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Original Research Article

EFFICACY OF THE LATEX AGGLUTINATION METHOD TO DETECT MRSA AND ESTIMATION OF THE PERCENTAGE OF INDUCIBLE CLINDAMYCIN RESISTANT STAPHYLOCOCCUS. AUREUS AMONG CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL, BANGALORE

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ABSTRACT

Background: Several commercial serological and molecular methods are available to detect Methicillin-resistant Staphylococcus. aureus (MRSA). This pathogen emerged soon after the introduction of Methicillin into clinical practice. In India, MRSA causes 30% to 70% of infections. MRSA strains consist of a mecA gene, which is responsible for the modified penicillin binding protein (PBP2a) having low affinity for methicillin and other β-lactum group antimicrobial drugs. For this reason, MRSA expresses resistance to all β-lactum antibiotics, leaving few treatment options in severe infections. The treatment of infections caused by these pathogens has become like a challenge in health care setups. Hence, rapid and accurate detection of MRSA is a needful action to immediately start the proper antimicrobial treatment and to avoid the spread of these infections. The Aim of the study is to evaluate the Latex agglutination method in the detection of MRSA in health care setups, and to estimate the percentage of inducible clindamycin MRSA isolates in the clinical samples received at a tertiary care hospital

Materials and Methods: The study was conducted in the Department of Microbiology in a tertiary care hospital. All Staphylococcus aureus(S.aureus) isolates from clinical samples were included in this study and processed as per the standard operating procedures. MRSA was detected by using the cefoxitin (30µg) disk diffusion method, the Latex agglutination method, and the standard PCR.

Results: In our study, among 100 staphylococcal species, 70 were S.aureus isolates, 30 were coagulase-negative staphylococcal species, and 60 were MRSA isolates. Among the 60 MRSA isolates, 20 (33%) were inducible clindamycin-resistant strains. Most of the S. aureus were isolated from pus samples 35% (n=35), followed by Blood samples 15% (n=15), sputum samples, 5%(n=5), and urine samples, 5%(n=5). The latex agglutination method can be used as a good screening method in routine diagnostic procedures of clinical practice.

Conclusion: MRSA plays a significant role in causing severe infections in both inpatients and outpatients. Serological methods like the Latex agglutination technique can be considered as a rapid screening method for the detection of methicillin resistance in S. aureus, as it can be done in less time, whereas conventional culture method or molecular method will take more time and technical expertise.

Keywords: Methicillin-resistant Staphylococcus aureus, Latex agglutination method, Inducible clindamycin resistance.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) causes various hospital and community-acquired infections, with limited treatment options leading to significant health impacts.[1] Rapid detection is crucial for effective treatment and infection control. Conventional methods like oxacillin disk diffusion, chrome agar screen, and MIC testing take 24 – 48 hours and are affected by factors like inoculum size, incubation conditions, and media composition. MRSA strains harbour the mecA gene, which encodes the modified penicillin binding protein (PBP2a) with low affinity for methicillin and all β-lactum antibiotics. Resistant to this antibiotic implies resistance to all β-lactum antibiotics, leaving few therapeutic options to treat such severe infections. The treatment of infections caused by these organisms has become problematic. Molecular testing is faster but costly and technically complex. So rapid and accurate identification of MRSA is required to immediately start the appropriate antimicrobial therapy and to avoid the spread of these infections.[2] Several studies have reported the significance of rapid phenotypic methods for the detection of MRSA. However, these methods are inadequate as the expression of resistance is subject to environmental and conditional expression of the PBP2a antigen. Discrepancies in interpretation are leading to an adverse effect on patient management. So, there is a need to highlight the importance of accuracy in detection within a short period of time.[2,3] Hence, the current study was conducted to find out the accuracy of the rapid latex agglutination method to detect MRSA in diagnostic centers of hospitals.

Objectives of the study

1. To evaluate the accuracy of the latex agglutination method for MRSA detection, by comparing it with the Polymerase chain reaction (PCR)
2. To estimate the percentage of inducible Clindamycin-resistant isolates among clinical samples received at a tertiary care teaching hospital.

MATERIALS AND METHODS

The study was conducted in the tertiary care hospital, Bangalore. Material & data that are required for the study were collected from the central laboratory of the Dr. B. R. Ambedkar Medical College & Hospital. Around 100 staphylococcal species isolates were collected from the clinical samples, which were positive for routine culture tests. Identification and isolation of Staphylococcus. aureus has been done by standard microbiological procedures. we included both inpatient samples and outpatient samples. The study was initiated by collecting the clinical samples from the patients, like pus or wound swabs, blood, sputum, and urine. These samples were immediately inoculated on blood agar, MacConkey agar nutrient agar, and after overnight incubation, developed colonies were observed for phenotypic properties like β-hemolysis, opaque, smooth, round, convex, easy to emulsify, and golden yellow diffused pigmentation, and those colony smears were stained by Gram's staining for Gram positive cocci in clusters. Selected colonies were tested for biochemical reactions like the glucose O/F test, catalase test, and tube coagulase test. The pure cultures of S.aureus were performed for cefoxitin disk diffusion by using the McFarland 0.5 standard tube. By observing the zone of inhibition, 22 mm.[3] A phenotypic method D-test was performed for all MRSA isolates to detect inducible clindamycin resistance. Antimicrobial susceptibility testing was done according to CLSI-2024 guidelines. Simultaneously, pure cultures of Staphylococcus aureus isolates were tested for MRSA detection by the rapid latex agglutination technique. The test procedure and the interpretation of the results were followed by the literature manual.

