

## Comparative Study of Cefoxitin Disc Diffusion Test, Oxacillin Diffusion Test and Oxacillin Screening Agar Test for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) in a Tertiary Care Centre

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Cefoxitin is increasingly recommended for the detection of Methicillin resistance in *S.aureus*, when using diffusion testing. The aim of the study was to evaluate and compare the efficacy of Cefoxitin disc diffusion test with Oxacillin Screen Agar, and Oxacillin disc diffusion test. 100 strains of *S.aureus* isolated from clinical samples were used in the study. Routine antibiotic susceptibility testing were performed including Oxacillin disc (1µg), Oxacillin Screening Agar with 4% NaCl and 6µg/ml of Oxacillin were inoculated and interpreted as per standard guidelines. Cefoxitin disc diffusion was performed using 30µg disc & zone sizes were measured. Out of 100 isolates, 44 were found to be Methicillin resistant by Oxacillin diffusion test, 50 were resistant by Oxacillin screen agar method and 70 were resistant with Cefoxitin disc diffusion. Results of Cefoxitin disc diffusion test is better than other phenotypic methods and can be used for routine susceptibility testing of MRSA, in resource constraint setups that cannot afford PCR testing for *mecA* as a confirmatory test.

**Key words:** MRSA, Cefoxitin, Oxacillin, Tertiary Care Centre.

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The incidence of both hospital acquired and community acquired infections caused by MRSA have steadily been increasing worldwide. Infections caused by MRSA result in lengthier hospital stays and raising health care costs and have a high attributable mortality rate<sup>1</sup>. Early recognition of patients colonised or infected with MRSA can have a direct impact on the selection of antibiotic therapy and the decision to initiate

isolation procedures. An ideal method for MRSA detection, should have a high sensitivity and a short time to the reporting of the results<sup>2</sup>.

In the recent past there have been multiple reports on the use of Cefoxitin as a surrogate marker for the detection of *mecA* gene mediated methicillin resistance<sup>3,4,5</sup>. Susceptibility to Oxacillin by disc diffusion has been used for the detection of MRSA strains in routine testing however some recent studies have reported low sensitivity and low specificity of Oxacillin, compared with Cefoxitin for the detection of Methicillin Resistant isolates<sup>8</sup>. Cefoxitin is a potent inducer of the *mecA* regulatory system<sup>5</sup>. The CLSI guidelines (2012) has recommended Cefoxitin disc diffusion method for the detection of MRSA. This is performed by using a 30µg Cefoxitin disc and an inhibition zone

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diameter of  $\leq 19$ mm is reported as methicillin resistant, and  $\geq 20$ mm is considered as methicillin sensitive<sup>6</sup>.

The aim of this study was to evaluate and compare the efficacy of Cefoxitin disc diffusion test with Oxacillin disc diffusion test and Oxacillin screening agar to detect Methicillin Resistance in *Staphylococcus aureus*.

## MATERIALS AND METHODS

A total of 100 strains of *Staphylococcus aureus* isolated from various clinical samples like post-operative wounds, abscess, cellulitis etc, that were referred to the department of microbiology Dr. B.R.Ambedkar Medical College, Bangalore, during the period of 1 year, from July 2010 to June 2011. Confirmation of the strains, were done using standard tests like catalase, slide and tube coagulase and growth on mannitol salt agar. Routine antibiotic susceptibility testing were performed by Kirby Bauer disc diffusion method for the following antibiotics, ampicillin (10 $\mu$ g), amoxicillin clavulanic acid (20 $\mu$ g), ciprofloxacin (5 $\mu$ g), erythromycin (15 $\mu$ g), clindamycin (2 $\mu$ g), gentamycin (10 $\mu$ g), and vancomycin (30 $\mu$ g) at 37°C and oxacillin (1 $\mu$ g) at 30°C and identified to species level.

### Oxacillin screen agar

Mueller Hinton agar plates containing 4% NaCl and 6  $\mu$ g/ml of Oxacillin were prepared,

plates were spot inoculated with a cotton swab dipped into a 0.5 Mcfarland standard suspensions of each isolate, according to the procedures outlined by CLSI(2012) Oxacillin resistance was confirmed by bacterial growth after 24hrs incubation at 35°C.

### Cefoxitin disc diffusion test

All the isolates were subjected to Cefoxitin disc diffusion test using a 30  $\mu$ g disc, a 0.5 Mcfarland standard suspensions of the isolates were made, and lawn culture done on MHA plates, and were incubated at 37°C for 18hrs and zone diameters were measured.

