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Evaluation of Phenotypic Methods for the Detection of Methicillin-resistant *Staphylococcus aureus* in Comparison with PCR

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Abstract

Background: The major cause of hospital- and community-acquired infections is Methicillin-resistant Staphylococcus aureus (MRSA). Early and specific identification is important for effective treatment and infection management. While PCR for mecA is the gold standard, its use is often restricted in routine laboratories due to high costs and technical limitations. **Objective:** The objective of this research was to assess the efficacy of widely used phenotypic approaches for MRSA identification and to compare them with PCR. **Methodology:** This cross-sectional study was conducted from March 2016 to February 2017 at the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. A total of 120 clinically confirmed Staphylococcus aureus isolates were collected from DMC, BIRDEM, and BSMMU microbiology laboratories. MRSA detection was performed using four phenotypic methods: oxacillin disk diffusion, cefoxitin disk diffusion (CLSI 2014 guidelines), MRSA chromogenic agar (HiCrome MeReSa), and latex agglutination test (Denka-Seiken PBP2a kit). PCR targeting the *mecA* gene, using the QIAamp DNA Mini Kit for extraction and electrophoresis on 3% agarose gel, served as the gold standard reference method. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) were calculated and compared across all methods. **Results:** PCR identified 70(58.3%) MRSA and 50(41.7%) MSSA among 120 *Staphylococcus aureus* isolates. Cefoxitin disk diffusion showed great performance of 92.9% sensitivity and 98.0% specificity, while MRSA chromogenic agar showed 90.0% sensitivity and specificity. The oxacillin disk diffusion test was the least sensitive test, with a sensitivity of 81.4%. Latex agglutination test is the best test, with 100% sensitivity, specificity and overall accuracy. **Conclusion:** Latex agglutination was the quickest and most accurate phenotypic approach, similar to the PCR finding exactly. Cefoxitin disk diffusion was also a good alternative. Using these strategies may help to identify MRSA and stop the spread of infections with limited resources. [*Bangladesh Journal of Infectious Diseases, December 2025;12(2):294-299*]

Keyword: *Staphylococcus aureus*; MRSA; Phenotype; PCR; Bangladesh

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Introduction

Staphylococcus aureus persists to be one of the most clinically related to human infections because it may easily become resistant to antimicrobial drugs. Over the years, it rapidly became resistant to almost all antibiotics that have been used to treat it, and this antibiotic resistance is a major worldwide health concern[1]. The earliest form of resistance emerged soon after the introduction of penicillin, when *Staphylococcus aureus* strains began producing β-lactamase enzymes that inactivated the drug[2]. In response, methicillin—a penicillinase-resistant penicillin was developed; however, by 1961, initially in hospitals and subsequently in community settings, the methicillin-resistant Staphylococcus aureus (MRSA) had already been reported[3].

The dissemination of MRSA has since evolved into a major global clinical and public health issue[4]. The microbes have a unique way of resisting β-lactam antibiotics, making it challenging to identify effective and broadly accessible treatments[5]. This resistance can be strengthened by the acquisition of the *mecA* gene, which encodes the penicillin-binding protein PBP2a, permitting the bacterium to maintain cell wall synthesis in the presence of β-lactams[6]. New mutations in penicillin-binding protein genes are resistant to methicillin in susceptibility testing but don't have the *mecA* gene. Consequently, vancomycin emerged as the preferred treatment for MRSA infections. However, the widespread use of vancomycin has resulted in the rise of vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant S. aureus (VRSA), hence presenting new treatment problems[7]. Timely and precise diagnosis of MRSA is crucial for directing suitable antibiotic treatment and for averting further dissemination in clinical and community settings[8]. Nonetheless, methicillin resistance is often exhibited heterogeneously, complicating phenotypic identification in standard laboratory environments[9]. These hetero-resistant strains may potentially develop into completely resistant phenotypes and, thus, should be considered noteworthy from both diagnostic and epidemiological perspectives[10].

