Original Article

Comparison of Phenotypic and Molecular Methods for Rapid and Accurate Detection of Methicillin-resistant *Staphylococcus aureus* from Clinical Specimens: A Cross-sectional Observational Study from a Tertiary Care Center in New Delhi

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Abstract

Background and Aim: Accurate and rapid identification of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical specimens is essential for timely decisions on patient isolation procedures and effective antimicrobial therapy. Hence, this study was carried out to evaluate the usefulness of methods such as MRSA latex agglutination and MRSA CHROMagar for rapid and accurate detection of MRSA. **Materials and Methods:** A total of 86 isolates of *S. aureus* identified using the VITEK-2 Compact system were included in this study. These isolates were further tested by MRSA CHROMagar and MRSA latex agglutination test for their MRSA status. Results of these phenotypic tests were compared against the gold standard molecular method, i.e., *mec*A *fem*B multiplex polymerase chain reaction. **Results:** MRSA CHROMagar was found to have 100% sensitivity and 100% specificity. The MRSA latex agglutination test had 100% sensitivity and 78.26% specificity; positive predictive value (PPV) was 92.65%, and the negative predictive value (NPV) was 100%. However, as compared to MRSA CHROMagar, which took 18–24 h, MRSA latex agglutination took only 10–15 min for the result. **Conclusion:** MRSA latex agglutination test was found to have very high sensitivity, specificity, PPV, and NPV and has a very short turnaround time for MRSA detection, and thereby very helpful in taking therapeutic decisions as well as infection prevention measures.

Keywords: CHROMagar, infection control, latex agglutination, methicillin-resistant *Staphylococcus aureus*, turnaround time

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of endocarditis, bacteraemia, osteomyelitis, and skin and soft-tissue infections. Penicillins initially offered an effective cure of *S. aureus* infection. However, MRSA emerged in a short time after the antibiotic era started. In fact, genomic evidence suggests that methicillin resistance even preceded the first clinical use of antistaphylococcal penicillins.[1] Methicillin resistance is mediated by *mec*A and acquired by horizontal transfer of a mobile genetic element designated staphylococcal cassette chromosome mec. The gene *mec*A encodes penicillin-binding protein 2a (PBP2a), an enzyme responsible for cross-linking the peptidoglycans in the bacterial cell wall. PBP2a has a low affinity for β-lactams, resulting in resistance to this entire class of antibiotics.[2] MRSA is considered resistant to multiple classes of antibiotics, including

Access this article online

Quick Response Code:

Website: https://www.ovid.com/jnls/armh

DOI: 10.4103/amhs.amhs_187_25

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Submitted: 27-Jun-2025 **Revised:** 09-Nov-2025
Accepted: 10-Nov-2025 **Published:** 07-Sep-2026

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How to cite this article: Tandel K, Mahajan P, Malik M, Swarnim V, Bhalla GS, Kumar M. Comparison of phenotypic and molecular methods for rapid and accurate detection of methicillin-resistant *Staphylococcus aureus* from clinical specimens: A cross-sectional observational study from a tertiary care center in New Delhi. Arch Med Health Sci 2026;14:414-8.

penicillins, β-lactam combination agents, cephems (with the exception of ceftaroline), and carbapenems.

MRSA is also a leading cause of healthcare-acquired infection and affects the most vulnerable patients with significant morbidity and mortality.[3] Despite the lack of convincing evidence, it is now accepted that a major aspect of controlling the spread of MRSA is the prompt identification of patients at risk of MRSA carriage.[4] Preemptive isolation of patients suspected of being MRSA positive is an important factor in reducing cross infections.[5]

Conventional methods for identification of MRSA are culture followed by antimicrobial susceptibility testing using a cefoxitin (30 μg) disc diffusion test or testing by the VITEK-2 Compact automated identification and antimicrobial susceptibility system. Currently, this method is the most used method for MRSA detection, but the minimum processing time of 48 h may result in the spread of MRSA if patients are not timely treated and isolated.[6]

Accurate and rapid identification of MRSA in clinical specimens is essential for timely decisions on isolation of patients and effective antimicrobial therapy. Numerous approaches that improve turnaround time (TAT) for the identification of MRSA have been described. These include CHROMagar™ MRSA, MRSA latex agglutination test, polymerase chain reaction (PCR), Xpert MRSA, BIOFIRE® FILMARRAY® System, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry.[7-10] Among these, methods such as Xpert MRSA and BIOFIRE® FILMARRAY® System are very rapid and accurate. However, they require very costly equipment and their costly consumables for performing the test. Hence, this study was carried out to compare cost-effective methods not requiring costly equipment for performing these tests against the gold standard molecular method for rapid and accurate detection of MRSA.

