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Comparative Diagnostic Performance of Phenotypic Methods for Detection of Methicillin-Resistant Staphylococcus aureus in a Resource-Limited Healthcare Setting

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important worldwide cause of infections related to healthcare and the community, especially in low- and middle-income countries (LMICs) where molecular diagnostics are limited. Reliable phenotypic methods are therefore essential for routine MRSA detection in resource-constrained laboratories. **Objective:** This study was aimed to compare the diagnostic performance of four commonly used phenotypic methods for MRSA detection and to evaluate their operational suitability in PCR-limited settings. **Methodology:** This cross-sectional analytical study was conducted from March 2016 to February 2017 in the Department of Microbiology and Immunology at Bangladesh Medical University, Dhaka, Bangladesh. A total of 120 confirmed *Staphylococcus aureus* isolates were tested using oxacillin disk diffusion, cefoxitin disk diffusion, MRSA chromogenic agar and PBP2a latex agglutination. Diagnostic performance indicators including sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard statistical methods. **Results:** Among the 120 isolates, 70 (58.3%) were identified as MRSA by different phenotypic methods. MRSA prevalence was significantly higher among indoor patients compared to outdoor patients ($p < 0.01$). Cefoxitin disk diffusion demonstrated high sensitivity (92.9%) and specificity (98.0%), while oxacillin disk diffusion, which showed lower sensitivity (81.4%). Chromogenic agar exhibited moderate diagnostic accuracy (90%). PBP2a latex agglutination showed 100% sensitivity, specificity, and overall accuracy, demonstrating perfect concordance with confirmed MRSA isolates. **Conclusion:** Cefoxitin disk diffusion and PBP2a latex agglutination are reliable as well as practical phenotypic methods for accurate MRSA detection in resource-limited laboratories, supporting effective clinical management and infection control.

Keywords: Methicillin-resistant *Staphylococcus aureus* (MRSA); Phenotypic detection; Cefoxitin disk diffusion; PBP2a latex agglutination; Chromogenic agar

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections is a worldwide public health issue. Since its first identification in 1961, following the introduction of methicillin, MRSA has evolved into a complex human pathogen. It has been responsible for a wide variety of infections, ranging from minor skin infections to life-threatening conditions, including pneumonia, bacteremia, endocarditis, and surgical site infections[1].

As MRSA is capable of acquiring resistance determinants and efficiently disseminating it within healthcare environments, the necessity of rapid and reliable diagnostic methods is very high[2].

The *mecA* gene is the hallmark of MRSA, which

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located on the staphylococcal cassette chromosome mec (SCCmec). The Penicillin-binding protein 2a (PBP2a) is encoded by this mecA gene and this protein is responsible for markedly reduced affinity for β-lactam antibiotics[3]. Consequently, MRSA strains are resistant to almost all β-lactam agents, including cephalosporins, methicillin and oxacillin. In the recent years, some other resistance patterns have emerged, showing intermediate or full resistance to vancomycin-making the prevailing therapeutic options limited[4]. Healthcare-associated MRSA (HA-MRSA) strains are predominant in overcrowded hospitals, while community-associated MRSA (CA-MRSA) strains containing Panton-Valentine leucocidin are posing new risks for community transmission. Strengthening MRSA diagnostic capacity is therefore essential not only for clinical care but also for infection prevention and control (IPC) programs[5].

Although molecular detection of *mecA* by PCR is the gold standard for confirming MRSA, most laboratories in low- and middle-income countries (LMICs) struggle to implement molecular diagnostics due to high cost of PCR reagents and platforms, the need for specialized technical expertise with strong biomedical infrastructure. Consequently, phenotypic methods remain the backbone of MRSA diagnosis in LMICs, despite known limitations such as heteroresistant expression, environmental dependency, and method-specific variability[6].

While PCR detection of *mecA* provides the most accurate diagnosis, its adoption remains limited across Asia and low-income regions due to resource constraints[7]. Consequently, clinical laboratories continue to rely heavily on phenotypic screening methods, despite their varied sensitivity and susceptibility to environmental factors such as salt concentration, incubation temperature, inoculum density, and heterogeneous gene expression[6].

This study presents a comparative evaluation of four commonly used phenotypic MRSA detection methods-oxacillin disk diffusion (ODD), cefoxitin disk diffusion (CDD), MRSA chromogenic agar, and latex agglutination for PBP2a-highlighting their diagnostic values and operational feasibility in PCR-limited settings.

Methodology

Study Setting and Design: This cross-sectional analytical study was conducted from March 2016 to February 2017 at the Department of Microbiology and Immunology, Bangladesh Medical University (BMU), Dhaka, Bangladesh. The study included 120 *Staphylococcus aureus* isolates based on colony morphology on blood agar, Gram staining, catalase test, slide and tube coagulase test and mannitol fermentation as per standard clinical microbiology guidelines[8].

Phenotypic MRSA Detection Methods: All confirmed *Staphylococcus aureus* isolates were screened for methicillin resistance using four phenotypic methods, including oxacillin disk diffusion (ODD), cefoxitin disk diffusion (CDD), PBP2a latex agglutination, and MRSA chromogenic agar.