Over time, several different ways to test for MRSA have been constructed in the lab. The phenotypic methods used for the detection of MRSA in lab are-oxacillin and cefoxitin disk diffusion test, agar dilution or E-test to measure the minimum inhibitory concentration (MIC). Specific chromogenic agars are also used for rapid screening. Latex agglutination test is an immunological technique that identifies the PBP2a protein, encoded by the *mecA* gene[11,12]. PCR is considered as the "Gold Standard" test with the highest sensitivity and specific in identifying *mecA* gene[13]. However, the use of molecular diagnostics is very limited due to their relatively higher cost, lack of modern apparatus and highly skilled staff. These limitations render the use of PCR in low-resource laboratories and peripheral healthcare settings.

Due to these constraints, phenotypic methods are still considered to be the most convenient screening tool for the detection of MRSA in low-resource laboratory settings[14]. So, this research work was conducted to evaluate the efficacy of certain phenotypic methods, oxacillin disk diffusion, cefoxitin disk diffusion, MRSA chromogenic agar and latex agglutination test in compared to PCR in detecting *mecA* gene. The objective was to find out a reliable, economical, and feasible test specifically to use in low-resource laboratory settings.

Methodology

Study Design and Bacterial Isolation: This cross-sectional study was conducted over one year, from March 2016 to February 2017, in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. A total of 132 isolates from different clinical specimens clinically suspected (Beta haemolytic blood agar colonies) as *Staphylococcus aureus* were collected from the microbiology laboratories of Dhaka Medical College (DMC), BIRDEM General Hospital, and BSMMU. Non-haemolytic, pinpoint colonies and suspected growth of fungus colonies in blood agar were excluded during sample collection. The isolates were inoculated onto blood agar plates and incubated aerobically at 37 °C for 24 hours[15]**.** Based on standard microbiological procedures, 120 isolates were identified as S. aureus by their Colony morphology, β-haemolysis, and golden pigment production, Gram-positive cocci in clusters on Gram stain, Positive catalase and coagulase tests (slide and tube methods) and Mannitol fermentation on mannitol salt agar[16,17]**.**

Quality Control, Preservation of Isolates and Antimicrobial Susceptibility Testing: An ATCC-25923 strain of *S. aureus* obtained from the BSMMU microbiology laboratory was used as a control for biochemical reactions (catalase, coagulase, and mannitol fermentation). Confirmed isolates were maintained on nutrient agar slants in screw-capped tubes. After overnight incubation at

37 °C, sterile liquid paraffin was layered over the surface to prevent dehydration. The tubes were stored at 4 °C for subsequent testing. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA) following CLSI 2014 guidelines.

Detection of Methicillin-Resistant S. aureus (MRSA)

Oxacillin Disk Diffusion Test: Isolates standardized to 0.5 McFarland were inoculated onto MHA. A 1 µg oxacillin disk was applied, and plates were incubated at 35 °C for 24 h. A zone ≤ 10 mm was interpreted as resistant (CLSI 2010)[18,19].

Cefoxitin Disk Diffusion Test (CDD): A 30 µg cefoxitin disk (Hi-Media) was placed on MHA inoculated with the test suspension and incubated at 37 °C for 24 h. A zone ≤ 21 mm was interpreted as MRSA, whereas isolates that showed an inhibition zone ≥ 22 were considered to be MSSA by following CLSI 2014.

MRSA Latex Agglutination Test: The Denka-Seiken (Japan) PBP2a latex agglutination kit was used[9]. After alkaline extraction and neutralization, the supernatant was mixed with latex reagents. Agglutination within three minutes with the test latex but not with control latex indicated PBP2a positivity (MRSA). Reactions were graded as 3+, 2+, 1+, or negative based on agglutination intensity.

MRSA Chromogenic Agar Test: The HiCrome MeReSa Agar Base (Hi-Media) was prepared according to manufacturer instructions[20]. A 0.5 McFarland suspension was streaked on the medium and incubated at 37 °C for 24–48 h. Bluish-green colonies were identified as MRSA.

Detection of mecA Gene by PCR: PCR was performed at the BSMMU PCR laboratory following standard protocol[21].DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany), according to the manufacturer's procedure for Gram-positive bacteria.

Primers Used

Gene	Primer sequence (5′–3′)	Amplicon size (bp)
femA	AAAAAAGCACATAACAAGCG GATAAAGAAGAAACCAGCAG	132
mecA	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	163

PCR amplification included: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation (94 °C, 2 min), annealing (57 °C, 2 min), extension (72 °C, 1 min), and final extension at 72 °C for 7 min.