Results were interpreted according to CLSI guidelines. An inhibition zone of  $\leq 19$ mm were reported as Oxacillin resistant and  $\geq 20$ mm was considered as Oxacillin sensitive<sup>7</sup>.

### Quality control strains

Methicillin Sensitive Staph aureus (MSSA) ATCC 25923 and Methicillin Resistant *S. aureus* (MRSA) ATCC 43300-were used as negative and positive controls, respectively.

## RESULTS

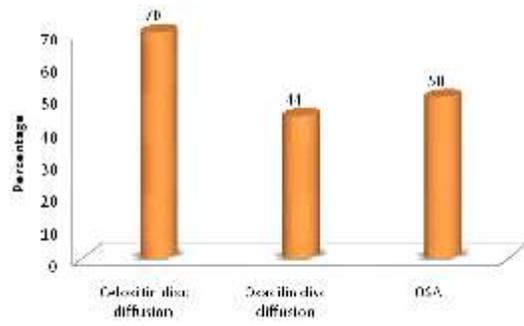
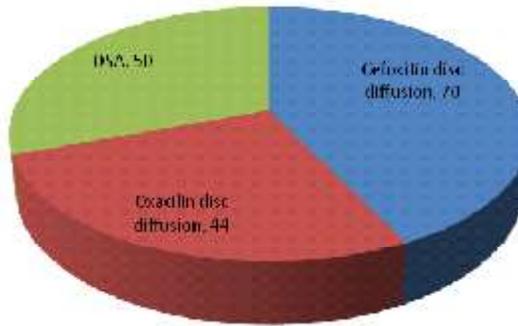
Out of 100 *S.aureus* isolates, 44 were MRSA and 56 were MSSA by routine disc diffusion test using Oxacillin disc. 50 were MRSA and 50 were MSSA in Oxacillin Screen agar. 70 were resistant with Cefoxitin disc diffusion test.

**Table 1.**

Methods for detection of MRSA	n=total no of isolates	No of isolates detected as MRSA	Proportion of MRSA isolates detected
Cefoxitin disc diffusion test	100	70	0.7
Oxacillin screen agar	100	50	0.5
Oxacillin disc diffusion test	100	44	0.44

**Table 2.**

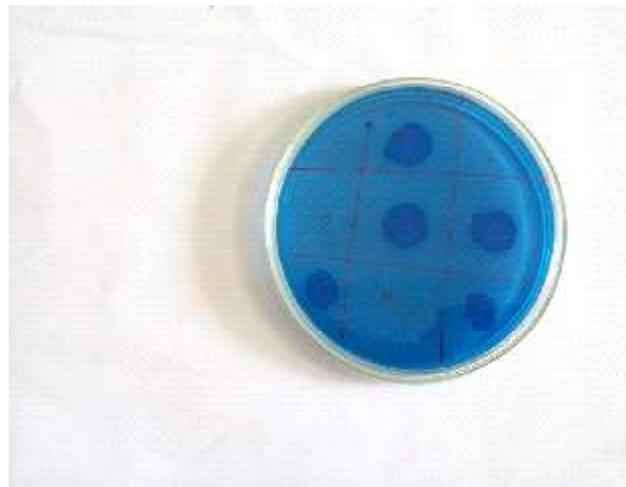
Comparision between proportion of MRSA detected	Difference in proportion	P-Value
Cefoxitin disc diffusion and Oxacillin disc diffusion	0.26	0.0043(<0.05)
Cefoxitin disc diffusion and Oxacillin screen agar	0.2	0.0206(<0.05)



Routine antibiotic susceptibility testing including oxacillin disc test



Cefoxitin Diffusion test



Oxacillin screening agar test

## DISCUSSION

Cefoxitin disc diffusion testing is now an accepted method for the detection of Methicillin Resistance in *S.aureus* by an increasing number of reference resistance groups, including CLSI<sup>1</sup>.

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<sup>8</sup>.

The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S.aureus*. In the 50 strains studied the proportion of MRSA detected by Cefoxitin disc diffusion is 70%, Oxacillin screen agar is 50%, Oxacillin disc diffusion is 44%.

When Cefoxitin disc diffusion method was compared with other 2 phenotypic methods, it is seen in this study that the P-value was 0.0043 and was found to be significant (Table 2). The accuracy of the detection of MRSA by the disc diffusion method may be affected by various components of MHA, temperature and duration of incubation.

## CONCLUSION

This study provides further evidence that Cefoxitin is an accurate surrogate marker for the detection of MRSA in routine susceptibility testing by disc diffusion. In other studies the results have shown 100% sensitivity and specificity as compared to *mecA* gene detection by PCR and it can be used, alternative to the technically demanding PCR. PCR was not done in the current study due to cost constraints.

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