Oxacillin Disk Diffusion Test: Oxacillin disk diffusion test was performed, and the results were interpreted according to CLSI guidelines[9]. A bacterial suspension was prepared equivalent to a 0.5 McFarland standard from each isolate and by using sterile swab the suspension was inoculated onto Mueller–Hinton agar (MHA). A 1 µg oxacillin disk (Hi-Media, India) was placed on the inoculated plate and was incubated at 35°C for 24 h. Zone diameters were measured and interpreted according to CLSI guidelines; the zone of inhibition ≤10 mm was considered as oxacillin-resistant which was indicative of methicillin resistance[10].

Cefoxitin Disk Diffusion Test: Cefoxitin disk diffusion test was performed according to CLSI guidelines. MHA plate was inoculated by bacterial suspension equivalent to 0.5 McFarland standard, and a 30 µg cefoxitin disk (Hi-Media, India) was used and incubated at 37°C for 24 h. Inhibited zone diameter ≤ 21 mm was considered cefoxitin-resistant MRSA[10].

MRSA Chromogenic Agar Media: Phenotypic detection was performed on HiCrome MeReSa Agar Base (Hi-Media, India) according to the manufacturer's provided selective supplement. The medium containing chromogenic substrate was cleaved by α-glucosidase, thus producing bluish-green colonies, which was characteristic of MRSA. High sodium chloride concentration makes the media selective by inhibiting the growth of accompanying microflora. Plates were made according to the manufacturer's instructions. A 0.5 McFarland suspension of each isolate was inoculated on the chromogenic medium and incubated at 37°C for 24–48 h. The appearance of bluish-green colonies was indicative of MRSA[11].

PBP2a Latex Agglutination Test: A commercial latex agglutination kit was used to detect PBP2a. This test employs monoclonal antibody-coated latex particles targeting PBP2a protein, encoded by mecA gene.

Visible agglutination with the sensitized latex within 3 minutes was indicative of the presence of MRSA. This method has been found for rapid and specific detection of MRSA[12]. The assay kit was obtained from Denka Seiken Co. Ltd., Japan. Protein extraction was carried out as described by Cavassini et al. (1999). Approximately 1.5 × 10^9 cells were suspended in 200 µL of 0.1 M NaOH, followed by heating for 3 min at 100°C, then cooled and finally neutralized with the help of 50 µL of extraction reagent 2. The suspension was centrifuged for 5 min at 3000 rpm and the remaining supernatant was taken as the test extract. Two circles on the test card were labeled as test and control. 50 µL of extract was dispensed into each circle. One drop of sensitized latex was added to the test circle, and one drop of control latex to the control circle. Mixtures were stirred separately and the card was gently rotated for 3 min. Agglutination with sensitized latex and no reaction with control latex was interpreted as PBP2a positive, reported as MRSA[12].

Statistical Analysis: All the data were entered into data base using SPSS (version 23). Results were presented in the form of tables. P-value was calculated by the Chi-square test and P <0.05 was considered significant. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of different methods for the detection of MRSA were calculated according to the formula mentioned by Harris and Taylor, 2004[13].

Results

Among 120 Staphylococcus aureus isolates, the majority were obtained from pus (59/120), followed by wound swab (31/120), blood (21/120), and urine (9/120). MRSA detection was highest in pus specimens (41.42%), followed by wound swabs (27.14%) and blood (24.29%). Only 7.14% of MRSA isolates were recovered from urine samples (Figure 1). MRSA was significantly more prevalent among in-patients (69.0%) compared to out-patients (42.9%), and the difference was statistically significant (Chi-square test, p < 0.01). This was suggested a higher burden of healthcare-associated MRSA among hospitalized patients (Table 1).

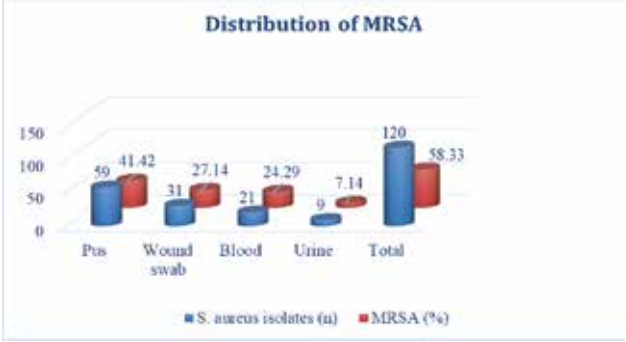

Figure I: Distribution of MRSA according to specimen (n = 120) (Percentage calculated against total MRSA, n=70)

Table 1. Distribution of MRSA and MSSA Isolates among Indoor and Outdoor Patients (n = 120)

Department	MRSA	MSSA	Total	Percent
OPD	21 (42.9%)	28 (57.1%)	49 (100.0%)	<0.01
IPD	49 (69.0%)	22 (31.0%)	71 (100.0%)	
Total	**70 (58.3%)**	**50 (41.7%)**	**120 (100.0%)**	

p value was determined by Chi-square test. P<0.05 considered as statistically significant; OPD: Out-patient Department; IPD: In-patient Department

