

COMPARISON OF CEFOTAXIME DISC DIFFUSION TEST, OXACILLIN SCREEN AGAR, AND PCR FOR *mecA* GENE FOR DETECTION OF MRSA

KB Anand, P Agrawal, *S Kumar, K Kapila

Abstract

Cefotaxime is a potent inducer of the *mecA* regulatory system. It is being recommended for detection of methicillin resistance in *Staphylococcus aureus* (MRSA) when using disk diffusion testing. The aim of our study was to evaluate the efficacy of cefotaxime disc diffusion test to characterize MRSA and compare it with oxacillin agar screening and detection of *mecA* gene by PCR. **Materials and Methods:** Fifty strains of *S. aureus* isolated from clinical samples were used in the study. Routine antibiotic susceptibility testing was performed including oxacillin disk. Oxacillin screen agar plates with 4% NaCl and 6 µg/ml of oxacillin were inoculated and interpreted as per standard guidelines. Cefotaxime disc diffusion test was performed using 30 µg disc and zone sizes were measured. PCR for amplification of the *mecA* gene was performed. **Results:** Out of the 50 isolates, 28 were found to be methicillin resistant by oxacillin disc diffusion test, 30 were resistant by oxacillin screen agar method, and 32 were resistant with cefotaxime disc diffusion. For these 32 isolates *mecA* gene was positive. **Conclusion:** Results of cefotaxime disc diffusion test is in concordance with the PCR for *mecA* gene. Thus, the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

Key words: *mecA* gene, MRSA, cefotaxime

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged soon after the introduction of methicillin into clinical practice. In addition to being a nosocomial pathogen, MRSA has become a community pathogen. Strains that possess *mecA* gene are either heterogeneous or homogeneous in their expression of resistance. The heterogeneous expression occasionally results in minimal inhibitory concentrations that appear to be borderline and consequently the isolates may be interpreted as susceptible.^[1]

In the recent past, there have been multiple reports on the use of cefotaxime as a surrogate marker for detection of *mecA*-gene-mediated methicillin resistance.^[2,3,4] Cefotaxime is a potent inducer of the *mecA* regulatory system.^[4] The Clinical and Laboratory Standards Institute (CLSI) guidelines (2006) has recommended cefotaxime disc diffusion method for the detection of MRSA. This is performed by using a 30 µg cefotaxime disc and an inhibition zone diameter of ≤19 mm is reported as methicillin resistant and ≥20 mm is considered as methicillin sensitive.^[5]

The aim of our study was to evaluate the efficacy of cefotaxime disc diffusion test to detect methicillin resistance in *S. aureus* and compare it with oxacillin agar screening

and detection of *mecA* gene by PCR, which is considered as the gold standard.

Materials and Methods

A total of 50 strains of *S. aureus* isolated from clinical samples were used in the study. Confirmation of the strains was done using standard tests like catalase, slide and tube coagulase, and growth on Mannitol salt agar. Routine antibiotic susceptibility testing was performed by Kirby–Bauer disc diffusion method for the following antibiotics: ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), ciprofloxacin (5 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), oxacillin (1 µg), and vancomycin (30 µg).

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 °C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant.^[6,7]

Cefotaxime disc diffusion test

All the isolates were subjected to cefotaxime disc diffusion test using a 30 µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37 °C for 18 h and zone diameters were measured. An inhibition zone diameter of ≤ 19 mm was reported as oxacillin resistant and ≥20 mm was considered as oxacillin sensitive.^[8]

*Corresponding author (email: <sat12kum@indiatimes.com>)
Department of Microbiology, Armed Forces Medical College,
Pune-440 040, India
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Quality control strains – methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC 43300 – were used as negative and positive controls, respectively.^[5]

PCR amplification of the *mecA* gene

DNA extraction was performed by QIAamp DNA minikit (QIAGEN). The *mecA* gene was amplified using the primers as described by Geha *et al.*,^[9] and given in Table 1. A 50 µl PCR reaction consisted of plus 45 µl of master mix containing PCR buffer (1X), dNTP mix (0.2 mM of each), primer (0.5 µM), Taq DNA polymerase (0.25 U), and MgCl₂ (1.5 mM) with 5 µL of template DNA. Cycling conditions were – hot start at 94 °C for 4 minutes followed by 30 cycles of denaturation at 94 °C for 45 seconds, annealing at 50 °C for 45 seconds, and extension at 72°C for 1 minute and final extension step at 72°C for 3 minutes. PCR products were visualized on 2% agarose gel with ethidium bromide dye under UV transilluminator. Amplicons of 310 bp were consistent with *mecA* gene amplification (Fig. 1).

