

Comparison of phenotypic methods with mecA gene based polymerase chain reaction for Methicillin-resistant Staphylococcus aureus detection

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Abstract

Objective: To determine methicillin resistance in staphylococcus aureus by different phenotypic methods, and to evaluate their accuracy with mecA gene polymerase chain reaction for methicillin-resistant staphylococcus aureus detection.

Methods: The descriptive cross-sectional study was conducted from January to December 2015 at the Post-Graduate Medical Institute, Lahore, Pakistan, and comprised consecutive, non-repetitive clinical isolates of methicillin-resistant staphylococcus aureus that were screened with oxacillin disk 1µg and ceftiofuran disk 30µg by Kirby-Bauer method using Clinical and Laboratory Standards Institute guideline. The isolates were cultured on oxacillin screen and mannitol salt agar, and subjected to latex agglutination for penicillin-binding protein 2a and polymerase chain reaction for mecA gene. Data was analysed using SPSS 20.

Results: All the 105 isolates were resistant on oxacillin and ceftiofuran disk diffusion test, but 95(90.47%) were positive for mecA gene by latex agglutination and polymerase chain reaction. The sensitivity of oxacillin salt agar, mannitol salt agar and latex agglutination was 94.31%, 96.73% and 98.95%, respectively. Keeping polymerase chain reaction as the gold standard, the specificity and diagnostic accuracy of latex agglutination were 77.77% and 97.14% respectively, which was the highest among all the phenotypic methods.

Conclusion: Latex agglutination method can be proposed as a swiftly reliable diagnostic technique for the detection of mecA gene in methicillin-resistant staphylococcus aureus isolates in resource-constrained settings where molecular methods are limited.

Keywords: Methicillin resistant staphylococcus aureus, Oxacillin, Ceftiofuran, Latex agglutination, PCR. (JPMA 70: 276; 2020) <https://doi.org/10.5455/JPMA.290732>

Introduction

Methicillin resistance is an outcome of modification in the assembly of bacterial cell wall by the penicillin binding protein 2a(PBP2a) conferred by the mecA gene.¹ This additional mecA gene is found in methicillin-resistant staphylococci, with no allelic equivalence in methicillin-susceptible staphylococci.²

The phenotypic expression of resistance can be variable even with genetic uniformity being affected by cultural conditions, temperature and osmolality of the medium. Phenotypic resistance to β -lactam antibiotic is demonstrated in terms of decreased zone of inhibition for oxacillin and ceftiofuran or increased minimal inhibitory concentration (MIC). The heterogeneous resistance pattern may cause difficulties in detecting methicillin-resistant staphylococcus (S.) aureus (MRSA) by phenotypic methods.³

The current study was planned to compare the precision

of different conventional phenotypic methods in detecting MRSA and to evaluate their sensitivity and specificity with polymerase chain reaction (PCR) as the gold standard for mecA gene.

Material and Methods

The descriptive cross-sectional study was conducted at the Pathology Department of the Post-Graduate Medical Institute (PGMI), Lahore, Pakistan, from January to December 2015, and comprised clinical specimens from patients admitted in different clinical wards of the Lahore General Hospital (LGH). All the samples were processed according to the standard operating guidelines in the PGMI microbiology laboratory.

All the specimens were inoculated on blood agar and MacConkey agar, prepared as per the instructions of the manufacturer. The plates were incubated at 35-37°C aerobically. Following standard microbiological techniques, preliminary identification of S. aureus isolates was done by observing the colony morphology on blood agar plates, finding β -positive cocci in clusters on gram staining and positive catalase test. Further, biochemical tests, like coagulase and deoxyribonucleic acid-ase (DNA-

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ase), were performed for the confirmation of *S. aureus*. For all the phenotypic and genotypic test runs, MRSAATCC 33591 and methicillin-susceptible *S. aureus* (MSSA)ATCC 25923 were used as positive and negative controls respectively.⁴

Initial screening was performed by disk diffusion test following the 2016 guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI).⁴ A bacterial suspension of each strain (0.5 McFarland standards) was inoculated on Mueller Hinton agar (MHA). Oxacillin disk 1 g was applied and plates were incubated at 35°C for 24 hrs. An inhibition zone of ≤ 10 mm was considered oxacillin (methicillin) resistance.⁵

