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RESEARCH ARTICLE

Comparison of Different Phenotypic Methods of Detection of Methicillin-Resistant *Staphylococcus aureus* with Polymerase Chain Reaction

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ABSTRACT

Objectives: The objective of the current study was to compare the diagnostic methods of Oxacillin Disk Diffusion, Cefoxitin Disk Diffusion, Oxacillin Resistance Screening Agar Base, and CHROM Agar MRSA with the gold-standard method of Polymerase Chain Reaction for detection of Methicillin-resistant *Staphylococcus aureus*.

Methods: Two hundred pus samples were included in the study from which Staphylococcus strains were evaluated. The isolates of Staphylococcus aureus were subjected to the Oxacillin Disk Diffusion test, Cefoxitin Disk Diffusion test, Oxacillin Resistance Screening Agar Base, and CHROM Agar MRSA to detect MRSA with PCR, the reference standard. The diagnostic techniques were compared to their sensitivity, specificity, positive predictive, and negative predictive values.

Results: The sensitivity of the Cefoxitin Disk Diffusion test was 100%, followed by CHROM Agar MRSA at 96.7%, Oxacillin Disk Diffusion at 90%, and Oxacillin Resistance Screening Agar Base at 86.7%. Most specific was the Cefoxitin Disk Diffusion test (99.4%), followed by Oxacillin Resistance Screening Agar Base (98.8%), CHROM Agar MRSA (97.7%), and the least specific was the Oxacillin Disk Diffusion test (96.5%).

Conclusion: The Cefoxitin Disk Diffusion test was the most sensitive and specific of all four methods, next to the Polymerase Chain Reaction. However, future multicentric studies are recommended to test this method across all prevalent centers of methicillin resistance. *J Microbiol Infect Dis 2022; 12(3):116-126.*

Keywords: *CHROM Agar, Methicillin-Resistant Staphylococcus aureus, Oxacillin, Cefoxitin, Polymerase Chain Reaction*

INTRODUCTION

Staphylococcus aureus is one of the most common causes of nosocomial and community-associated infections, leading to serious infectious diseases with high morbidity and mortality rates [1]. After the introduction of penicillin, resistance in strains of *Staphylococcus aureus* was reported due to the production of the enzyme penicillinase. This was relatively uncommon at the beginning, but its rapid spread along with widespread use of semisynthetic penicillinase-resistant penicillins such as Methicillin, Oxacillin, Cloxacillin, and Dicloxacillin, and further led to the development of certain strains of *Staphylococcus aureus* that were resistant to these newer agents. These strains were called Methicillin-resistant *Staphylococcus aureus* (MRSA) [2].

Methicillin resistance is now becoming recognized increasingly in the community, and the proportion of MRSA has risen worldwide

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during the last two decades. In India, the incidence of MRSA varies from 25% in the western states to 50% in the southern parts [3]. MRSA most frequently affects people with certain predisposing risk factors such as advanced age, prolonged hospital stays, history of antibiotic intake within the last three months, and the presence of an immunocompromised state [4]. It is associated with a high rate of virulence, causing a host of healthcare and community-acquired infections. Healthcare-acquired staphylococcal infections include surgical wound infections, urinary tract infections, and bloodstream or catheter-related infections [5]. Community-acquired MRSA infections include skin, soft tissue infections, and necrotizing pneumonia, and the high mortality associated with some of these community-acquired MRSA infections is a cause of grave concern [6].

Resistance in *Staphylococcus aureus* is conferred by acquiring the *mecA* gene, which is part of a genomic island designated staphylococcal cassette chromosome mec (SCC mec). *MecA* is responsible for synthesizing penicillin-binding protein 2a (PBP 2a, also called PBP 2'), a 78 k Dalton protein. PBP 2a substitutes for other PBPs and, because of its low affinity for all beta-lactam antibiotics, enables staphylococci to survive exposure to high concentrations of these agents. Thus, methicillin resistance confers to isoxazolyl penicillin, such as oxacillin, cloxacillin, and dicloxacillin, as well as cephalosporins and carbapenems. Other mechanisms include the production of a beta-lactamase enzyme by the bacteria that breaks down the beta-lactam ring of the antibiotic, thus inactivating them [7]. The existence of borderline-resistant *Staphylococcus aureus* (BORSA) produced due to the subtle modifications in PBPs has made detecting MRSA more complicated. BORSA isolates are characterized as a small population that exhibits resistance to oxacillin, have an intermittent MIC ranging from 4-8 mg/ml, and have an absence of the *mecA* gene [8].