Results

Among 50 *S. aureus* strains, 28 were MRSA and 22 were MSSA by routine disc diffusion test using oxacillin disk. Thirty were MRSA and 20 were MSSA in oxacillin agar screening. Thirty two were resistant with cefoxitin disc diffusion test and in these 32 isolates *mecA* gene was detected. The results for inhibition zone diameters by cefoxitin disc diffusion are given in table 2. The sensitivity and specificity of the three phenotypic tests as compared with genotypic test are given in Table 3.

Discussion

Detection of *mecA* gene or its product, penicillin binding proteins (PBP2a), is considered the gold standard for MRSA confirmation.^[10] Recent studies indicate that disc diffusion testing using cefoxitin disc is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA by many reference groups including CLSI.^[11] The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. In this

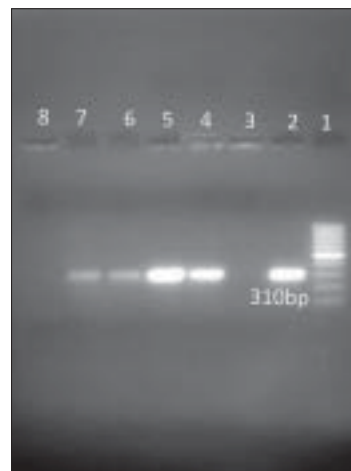


Figure 1: Results of *mecA* gene PCR (310 bp product)

Lane 1: 100 bp molecular weight marker

Lane 2: MRSA ATCC 43300

Lane 3: MSSA ATCC 25923

Lane 4, 5, 6, and 7: MRSA isolates from clinical samples

Lane 8: MSSA isolates from clinical sample

Table 1: Primers for amplification of *mecA* gene

Primer	Sequence	Position	Product size (bp)
Forward	5'-GTAGAAATGACTGAACGTCCGATAA - 3'	318 to 342	310

Table 2: Cefoxitin* inhibition zone diameters and result of *mecA* gene PCR

<i>mecA</i> result	n	Results at a zone diameter (mm) of															
		≤14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Positive	32	14	10	5	3												
Negative	18								2	8	8						

*30 µg cefoxitin disks

Table 3: Comparison of three phenotypic methods with genotypic method of detection of MRSA

Test method	Detected as MRSA*	Sensitivity (%)	Specificity (%)
Oxacillin disc diffusion (1 µg)	28	87.5	100
Oxacillin agar screen (6 µg)	31	96.8	100
Cefoxitin disc diffusion (30 µg),	32	100	100
PCR for <i>mecA</i> gene	32	100	100

*Total number of samples N = 50

study, we attempted to evaluate different methods for detection of *mecA*.

During the last several years, the CLSI-AST has attempted to improve the accuracy of detecting *mecA*-positive strains of *S. aureus*. In our study, the observations using the CLSI disc diffusion criteria to define resistance (cefoxitin zone diameters of ≤ 19 mm for resistance and ≥ 20 mm for sensitivity), the sensitivity and specificity were 100% in the 50 strains tested in the study whereas the results of oxacillin screen agar were not so accurate. Results of cefoxitin disc diffusion test is in concordance with the PCR for *mecA* gene [Table 2], and thus the cefoxitin disk diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings.

From a clinical perspective, it is important to differentiate isolates that have *mecA*-positive resistance from the infrequently encountered isolates that have borderline resistance because it may affect therapy. Strains that possess *mecA*-classic resistance are either heterogenous or homogenous in their expression of resistance. It is the testing of heteroresistant isolates which may appear as susceptible. The presence of resistance in *S. aureus* isolates on an oxacillin screen agar plate generally means that the isolate is *mecA* positive. Occasionally, however, heteroresistant *mecA*-positive strain is not detected due to low expression of resistance. Oxacillin agar screen generally does not detect borderline resistant strains, when studies have included strains whose resistance is heterogeneous the test has been shown to perform less well. Also, agar dilutions and oxacillin disc diffusion method may be affected by various components of MHA, temperature, and duration of incubation.^[12]

Conclusion

Although the number of isolates are less, this study provides an evidence that cefoxitin can be used as an accurate surrogate marker in routine susceptibility testing at 37 °C for 18–24 hours. In addition, the results have shown 100% sensitivity and specificity as compared to *mecA* gene detection by PCR. Hence, it can be used as an alternative to the technically demanding PCR.

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