The phenotypic resistance to methicillin was ascertained by modified Kirby-Bauer using 30µg cefoxitin disc (Oxoid) on MHA, according to the CLSI guiding principles.⁴ For each strain, a bacterial suspension, adjusted according to 0.5 McFarland turbidity standards, was prepared and inoculated on MHA. The plates were incubated at 35°C and zone of inhibition was determined after 24 hours. The results were interpreted according to CLSI criteria, i.e. zone of ≤ 21 mm was considered resistant and ≥ 22 mm was considered sensitive.⁵

MHA containing 4% sodium chloride (NaCl) and 6 g/ml oxacillin was prepared. Each isolate was inoculated in one quadrant (10 l of 0.5 McFarland standards) and incubated at 35°C for 24 hours. The plates were inspected carefully in transmitted light for any growth. Any growth after 24 hours indicated oxacillin resistance.⁶

Mannitol salt agar (MSA) containing cefoxitin (8µg/ml) was prepared according to instructions given by the manufacturer. All the isolates were inoculated on the prepared plates along with positive and negative controls. The plates were incubated at 35°C and first checked after 24 hours. Yellow coloured colonies produced due to mannitol fermentation indicated methicillin resistance. The results were recorded at 48 hours.^{7,8}

All the *S. aureus* isolates showing resistance by disk diffusion method were tested for mecA gene product PBP2a using latex agglutination (LA) kit (Oxoid, DR0900). The procedure was carried out according to the manufacturer's instructions using colonies on MHA. Latex particles activated with monoclonal antibodies directed against PBP2a particularly bind with methicillin-resistant staphylococci to produce clumping noticeable to the naked eye. MSSA does not agglutinate the latex particles. All the reagents were stored at 2-8°C. Each strain was tested simultaneously with a negative control latex suspension.⁹

All the MRSA isolates were grown in nutrient broth by incubating in a shaking incubator at 37°C for 24 hours. 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 MRSA. The mecA gene was detected using primers for mecA gene (Table-1). DNA amplification was performed as follows: An initial denaturation step of 5min at 94°C; followed by 35 cycles of denaturation step at 95°C for 45s, annealing step at 58°C for 45s, and extension step at 72°C for 45s; and a final extension at 72°C for 5min. The PCR amplification products (310 bp) were analysed by electrophoresis on 1.2% agarose gel stained with ethidium bromide (10mg/ml), using DNA ladder (1kb) and visualised under ultraviolet (UV) light.¹⁰

Data was analysed using SPSS 20. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA) of all the phenotypic methods was calculated using mecA gene PCR as the gold standard.

Results

All the 105(100%) isolates were (resistant to oxacillin and cefoxitin by disk diffusion method. Among these isolates, 92(87.61%), 95(90.47%), and 97(92.38%) isolates produced positive results for oxacillin screen agar (OSA), MSA (Figure-1) and LA (Figure-2), respectively. The PCR amplification for the mecA gene detected 95(90.47%) mecA-positive and 10(9.52%) mecA-negative isolates. Out

Table-1: Primers for mecA gene by PCR amplification.

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

PCR: Polymerase chain reaction.

Table-2: Comparisons of Oxacillin Screen Agar, Mannitol Salt Agar and Latex Agglutination (LA) for MRSA Detection.

Parameters	Oxacillin Screen Agar	Mannitol Salt Agar	Latex Agglutination
True positive	83	89	95
False positive	4	3	2
False negative	5	3	1
True negative	11	10	7
Sensitivity (%) *	94.31	96.73	98.95

* 95 % confidence interval

MRSA: Methicillin-resistant staphylococcus aureus.



Figure-1: Growth of *Staphylococcus aureus* on Mannitol Salt Agar showing Methicillin resistance (MRSA).

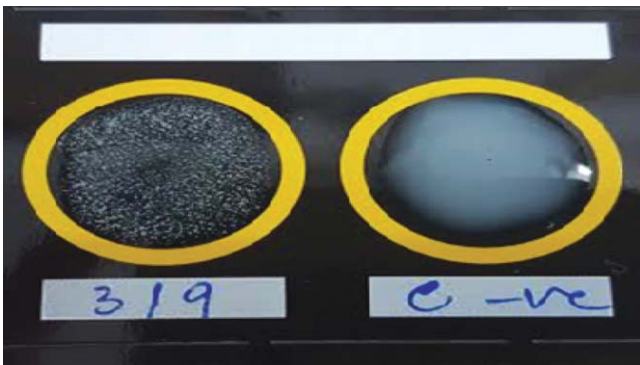


Figure-2: Latex agglutination (LA) test showing Positive Reaction for methicillin-resistant *Staphylococcus aureus* (MRSA).

of the 95 *mecA* PCR-positive isolates, 83(87.4%), 89(93.6%) and 95(100%) strains were correctly identified as MRSA by OSA, MSA and LA, respectively.