The phenotypic expression of methicillin resistance among MRSA isolates varies widely. This phenomenon is called heterogeneous resistance. This occurs when a small proportion of the bacterial population in a given culture manifests a high level of resistance under standard conditions. Therefore, heterogeneous resistance, along with high virulence, transmissibility, and resistance to multiple antibiotics, mandate its early detection and management [9]. The emergence of *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides, vancomycin, and teicoplanin, widely regarded as the definitive therapy for multi-drug resistant MRSA, is concerning [10,11]. The continuous evolution of *Staphylococcus aureus* into developing resistance to the newer group of antibiotics is a severe threat to humanity, which could invalidate the use of antibiotics, considered life-saving drugs. Accuracy and promptness in the detection of MRSA are crucial to warrant the selection of an appropriate antibiotic regimen in MRSA-infected patients to decrease MRSA-associated mortality and prevent the spread of MRSA isolates in hospital environments and further development of antimicrobial resistance due to the unjustifiable use of antibiotics.

Various methods have been proposed to detect methicillin resistance in *Staphylococcus aureus*. Polymerase Chain Reaction (PCR) is considered the gold standard for detecting MRSA, which involves the identification of the *mecA* gene in the isolated strains [12]. Other methods include determination of the minimum inhibitory concentrations (MICs) by agar dilution method, broth dilution method, breakpoint method, and E-test, disk diffusion tests using oxacillin, Cefoxitin, and disks screening techniques with a solid culture medium containing oxacillin, CHROM Agar MRSA, latex agglutination test for detection of PBP 2' protein, automated methods, quenching fluorescence method, Velogene rapid MRSA identification assay, and BBL Crystal MRSA ID system [13]. This study aims to determine the diagnostic accuracy of Oxacillin Disk Diffusion and Cefoxitin Disk Diffusion, Oxacillin Resistance Screening Agar Base, and CHROM Agar MRSA methods of detecting MRSA and compare it with PCR which is considered the current gold standard concerning their sensitivity and specificity.

METHODS

The study was conducted in the Department of Microbiology of a central government medical institute and its associated hospitals. The study subjected two hundred pus samples from various clinical disciplines for microbiological evaluation in the Department of Microbiology, and *Staphylococcus aureus*

strains were isolated from pus samples. The isolates used for the study were obtained as a part of routine patient care. Therefore, patient consent was not sought.

Identification of Isolates:

Inoculation of pus samples on Blood agar and Mac Conkey agar was conducted. Following tests were performed to detect *Staphylococcus aureus* to obtain a primary insight into the likely organism present: Gram stain, Culture on Blood agar and Mac Conkey agar, Catalase test, and Coagulase test (Slide and Tube Coagulase tests). Smooth glistening, opaque and beta-hemolytic colonies on Blood agar with a low convex surface, butyrous consistency, positive for catalase, slide and tube coagulase tests, and morphologically showing Gram-positive cocci arranged in grape-like clusters were identified and subjected to a battery of tests to detect MRSA.

The strains were subjected to the MRSA Oxacillin Disk Diffusion test, Cefoxitin Disk Diffusion test, Oxacillin Resistance Screening Agar Base, and CHROM Agar MRSA to detect MRSA isolate with PCR as the reference standard.

Oxacillin Disk Diffusion test and Cefoxitin Disk Diffusion test:

Oxacillin and Cefoxitin susceptibility was determined by disk diffusion test on Mueller Hinton agar plate (Hi-Media India) using a bacterial suspension with turbidity adjusted to 0.5 McFarland standard (Figure 1,2). Plates were incubated at 35 ^{0}C for 24 hours. Results were interpreted according to Clinical and Laboratory Standards Institute: Guidelines (CLSI) [14].

Oxacillin Resistance Screening Agar Base

All strains of *Staphylococcus aureus* were screened for resistance by the agar screening method recommended by CLSI. 4% w/v; 0.68 mol/L of NaCI (Sodium chloride) was added to 1 liter of Mueller Hinton agar. This medium was distributed into 250 ml quantities and sterilized by autoclaving. For the preparation of the plates, oxacillin powder was added after the molten medium was cooled to 50°C-55°C to give a final concentration of 6 µg/ml. The medium was poured into sterile plates at a depth of 4 mm on a flat horizontal surface. The test strains were inoculated into 1 ml quantities of sterile normal saline, confirming the 0.5 McFarland turbidity standard. Using a 1 uL loop dipped in the suspension, an area of 10-15 mm in diameter was spotted. Incubation was done at 35 ^{0}C x 24 hrs. The growth of more than one colony was positive for methicillin resistance. Known positive and negative controls were included in every test run (Figure 3).

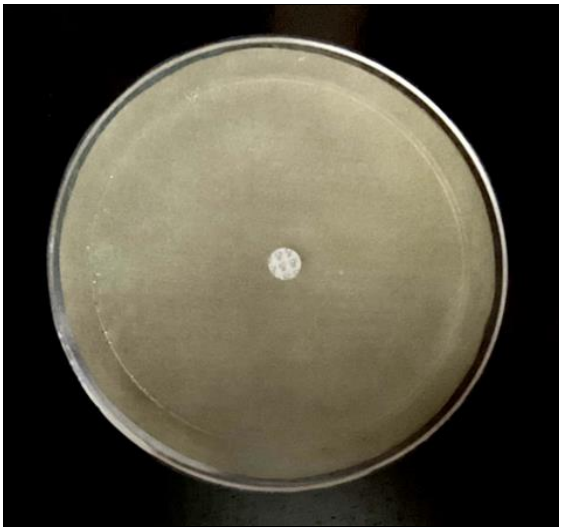

Figure 1. Oxacillin (1 µg) disk diffusion test with 2 % NaCl showing no zone of inhibition.

