Design and Setting

Research was performed at Government Medical College (GMC) Department of Microbiology for six months from December 2023 through June 2024. The analysis of Staphylococcus aureus isolates from clinical samples proceeded during six months through the observation period at Government Medical College Anantapuramu.

### Sample Collection and Processing

- **Clinical Samples**: A total of 314 clinical samples that yielded Staphylococcus aureus upon culture were included in the study. Samples were obtained from both inpatient and outpatient departments.
- **Isolation and Biochemical Identification**: The laboratory personnel conducted standard media subculture for each suspected S. aureus colony. The identification process relied on traditional biochemical tests like catalase assessment with slide and tube coagulase tests as well as mannitol salt agar growth tests. The study processed further testing exclusively for confirmed S. aureus isolation results.

### Detection of MRSA by Conventional Methods

1. **Cefoxitin Disc Diffusion Method**
   - **Inoculum Preparation**: Each isolate was adjusted to a 0.5 McFarland standard using sterile saline.
   - **Media and Conditions**: Mueller–Hinton agar (MHA) plates containing 2% NaCl were used to enhance the expression of mecA-mediated resistance.
   - **Disc Application**: Cefoxitin discs (30 µg) were placed on the inoculated agar surface. Plates were incubated at 37°C for 24 hours.
   - **Interpretation**: Inhibition zone diameters were measured and interpreted using CLSI 2023 guidelines. Isolates with a zone of ≤21 mm were reported as MRSA, while those ≥22

mm were reported as methicillin-sensitive S. aureus (MSSA). Standard MRSA (ATCC 43300) and MSSA (ATCC 29213) strains served as controls.

**2. Mannitol Salt Agar with Cefoxitin (MSA-CFOX)**

- **Media Preparation**: Mannitol salt agar plates were incorporated with cefoxitin at a final concentration of 8 mg/L.
- **Inoculation and Incubation**: Each S. aureus isolate was inoculated onto MSA-CFOX plates, followed by incubation at 37°C for 24 hours.
- **Interpretation**: A yellowish transformation of the agar (reflecting mannitol fermentation) with growth in the presence of cefoxitin was taken as presumptive evidence of MRSA.

**Minimum Inhibitory Concentration (MIC) Determination by VITEK 2**

All isolates reported as MRSA by either cefoxitin disc diffusion or MSA-CFOX were subjected to MIC testing on the VITEK 2 automated system. The system interprets the MIC breakpoint for oxacillin or cefoxitin as per the CLSI criteria.

- **Inoculum Standardization**: Cultured pure bacteria colonies got suspended in sterile saline solution before reaching a turbidity level equivalent to 0.5 McFarland.
- **Card Loading and Analysis**: Each isolate was loaded into appropriate antimicrobial sensitivity testing cards (AST) in the VITEK 2 system.
- **Interpretation**: Isolates were considered methicillin-resistant if their MIC for oxacillin or cefoxitin was >4 µg/mL, confirming MRSA status.

**Ethical Considerations**

Institutional ethical clearance was obtained. Patient confidentiality was maintained by de-identifying all samples before analysis.

**Data Analysis**

Data were entered in a structured format and analyzed for prevalence, sensitivity, specificity, and overall concordance between each conventional method and the VITEK 2 reference. Descriptive statistics were presented as frequencies and percentages.

## RESULTS

**Overview of Study Isolates**

During the six-month duration researchers acquired 314 clinical isolates of S. aureus from different medical samples. The primary sources for S. aureus sample collection consisted of wound swabs and pus which accounted for 40% while blood and urine sources added up to 45% of the total number of cases. Miscellaneous samples such as sputum along with other fluids made up 15% of the cases. A summary of these isolates can be found in Table 1.

Table 1. Distribution of *S. aureus* Isolates by Sample Type

| Sample Type | Number of Isolates | Percentage (%) |
|---|---|---|
| Wound Swabs/Pus | 126 | 40.1 |
| Blood | 78 | 24.8 |
| Urine | 63 | 20.1 |
| Sputum/Respiratory | 31 | 9.9 |
| Other Body Fluids | 16 | 5.1 |
| **Total** | **314** | **100** |

**Detection of MRSA Using Cefoxitin Disc Diffusion and MSA-CFOX**

**1. Cefoxitin Disc Diffusion Method**

Out of 314 S. aureus isolates, 90 (29%) showed inhibition zones ≤21 mm, indicating MRSA. The remaining 224 (71%) isolates were deemed MSSA with inhibition zones ≥22 mm.