Latex agglutination test (MRSA Screen test, Denka Seiken, Co., LTD., Japan).[4-6]

PBP2' extraction

- 4 drops of extraction reagent-1 were placed into a micro-centrifuge tube. 4 large colonies of fresh staph. aureus pure culture from a blood agar plate was suspended in the tube, closed the tube, and kept for boiling at 95°C for 3 minutes.
- Then added a drop of extraction reagent-2 into the tube, mixed well, and centrifuged at 3000 rpm with a 15 cm rotor radius. The supernatant was collected and used as the sample to test.

Latex agglutination

- For each sample, 2 circles were selected and labeled on the slide, as a test and the other one as a control. 50 µl of the sample was placed onto each of the test and the control circles. To the test circle, 1 drop of sensitized latex mixture was added, and to the control circle, 1 drop of control latex mixture was added. With separate sterile applicator sticks, each reagent was thoroughly mixed with the sample over the area of the circle. The testing slide was rotated by the mechanical rotator for 3 minutes. And then it was placed on the bench and observed for agglutination pattern with the naked eye.

Standard Polymerase Chain Reaction (PCR) method to detect MRSA.[7-11]

The PCR method to detect the mecA gene, considered as Gold standard method. The positive control was Staphylococcus aureus ATCC 43300 (MRSA), while the negative control was ATCC 25923 (MSSA). The chromosomal DNA of the isolates was extracted by a simple lysis method. A single large colony of the isolate was inoculated in 1 ml of Luria Bertani Broth (LB Broth Difco) in small Eppendorf tubes and incubated overnight at 37◦C. Then turbid tubes were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded, and the cell pellet was collected and resuspended in 400 µl of sterile

nuclease-free water. Then heated at 85°C for 15 minutes in a water bath and immediately transferred to -20°C, which causes cell lysis. Master Mix of a single reaction: A 20µL PCR reaction consisted of PCR buffer (2µL), MgCl2 (2µL), dNTPs (1.5µL), MecA F primer (0.5µL), MecA R primer (0.5µL), Taq polymerase (0.3µL), Template DNA (1µL), and MilliQ H2O (12.2µL). The mecA F primer (5'-AAAATCGATGGTAAAGGTTGGC-3'), which corresponds to nucleotides 1282 to 1303, and the mecA R primer (5'-AGTTCTGCAGTACCGGATTTGC-3'), which is complementary to nucleotides 1581 to 1598 within the coding frames, were used for the amplification of the 533-base pair (bp) fragment of the mecA gene. The PCR technique included a 5-minute denaturation stage at 95ºC, followed by 30 amplification cycles. Each cycle consisted of a 60-second denaturation at 94ºC, a 60-second annealing at 55ºC, and a 90-second extension at 72ºC. The programme ended with a 10-minute extension at 72ºC. The PCR products are observed on a 1% agarose gel containing ethidium bromide dye under a UV transilluminator (Gel Doc, Bio-Rad US). A 100-base-pair DNA ladder serves as the molecular-weight size marker.

RESULTS

Table 1: Percentage of Staphylococcal isolates detected in the clinical samples.

Name of the isolate	Percentage
Staphylococcus aureus isolates	70
MRSA Isolates	60
Inducible Clindamycin-Resistant Strains among MRSA Isolates	20
Coagulase-negative Staphylococcus species	30

Table 2: Detection of MRSA isolates by various methods.

Employed method	Conventional Culture method	Standard PCR	Latex agglutination method
No. of MRSA isolates detected	60	60	58

Table 3: Evaluation of the Latex agglutination test to detect MRSA:

Sensitivity	97%
Specificity	100%
Positive predictive value	100%
Negative predictive value	61%
Accuracy	97%

Statistical analysis: The Latex agglutination method was considered as a test for evaluation, and the PCR test was considered as the Gold standard method to detect MRSA among clinical isolates, and a 2x2 contingency table was used for accuracy calculation.

DISCUSSION

The Latex agglutination method for MRSA detection was evaluated by some previous studies. Van Griethuysen et al reported a sensitivity of 98.5% and specificity of 100% for the MRSA screen test.[12,13] Another similar study done by Gupta N et al,[14] reported 100% sensitivity and 97% specificity. In our study, we found 97% sensitivity and 100% specificity. The results of our study for the evaluation of the latex agglutination method to detect MRSA in clinical samples are positively correlated with the studies of van Griethuysen et al. & Gupta N et al. Apart from methicillin resistance, some of the MRSA isolates are expressing Inducible clindamycin resistance day by day, the percentage of Inducible clindamycin-resistant bacteria in Staph. Aureus isolates are increasing worldwide. In the previous study, Helmi HA et al reported 27.8% of Inducible clindamycin-resistant isolates among MRSA isolates.[11] In the current study, we got 33% of clindamycin resistance among MRSA isolates. Through this study, we suggest that the latex agglutination technique (MRSA Screen test) is highly accurate, more reliable, and easy to perform in health care settings where costly molecular methods are unavailable.

CONCLUSION

MRSA with inducible clindamycin resistance is a clinically significant pathogen playing a major role in causing severe infections in both inpatients and outpatients. The latex agglutination test can be considered a more useful method for the detection of MRSA as it is highly accurate, specific, and an easy-to-perform test for MRSA detection. and can be done in less time compared to conventional bacteriological culture and molecular methods.

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