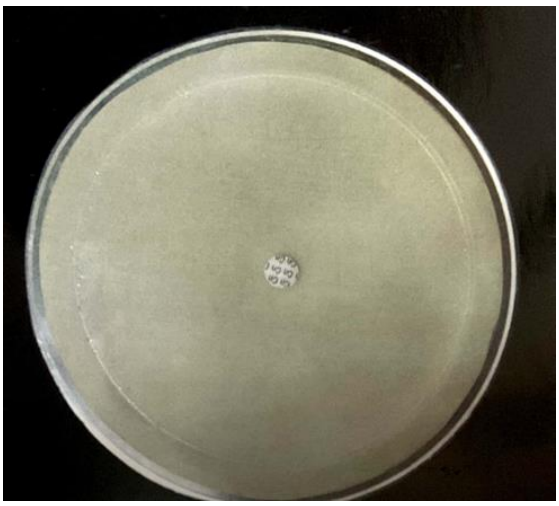

Figure 2. Cefoxitin (30 µg) disk diffusion test shows no inhibition zone.

HiCrome Aureus agar base (Hi-Media India) is a new chromogenic plate medium for identifying *Staphylococcus aureus* and detecting MRSA. The criteria for medium evaluation included colony growth reaction, color reproducibility for identifying *Staphylococcus aureus*, and ease of color detection of MRSA when the medium was supplemented with methicillin or oxacillin. The medium was prepared as per manufacturer instructions by avoiding heating at over 100

^{0}C. Concentrated egg yolk tellurite emulsion was added aseptically; after the medium was cooled to 50 ^{0}C. Oxacillin (4 µg/ml) was added when the agar was cooled at 48°C. Each plate contained 20 ml of agar medium dispensed into 90 mm diameter Petri dishes.

Two hundred samples of *Staphylococcus aureus* were cultured on nutrient agar slopes and sent to the All India Institute of Medical Sciences, New Delhi, to detect the *mecA* gene by PCR. Rapid DNA extraction was carried out after overnight culture on BHIA (brain heart infusion agar) plates (Difco Laboratories), and 5 ul were used directly as the template for amplification from the suspension obtained. Oligonucleotide primers were obtained from a commercial source (Roche Diagnostics): Predicted size of PCR products was 310 base pairs for the *mecA* gene, 456 basepairs for ileS2, and 651 base pairs for the femB fragment: FemB1 (5'-TTA CAG AGT TAA CTG TTA CC-3') and FemB2 (5'-ATA CAA ATC CAG CAC GCT CT-3') (for femB, *MecA*1 (5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3') and *MecA*2 (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3') for *mecA*, and MupA (5'-TAT ATT ATG CGA TGG AAG GTT GG-3') and MupB (5'-AAT AAA ATC AGC TGG AAA GTG TTG-3') for ileS2.

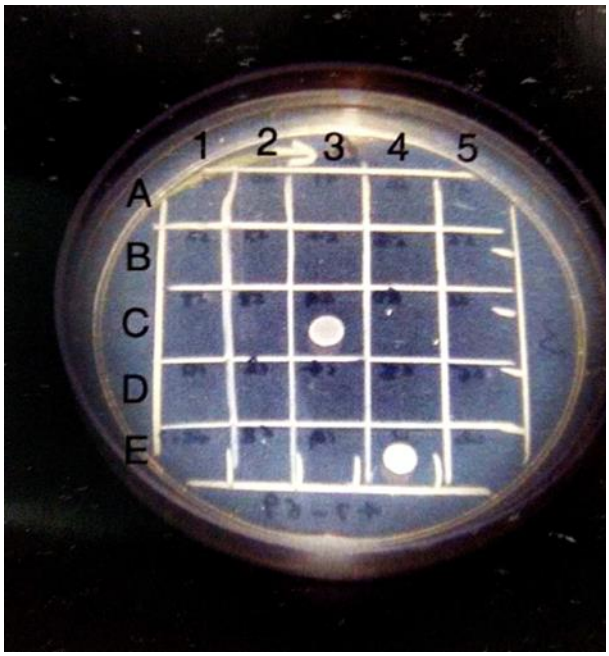

Figure 3. Oxacillin Resistance Screening Agar Base with six µg/ml of oxacillin. Strain C3 and E4 showing growth are MRSA.

Staphylococcus aureus ATCC positive and negative controls were used to monitor batch viability. The organism was subcultured onto these plates, and the growth characteristics were observed after an incubation of 24-48 hours at 35-37 ^{0}C. MRSA gives brown-black colonies with a clear zone around the colony, whereas Methicillin sensitive Staphylococcus aureus (MSSA) is inhibited (Figure 4).