Materials and Methods

This cross-sectional observational study was carried out at a tertiary care center in New Delhi for 1 year, from January 2018 to December 2018. A total of 86 consecutive nonrepeat isolates of *S. aureus* grown from different clinical specimens and identified by the VITEK-2 Compact system (bioMérieux, France) were selected. All these 86 isolates were tested for their MRSA status using the *mec*A *fem*B multiplex PCR. All these isolates were further analyzed using CHROMagar™ MRSA and the MRSA latex agglutination test for determining the MRSA status and were compared with the gold standard *mec*A *fem*B multiplex PCR.

*mec*A *fem*B multiplex polymerase chain reaction

The *mec*A gene encodes the PBP2' (PBP2a), which is unique to methicillin-resistant Staphylococci. The *fem*B gene was used to identify the *S. aureus* species. The primer sequences used in this study are given in Table 1. DNA extraction was done using QIAamp® DNA Mini Kit as per the manufacturer's instructions. The amplification was performed in a 25 μL volume mix containing 12.5 μL of the PCR enzyme/dNTP mix (DreamTaq Green PCR Master Mix (2X), ThermoFisher Scientific, USA), 2.5 pmol each of the *mec*A gene primers and 7 pmol each of *fem*B gene primers, 5 μl of the DNA, and nuclease-free water. The PCR cycles were programmed with initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 60 s, followed by a final extension of 2 min at 72°C. The amplified products were electrophoresed on a 1% agarose gel at 100 V for 20 min and photographed on an ultraviolet transilluminator. The target size of *mec*A gene was 310 bp, and the size of *fem*B gene was 651 bp [Figure 1].

CHROMagar™ methicillin-resistant *Staphylococcus aureus*

CHROMagar™ MRSA (CHROMagar, France) was used as a rapid phenotypic test to identify MRSA. The media was prepared as per the manufacturer's instructions. All the study isolates were inoculated on the CHROMagar™ MRSA plates and were incubated at 37°C for 18–24 h. After the incubation period, the plates were observed for the growth of bacteria. Along with the clinical isolates, ATCC 25923 *S. aureus* was also tested every time on this CHROMagar as a negative control. MRSA isolates showed pink to mauve colored colonies after overnight incubation, whereas the ATCC 25923 *S. aureus* strain, as well as clinical methicillin-sensitive *S. aureus* (MSSA) strains, were inhibited and did not show any growth [Figure 2].

Methicillin-resistant *Staphylococcus aureus* latex agglutination test

MRSA-Screen (Denka Seiken Co. Ltd.; Tokyo, Japan) is a qualitative slide latex agglutination test for the detection of PBP2' (PBP2a) present in the MRSA isolates. This kit contains a latex reagent sensitized with monoclonal antibody against PBP2' (PBP2a) together with reagents to rapidly extract PBP2' (PBP2a) from the bacterial membranes of MRSA. Extracts are prepared by boiling a suspension of the *S. aureus* cells suspension under alkaline conditions, followed by neutralization and centrifugation step. The supernatant is then mixed with the latex reagent on a test card, and visible clumping or agglutination within 3 min indicates the presumptive presence of PBP2' (PBP2a) [Figure 3].