**2. Mannitol Salt Agar with Cefoxitin (MSA-CFOX)**

On MSA-CFOX plates, 96 isolates (31%) produced colonies capable of fermenting mannitol (yellow color) in the presence of cefoxitin. The other 218 isolates (69%) did not grow or did not produce typical color change in the presence of the antibiotic, hence were considered methicillin-sensitive by this method.

Table 2. Comparison of Cefoxitin Disc Diffusion and MSA-CFOX Methods

| Method | MRSA (n) | MSSA (n) | Total |
|---|---|---|---|
| **Cefoxitin Disc Diffusion** | 90 | 224 | 314 |
| **MSA-CFOX** | 96 | 218 | 314 |

**Confirmation of MRSA by VITEK 2 and False Positives**

All isolates classified as MRSA by either method were further tested using the VITEK 2 system to determine their MIC values. Isolates with MIC values >4 µg/mL were confirmed as MRSA.

- **VITEK 2 Confirmation**:
  - Of the 90 isolates identified as MRSA by the cefoxitin disc diffusion, all 90 were confirmed as MRSA by VITEK 2.
  - Of the 96 isolates identified as MRSA by MSA-CFOX, 90 matched the VITEK 2 MRSA criteria, while 6 were identified as false positives (MIC ≤4 µg/mL)

Table 3. Final MRSA Classification Based on VITEK 2 MIC Testing

| Method | True MRSA (MIC >4 µg/mL) | False Positives | Total MRSA Identified by Method |
|---|---|---|---|
| **Cefoxitin Disc Diffusion** | 90 | 0 | 90 |
| **MSA-CFOX** | 90 | 6 | 96 |

These findings indicate that cefoxitin disc diffusion correlates more precisely with the VITEK 2 results, while MSA-CFOX, despite its ease of use, may yield a small number of false positives.

**MIC Distribution**

Figure 1 illustrates the MIC value distribution for the confirmed 90 MRSA isolates on the VITEK 2 system. The majority of these isolates showed MICs ranging between 4 and 8 µg/mL, underscoring the high-level resistance among MRS

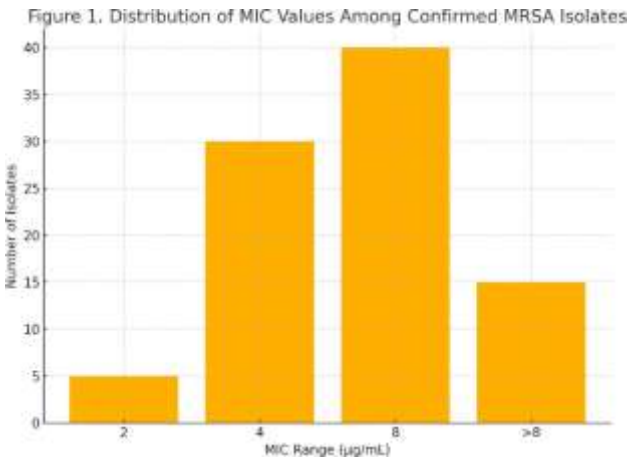
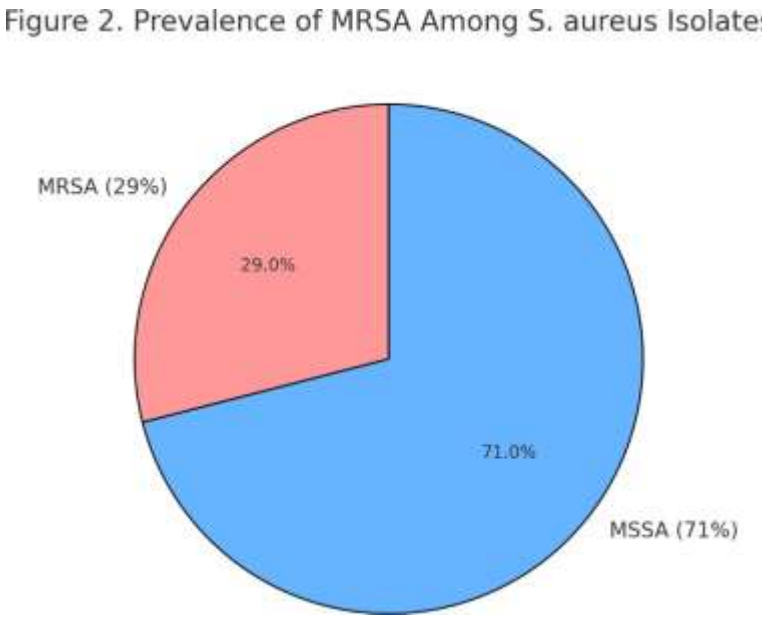