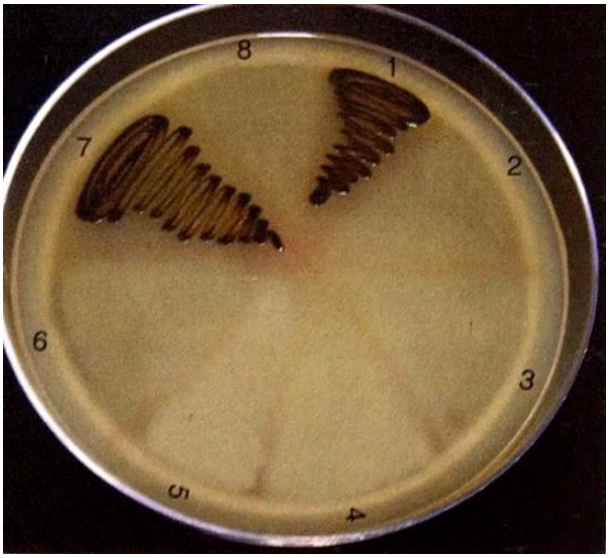

Figure 4. CHROM Agar Staphylococcus aureus with four µg/ml of oxacillin. Strain 1 and 7 with black colonies depict MRSA.

Multiplex PCR assays were directly performed from the bacterial suspension obtained after rapid DNA extraction. For each sample, one reaction was performed with the femB pair of primers to identify *Staphylococcus aureus* strains and with the *mecA* and ileS2 primers to detect both resistance markers. Hot start PCR protocol was used to reduce the formation of nonspecific extension products. All multiplex PCR assays were carried out with a negative control containing all the reagents without a DNA template. DNA amplification was carried out with the thermal cycling profile. The amplified product was subjected to agarose gel electrophoresis and was compared with a 100-bp molecular size standard ladder. The gel was stained with ethidium bromide, and the amplicons were visualized using a UV lightbox (Figure 5).

Analysis

Derivation of various parameters used to compare different methods of detection of MRSA with PCR as the gold standard is given in Table 1.

Sensitivity, Specificity, Positive predictive values, and Negative predictive values were calculated for each test to evaluate their diagnostic accuracy.

- Sensitivity = a/(a+c) x 100
- Specificity= d/(b+d) x 100
- PPV= a/(a+b) x 100
- NPV= d/(c+d) x 100

Table 1: Criteria for evaluation of screening tests in the detection of MRSA.

ScreeningTest Results	Diagnosis by PCR (Gold Standard)		Total
	PCR Positive	PCR Negative	
Positive	a (True positive)	b (False positive)	a+b
Negative	c (False negative)	d (True negative)	c+d
Total	a+c	b+d	a+b+c+d

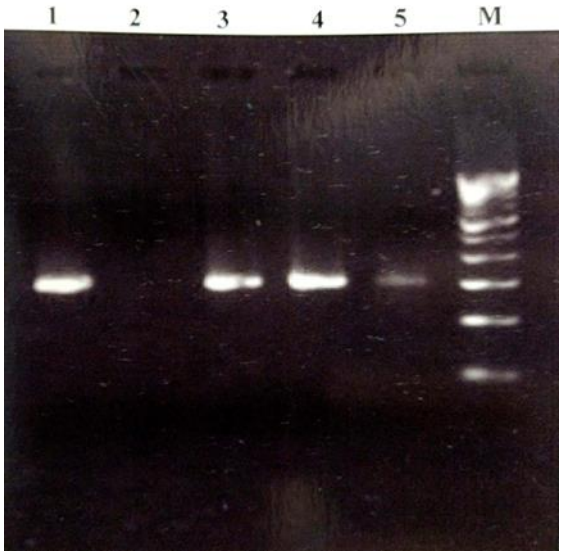

Figure 5. Polymerase Chain Reaction.
Lane1 shows positive control (ATCC 43300), Lane2 shows negative control (ATCC 29213), and Lane3,4,5 are test strains that are positive for the *mecA* gene at 310 base pair, M: Markers.

RESULTS

Prevalence of *Staphylococcus aureus* and MRSA:

A total of two hundred confirmed clinical isolates of *Staphylococcus aureus* were obtained from pus samples from various clinical disciplines. The distribution of *Staphylococcus aureus* according to various clinical disciplines is depicted in the figure (Figure 6).

The *mecA* PCR assay allowed us to classify 30 *Staphylococcus aureus* isolates as *mecA*-positive, i.e., MRSA (15%), and 170 as *mecA*-negative, i.e., MSSA (85%). The distribution of MRSA, according to different clinical disciplines, is depicted in the table: (Table 2). Moreover, two isolates of BORSA were also identified as per their criteria of MIC for oxacillin ranging from 4-8 mg/ml and the absence of the *mecA* gene.

The prevalence rate of MRSA from various clinical disciplines ranged from 8.33% to 16.50%. The highest percentage of *Staphylococcus aureus* and the MRSA strains was isolated from the general surgical ward (51.5% and 16.5%, respectively).