Amplicon Detection: Amplicons were resolved on 3% agarose gel containing ethidium bromide, electrophoresed at 100 V for 70 min in 1× TAE buffer. Bands were visualized under UV transilluminator and photographed. Bands at 163 bp (*mecA*) and 132 bp (*femA*) confirmed MRSA and S. aureus, respectively.

Statistical Analysis: Categorical data were expressed as counts and percentages and were analyzed by using SPSS version 20. Results were presented in tables. Chi-square test was done to measure the p value and $p < 0.05$ was considered statistically significant. Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of phenotypic methods were calculated.

Results

A total of 120 clinical isolates of S. aureus were tested by PCR for the identification of *mecA* gene. Among them, 70 isolates (58.3%) were *mecA*-positive (MRSA), while 50 isolates (41.7%) were *mecA*-negative (MSSA). Figure I shows identification of 66 MRSA isolates (55.0%) by cefoxitin disk diffusion, followed by MRSA chromogenic agar (68 isolates, 56.7%). Oxacillin disk diffusion demonstrated the lowest, only 58 isolates (48.3%) as MRSA. Latex agglutination test (LAT) result completely coincides with PCR by detecting all 70 *mecA*-positive isolates (58.3%) as MRSA

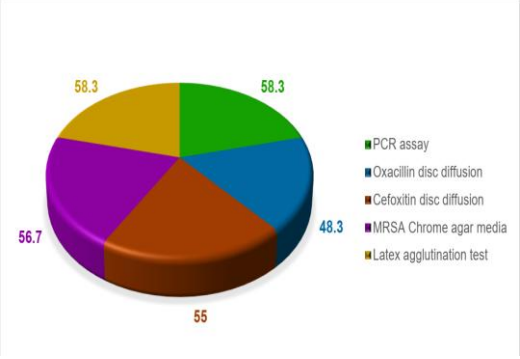

Figure I: The detection rate of MRSA by Oxacillin and cefoxitin disk diffusion method, MRSA chromogenic agar media, Latex agglutination test and PCR

Table 1: Performance of Phenotypic Methods of MRSA Detection Compared to PCR

Methods	PCR assay	
	MRSA (n=70)	MSSA (n=50)
Oxacillin Disk Diffusion		
MRSA	57(81.4%)	1(2.0%)
MSSA	13(18.6%)	49(98.0%)
Cefoxitin Disk Diffusion		
MRSA	65(92.9%)	1(2.0%)
MSSA	5(7.1%)	49(98.0%)
MRSA Chromogenic agar		
MRSA	63(90.0%)	5(10.0%)
MSSA	7(10.0%)	45(90.0%)
Latex agglutination		
MRSA	70(100.0%)	0 (0.0%)
MSSA	0(0.0%)	50(100%)

Only one *mecA* negative *S. aureus* was resistant to both oxacillin and cefoxitin disc diffusion; These 5 isolates were also sensitive in oxacillin disc diffusion method. Out of 70 MRSA isolates detected by PCR, oxacillin disk diffusion identified 57 (81.4%) and cefoxitin disk diffusion identified 65 (92.5%) as MRSA, with 13 (18.6%) and 5 (7.1%) *mecA*-positive isolates, respectively, appearing falsely sensitive. Only one *mecA*-negative isolate showed false resistance to both oxacillin and cefoxitin.

Among the *mecA*-positive isolates, MRSA chromogenic agar detected 63 (90.0%) as MRSA, yielding seven (10.0%) false negatives and five (10.0%) false positives, whereas latex agglutination demonstrated perfect performance, detecting all 70 *mecA*-positive isolates as MRSA and all 50 *mecA*-negative isolates as MSSA, with strong agglutination reactions (3+ in 43 isolates and 2+ in 27 isolates).

