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Research Article

COMPARISON *OF DIFFERENT PHENOTYPIC METHODS WITH PCR DETECTION OF MEC A GENE FOR DETECTION OF* METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

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ABSTRACT

Emergence of Methicillin-resistant Staphylococcus aureus (MRSA) as leading cause of nasocomial infection is major concern in clinical practice. Molecular identification of Mec A gene is considered gold standard and preferred on other phenotypic identification of MRSA. Aim of our study to compare different phenotypic methods for MRSA with molecular identification of Mec A gene. 63 clinical MRSA strain were used in this study to evaluate different test like cefoxotin disc diffusion test, Oxoid PBP2′ latex agglutination test kit, Slidex staph plus by Biomeurix, oxacillin agar screening test and compare it with molecular detection of Mec A gene by PCR. Cefotoxin disc diffusion test and PBP2' latex test assay have high sensitivity and specificity i.e 100 % compared to Gold standard PCR for MRSA identification.

Keywords: Methicillin-resistant Staphylococcus aureus (MRSA), Oxacillin screen agar, Cefoxitin disc diffusion test, PBP2' latex kit

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) was emerged soon after once methicillin introduced in regular clinical practice and has become a one of leading cause of nasocomial infections as well as community pathogen [1]. Most of the times accurate detection of methicillin resistance *S.aureus* becomes difficult due to the presence of either heterogenous or homogenous in their gene expression of resistant[2]. Also heteroresistance grow more slowly than homogenous and that is why CLSI recommended incubating isolates tested against these antibiotics, for full 24 hours [3].The gold standard for identifying MRSA is to detect the *mecA* gene, or its product, PBP2a, by latex agglutination [4]. However performing this test is costly and may not be possible to be done at all the labs mainly in developing countries.

In recent years there are multiple published report suggest the use of cefoxitin as surrogate marker for the detection of Mec A gene mediated Methicillin-resistant Staphylococcus aureus. CLSI guidelines recommended cefoxitin to be used to identify MRSA. According to CLSI recommendation a 30 ug of cefoxitin disk is used and a zone of less than 19 mm or equal is considered as resistant strain [3].

The aim of our study was to evaluate the efficacy of cefoxotin disc diffusion test as phenotypic marker for MRSA and compare it with molecular detection of Mec A gene by PCR, considered as Gold standard test, Oxoid PBP2′ latex agglutination test kit, Slidex staph plus by Biomeurix and oxacillin agar screening test.

MATERIALS AND METHODS

A total of 63 isolates of *S. aureus* were collected from clinical samples for this study. Confirmation of all the isolates was done by using standard prescribed tests like catalase, tube and slide co aggulase[5].

Cefoxitin Disc diffusion test

All the isolates were tested for the cefoxitin disc diffusion as per CLSI protocol. A 0.5 Mac farland suspension of isolates were pleated on MHA and a 30 ug potency disc of cefoxitin was placed plates. Zone diameter was measured after 18 hours of incubation. An inhibition of zone diameter of equal or less than 19 mm was considered as resistant and equal or more than 20mm was considered as susceptible.

Quality control strain ATCC 25923 MSSA as negative control and ATCC 43300 MRSA as positive control was used.

Oxacillin screen agar

Mueller-Hinton agar (MHA) plates containing 4% NaCl and 6 µg/ml of oxacillin were prepared. Plates were inoculated with 10 µL of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35 ^{0} C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant [6].

Molecular detection of Mec A gene by PCR

DNA extraction was performed by using Gene Elute ™ Bacterila Genomic DNA Kit provided by Sigma Aldrich[R]. The target gene was amplified Using the primer as described (5). A 25ul of reaction prepared of 22.5 of master mix which contain PCR buffer (1X), Mgcl2 (3.5mM), dNTPs (200uM), Taq DNA polymerase (0.5u) with 2.5 ul of DNA extract. Cycling condition were pre denaturation at 95^0 C for 5 minutes followed by 35 cycle of denaturation at 94 ^{0}C for 60 seconds, annealing at 56^0 C for 60 seconds, extension at 72 ^{0} C for 60 seconds and final extension at 72^0 C for 7 minutes. PCR products were visualized using 1.5 % of agarose gel with ehtidium bromide under UV transilluminator using Gel documentation from BioRad. A product of 310 bp size was considered a positive for Mec A gene.

All the isolates were tested with PBP2a latex agglutination test kit supplied by (Oxoid, Hampshire, United Kingdom) and Slidex staph plus by Biomeurix to detect MRSA and MSSA as per manufacturer protocol and instructions.