Comparison of Oxacillin Disk Diffusion and Cefoxitin Disk Diffusion tests

For oxacillin disk, 149 (74.5%) strains were sensitive with zone diameters more than or equal to 13 mm (MSSA), 18 (9%) strains yielded zone diameters ranging between 11-12 mm and were labeled as intermediately MSSA, 33 (16.5%) strains with zone diameters less than 10 were resistant (MRSA). For the cefoxitin disk, ranges of the inhibition zones around the antibiotic disk for the isolates were as follows: 169 (84.5%) isolates yielded zone diameters ≥22 mm (MSSA), whereas 31 (15.5%) strains yielded inhibition zones ≤22 mm (MRSA). There is no definable criterion for intermediate sensitive strains in Cefoxitin disks as per CLSI standard (Table 3).

Comparison of the detection methods of MRSA

All free coagulase-producing *Staphylococcus aureus* strains were subjected to various laboratory methods of detecting methicillin resistance. The number and percentage of methicillin-resistant and methicillin-sensitive strains isolated by these methods in comparison with PCR which is considered to be the gold standard is given in the table (Table 4).

The evaluation of different methods for detecting MRSA was done compared to PCR. The comparison of the methods concerning their sensitivity, specificity, PPV, and NPV has been depicted in Table 5.

The sensitivity of the Cefoxitin Disk Diffusion test (100%) was found to be the highest, followed by the CHROM Agar MRSA (96.7%). The lowest sensitivity was demonstrated by Oxacillin Resistance Screening Agar Base (86.7%). The Cefoxitin Disk Diffusion test was the most specific (99.4%), followed by the

Oxacillin Resistance Screening Agar Base (ORSAB) test (98.8%). The least specific was the Oxacillin Disk Diffusion test (96.5%) (Figure 7). The positive predictive value for the Cefoxitin Disk Diffusion test was maximum (96.8%) and lowest for the Oxacillin Disk Diffusion test (81.9%). The negative predictive value of the Cefoxitin Disk Diffusion test (100%) was the highest and lowest for the ORSAB test (97.7%) (Figure 8).

Table 2. Distribution of MRSA according to the clinical discipline

Clinical discipline	Total number of samples screened	No. of MRSA isolated	Percentage of isolation of MRSA
Surgery	103	17	16.5
Skin ward	41	06	14.6
Orthopedics	19	03	15.8
Ophthalmology	12	01	8.3
ENT	10	01	10
Pediatrics	15	02	13.3
Total	200	30	100

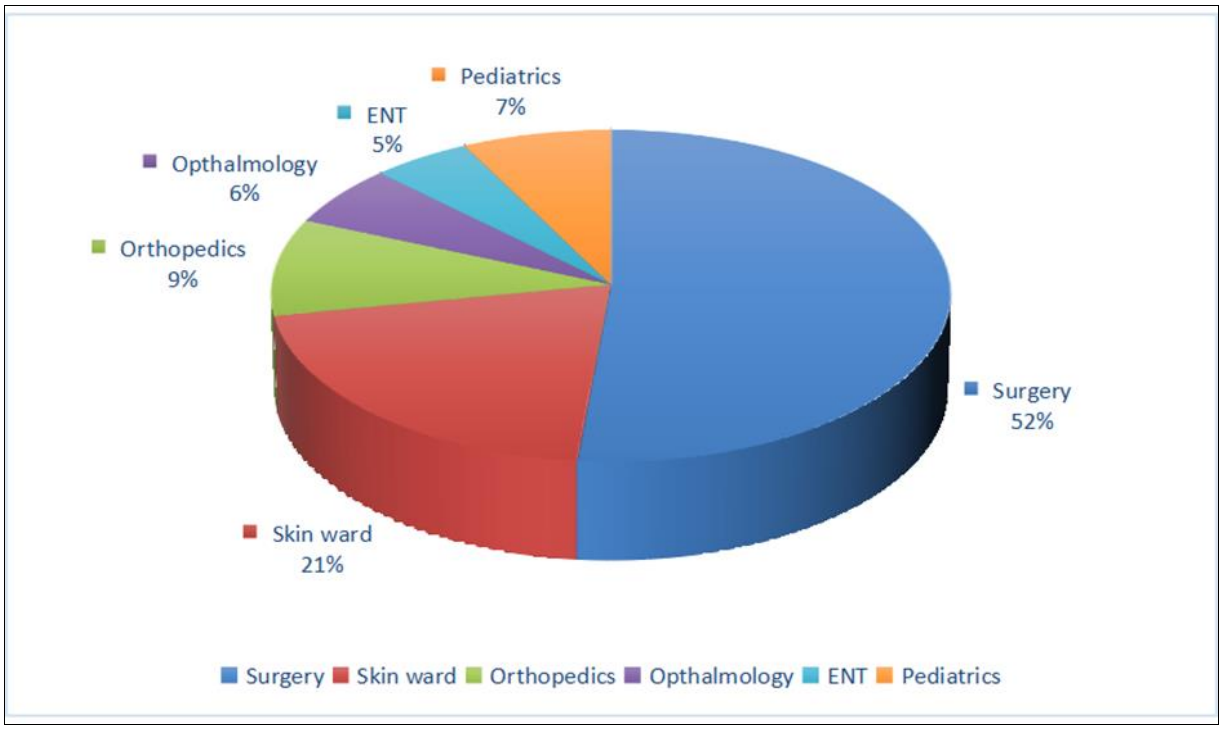

Figure 6. Distribution of Staphylococcus aureus according to clinical discipline.