Results

A total of 86 isolates of *S. aureus* identified by the VITEK-2 Compact system (bioMérieux, France) were included in this

Table 1: Primer sequences used for *mec*A *fem*B multiplex polymerase chain reaction

Primer names	Primer sequence
*mec*A Forward	5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3'
*mec*A Reverse	5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3'
*fem*B Forward	5'-TTA CAG AGT TAA CTG TTA CC-3'
*fem*B Reverse	5'-ATA CAA ATC CAG CAC GCT CT-3'

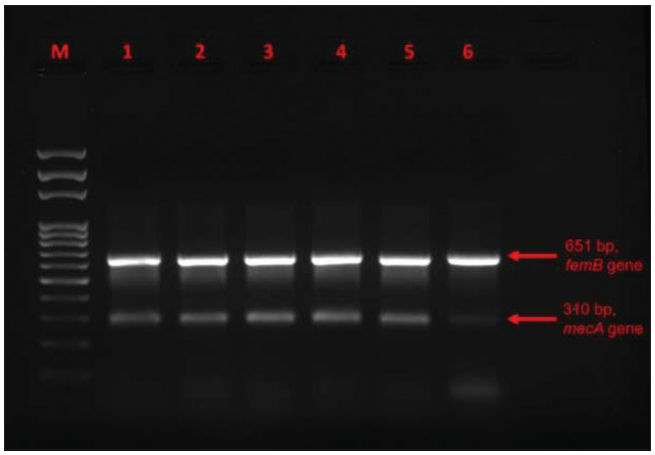

Figure 1: A *mecA femB* multiplex polymerase chain reaction was performed, and products were separated on an agarose gel. Lane 1-6 showing the result of six different methicillin-resistant *Staphylococcus aureus* isolates, showing both bands of *mecA* gene and *femB* gene

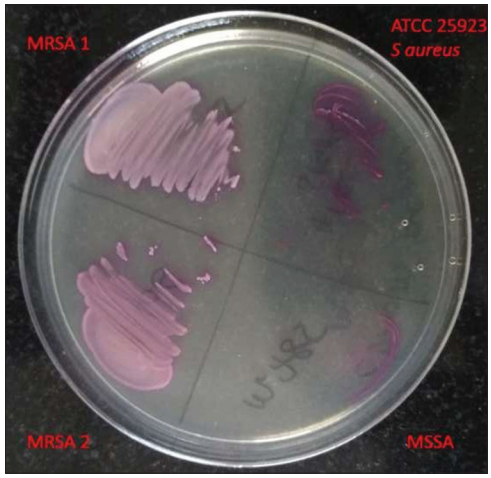

Figure 2: Pink colored colonies of methicillin-resistant *Staphylococcus aureus* (MRSA) (two different isolates, MRSA-1 and MRSA-2) on CHROMagar™ MRSA (CHROMagar, France). Growth of ATCC 25923 *S. aureus* was inhibited. Similarly, the growth of a clinical methicillin-sensitive *S. aureus* isolate was also inhibited by this media

Figure 3: Agglutination of methicillin-resistant *Staphylococcus aureus* (MRSA) isolate (isolate no. 02). No agglutination was observed for methicillin-resistant *S. aureus* isolate (isolate no. 142)

study. These isolates were grown from different body fluids including 54 pus specimens, 19 respiratory tract specimens including sputum, tracheal aspirates, and bronchoalveolar lavage fluid, 9 urine specimens and 4 blood specimens.

*mec*A *fem*B multiplex polymerase chain reaction

All the 86 samples, which were identified as *S. aureus* by the VITEK-2 system, were further tested using the *mec*A *fem*B multiplex PCR. A total of 63 samples were found to be positive by PCR for *mec*A gene, whereas the remaining 23 samples were found to be negative for *mec*A gene. All the isolates were positive for *fem*B gene, confirming their identification as *S. aureus*.

CHROMagar™ methicillin-resistant *Staphylococcus aureus*

All the 86 *S. aureus* isolates were inoculated on the CHROMagar™ MRSA. Sixty-three isolates were found to be positive, whereas the remaining 23 isolates were found to be negative. When compared with PCR, the sensitivity and the specificity were found to be 100%.

