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Comparison of Latex Agglutination Test with Polymerase Chain Reaction for Detection of mecA Gene in Methicillin Resistant *Staphylococcus aureus*

Aneela Khawaja[1]*, Faiqa Arshad[2], Ambreen Tauseef[3], Liaqat Zia[4], Sharjeel Ahmad[5], Shahmir Altamash[5] and Rana Harris Farooq[5]

[1] Department of Pathology, Rahbar Medical and Dental College, Lahore, Pakistan

[2] Department of Pathology, Gujranwala Medical College, Gujranwala, Pakistan

[3] Department of Physiology, CMH Lahore Medical College and Institute of Dentistry, Lahore, Pakistan

[4] Department of Surgery, Gujranwala Medical College, Gujranwala, Pakistan

[5] The Lahore Alma, Pakistan

**Corresponding e-mail: draneelakh@gmail.com*

ABSTRACT

Background: *Antimicrobial resistance is a devastating question that threatens health globally. The extensive, indiscriminate and unnecessary consumption of antibiotics for humans, as well as wildlife and in agriculture; lead to the development of notoriously resistant Staphylococcus aureus; through possession of mecA gene, encoded by modified Penicillin binding protein (PBP2a); being labeled "Methicillin resistant Staphylococcus aureus". Conventional phenotypic techniques for MRSA detection rely on standardization of cultural characteristics. The latex agglutination method can be adopted as an accurate strategy for rapid detection of MRSA.* ***Methodology:*** *A total of 713 consecutive, non-duplicate isolates of Staphylococcus aureus were processed. Methicillin resistance was determined using cefoxitin (30 µg) by Kirby-Bauer method following Clinical and Laboratory Standards Institute (CLSI) guideline, latex agglutination method; and PCR for mecA gene.* ***Results:*** *The results showed that out of 713 Staphylococcus aureus isolates, 12.90% isolates were detected as MRSA due to resistance to cefoxitin. By latex agglutination method, 87 (94.50%) were positive for PBP2a; while on PCR mecA gene was detected only in 82 (89.10%) MRSA isolates. When assessed with PCR (gold standard) the sensitivity and diagnostic accuracy of latex agglutination was 100% and 94.57%, respectively.* ***Conclusion:*** *Latex agglutination test can be used as a prompt and reliable diagnostic technique for mecA gene detection in MRSA isolates, where molecular methods are limited. This can effectively minimize the misdiagnosis of resistant strains, and over/misuse of antibiotics.*

Keywords: Methicillin resistant *Staphylococcus aureus* (MRSA), Cefoxitin, Latex agglutination, mecA gene, Polymerase chain reaction (PCR)

INTRODUCTION

Infectious diseases caused by drug-resistant microorganisms have turned out to be a major contributor to human debility and death [1]. The genetic abilities of bacteria and indiscriminating use of antibiotics have critically advanced the progress of resistance; thus, impeding the use of antibiotic therapy [2]. The crisis of Antimicrobial resistance (AMR) is a grave concern budding globally and imposing a serious threat for public health as well as to infection control [3].

The acquirement of antibiotic resistance has led to progression of *Staphylococcus aureus* infections in this antibiotic era [4]. The alarming frequency of resistance patterns in *Staphylococcus aureus* has turned out to be world-wide distress. Methicillin resistance outcomes after the assembly of changed synthetic bacterial cell wall penicillin binding

protein known as PBP2a conferred by mecA gene, which has decreased affinity for most beta-lactam antibiotics [5]. The resistance to β-lactams can be accomplished due to an additional low-affinity PBP, the overexpression of an endogenous low-affinity PBP, and/or the alteration of endogenous PBPs by point mutations [6].

Conventional methods for identifying MRSA are at all times not steady as the phenotypic manifestation of methicillin resistance may be heterogeneous; and influenced by incubation period, temperature and salt (NaCl) concentration. The accurate and timely recognition of resistance among staphylococcal strains is vital in obtaining a prompt confirmation of antibacterial option, leading to a reduction in mortality, lessening use of vancomycin, decreases hospital stay and therapeutic expenses [7].

For identification and detection of MRSA, a simple, swift and more appropriate method is necessary to identify the gene encoding for methicillin resistance as well as its product, PBP2'. Latex agglutination assay is a rapid extraction procedure, which directly detects PBP2' from isolates of *Staphylococcus aureus*. The Latex agglutination procedure involves no extraordinary apparatus and the test is very precise and profound [8,9]. Studies have revealed that the MRSA-Screen test (slide latex agglutination experiment) detected the same number of MRSA as the mecA PCR when compared with traditional susceptibility test methods [10]. Detection of MRSA at molecular level has the potential to facilitate stewardship efforts by avoiding use of broad-spectrum antimicrobials as well as reducing antibiotic utilization by 60%-80% [11].