Table 2: MRSA Detection Rate by Different Phenotypic Methods

Method	Frequency	Percent
Oxacillin disk diffusion	58	48.3
Cefoxitin disk diffusion	66	55.0
Chromogenic agar	68	56.7
Latex agglutination	70	58.3

In this study, the Sensitivities of oxacillin disc diffusion, cefoxitin disc diffusion and MRSA chromogenic agar media were found to be 81.43%, 92.86% and 90.0% respectively. Specificities of oxacillin disc diffusion, cefoxitin disc diffusion, MRSA chromogenic agar media were found to be

Table 3: Comparative Diagnostic Accuracy of Phenotypic Methods

Method	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	PPV (95% CI)	NP (95% CI)
Oxacillin disk diffusion	81.4%	98.0%	88.3%	98.2%	79.0%
Cefoxitin disk diffusion	92.9%	98.0%	95.0%	98.5%	90.7%
Chromogenic agar	90.0%	90.0%	90.0%	92.6%	86.5%
Latex agglutination	100.0%	100.0%	100.0%	100.0%	100.0%

NPV= Negative Predictive value, PPV= Positive predictive value, CI= Confidence Interval

98.0%, 98.0% and 90%, respectively. The latex agglutination test showed 100% sensitivity and specificity. Accuracies of oxacillin disc diffusion, cefoxitin disc diffusion, MRSA chromogenic agar media and latex agglutination test were 88.33%, 95.0%, 90.0% and 100% respectively. Positive predictive values of oxacillin disc diffusion, cefoxitin disc diffusion, MRSA chromogenic agar media and latex agglutination test were 98.28%, 98.48%, 92.6% and 100% respectively and negative predictive values were 79.03%, 90.74%, 86.5% and 100% respectively (Table 3).

Discussion

Methicillin resistance in *Staphylococcus aureus* remains a major concern in clinical microbiology, particularly in regions where laboratory resources are limited. The widespread circulation of methicillin-resistant *Staphylococcus aureus* (MRSA) has significantly increased the burden of hospital-associated infections, presenting considerable challenges for treatment and infection prevention[1]. In settings where molecular detection of the *mecA* gene is not feasible, it becomes essential to identify phenotypic methods that can generate reliable diagnostic information. The present study demonstrated four commonly used methods, compared their important performance differences and also highlighted their implications for low-resource laboratory settings.

The present study revealed a high MRSA detection rate, with more than half of the Staphylococcus aureus isolates identified as MRSA (Figure 1). This finding is consistent with reports from tertiary healthcare facilities in Bangladesh and neighboring South Asian countries, where MRSA prevalence remains high. Factors such as overcrowding, prolonged hospital stays, and inappropriate use of broad-spectrum antibiotics may contribute to MRSA transmission[5,6]. The significantly higher MRSA prevalence among inpatient samples compared to outpatient samples supports the association of MRSA with healthcare exposure and the predominance of hospital-associated strains[5].

Cefoxitin demonstrated high sensitivity in MRSA detection, likely due to its strong induction of *mecA*-mediated resistance, and its performance aligns with international recommendations for its use as a surrogate marker[10]. In contrast, oxacillin showed lower reliability, as its results are influenced by test conditions, which may lead to false susceptibility. Therefore, cefoxitin is preferred for routine MRSA screening[6,10].

Chromogenic agar showed good diagnostic performance and allowed rapid presumptive identification of MRSA through distinct colony coloration, although occasional false results highlight the need for confirmatory testing[11]. Latex agglutination targeting PBP2a showed excellent performance and complete agreement with confirmed MRSA isolates, supporting its value as a rapid confirmatory method, despite cost and availability limitations in resource-limited settings[12].

These findings demonstrated considerable variation among phenotypic methods used for MRSA screening. Cefoxitin disk diffusion and latex agglutination test appeared to be the most reliable methods that can be recommended for primary diagnostic application in laboratories that lack advanced molecular tools. Chromogenic media can be considered as an effective screening tool, whereas oxacillin disk diffusion should be interpreted carefully due to its lower sensitivity. The significantly higher burden of MRSA among inpatient specimens further highlights the importance of increasing the diagnostic capacity to prevent infections, implement antimicrobial stewardship and reduce healthcare-associated transmissions[5].

Although the current study provides practical evidence for routine laboratory use, some limitations must be acknowledged. The work was conducted in a single tertiary center and relied solely on phenotypic approaches, which may limit the applicability of findings to other context. Future work incorporating multicenter sampling, genotyping of MRSA clones, and cost-effectiveness comparison of phenotypic strategies would help national policymakers determine scalable diagnostic solutions for routine use.

Conclusion

This study underscores significant variability in the diagnostic performance of phenotypic MRSA detection methods. Oxacillin disk diffusion was unreliable and should not be used in isolation. Cefoxitin disk diffusion emerged as a robust and highly reliable method, while chromogenic media demonstrated moderate accuracy suitable for screening. Latex agglutination showed perfect concordance with PCR and stands out as the preferred phenotypic method in low-resource settings due to its speed, reliability, and operational simplicity.

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Conflict of Interest
The authors declare no conflict of interest.

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Authors' contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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