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Evaluation of Penicillin Binding Protein 2a (PBP-2a) Latex Agglutination Assay for Rapid Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Blood Culture Growth

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ABSTRACT

***Objective*:** To determine in vitro activity of Penicillin Binding Protein 2a Latex Agglutination Assay for Rapid Identification of Methicillin-Resistant Staphylococcus aureus isolated from positive blood culture growths.

***Study Design*:** Cross-sectional study.

***Place and Duration of Study*:** Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi Pakistan, Jan to Jun 2022.

***Methodology*:** *Staphylococcus aureus* isolates were taken from a subculture of blood culture samples flagged positive by the BactAlert automated blood culture system. A total of 107 isolates were included in the study. A latex agglutination assay was used to identify Methicillin-Resistant Staphylococcus aureus and non-Methicillin-Resistant Staphylococcus aureus isolates. The Cefoxitin disc diffusion test also tested the isolates, keeping it the reference method.

***Results*:** Out of 107 samples, 92 isolates were identified as Methicillin-Resistant Staphylococcus aureus by latex agglutination and Cefoxitin disc diffusion. The rest of the isolates were identified as Methicillin-Susceptible Staphylococcus aureus.

***Conclusion*:** The latex agglutination assay is a highly sensitive, cost-effective, less time-consuming, and accurate test that may be used in routine laboratories for rapid identification of staphylococcus aureus isolates as Methicillin-Resistant *Staphylococcus aureus*.

Keywords: Cefoxitin, Latex agglutination, *mecA* gene, Methicillin-Resistant Staphylococcus aureus (MRSA), Penicillin Binding Protein 2a (PBP2a).

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INTRODUCTION

The incidence and perseverance of Methicillin-Resistant Staphylococcus aureus (MRSA) within community and hospital environments persists to be a significant menace to human health.[1,2] According to a recent report, more than 100,000 deaths cases have been reported due to MRSA alone.[3] The *mecA* gene is responsible for resistance in MRSA as acquiring this particular gene helps encode a modified Penicillin-Binding Protein 2a (PBP2a); eventually, all beta-lactam antibiotics have low binding affinity with this protein.[4]

Various phenotypic methods are available for detecting MRSA but differ in specificity and sensitivity.[5] Most MRSA testing strategies are either phenotypic or molecular. Some methods include determining minimum inhibitory concentrations (MICs) using disk diffusion tests using Cefoxitin or Oxacillin, agar dilution method, E-test, breakpoint method, and broth dilution method. Other methods include disk screening techniques like latex agglutination test for detecting PBP2a protein, CHROM Agar MRSA and solid culture medium with Oxacillin.[6] Last but not least, some automated methods exist, such as the BBL Crystal MRSA ID system, Velogene rapid assay, and quenching fluorescence method.[7] Most of these phenotypic tests can ensure timely and appropriate treatment of MRSA-infected patients.[8] Polymerase chain reaction (PCR) is the gold standard for the detection of the mecA gene of MRSA. However, it is not affordable and is only sometimes available in all laboratories.[9] Additionally, many methods require a high level of sophistication, such as unique instrumentation or operator skills, and are also very costly. Thus, a laboratory with limited resources is unable to afford such methods, and these constraints make the usage of specific methods inappropriate.[10] However, the LA test is an accurate, rapid and sensitive method for identifying mecA gene product PBP2a and is available in routine in laboratories.[5]

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This study aims to determine the diagnostic accuracy of the latex agglutination assay and compare it with the Cefoxitin Disk Diffusion method, keeping it as the reference method concerning its sensitivity and specificity to detect MRSA. The isolates were taken from the subculture of positive blood culture samples. This research study opted for Cefoxitin instead of Oxacillin as it acts as a surrogate maker for detecting *mecA*-mediated resistance in *S. aureus*. Cefoxitin was also used as it is a more effective inducer of the *mecA* regulatory system; it is more reliable than Oxacillin, and no particular incubation temperature or unique medium is required for the Cefoxitin method.The outcomes of this study will be helpful to for laboratories in which screening of the considerable number of isolates can be carried out at low cost.

METHODOLOGY

The cross-sectional descriptive study was conducted at the Microbiology Department of the Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from January to June 2022, IRB approval no:-IRB/21/1783. The sample size was calculated using the WHO calculator, with the anticipated population proportion of 16%.

Inclusion Criteria: Growth of *Staphylococcus aureus* isolated from the subculture of positive blood culture samples of patients of all ages and genders was included.