Table 2: Performance of Diagnostic Phenotypic MRSA Detection Methods

Methods	Sensitivity	Specificity	Accuracy	PPV	NPV
Oxacillin Disk Diffusion	81.4%	98.0%	88.3%	98.3%	79.0%
Cefoxitin Disk Diffusion	92.9%	98.0%	95.0%	98.5%	90.7%
MRSA Chromogenic agar	90.0%	90.0%	90.0%	92.6%	86.5%
Latex Agglutination Test	100.0%	100.0%	100.0%	100.0%	100.0%

Diagnostic performance indices of all phenotypic methods are presented in Table 2. Oxacillin disk diffusion demonstrated 81.43% sensitivity, 98.0% specificity and overall accuracy of 88.3%. Cefoxitin disk diffusion showed a better diagnostic ability, with 92.86% sensitivity, 98.0% specificity, and 95.0% accuracy. MRSA chromogenic agar showed a balanced performance with an overall 90.0% of sensitivity, specificity, and accuracy. Whereas, among all the methods, Latex agglutination demonstrated the highest diagnostic utility with 100% of sensitivity & specificity, PPV & NPV, and accuracy.

Discussion

Since the incidence of healthcare-associated infections are rising worldwide, it is critically important to detect MRSA rapidly and accurately[22]. Although genotypic techniques like PCR most accurately find out methicillin resistance, they aren't commonly available in many regular diagnostic labs due to their highly technical process and higher expense[23]. In resource-limited settings, phenotypic methods are still considered as the primary diagnostic tools[24]. In this context, the present study was performed to evaluate the commonly used phenotypic methods of MRSA detection and compare them with the PCR-based *mecA* gene assay.

In this study, the prevalence of MRSA was 58.3%, which is almost similar with previous findings of Bangladesh reporting about 32%–63% prevalence range in tertiary-care settings[25]. Overcrowding, lack of hygiene, cross-transmission within wards, and the irrational use of antibiotics were found as the contributing factors for a higher proportion of MRSA in referral hospitals.

About 55% MRSA isolates were detected by the cefoxitin disk diffusion (CDD) method, and compared to PCR, CDD identified 92.9% *mecA*-positive isolates (Figure 1 and Table 1), which is almost similar to previous studies reporting comparatively higher sensitivity of cefoxitin due to its strong induction of *mecA* expression[19,26]. The heterogeneous expression of methicillin resistance

may have contributed to 7.1% false-negative result, previously documented among different MRSA strains[27]. Only one *mecA*-negative isolate was found as resistant on CDD, and this false-positive result may be due to hyperproduction of β-lactamase, which can occasionally mimic borderline resistance[18].

About 56.7% (68/120) of MRSA isolates were detected by chromogenic agar, including 90% *mecA*-positive strains. These findings show moderate sensitivity of chromogenic agar, particularly after incubation of 48 hours[28]. False-negative results can be found as methicillin present in the medium causes lower expression of α-glucosidase in some MRSA strains and prevent the formation of green colonies[29]. Conversely, heavy inoculum or nonspecific colony pigmentation may lead to 10% false positives among *mecA*-negative isolates.

The detection rate of *mecA*-positive isolates and *mecA*-negative isolates of MRSA are 100% by Latex agglutination test (LAT). There were no false-positive or false-negative results, which coincides with the earlier studies indicating highly accurate detection of PBP2a[30]. Some reports showed a small percentage of false positives[10].

There are clear differences in performance among the phenotypic methods when compared with PCR. Latex agglutination has 100% sensitivity, specificity, and accuracy, similar to previous studies that highlight its excellent reliability for detecting PBP2a[10,30]. Where Cefoxitin disk diffusion showed high sensitivity (92.86%) and specificity (98.0%), supporting earlier evidence that cefoxitin is a stronger *mecA* inducer than oxacillin and provides better MRSA detection[19,26].

Overall, the study demonstrates that latex agglutination shows complete agreement with PCR and is the most accurate phenotypic method for MRSA detection.

Conclusion

This research has several flaws, such as the fact that it was done at just one facility and with a limited number of people. Variations in the inoculum and subjective interpretation may have also affected the chromogenic agar and disk diffusion procedures. Even with these problems, the data clearly reveal that latex agglutination is the most accurate and fastest phenotypic test, matching PCR results perfectly. Using these strategies can make it easier to find MRSA in low-resource settings and thus contribute in controlling infections.

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Conflict of Interest
The Author has declared no conflict of interest in this work.

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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, BSMMU. All methods were performed in accordance with the relevant guidelines and regulations.

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