MRSA latex agglutination test

All the 86 *S. aureus* study isolates were tested by the latex agglutination method. Sixty-eight isolates were found to be positive, whereas the remaining 18 isolates were found to be negative. When compared with PCR, the sensitivity was 100%, and the specificity was 78.26%. The positive predictive value (PPV) was 92.65%, and the negative predictive value (NPV) was 100%. However, as compared to CHROMagar™ MRSA, which took 18–24 h (overnight incubation) for MRSA identification, MRSA latex agglutination took only 10–15 min.

Discussion

S. aureus is an important pathogen causing community-acquired infection as well as hospital-acquired infections. Among *S. aureus* species, MRSA are even more dangerous due to their resistance to various groups of antimicrobials and its association with more severe and prolonged infections. The clinical advantages of rapid detection and differentiation between MSSA and MRSA are well documented in literature. Its significance can be understood by the fact that two meta-analyses have found an increased mortality risk of 1.93 (95% confidence interval [CI]: 1.54–2.42) and 2.03 (95% CI: 1.55–2.65) associated with MRSA bacteremia compared with MSSA.[11] Another meta-analysis observed that bacteremia caused by MRSA was associated with significantly higher mortality rates than bacteremia caused by MSSA (29% vs. 12%; $P < 0.001$).[12] The prognosis of these infections can be positively impacted by early diagnosis and effective antimicrobial therapy.[13-15]

Moreover, the cornerstone of any infection control strategy is the rapid identification of patients who are contagious to others and taking appropriate control measures. Microbiological culture and identification of pathogens can take a long time, from several hours to days. Currently, MRSA is one of the most important pathogens among hospital-acquired nosocomial infections, and its detection using conventional cultures takes

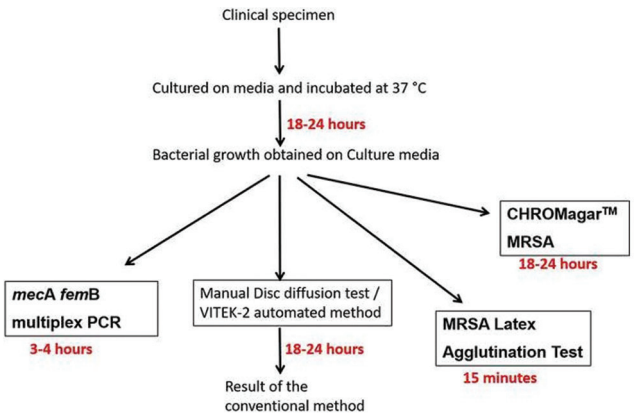

Figure 4: Workflow timelines of the various methods adopted in the identification of methicillin-resistant Staphylococcus aureus

2–3 days. This delay in culture results leads to a suboptimal hospital infection control strategy [Figure 4].

In the present study, we evaluated two methods for the accurate detection of MRSA and compared it with the gold standard molecular method. Among these methods, the MRSA latex agglutination test reduces the final identification time for MRSA by 18–24 h, giving an edge over the conventional methods like the disc diffusion method using a cefoxitin (30 µg) disc, in treatment as well as in infection control [Figure 4].

Both the MRSA latex agglutination test and CHROMagar™ MRSA were found to have 100% sensitivity. CHROMagar™ MRSA was found to have 100% specificity, whereas the MRSA latex agglutination test had a specificity of 78.26% as compared to the gold standard PCR. However, CHROMagar™ MRSA took 18–24 h (overnight incubation) for MRSA identification, whereas MRSA latex agglutination took only 10–15 min for MRSA identification. The gold standard PCR took a slightly longer time of around 2–3 h as compared to the MRSA latex agglutination test [Figure 4].

The MRSA latex agglutination test was found to be highly sensitive in this study. However, the specificity was found to be low compared to other studies available in the scientific literature. This same kit was also evaluated by Alipour *et al*., where they reported 97% sensitivity and 95% specificity for this kit while detecting methicillin resistance among *S. aureus*.[16] Nair *et al*. also reported very high sensitivity and specificity of 98.9% and 98.3% using MRSA-Screen latex agglutination when compared it with PCR as the gold standard test.[17] Other rapid tests based on different principles such as immunochromatographic assay, have been developed for the detection of methicillin resistance by Alere (PBP2a SA culture colony test; Abbott Diagnostics Scarborough, Inc., USA). It is a rapid, reliable, and easy-to-use test for the detection of PBP2a-mediated methicillin resistance in *Staphylococci* spp. Few studies have used this rapid test and reported 100% sensitivity and 100% specificity in detecting PBP2a-mediated β-lactam resistance in *S. aureus*, as well as in Coagulase negative *Staphylococci* (CoNS) isolates.[18,19] Delport *et al*. also evaluated this kit and found it to be very useful with a sensitivity of 87.5%, specificity of 100%, PPV of 100% and NPV of 94.3%.[20]