The sensitivity of OSA, MSA and LA was 94.31, 96.73 and 98.95%, respectively. Among the 10 *mecA* negative isolates; 4(40%), 3(30%) and 2(20%) isolates were incorrectly identified by OSA, MSA and LA, respectively. LA gave the lowest false negative result compared to MSA and OSA, while LA had the highest sensitivity (Table-2).

Discussion

MRSA is one of the most challenging bacterial pathogens that presently affect patients in hospitals as well as in the community.¹¹ Worldwide, dramatic increase in MRSA burden has started to pose a great difficulty in the selection of antibiotics in the management and treatment of infections caused by this super bug.¹² Here comes the importance of rapid and

accurate detection of MRSA in clinical microbiology laboratories not only for choosing the appropriate antibiotic therapy for the individual patient, but also to avoid treatment failure as well as to control the spread of MRSA.¹³

A wide range of phenotypic methods have been developed to detect MRSA, but they vary in sensitivity and specificity.¹⁴ Moreover, these tests may not ensure appropriate and timely treatment of all the patients suffering from MRSA infections.¹⁵ Detection of the *mecA* gene by PCR is the gold standard, but not always available in routine laboratories and is not affordable.¹⁶ LA test to identify *mecA* gene product PBP2a is a sensitive, rapid and accurate method for the routine use in resource-constrained laboratories.⁹

The current study evaluated the diagnostic ability of different phenotypic methods in detecting MRSA. These methods were OSA, MSA and LA tests. Sensitivity of these phenotypic methods was evaluated. Comparison was done against the PCR assay results.

Data showed that the OSA test gave the lowest performance values in comparison to the other phenotypic methods. MRSA detection by OSA had sensitivity of 94.31%. The sensitivity of OSA was the lowest among all phenotypic methods. Similar results have been shown by other studies.^{17,18} Another study¹⁴ showed high sensitivity and specificity of OSA, mentioning that incubating the plate up to full 48 hours increases the sensitivity but the delay in getting results reduces the efficacy of this method.

The current study found MSA sensitivity of 96.73% more suitable than OSA's 94.31%, but it was less than LA which showed 98.95%. Other studies have shown good sensitivity and specificity of MSA as well.^{19,20} However, the present results are not in concordance with a study⁷ in which MSA showed lower sensitivity 89.13% than OSA, suggesting that several strains of MRSA might fail to grow on MSA due to its high salt concentration.

By analysing the performance of different phenotypic tests, the current study found that best overall performance of LA was comparable with PCR. LA test was found to be more sensitive and specific (98.95% and 77.77% respectively) for the detection of MRSA. Moreover, PPV, NPV and DA of LA were the highest among all the tests i.e., 97.93%, 87.50% and 97.14% respectively. Several studies have shown comparable results with LA sensitivity being $\geq 97\%$.^{6,15,21-23} A study proved that the combination of cefoxitin disk diffusion

with LA improves sensitivity and specificity to detect MRSA isolates.²⁴ However, two strains that were tested positive in the LA test showed negative results for mecA by PCR in the current study. Similar discrepancy was reported with one strain earlier as well.²⁵

The present study found that out of 105 cefoxitin resistant MRSA strains, 95 were mecA gene-positive on PCR and 10 were mecA-negative. The results are in concordance with the study in India that detected 92 isolates mecA-positive and 10 isolates as mecA-negative out of 102 cefoxitin resistant MRSA stains.⁷ Other studies have shown even higher mecA-negative isolates.^{26,27} A novel mecA homologue called mecC have been reported in different parts of the world.²⁸⁻²⁹ These isolates are resistant to oxacillin or cefoxitin, but show negative results for mecA on PCR.²⁶

Conclusion

OSA and MSA tests were less sensitive and specific than LA test. The results of LA test more in line with PCR. For the early and precise identification of MRSA, cefoxitin disc diffusion method must be used with another reliable method, preferably LA test, in routine clinical microbiology laboratories in resource-limited settings as an alternative to PCR. This will help enhance and facilitate MRSA detection.

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Conflict of Interest: None.

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