Table 3. Comparison of Oxacillin Disk Diffusion and Cefoxitin Disk Diffusion tests.

Antibiotic disk used	**Defining Criteria**	**Sensitive (MSSA) No. (%)**	**Intermediate (MSSA) No. (%)**	**Resistant (MRSA) No. (%)**
Oxacillin	S (≥ 13 mm) I (11-12 mm) R (≤ 10 mm)	149 (74.5)	18 (9)	33 (16.5)
Cefoxitin	S (≥ 22 mm) I (-) R (≤ 21 mm)	169 (84.5)	0 (0)	31 (15.5)

Table 4. MRSA and MSSA strains as detected by different methods.

Detection Method	Total Sample Screened	MRSA	MSSA
		Number detected (%)	Number detected (%)
DD-Ox	200	33 (16.5)	167 (83.5)
DD-Cn	200	31 (15.5)	169 (84.5)
ORSAB	200	28 (14.0)	172 (86.0)
CHROMagar	200	33 (16.5)	167 (83.5)
PCR	200	30 (15.0)	170 (85.0)

DD-Ox=Oxacillin Disk Diffusion test, DD-Cn=Cefoxitin Disk Diffusion test, ORSAB=Oxacillin Resistance Screening Agar Base, CHROMagar-CHROM Agar MRSA, PCR= Polymerase Chain Reaction

Table 5. Comparison of the laboratory methods for detection of MRSA.

Detection method of MRSA	True negative	True positive	False positive	False negative	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Oxacillin Disk Diffusion (1 µg)	27	164	6	3	200	90	96.5	81.8	98.2
Cefoxitin Disk Diffusion (30 µg)	30	169	1	0	200	100	99.4	96.8	100
Oxacillin Resistance Screening Agar Base	26	168	2	4	200	86.7	98.8	92.9	97.7
CHROMagar MRSA	29	166	4	1	200	96.7	97.7	87.9	99.4

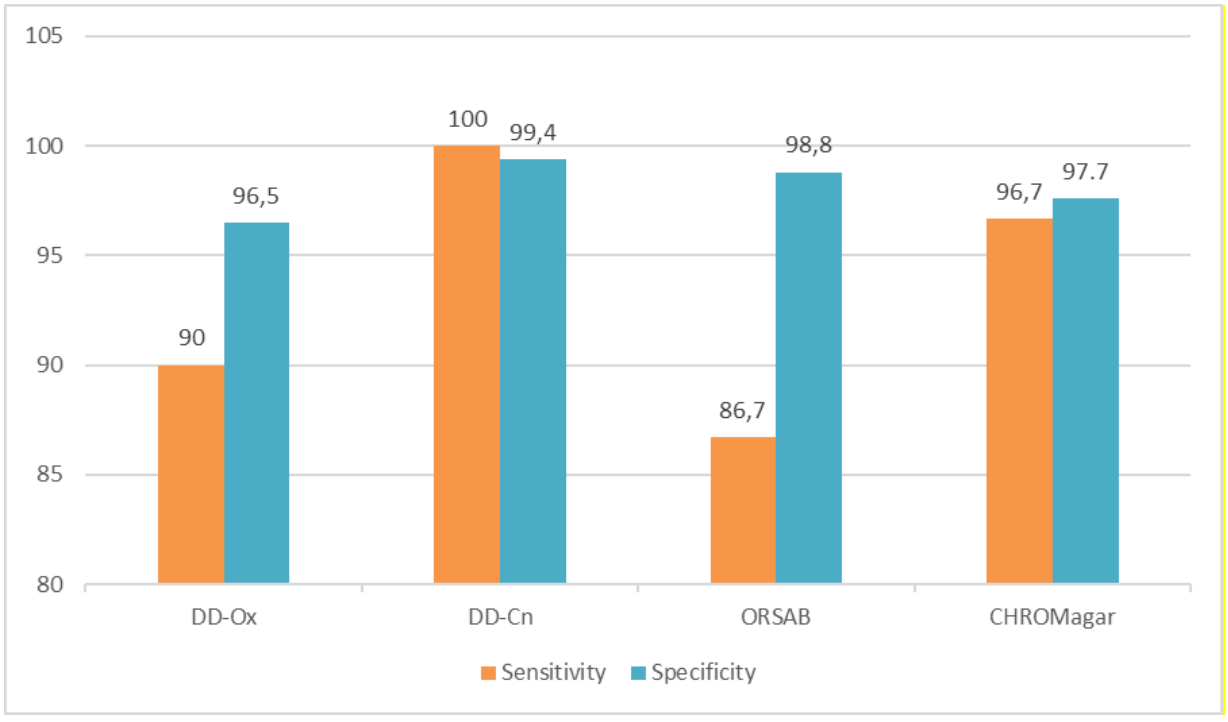

Figure 7. Comparison of sensitivity and specificity of different methods of detection of MRSA.