CHROMagar has been used by many researchers for culturing the nasal swabs for screening healthcare workers for nasal *S. aureus* carriage. Poojary and Bhandarkar reported that the overall sensitivity and specificity of the CHROMagar when compared with the conventional techniques were 100% and 98.4%, respectively. The strength of agreement between the two methods, as assessed by the kappa value, was 0.957, indicating very good agreement of the chromogenic medium with the conventional methods.[21] In their study, which was performed directly on clinical samples, the earliest TAT for CHROMagar was 24 h as compared to a minimum of 48 h with the conventional methods. Other authors like Van Hoecke *et al.* also reported very high sensitivity and specificity for the CHROMagar in MRSA identification.[22] Despite the increase in the cost per specimen using the CHROMagar, the time savings of almost 24 h with the use of the chromogenic medium go a long way as a cost-saving measure in preventing the control and spread of MRSA outbreaks in the hospital.[21] In this study, we used CHROMagar on culture isolates, and hence, we could not achieve the reduction of TAT, which could have been achieved provided we had used it directly on clinical samples. Hence, the CHROMagar should be used directly on the clinical specimens to reduce the TAT with very high sensitivity and specificity.

Molecular technique like PCR has a major advantage of low TAT from the time of sampling to the declaration of results. However, setting up a molecular laboratory is costly, and this facility is not available in many laboratories. In this study, the reduction of TAT was around 18–24 h for PCR as compared to the chromogenic media. The mean TAT for screening of MRSA from clinical samples ranged from 13.2 h to 21.6 h with PCR versus 46.2 h to 79.2 h using chromogenic agar, across all studies.[23-25] Clay *et al.* reported a very high NPV (98.4%) for MRSA PCR, making it an ideal tool for rapid exclusion of MRSA directly from clinical specimen and thereby having the potential to be a vital tool in de-escalating antimicrobial therapy.[26]

Preemptive isolation of all patients suspected of carrying MRSA infection is an important factor in reducing cross infection.[5] Phenotypic methods such as disc diffusion methods take a long time and cannot help in the prevention or reduction of these preemptive isolations. Poojary and Bhandarkar mentioned that the earliest TAT for MRSA identification with the conventional methods is 48 h. For nasal specimens and some pus specimens, which were mainly polymicrobial in origin, additional subcultures and biochemical testing caused delays in reporting beyond 48 h and sometimes up to 120 h.[21] However, studies showed that rapid screening for MRSA reduced the need for preemptive isolation.[27,28] While the best available option is to place all newly admitted patients in isolation until MRSA screening results are available, this is practically not possible in limited resource countries with very high patient load, and hence, the rapid tests for detection of MRSA have a big role to play.

The strength of this is that most of the available phenotypic and genotypic methods were performed and compared for the rapid and accurate detection of MRSA. The limitation of this study is that the clinical profile and outcome of the MRSA-positive cases were not studied in this study, as it is very important to know the impact of the early diagnosis. Future studies should be carried out on the clinical impact of rapid and accurate diagnosis of MRSA. Moreover, the impact and need for isolation of patients harboring MRSA needs to be studied.

Conclusion

Rapid and reliable detection of MRSA plays a huge role not only in the treatment of infected patients but also in the effective implementation of infection control practices. In this study, both the CHROMagar™ MRSA and the MRSA latex agglutination test were found to have high sensitivity and PPV when compared to the PCR. In addition, the MRSA latex agglutination test was found to have a short TAT which is important in taking therapeutic decisions as well as for infection control measures.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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