RESULTS

A total of 63 isolates were collected and identified as *S.aureus* of which 48 were harvested Mec A gene and also resistant for cefoxitin disc diffusion test. Rests 15 were susceptible to cefoxitin and shown to be negative for PCR. Comparision of phenotypic methods with PCR detections of Mec A gene with sensitivity and susceptibility is given in table 1 below. Zone of inhibition for cefoxotin is shown in table .2

DISCUSSION

In a context of worldwide emergence of resistance among *S.aureus* strains, early detection of strains with decreased susceptibility to β-lactam antibiotics is important for clinicians [7]. CLSI 2007 recommended use of cefotoxin as preferred method for testing S.aureus as surrogate marker for detecting oxacillin resistant, and reporting oxacillin resistant or susceptible is based on cefoxitin results only. Same time CLDSI also mentioned Test for Mec a gene or protein expressed by Mec A gene that called PBP2' is most accurate test to identify MRSA (A). Isolates which harvest any one of this should be reported oxacillin resistant as very rare mechanism other than Mec A cause oxacillin resistant [8]. Cefoxitin results are easier to interpret compare to oxacillin and preferred on another.

Table 1: Comparision of phenotypic methods with PCR detections of Mec A gene

	Detected as MRSA	Sensitivity*	Specificity*
Oxacillin Disc diffusion (1ug)	41	85	100
Cefoxotin Disc diffusion (30ug)	48	100	100
Oxacillin screening agar (6ug)	43	90	100
Oxoid PBP2′ latex agglutination	48	100	100
Slidex staph	45	94	100
PCR for Mec A gene	48	100	100

*considering PCR detection of Mec A gene as Gold stanadred.

Table 2: Cefoxitin Inhibition zone and PCR Mec A gene Results

Results for Mec A gene	N=63	Results of Zone diameter															
		<14	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Positive	48	29	11	4	4												
Negative	15								2			4		9			

N= 63, *MRSA=48, MSSA=15

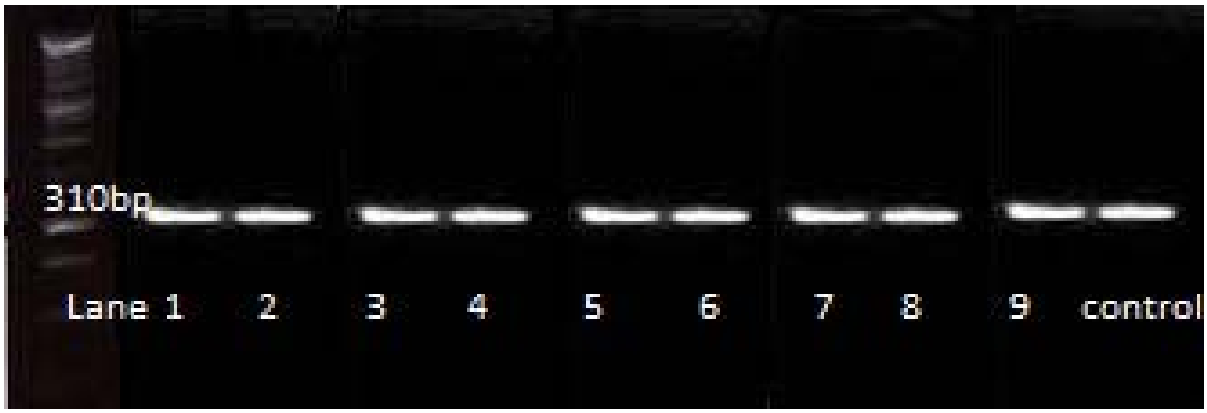

Fig. 1: Results for mec A gene PCR

Many laboratories use multiple tests and often get a conflicting oxacillin and cefoxitin susceptibility results which are most likely to occur for isolates with reduced susceptibility to oxacillin by a non–*mec A* mediated mechanism or are *mec A* positive but are very hetero-resistant [8]. The CLSI 2007 breakpoints for the cefoxitin disk diffusion test for *S. aureus* were modified. Few studies has validated the new cefoxitin breakpoints for the detection of *mecA*-mediated resistance in *S. aureus* and cefoxitin sensitivity and specificity compared to those of oxacillin were 97.3% and 100%, respectively [9].

In our study all the test were having 100 % specificity and but sensitivity was 100 % only for PCR and latex test for PBP2' binding protein Table 1.

The product of *mecA gene*, an altered penicillin binding protein (PBP2a), identification for methicillin resistance in staphylococci [4]. The PBP2a Latex test kit assay is faster and less complicated than PCR for *mecA* and has been shown to be more sensitive than other phenotypic methods, such as the use of oxacillin screen agar [10]. Latex Test kit can also be used for blood culture supernatant but shows very poor sensitivity (33 %) but high specificity (99%) in our study (Data is not shown here for this).

Slidex Staph Plus is based on a triple detection system: blue latex particles sensitized with human fibrogen and monoclonal antibodies simultaneously detect clumping factor, Protein A and group-specific antigen bound to the S. aureus-specific peripheral structure. This kit has less sensitivity than the one described before.

CONCLUSION

PCR is preferred and recommended for identification of MRSA as testing Oxacillin takes long time need enough care to be taken. Comparably latex test assay is rapid test with high sensitivity and specificity. Cefoxitin disc diffusion test is very economical with high sensitivity and specificity but require another day for interpretation. For small lab performing PCR and latex test may not be possible but it can be an alternate option to confirm the doubtful results.

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