Exclusion Criteria: The study excluded duplicate samples and all agar plates showing mixed or contaminated growths.

The standard operating guidelines were strictly followed to process the received blood culture samples at the AFIP laboratory. According to the manufacturer's instructions, MacConkey (Oxoid, UK) and blood agar (Oxoid, UK) were used to inoculate the specimens. Incubation of the plates was carried out aerobically at 35-37°C. *S. aureus* isolates were preliminarily identified by following standard microbiological techniques, such as using blood agar plates to observe the colony morphology, gram stain, and a positive catalase test. Furthermore, *S. aureus* was confirmed by performing deoxyribonucleic acid-ase (DNAase) and coagulase biochemical tests. Methicillin-susceptible *S. aureus* (MSSA) ATCC 25923 and MRSAATCC 33591 were used as negative and positive controls for all genotypic and phenotypic tests.[12,13]

Disk diffusion test was used for initial screening of MRSA after following CLSI instructions.[14] Mueller Hinton agar (MHA) was used for inoculation of bacterial suspension of each strain (0.5 McFarland standards).[15] As per CLSI principles, modified Kirby-Bauer was used for determining phenotypic resistance to Methicillin, employing Cefoxitin disc (30µg Oxoid) on MHA.[15] The zone of inhibition was ascertained after 24 hours of aerobically incubating the plates at 35°C. CLSI criteria were followed to interpret the results; that is, a zone diameter of >22mm was considered as sensitive, and the isolates were regarded as methicillin-sensitive Staphylococcus aureus (MSSA) and a zone diameter of <21mm was taken as resistant, and the isolates were labelled as MRSA.[16] This was the routine protocol for identifying *S. aureus* in the lab.

All of these *S. aureus* isolates were then tested using a latex agglutination (Oxoid, DR0900) kit for PBP2a *(mecA* gene product). The manufacturer's instructions for the procedure were followed. Colonies were taken from enriched agar plates obtained from the subculture of positive blood culture bottles. Clumping was noticed through the naked eye when latex particles bind with methicillin-resistant *Staphylococci aureus*. The monoclonal antibodies activate these latex particles, which are directed specifically against PBP2a. Contrary to MRSA, the latex particles are not agglutinated by MSSA. The storage temperature for all reagents was kept at 2-8°C. The testing of each strain was carried out concurrently with a negative control latex suspension.[17,18]

The collected data was processed through the Statistical Package for the Social Sciences (SPSS) version 23 using standard protocol analysis. Baseline variables were analyzed descriptively using frequencies and percentages for qualitative variables. Diagnostic parameters were calculated using a 2x2 contingency table. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were determined by using the standard formulae.

RESULTS

The study included 107 blood culture samples that isolated the growth of *S. aureus* on subculture. Of these, 77(72.0%) samples were from male patients, and 30(28.0%) samples were from female patients.

Out of all isolated *S. aureus*, 95(88.8%) latex agglutination tests were positive, and 12(11.2%) were negative. 99(92.5%) samples were MRSA positive by Cefoxitin disk test, and 8(7.5%) were negative. Overall,

92 samples were positive for both latex agglutination and Cefoxitin disk, and five were negative, as shown in Table-I. On statistical analysis, the latex agglutination test was found to be 96.84% sensitive (95% CI: 91.05% - 99.34%) and 41.67% specific (95% CI: 15.17% to 72. 33%). The positive predictive value was 92.93% (95 CI:85.97% to 97.11%), and the Negative Predictive Value was 62.50% (95% CI: 24.49% to 91.48%). The Diagnostic Parameter is shown in Table-II.

Table-I: Frequency of Cefoxitin Disk Test and Latex Agglutination (n=107)

	Latex Agglutination (Positive)	Latex Agglutination (Negative)	Total
Cefoxitin Disk Test (Positive)	92(86.0%)	7(6.5%)	99(92.5%)
Cefoxitin Disk Test (Negative)	3(2.8%)	5(4.7%)	8(7.5%)
Total	95(88.8%)	12(11.2%)	107(100.0%)

Table-II: Diagnostic Parameters (n=107)

Parameters	Values	95% CI
Sensitivity	96.84%	91.05% to 99.34%
Specificity	41.67%	15.17% to 72.33%
Disease prevalence (*)	88.79%	81.23% to 94.07%
Positive Predictive Value (*)	92.93%	85.97% to 97.11%
Negative Predictive Value (*)	62.50%	24.49% to 91.48%
Accuracy (*)	90.65%	83.48% to 95.43%