Molecular techniques are not affordable by every laboratory especially in developing countries, so it is essential to evaluate an accurate sensitive method that can provide equivocal results with molecular methods [12]. The reason to conduct the study was to compare latex agglutination test with polymerase chain reaction for detection of mecA gene in Methicillin-resistant isolates of *Staphylococcus aureus*.

METHODOLOGY

This descriptive cross-sectional study was conducted in the Pathology Department of PGMI, Lahore, from 1st January 2018 till 31st December 2018. All the isolated strains of *Staphylococcus aureus* from different clinical specimens were processed in the study project. Clinical isolates of *Staphylococcus aureus*; sensitive to cefoxitin were not included in this study.

Sampling Technique, Collection, and Processing

Sample selection was done by simple convenient sampling. Clinical specimens were received from patients admitted in different clinical wards of Lahore General Hospital (LGH). The specimens were processed according to standard operating procedures in Microbiology test center of the Pathology department, PGMI, Lahore. After processing a total of 713 isolates of *Staphylococcus aureus* isolates were recovered.

All the specimens were inoculated on blood agar and MacConkey agar (prepared as instructions given by the manufacturer). The plates were incubated at 35-37°C aerobically for 24 hours. Following standard microbiological techniques; preliminary identification of *Staphylococcus aureus* isolates was done by observing the colony morphology on agar plates, finding gram-positive cocci in clusters on Gram stain and positive Catalase test. Additional biochemical tests i.e., coagulase and DNA-ase were carried out for the confirmation of *Staphylococcus aureus* [13].

Screening for methicillin resistance was implemented on all isolates of *Staphylococcus aureus*. The phenotypic resistance to methicillin was ascertained by Modified Kirby-Bauer Disc Diffusion Method using 30 µg cefoxitin disc (Oxoid) on Muller Hinton agar according to CLSI (2016) guiding principles. For each strain, a bacterial suspension adjusted according to 0.5 McFarland turbidity standards was prepared and inoculated on Mueller Hinton agar. The plates were incubated at 35°C and zone of inhibition were determined after 24-hour time. The outcomes were read agreeing to CLSI criteria, i.e. zone of ≤ 21 mm was considered as resistant and ≥ 22 mm was considered to be sensitive. Standard positive and negative control strains were employed accordingly [14].

PBP2a' Latex Agglutination

Isolates of *Staphylococcus aureus* showing resistance to cefoxitin (MRSA) were examined for mecA gene outcome (PBP2a) employing latex agglutination kit (Oxoid, DR0900). The procedure followed according to manufacturer's instruction using colonies on Mueller Hinton agar. Latex particles activated with monoclonal antibodies were bound

to PBP2a particularly to produce clumping noticeable to the unaided eye. Methicillin susceptible *Staphylococcus aureus* (MSSA) does not clump the latex particles. All the components were kept at 2-8°C. Each strain was tested simultaneously with a negative control latex suspension.

Detection of mecA Gene by PCR

All the MRSA isolates were grown in nutrient broth by incubating in a shaking incubator at 37°C for 24 hrs. The boiling method was used for DNA extraction. The supernatant was collected and stored at -20°C for PCR reaction.

PCR was carried out to confirm the existence of mecA gene in Methicillin resistant isolates of *Staphylococcus aureus*. The mecA gene was detected using mecA forward 5' GTA GAA ATG ACT GAA CGT CCG ATA A 3' and mecA reverse 5' CCA ATT CCA CAT TGT TTC GGT CTA A 3' primers. DNA Amplification was performed as follows: An initial denaturation step of 5 min at 94°C; followed by 35 cycles of denaturation step at 95°C for 45 s, annealing step at 58°C for 45 s, and extension step at 72°C for 45 s; and a final extension at 72°C for 5 min. The PCR amplification products (310 bp) were analyzed by electrophoresis on 1.2% agarose gel stained with ethidium bromide (10 mg/ml) and visualized under UV light [15].

For all tests run, Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 33591 and Methicillin Sensitive *Staphylococcus aureus* (MSSA) ATCC 25923 were used as positive and negative controls, respectively.

Data Analysis

Data was entered and analyzed by using SPSS Version 20.0. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA) of latex agglutination method were calculated using mecA gene PCR as a gold standard.

RESULTS

Among 713 isolates of *Staphylococcus aureus*, 92 strains exhibited cefoxitin resistance. The study showed distribution of MRSA species recovered from different clinical wards (n=92). According to Table 1, most of the samples were recovered from surgical wards 43 (46.74%), followed by medicine 15 (16.30%), orthopedics 13 (14.13%), 9 (9.78%) each from neurology and pediatrics, and 3 (3.26%) from obstetrics and gynecology departments.

