



DETECTION OF *mecA* GENE BY LATEX AGGLUTINATION AND ITS MOLECULAR ANALYSIS BY PCR IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS.

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INTRODUCTION

The acquisition of antibiotic resistance has led to progression of *Staphylococcus aureus* infections in this antibiotic era. Evolution of lethal superbugs and loss of “miracle drug” effectiveness worsens the battle against serious bacterial infections, further complicating the treatment options. The loss of efficacy against common pathogens has led to an option towards more expensive antibiotic drugs in high-income countries, as well as increased morbidity and mortality in low-income and middle-income countries. Antibiotic drug consumption is a major driver of antibiotic resistance.^{1,2}

Antimicrobial resistance develops as a natural phenomenon when the infecting germ is not

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ABSTRACT... Objectives: To compare detection of *mecA* gene in methicillin resistant isolates of *Staphylococcus aureus* by latex agglutination and PCR; by assessing the sensitivity and specificity of both methods. **Study Design:** Descriptive Cross-Sectional study. **Setting:** Pathology Department, Post Graduate Medical Institute, Lahore. **Period:** From January 2015 to December 2015; according to standard operating procedures at Microbiology laboratory. **Material & Methods:** A total 713 consecutive, non-duplicate isolates of *Staphylococcus aureus* were processed. Methicillin resistance was determined using cefoxitin (30µg) by Kirby-Bauer method using CLSI guideline (2016), latex agglutination method; and PCR for *mecA* gene. **Results:** The results showed that out of 713 *Staphylococcus aureus* isolates, 92 (12.90%) isolates were resistant to cefoxitin and were labelled as MRSA. majority MRSA isolates recovered from pus (44.57%) and wound swab (20.65%), followed by blood (13.04%), fluid (8.70%), CSF (4.35%), CVP (3.26%), HVS (3.26%) and tracheal secretion (2.17%). 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 employed as rapid and reliable diagnostic technique in MRSA isolates for *mecA* gene detection, where resources for molecular methods are inadequate. This can effectually lessen the misdiagnosis of resistant strains, and over/ ill-use of antibiotics.

Key words: Cefoxitin, Latex Agglutination, *mecA* Gene, Methicillin Resistant *Staphylococcus Aureus* (MRSA), Polymerase Chain Reaction (PCR).

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killed or inhibited by the drug being used by the patient at required therapeutic levels. Infections with resistant pathogens are challenging to treat, demanding costly and at times toxic substitutes.³

The World Health Organization (WHO) defines antimicrobial resistance to human pathogens as a universal health challenge. Antimicrobial resistance makes diseases harder and more expensive to treat not only in developed countries but progressing towards financial crisis in undeveloped countries. Drug resistant infections are treating the economic future of the biosphere. Human action is making AMR worse. The reasons are: Poor infection control in hospitals; inappropriate food handling; Poor sanitary conditions; and most important misuse

and/or overuse of antimicrobial drugs in humans and in breeding crops and animals.^{4,5}

Among multidrug resistant infections, methicillin resistant *Staphylococcus aureus* (MRSA) is the foremost challenging pathogen, accounting for 40–70% of the *Staphylococcus aureus* infections worldwide.⁶

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are recognized globally both in developed and developing countries as a cause of frequent hospitalizations and invasive infections associated with remarkable high morbidity, high mortality and increased treatment costs. In recent years, developing multidrug-resistant strains of *Staphylococcus aureus* (MDRSA) have made treatment of *Staphylococcus aureus* infections more prolonged, troublesome and distressing.⁷

It is stated that methicillin resistance in *Staphylococcus aureus* has been associated with modifications in the penicillin binding proteins (PBPs) subsequently generating an additional penicillin-binding protein, PBP2a or PBP2'. This PBP2a is encoded by the *mecA* gene which is carried on a large genomic island labeled SCCmec. Methicillin resistance in *Staphylococcus aureus* is due to the acquisition of *mecA* gene to a mobile genetic element called Staphylococcal cassette chromosome *mec* (SCCmec). Integration and acquisition of a staphylococcal cassette chromosome *mec* (SCCmec) element into the chromosome converts drug-sensitive staphylococcal lineages into the notorious methicillin-resistant *staphylococcus aureus* (MRSA).⁸