DISCUSSION

A spectacular increase in the MRSA burden is affecting patients' health in hospitals as well as in the community and also posing a great deal of intricacy in selecting antibiotic regimes along with treating and managing its related infections.[8] That is why accurate and rapid detection of MRSA is of great significance, not only for choosing the suitable antibiotic therapy but also for avoiding failure of treatment procedures to control the spread of MRSA.[9]

The results of the current study showed that the latex agglutination test is not only sensitive but also specific for detecting MRSA strains. The accuracy of this test for diagnosing MRSA in a laboratory is significant and time-saving as well where patient management is concerned, as it can detect MRSA in 24 hours less than the time required by conventional disc diffusion methods. A study by Sanchini *et al.,* demonstrated that the detection of MRSA can be commercially carried out by two assays, i.e. Alere PBP2a SA culture colony assay and PBP2a latex agglutination assay. These two methods are sensitive for the detection of MRSA strains.[8] In addition, another study by Khawaja *et al.,* evaluated the accuracy of different phenotypic methods for determining Methicillin resistance in *S. aureus* and compared the results with the *mecA* gene PCR method. The phenotypic methods evaluated were Cefoxitin and oxacillin disk diffusion and LA assay. The research found that the sensitivity of latex agglutination, Cefoxitin and oxacillin salt agar were 98.95%, 96.73% and 94.31%, respectively. The diagnostic accuracy and specificity of LA was 97.14% as compared to PCR (77.77%). The study concluded that LA PB2a assay can be considered a reliable and accurate diagnostic technique for detecting MRSA strains in laboratories where molecular methods are limited and settings that are resource-constrained, such as ours.[5]

Although Polymerase Chain Reaction (PCR) has been considered a gold standard among diagnostic procedures for detecting MRSA isolates, it is time-consuming, labour-intensive and expensive.[12] Due To this and keeping the limited resources of our setups in mind, our study also did not employ the PCR method for MRSA detection. As a substitute, we used the phenotypic methods, i.e. Cefoxitin disc diffusion and LA assay. In agreement, a study by Sultana *et al.,* described that PCR, being a costly method, is only sometimes available in routine laboratories. This study proved that the Cefoxitin disc diffusion method showed 100% specificity and sensitivity compared to the detection of the mecA gene by PCR. Hence, the study established that phenotypic methods like Cefoxitin disc diffusion could be used instead of technically demanding PCR methods for diagnosing MRSA strains.[18,19] Similarly, a study by Panda *et al.,* also compared conventional phenotypic methods with PCR and determined that Cefoxitin disc diffusion cheaper, simple and rapid method and can be used as an alternative to PCR in routine laboratories.[20] Although our lab also employs the Cefoxitin disc diffusion method to identify MRSA, keeping these standards in mind, we evaluated the LA assay against this method to find a reliable, time- and cost-saving alternative.

There are many reasons for the complicated detection of MRSA. In most strains, MRSA is heterogeneous, and these isolates appear phenotypically sensitive to Methicillin. One study elucidated that accurately identifying MRSA using conventional methods is difficult. This is because some isolates resistant to one method may appear sensitive to

another. This study affirmed that the detection of Methicillin resistance is complex as positive strains of the *mec-A* gene have different levels of expressing resistance.[9] On the contrary, our study ascertained that conventional methods like latex agglutination assay can accurately diagnose MRSA strains because the *mecA* gene in MRSA encodes a low affinity to penicillin-binding protein PBP2a. A similar study by Khawaja *et al*., also indicated that for *mecA* gene detection in MRSA isolates, the latex agglutination test is the most consistent and quick diagnostic technique. Using this method in routine laboratories can effectively decrease the misdiagnosis of resistant strains of MRSA and eventually lessen the ill-usage of antibiotics.[19]

LIMITATION OF STUDY

This study has several limitations. A correlation documenting timely patient treatment and clinical response as a result of early identification of MRSA may also add to the benefits of using this kit which couldn't be done in our study due to limited resources.

CONCLUSION

This study concluded that latex agglutination is a time-effective, reliable, and highly sensitive alternative to PCR or Cefoxitin disc diffusion. It can easily be performed at resource-constrained laboratories. Because of its high specificity, this simple and accurate method can be used in routine laboratories. In this way, MRSA infections can be timely identified and managed accordingly.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

RB & SHN: Data acquisition, critical review, approval of the final version to be published.

IAM & AI: Conception, study design, drafting the manuscript, approval of the final version to be published.

RS & MMG: Data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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