Table 1 Distribution of Methicillin-Resistant *Staphylococcus aureus* isolates from different medical wards (n=92)

Ward	MRSA isolates	Percentage (%)
Surgery	43	46.74%
Medicine	15	16.3%
Ortho	13	14.13%
Neuro	9	9.78%
Paeds	9	9.78%
Gynae	3	3.26%
Total	92	99.99%

By latex agglutination technique 87 (94.50%) MRSA isolates were positive for mecA gene product (PBP2a). So, 5 (5.43%) mecA –ve isolates were inaccurately recognized as MRSA by the cefoxitin disk diffusion method. PCR amplification of all the MRSA isolates was carried out. mecA gene was detected in 82 (89.10%) MRSA isolates. So, 10 (10.90%) mecA –ve isolates were falsely recognized as MRSA by cefoxitin disk diffusion method (Table 2).

Table 2 Frequency of mecA gene positivity by latex agglutination technique and PCR technique among MRSA isolates (n=92)

Technique	mec A gene positive	
	No.	Age percentage (%)
Latex Agglutination	87	94.5%
PCR	82	89.1%

When assessed with PCR as a gold standard, latex agglutination showed 100% sensitivity, and the specificity was 50%. The overall diagnostic accuracy of the latex agglutination test was 94.57%.

DISCUSSION

AMR has accelerated the risk of failure of standard therapies as well as fast-tracked the morbidity, mortality, length of hospitalization and costs of treatment. Today, we are facing an era of decreased susceptibility to conventional antibiotics [16]. The lack of new antibiotic classes is a warning call for a vigilant use of existing agents [17]. The main intervention which can be helpful in combating drug-resistant infections especially MRSA is the promotion of new, rapid and low-cost techniques for definitive diagnosis [18]. Errors in the detection of methicillin resistance i.e., reporting false susceptibility may result in treatment failure. False resistance may not only raise health care cost but also increases the risk of emergence of clinically resistant isolates [19].

Studies by several researchers have revealed that the traditional methods for recognition of methicillin resistance like disc diffusion are economical but time-consuming and are subjective to distinct ecological circumstances [20]. Regarding accurate detection of MRSA, the latex agglutination is a simple and rapid test for identification of mecA gene. It can assist as a suitable investigation with eminent sensitivity and specificity comparable to PCR. It can be implemented easily in majority of the test centers in our setup.

Researchers observed that latex agglutination test was precise and swift technique and could be a reliable predictor for MRSA identification when the molecular means are not accessible. A similar study by Arshad et al. is in comparison with our study. According to the results, 54 isolates of *Staphylococcus aureus* were resistant to cefoxitin (MRSA), but 4 strains were negative for mecA gene product (PBP2a) by latex agglutination test kit [21]. In another study, Shaima'a, et al., has reported 57 (91.9%) isolates to be mecA gene positive among 62 MRSA isolates by latex agglutination method [22]. The study carried out by Oberoi, et al. is also comparable with our study. According to the results, among 46 cefoxitin resistant MRSA isolates; 44 isolates were positive for mecA gene product by latex agglutination technique [23].

PCR is a gold standard assenting test with eminent sensitivity and specificity but costly and is not offered in most of the accustomed test centers. Many researchers have reported that some of the phenotypically resistant isolates, turned out to be mecA gene negative when amplified using PCR technique. This can occur due to the hyperproduction of β-lactamase, thus normal PBP is produced with altered binding capacities due to amino acid substitutions in the transpeptidase domain [24].

Some researchers have stated the isolates which are hyper-producers of penicillinase produce large quantities of β-lactamase, and/or hetero-resistant strains; are falsely reported as MRSA by culture screening method [25].

A similar study carried out by Demir, et al. has shown that among 80 cefoxitin resistant MRSA isolates, 77 were positive for mecA gene by PCR technique [26]. Another study by Jindamwar, et al., has also reported that mecA gene was present in 72 (90%) out of 80 MRSA isolates by genotypic analysis [27]. Evaluation study by Kali, et al., has documented that among 102 cefoxitin resistant MRSA isolates, only 92 (90.1%) isolates were positive for the mecA gene by PCR [28].

According to our study sensitivity of latex agglutination is 100% and specificity is 50%; PPV is 94.25% and NPV is 100%. The diagnostic accuracy of the test is 94.57%. Many researchers have reported 100% sensitivity in accordance with our study. Similar studies by Diab, et al., Mohanasoundaram and Lalitha, and by Palavecino, have reported 100% sensitivity of latex agglutination test for recognition of PBP2a in *Staphylococcus aureus* [29-31]. The findings are in coherence with our study. The specificity reported in our study is 50% which incriminates selectively taking MRSA strains for the study, as well as the very small number of mecA gene negative isolates.

CONCLUSION

Latex agglutination tests can be used as an accurate and reliable diagnostic technique for the detection of mecA gene in MRSA isolates, where molecular methods are not available. The precision of diagnostic method will decrease the treatment cost, minimize the time to effective therapy, and misdiagnosis of resistant strains.

DECLARATIONS

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Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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