Prevention and control of MRSA transmission and infection are vital in decreasing the resistant infections, mortality and medical cost. Beside the standard precautions, the effective approaches helpful in reducing MRSA infection rate are active surveillance, decolonization, screening and isolation. Strict implementation of standard infection control practices is helpful in diminishing the carriage and transmission of MRSA in both hospital and community setting.⁹

At present, methicillin resistant strains of *Staphylococcus aureus* are resistant to all presently existing β -lactam agents, including semisynthetic penicillin's, i.e., methicillin, oxacillin and nafcillin, the combination of penicillin and β -lactamase inhibitors and as well as all of the cephalosporins, carbapenems, aminoglycosides, macrolides, chloramphenicol, tetracyclines and fluoroquinolones.¹⁰

Currently, the commonly used phenotypic methods for detection of MRSA are conventional disc diffusion method (Modified Kirby–Bauer and Stokes methods); broth microdilution method determining minimal inhibitory concentration (MIC); E Test method; breakpoint method; agar dilution method (oxacillin/methicillin screen agar, mannitol salt agar, isosensitest agar, chromogenic agar).¹¹ Latex agglutination method detects *mecA* gene product i.e., PBP2a.¹² The genotypic methods confirm the presence or absence of *mecA* gene in methicillin resistant *Staphylococcus aureus* isolates by polymerase chain reaction.¹³

MRSA is considered to be a potential “Super Bug” which is a major threat to hospital infection control. Laboratory diagnosis and susceptibility testing are essential steps in accurate detection, treatment, control and prevention of MRSA infections. Tests with cefoxitin do not appear to be affected to the extent as oxacillin by hyper-production of penicillinase. In disc diffusion tests, hyper-producers of penicillinase may show small methicillin or oxacillin zones of inhibition, whereas most true methicillin/oxacillin-resistant isolates give no zone.¹⁴

External parameters that influence the degree of heterogeneity and resistance include inoculum size, salt concentration, pH, composition of medium, osmolarity and temperature.¹⁵

Latex agglutination test is considered better than the conventional phenotypic methods as it is not generally biased by the environmental conditions. In this test latex particles sensitized with specific monoclonal antibodies directed against PBP2a present in MRSA cause agglutination. The latex

agglutination is a rapid test and provides good preliminary screening with high sensitivity and specificity.¹⁶

PCR is a gold standard confirmatory test with high sensitivity and specificity but is expensive and is not available in most of the routine laboratories. Regarding accurate detection of MRSA, the latex agglutination is a simple and rapid test for identification of *mecA* gene. It can serve as a good preliminary screen with high sensitivity and specificity equivalent to PCR. It can be easily performed in most of the laboratories in our setup.

This study has been organized to evaluate the methicillin resistance among clinical isolates of *Staphylococcus aureus* by latex agglutination and PCR as a gold standard; by assessing the sensitivity and specificity of both methods.

MATERIAL & METHODS

This descriptive cross-sectional study was conducted in Pathology Department of PGMI, Lahore; during the period from January 2015 to December 2015.

This study has been structured to compare detection of *mecA* gene in methicillin resistant clinical isolates of *Staphylococcus aureus* by latex agglutination and PCR; by assessing the sensitivity and specificity of both methods.

Clinical isolates of *Staphylococcus aureus* resistant to cefoxitin were only included in the study project. Clinical isolates of *Staphylococcus aureus*; sensitive to cefoxitin were not included in the study project.

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 Pathology department, PGMI, Lahore.

All the specimens were inoculated on blood agar and McConkey agar (prepared as instructions given by the manufacturer). The plates were incubated at 35-37°C aerobically. Following

standard microbiological techniques; preliminary identification of *Staphylococcus aureus* isolates was done by observing the colony morphology on blood 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 ran for the affirmation of *Staphylococcus aureus*.¹⁷

Screening was implemented on all isolates of *Staphylococcus aureus*. The phenotypic resistance to methicillin was ascertained by modified Kirby-Bauer using 30µg cefoxitin disc (Oxoid) on Muller Hinton agar according to the Clinical and Laboratory Standards Institute (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 was 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. Two standard strains, MRSA ATCC 33591 and MSSA ATCC 25923 were employed as inclusion and exclusion check respectively.¹⁸

Isolates of *Staphylococcus aureus* showing resistance to cefoxitin (MRSA) were examined for *mecA* gene outcome (PBP2a) employing latex agglutination kit (Oxoid, DR0900). Procedure followed according to manufacturer's instruction using colonies on Mueller Hinton agar. Latex particles activated with monoclonal antibodies opposed to PBP2a particularly bind with methicillin resistant staphylococci to produce clumping noticeable to the un-aided 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.¹⁹

All the MRSA isolates were grown in nutrient broth by incubating in a shaking incubator at 37°C for 24 hrs. 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* using following primers:

Primers	Oligonucleotide sequence (5' – 3')	Specificity	Product size (bp)
MecA1 (F) MecA2 (R)	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	<i>mec A</i>	310

Table-I. Primers for *mecA* gene by PCR Amplification.

