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Detection of Methicillin Resistance in *Staphylococcus aureus:* Comparison of Disc diffusion and MIC with *mecA* gene detection by *PCR*

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ABSTRACT
The use of cefoxitin disc test to detect Staphylococcus aureus that are likely to contain the mecA gene has been widely advocated since test was first suggested. The aim of our study was to evaluate the efficacy of cefoxitin disc diffusion test and compare it with oxacillin disc diffusion and detection of mecA gene by PCR. Three hundred strains of S.aureus isolated from clinical samples were included in the study. Antibiotic susceptibility testing was done for oxacillin (1 µg) and cefoxitin (30 µg). PCR for mecA gene was performed. Out of 300 isolates, 45 were found to be methicillin resistant, and 50 were resistant for cefoxitin. For all the 50 isolates mecA was positive. Results of cefoxitin disc diffusion test are in concurrence with the PCR for mecA gene. Thus, cefoxitin disc diffusion test can be used as an alternative to PCR.

KEYWORDS: *MRSA, mecA gene, oxacillin, cefoxitin*

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) strains and healthcare associated MRSA strains are growing threat to patients as well as to the general public. Accurate detection of MRSA is of utmost importance to ensure effective treatment of the patient.

S. aureus strains possessing *mecA* produce an altered penicillin binding protein 2a(PBP2a) and this altered enzyme has reduced affinity for betalactamase drugs[1,2]. This study evaluated the new clinical laboratory standard institute (CLSI) breakpoint for the cefoxitin disc diffusion test for determining *mecA* mediated resistance in *S. aureus*[3].

CLSI recommends usage of cefoxitin instead of oxacillin while using the disc diffusion method to detect resistance against methicillin for *S. aureus*[3] . Cefoxitin disc diffusion results are more sensitive for detection of *mec*A mediated resistance than oxacillin disc diffusion and oxacillin minimum inhibition concentration(MIC)[4-8]. The CLSI guidelines 2006 recommended cefoxitin 30 µg disc for disc diffusion method for detection of MRSA, inhibition zone of ≤ 19 mm is considered as MRSA and ≥ 20 mm is considered as MSSA[9].

The aim of the present study is to evaluate the efficacy of cefoxitin disc diffusion test to detect methicillin resistance in *S. aureus* and compare it with oxacillin disc diffusion, MIC and detection of *mecA* gene by PCR methodology which is considered as the gold standard.

MATERIALS AND METHODS

Between April 2009 and March 2011, a total of 300 *S. aureus* strains were isolated, 200 from the patient specimens and 100 from the carriers of hospital healthcare workers. Confirmation of the strains was done by using standard tests like catalase, tube coagulase and mannitol fermentation.

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method for oxacillin 1 µg disc and cefoxitin 30 µg disc recommended by CLSI guidelines. MIC of oxacillin was done recommended by CLSI guidelines.

All the isolates were subjected to oxacillin disc diffusion test using a 1 µg disc and cefoxitin disc diffusion test by using 30 µg disc on Mueller-Hinton agar (MHA) with 4 % NaCl. The plates were incubated at 37°C for 18 hours and zones of inhibition were measured. An inhibition zone of ≥13 mm was considered as susceptible, 11-12 mm was considered as intermediate and ≤ 10 mm as resistant for oxacillin. An inhibition zone of ≥ 20 mm was considered as susceptible and ≤ 19 mm resistant for cefoxitin. MIC testing was performed for oxacillin by agar dilution method, results were interpreted as susceptible if the MIC was ≤ 2 µg/ml and resistant if the MIC was ≥ 6 µg/ml[10].

PCR amplification of *mec*A gene

The *S.aureus* isolates were grown on blood agar for 18 hours. A single colony from each plate was used to inoculate into trypticase soy broth after 18 hrs of incubation at 37°C, 1.8 ml of each liquid culture was used for preparation of genomic DNA. Extraction of DNA was performed by using Pure Link Genomic DNA Mini Kit based on manufacturer's recommendation. Nucleic acids from each isolate were eluted in 50 µl of elution buffer and kept at –20 °C until tested.

PCR reaction consists of 45 µl of master mix containing PCR buffer (1X), dNTP mix (2.5 mM of each), primer (10pmol/µl), Taq DNA polymerase (0.25 U) and $MgCl_2$ (1.5 mM) with 5 µl of template DNA. Cycling conditions were- hot start 94°C for 4 minutes followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension step at 72°C for 2 minutes. PCR products were visualized on 1% agarose gel with ethidium bromide dye under UV transilluminator. Amplicons of 310 basepairs (bp) were consistent with *mec*A gene amplification.

RESULTS

Among 300 *S.aureus* strains, 45 were MRSA and 255 were methicillin sensitive *Staphylococcus aureus* (MSSA) by routine disc diffusion test using oxacillin disc. Forty eight were MRSA and 252 were MSSA by oxacillin agar screening. Fifty were resistant with cefoxitin disc diffusion test and in these 50 isolates *mecA* gene was detected. The results of disc diffusion are given in **table 1 and 2.** The sensitivity and specificity of the three phenotypic methods are compared with genotypic tests and are given in **table 3.**

Table 1: Primers for amplification of *mecA* gene

Primer	Sequence
Forward	**5'-TGCTATCCACCCTCAAACAGG-3'**
Reverse	**5'-AACGTTGTAACCACCCCAAGA-3'**

Table 2: Cefoxitin inhibition zone and results of *mecA* gene

mecA results	n	Results of disc diffusion(mm)
Positive	**50**	50(≤ 19 mm)
Negative	**250**	250 (≥ 20 mm)

Table 3: Comparison of two phenotypic methods with genotypic detection of MRSA

Test method	Detection of MRSA	Sensitivity (%)	Specificity (%)
Oxacillin disc diffusion (1µg)	45	90	100
Cefoxitin disc diffusion (30µg)	50	100	100
PCR for *mecA* gene	50	100	100

Total number of samples n=300

DISCUSSION

Recent studies indicate that disc diffusion test using cefoxitin is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar and is now an accepted method for detection of MRSA by CLSI[11]. The early and accurate detection of methicillin resistance is of importance in the prognosis of *S.aureus* infections. In this study, different methods are evaluated for detection of *mecA*. Detection of *mecA* is considered as the gold standard for MRSA confirmation.

In our study, CLSI disc diffusion criteria to define resistance (cefoxitin zone diameter ≤21mm for resistance and ≥22mm for susceptibility, oxacillin ≤10mm for resistance and ≥13mm susceptibility), the sensitivity and specificity were 100% in all 300 strains for cefoxitin and where as oxacillin disc diffusion and agar screening test were not so accurate. Results of cefoxitin disc diffusion test is in concurrence with the PCR for *mecA* gene, and thus the cefoxitin disc diffusion method is very suitable for detection of MRSA and it can be alternative to PCR.

CONCLUSION

This study provides evidence that cefoxitin disc diffusion can be used as an accurate method to detect MRSA. The results have shown 100% specificity and sensitivity as compared with *mecA* gene detection by PCR. Hence it can be used as an alternative to the PCR.

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