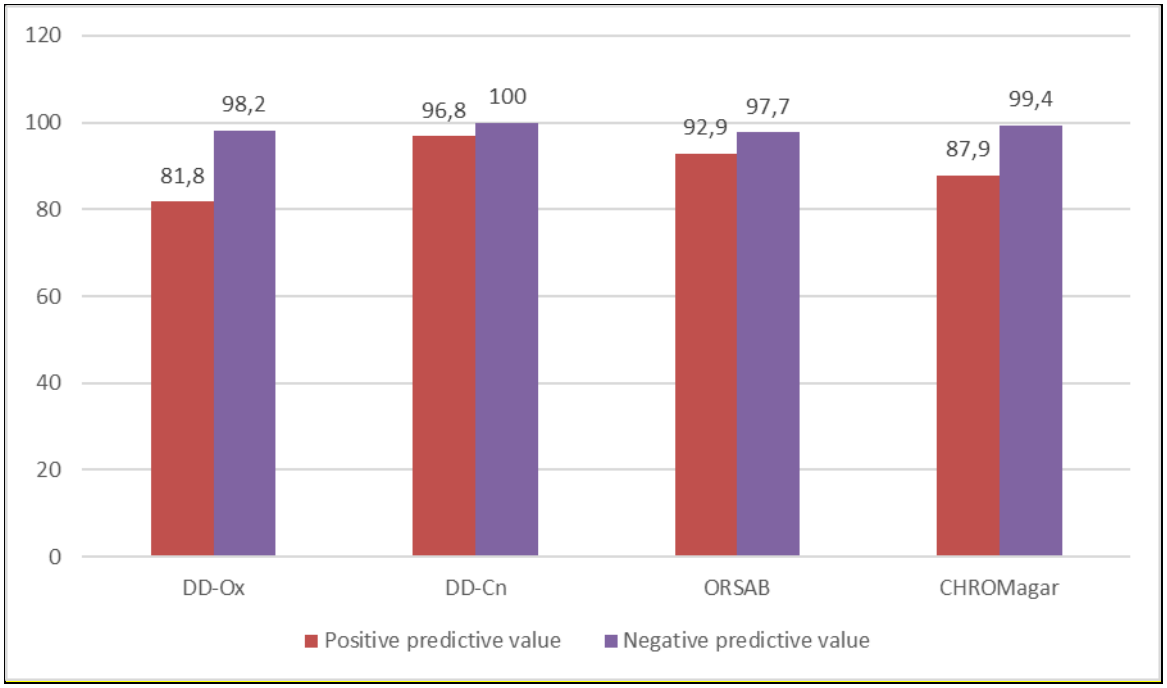

Figure 8. Comparison of PPV and NPV of different methods of detection of MRSA.

DISCUSSION

Staphylococcus aureus is one of the most frequently encountered bacterial pathogens responsible for various mild to life-threatening infections. MRSA is an important nosocomial pathogen, overcoming most therapeutic agents developed in recent years. Hence the antimicrobial chemotherapy for this species is empirical. The prevalence of MRSA is usually more in the surgical wards when compared to the medical units [15]. However, in the present study, MRSA was uniformly distributed across surgical and medical disciplines, thus pointing to an increase in the isolation rate of MRSA in medical units. Routine detection of MRSA is difficult using standard media with disk diffusion, MIC determination, or agar breakpoint methods. This has been ascribed to the heterogeneous expression of methicillin resistance in many strains. These strains seem to be increasing, in number and in level of heterogeneity, which is a challenge even with specialized methods.

Detection of the *mecA* gene by PCR is considered the gold standard for exposing methicillin resistance in staphylococci and therefore has been taken as the reference method in the present study [16]. It is a rapid, molecular-based assay detecting a specific gene, reproducible and for the direct detection of MRSA from nasal specimens. Compared to culture methods requiring up to 5 days, the rapid turn-around time of the MRSA PCR test enables hospitals to dramatically reduce the time required to identify an outbreak enabling improved prevention and control [17]. However, the expensive nature of this confirmatory test and its requirement of standard reference laboratories make it difficult to perform in developing centers. Therefore, this study aims to explore alternative cost-effective and more uncomplicated phenotypic “methods to PCR to detect MRSA”.