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 (10mg/ml), and visualized under UV light.²⁰

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.

All the 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 was calculated using *mecA* gene PCR as a gold standard.

RESULTS

The outcomes of the study revealed distribution of MRSA species recovered from different clinical specimens (n=92). Table-II of our study reveals distribution of MRSA isolates among different clinical specimens. Statistically the difference was significant ($p < 0.05$) among percentage of MRSA isolates from different clinical specimens.

Among 713 isolates of *Staphylococcus aureus*, 92 strains exhibited cefoxitin resistance. By latex agglutination technique 87 (94.50%) MRSA isolates were positive for *mecA* gene product (PBP2a). So, 5 (5.43%) *mecA* –ve isolates were

mistakenly recognized as MRSA by 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-III).

Specimen	No. of <i>Staphylococcus Aureus</i> Isolates	MRSA	
		No.	%age
Pus	328	41	12.50
Wound Swab	42	19	45.24
Blood	114	12	10.53
Fluid	15	8	53.33
CSF	22	4	18.18
CVP Tip	18	3	16.67
HVS	141	3	2.13
Tracheal Secretion	33	2	6.06
Total	713	92	12.90

Table-II. Distribution of methicillin resistant *staphylococcus aureus* isolated from different clinical specimen (n=713).

Chi-square = 78.2

Probability = 0.000 (statistically significant difference)

Technique	Mec A Gene Positive	
	No.	%age
Latex Agglutination	87	94.50
PCR	82	89.10

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

Table-IV shows the comparison of latex agglutination technique with PCR technique for detection of *mecA* gene in MRSA isolates. When compared with PCR (gold standard), latex agglutination showed 100% sensitivity, and the specificity was 50%. The positive predictive value of the test was 94.25% and the negative predictive value was 100%. The overall diagnostic accuracy of latex agglutination test was 94.57%.

		PCR		Total
		Positive	Negative	
Latex Agglutination	Positive	82	5	87
	Negative	0	5	5
Total		82	10	92

Table-IV. Comparison of Latex Agglutination technique with PCR technique for detection of mecA gene in MRSA isolates (n=92).

Statistics:

Parameter	Value (%)	Confidence Interval (95% CI)
	100.00	100.00% - 100.00%
Specificity	50.00	39.78% - 60.22%
Positive predictive value	94.25	89.49% - 99.01%
Negative predictive value	100.00	100.00% - 100.00%
Diagnostic Accuracy	94.57	89.94% - 99.2%

DISCUSSION

MRSA is probably the most disputing bacterial pathogen that presently distresses patients both in community and hospital settings. Globally, vivid rise in MRSA burden has proposed a serious difficulty in selection of antibiotics used for the treatment of infections caused by this super bug. In coming days, we will be in danger of untreatable infections because of resistance to available antibiotics along with continuous decline in the development of anti-bacterial to an unacceptable level globally. The new antibiotics are required to fight with the emerging threat of infections caused by resistant strains. Deliberate and coordinated action is needed to ensure constant availability of effective antibiotics.^{21,22}

Here comes the importance of swift and precise detection of methicillin resistance in *Staphylococcus aureus* (MRSA) in clinical microbiology laboratories not only for opting the appropriate antibiotic therapy for the individual patient but also to avoid treatment failure as well as to control the endemicity of MRSA.²³

Phenotypically, a wide-range of techniques have been established for detection of methicillin resistance in *Staphylococcus aureus* that may vary in sensitivity and specificity. Additionally, these methods may not certify proper and timely treatment of all the patients suffering from MRSA infections. Detection of the mecA gene by PCR is the “gold standard”, but its availability and affordability is not ascertained in routine laboratories.²⁴ Latex agglutination test for identification of mecA gene product i.e., PBP2a is rapid, sensitive and precise technique for the routine practice in facility constrained laboratories.¹⁹

In our study we evaluated the diagnostic capacity of latex agglutination method in detecting methicillin resistance in *Staphylococcus aureus*. Sensitivity, specificity, negative positive predictive values and diagnostic accuracy of this phenotypic method were estimated. Comparison was done against the PCR assay as a gold standard.