Figure 1. Distribution of MIC Values among Confirmed MRSA Isolates

**Prevalence of MRSA**

Overall, the confirmed prevalence of MRSA (by VITEK 2) in this study population was 29%. Figure 2 shows the overall prevalence among the 314 isolates.

Figure 2. MRSA prevalence rates for S. aureus isolate populations.




**DISCUSSION**

The current study highlights the importance of reliable phenotypic methods for the prompt detection of MRSA in a clinical microbiology setting. The prevalence of MRSA in our study population was found to be 29% using the cefoxitin disc diffusion method, which aligns closely with prior reports from similar healthcare facilities, where MRSA prevalence ranges between 25% and 40% [9,10]. Such variability in reported MRSA prevalence often reflects regional differences in antibiotic prescribing practices, infection control measures, and patient populations [11].

Cefoxitin disc diffusion has been recognized as a robust surrogate for oxacillin resistance screening, due to cefoxitin's ability to induce the mecA gene in Staphylococcus aureus [12]. In agreement with earlier studies, our findings demonstrate that cefoxitin disc diffusion showed a high concordance with the MIC results obtained from the VITEK 2 system, serving as an effective and cost-efficient approach in resource-limited settings [13].

On the other hand, mannitol salt agar supplemented with cefoxitin (MSA-CFOX) offers certain advantages, such as combining selective isolation and presumptive identification in a single step. However, our study observed a 31% positivity rate for MRSA on MSA-CFOX, of which six isolates were determined to be false positives upon VITEK 2 confirmation. These false positives might stem from variations in media preparation, inoculum density, or subtle differences in the expression of mecA and its regulators [14]. Despite its user-friendliness, laboratory personnel should be aware of the potential for inflated MRSA rates when relying solely on MSA-CFOX.

Confirmatory testing for MRSA often involves determining MIC values using automated systems like VITEK 2. This platform allows for precise quantification of antibiotic susceptibility and offers rapid turnaround times, which are crucial for guiding clinical decision-making, especially in severe infections like bacteremia and pneumonia. Early and accurate detection of MRSA is vital to optimize patient management, minimize the use of inappropriate antibiotics, and reduce selection pressure that drives resistance [15].

Furthermore, from an infection control standpoint, MRSA surveillance data help policymakers and hospital administrators target high-risk wards or patient populations. Implementing contact precautions, isolation protocols, and active surveillance can significantly curtail MRSA transmission [16]. Additionally, continuous monitoring of antibiotic susceptibility patterns remains indispensable for updating

hospital antibiotic guidelines to ensure efficacy against emerging resistant strains.

In conclusion, our data support the ongoing utility of cefoxitin disc diffusion as a primary screening test for MRSA. Although MSA-CFOX can be useful for rapid, high-volume screening, it should be complemented by a confirmatory test such as VITEK 2 to avoid overestimating MRSA prevalence. The synergistic combination of traditional phenotypic methods with automated MIC determination ensures high diagnostic accuracy, facilitating timely and appropriate antimicrobial therapy as well as enhancing infection control measures.

## CONCLUSION

In this study, methicillin-resistant Staphylococcus aureus constituted approximately 29% of all S. aureus isolates when assessed by cefoxitin disc diffusion and confirmed by VITEK 2 MIC testing. MSA-CFOX was convenient but had a slightly higher false-positive rate. Accurate detection of MRSA is paramount to guide appropriate antimicrobial therapy, prevent outbreaks, and minimize the spread of resistance within healthcare facilities. While phenotypic methods provide rapid initial results, confirmatory testing via automated systems remains crucial. The findings reinforce the need for vigilant screening policies and ongoing surveillance to mitigate the clinical and public health burden of MRSA.

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