In the present study, the Oxacillin Disk Diffusion test identified 33 (16.5%) samples positive for MRSA, whereas the Cefoxitin Disk Diffusion test accurately identified 31 (15.5%) isolates as per CLSI guidelines. It could correctly identify all the *mecA*-positive MRSA strains and missed none. There was only one false positive by this method. Previous analysis of diagnostic studies has described pooled sensitivity and specificity of the Oxacillin Disk Diffusion test as 81.7% (64.3-91.8) and 92.1% (80.4-97.1), respectively, whereas sensitivity and specificity for the Cefoxitin Disk Diffusion test has been reported as 95.5% (81.4-99.0) and 81.4% (46.6-95.6) respectively [18]. The Cefoxitin Disk Diffusion method is proposed to be the best in performing routine detection of all classes of MRSA. The evaluation for low-level methicillin resistance in MRSA isolates, including the disk

diffusion method with the antibiotics cephamycin, Cefoxitin, and moxalactam, has produced results in favor of Cefoxitin with 100% sensitivity and specificity [19]. Cefoxitin disk is the best predictor of methicillin resistance in *Staphylococcus aureus* without utilizing molecular biology techniques [11].

Cefoxitin and Oxacillin Disk Diffusion methods displayed comparable sensitivity and specificity in this study. However, Cefoxitin is still a better predictor than oxacillin for detecting intermediate resistant strains of *Staphylococcus aureus*. Cefoxitin is also a more potent inducer of PBP2' in vitro in MSSA and has a high affinity for staphylococcal PBP4, which is involved in cell wall cross-linkage. Testing with oxacillin and cefoxitin disks would give better sensitivity than the cefoxitin test alone, but at the expense of specificity [20]. The present study showed that the Cefoxitin (30 µg) disk performed better than the oxacillin (1 µg) disk with a sensitivity of 100% and specificity of 99.4%, and only one false positive as compared to six by the oxacillin disk test, thereby adding to the evidence from previous studies. The slight differences could be related to the difference in agar type and the inclusion of many rugged (low-level resistant) isolates. Cefoxitin disk susceptibility test appears to be a helpful procedure in that it is easy to perform routinely in laboratories, is more reliable, and has greater accuracy than oxacillin disk tests. It does not require any modification of conditions to improve the expression of resistance, hence eliminating the need for separate inoculum, media, and incubation time and temperature. The cost and workload are thereby reduced. Hence, the cefoxitin disk test has the potential for broader use in diagnostic laboratories.

CHROM Agar MRSA achieved 96.7% sensitivity and 97.7% specificity, while the ORSAB was far less sensitive (86.7%) but more specific (98.8%) in this study. According to previous reports, sensitivity and specificity for CHROM Agar MRSA were found as 88.1% (77.4–94.1) and 96.4% (91.3–98.5), respectively. On the other hand, the sensitivity and specificity for ORSAB are described as 82.9% (69.5–91.2) and 91.8% (82.4–96.4), respectively [16]. Previous studies that have compared the two tests have found CHROM Agar MRSA more accurate than ORSAB in identifying *Staphylococcus aureus* because of its significantly higher specificity, different from the findings in the present study. Although ORSAB performs better than CHROM Agar MRSA in screening methicillin resistance, its usefulness in clinical practice is limited because of its lower sensitivity [21]. Furthermore, non-multi-drug resistant community-acquired MRSA has been shown to grow inconsistently on the chromogenic medium in CHROM Agar MRSA. Therefore, it is suitable for identifying hospital-acquired MRSA, which shows a multi-drug resistant profile but community-acquired MRSA with a non-multi-drug resistant profile requires further evaluation [22]. CHROM Agar MRSA was also more sensitive than the Oxacillin Disk Diffusion test. It reduces the number of susceptibility tests performed on non-MRSA isolates, and less labor is required than the traditional dilution methods, which use multiple plates and reagents. However, the CHROM Agar was not more sensitive and specific than the Cefoxitin Disk Diffusion method.

The literature on the detection and identification of MRSA is often conflicting in recommendations regarding the most reliable method for routine use. Accurate detection of methicillin resistance in *Staphylococcus aureus* by routine methods is difficult due to heterogeneity in the subpopulations of *Staphylococcus aureus*. The current study involves isolates from a single healthcare center. With an increase in the prevalence of MRSA worldwide and a rise in multi-drug resistant strains of *Staphylococcus aureus*, a multicentric study with large sample size is recommended for establishing an early and accurate detection method, thus ensuring its efficient management while preventing the development of more resistant strains.

CONCLUSION

In the present study, the Cefoxitin Disk Diffusion phenotypic method was found to be the most sensitive and specific among all four methods of detection of methicillin resistance in *Staphylococcus aureus*. It is easy to perform in routine laboratories, is more reliable, and has greater accuracy. CHROM Agar MRSA was better than the ORSAB among the screening methods. Based on their sensitivity, specificity, cost, and convenience, the Cefoxitin Disk Diffusion test is the first choice for screening MRSA, followed by CHROM Agar MRSA.

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Data Availability: The datasets generated during and/or analyzed during the current research are available from the authors upon reasonable request.

Conflicts of interest: The authors declare no potential conflict of interest relating to regarding article's research, authorship, and publication.

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