Distribution of methicillin resistant *Staphylococcus aureus* isolates among different clinical specimens is shown in Table-II. Similar studies have been conducted locally as well as abroad. Many researchers have reported higher frequency of MRSA isolated from pus and wound swabs.^{25,26} Maximum number of MRSA (57.69%) isolated from endotracheal secretions and CV catheters documented by Mir et al., (2017) from Lahore. These findings are in contrast with our study.²⁷

Frequency of mecA gene as detected by latex agglutination technique and PCR technique among MRSA isolates (n=92) is shown in Table-III. According to this table, among total 92 MRSA isolates 87 were mecA gene positive by latex agglutination (94.50%) while on PCR 82 were detected positive for mecA gene (89.10%). However, statistically the difference was non-significant ($p>0.05$). So, among total 92 cefoxitin resistant MRSA isolates, 10 (10.86%) mecA negative isolates were falsely identified as resistant. These 10 false positive results could probably be liable to the existence of alternate resistance mechanisms i.e., MODSA are the moderately resistant *Staphylococcus aureus*

isolates with alterations in the existing PBPs and / or BORSA are the penicillinase hyper-producer strains referred to as borderline oxacillin resistant *Staphylococcus aureus*.

Table-IV of our study demonstrates the comparison of latex agglutination technique with PCR for detection of *mecA* gene in MRSA isolates (n=92). The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of latex agglutination and PCR are shown below Table-IV. According to our study sensitivity is 100% and specificity is 50%, PPV is 94.25% and NPV is 100%. The overall diagnostic accuracy of the tests is 94.57%. Many researchers have reported 100% sensitivity in accordance to our study.

Similar study by Palavecino, (2014) have reported 100 % sensitivity of latex agglutination test for detection of PBP2a in *Staphylococcus aureus*. These findings are in comparison with our study.²⁸

The findings of Hassoun et al., (2017), have revealed 97% sensitivity and 100% specificity of latex agglutination test for correctly identifying MRSA from MSSA. 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.⁶

Study carried out by Panda et al., (2016) and Anand et al., (2009), have shown 100% sensitivity of phenotypic methods when evaluated by genotypic methods for detection of *mecA* gene in MRSA. Findings of these researchers are in accordance with our study.^{29,30}

In the present study, we found that out of 92 cefoxitin resistant MRSA strains, 82 stains were *mecA* gene positive on PCR and 10 strains were *mecA* negative. Similar study carried out by Demir et al., (2016) and Jindamwar et al., (2016) have shown 93% and 90% MRSA isolates to be *mecA* gene positive by PCR technique.^{31,32}

Evaluation study by Kali et al., (2014) has documented that among 102 cefoxitin resistant

MRSA isolates, only 92 (90.1%) isolates were positive for the *mecA* gene by PCR.³³

Other studies have shown even higher *mecA* negative isolates.³⁰ It has been shown in various studies that MRSA with a novel Mec A homologue called as *mecC* have been recorded in different parts of the world. These isolates are resistant to cefoxitin but show negative results for *mecA* on PCR.³⁴

CONCLUSION

Effectual scrutiny of the pathogen is required to deal with its emerging public health threat worldwide. The development of diagnostic technologies will have fundamental position in providing faster results [often referred to as 'fast clinical microbiology' (FCM)] to support antimicrobial stewardship programs (ASPs). For the early and precise identification of MRSA, Cefoxitin disc diffusion method must be used with another reliable method preferably latex agglutination test in routine clinical microbiology laboratories with resource limited settings as an alternative to PCR. This will help us to enhance and facilitate the detection of methicillin resistance in *Staphylococcus aureus* and finally aid for its control of spread.

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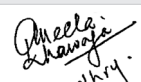
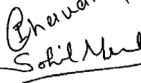
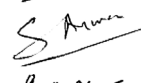
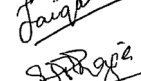
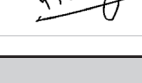
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2	Iffat Javed	Supervision of whole research project & help in write up.	
3	Sohaila Mushtaq	Help in write up.	
4	Saeed Anwar	Result compilation & help in data analysis.	
5	Faiqa Arshad	Proof reading.	
6	Naseer Ahmad Raja	Proof reading.	