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Original Research Article

Comparison of Cefoxitin Disc Diffusion with Oxacillin E-Test for Detection of MRSA

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Abstract

Infections caused by MRSA are worldwide, resulting in increased mortality and morbidity. Detecting the *mec*A gene or its product by PCR is recognized as a gold standard for detection of MRSA. In resource limited clinical settings phenotypic method which is simple, rapid, accurate and cost effective is required. *mec A* gene detection considered as gold standard for MR isolates. The aim of this study was to do a comparative evaluation of E-test MIC and Cefoxitin disc diffusion for detection of Methicillin resistant *Staphylococcus aureus* (MRSA). A total of 94 *S. aureus* isolates were identified, which were subjected to Cefoxitin disc diffusion and Oxacillin MIC by E-test. A total of 53 isolates were identified as MRSA by E-Test strip and 51 by Cefoxitin disc diffusion test. In this study sensitivity and specificity of E-Test is 100% while sensitivity and specificity of Cefoxitin disc as 96.23%.

Keywords: *mec*A gene, E- test MIC.

Introduction

Staphylococcus aureus is one of the most common bacteria encountered in the clinical practice. [1,2] Increase in the number of bacterial strains that show resistance to methicillin (MRSA) has become a serious clinical and epidemiological problem.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are significant pathogens that have emerged over the past several years to cause both Nosocomial and Community acquired infections. [3]

Infections caused by MRSA are worldwide, resulting in increased mortality and morbidity [4]. In India, the prevalence of Nosocomial infections caused by MRSA varies between 30-70%.

Methicillin resitance in *S. aureus is* based on the production of an additional penicillin binding protein, PBP 2a or PBP 2', which is mediated by the *mecA* gene. [5]

Infections caused by MRSA are serious and are difficult to treat. Only a few antimicrobial agents are available for treatment of such infections.

For these reasons, simple, rapid, accurate and sensitive method for the detection of methicillin resistance is of key importance to ensure correct antibiotic treatment in infected patients as well as control of MRSA isolates in hospital environments, to prevent their spread.

Material and Methods:-

Sample size: Total of 94 Non duplicate strains of *S.aureus* were collected

Study period: 4 months from July-Oct 2013

Samples: Various clinical specimens such as pus, blood, urine, wound swab, throat swab etc.

Sample processed at Dept. of Microbiology in tertiary care hospital in Bhopal.

Detection of Methicillin resistance by phenotypic methods

All *S. aureus* isolates were subsequently tested for methicillin resistance by Cefoxitin (30 µg) disc diffusion tests and E- test Oxacillin MIC. *S. aureus* ATCC 25923 was used as control strain

a) Cefoxitin disc diffusion test [6]

It was done using Cefoxitin (30µg) antibiotic disc. Inoculum of test isolate was prepared and incubated for 2 -3 hours. The turbidity after incubation was matched to 0.5 McFarland standard. After the standardization of the inoculum, a freshly prepared, dried MHA plate was inoculated for lawn culture

using a sterile cotton swab stick. Cefoxitin 30µg disc was placed in the center and the plate was incubated aerobically at 35ºC ± 2ºC for 24 hours. The zone size was measured in reflected light and was interpreted as Resistant ≤ 21mm and Sensitive ≥ 22 mm as per CLSI guidelines.(Fig: 1 & 2).

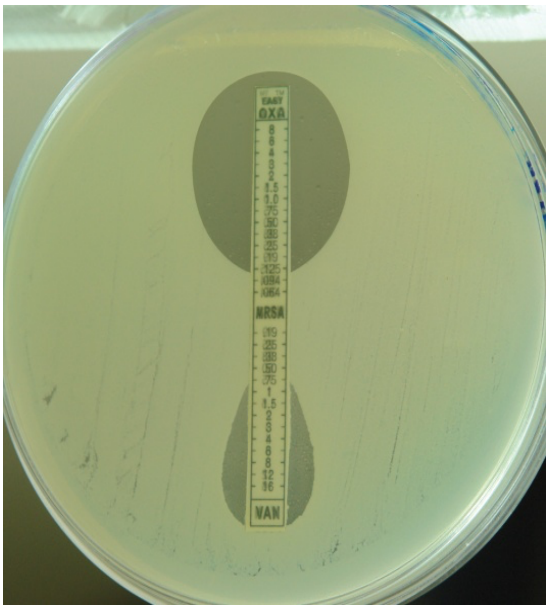
Fig1: showing cefoxitin resistant strain

Fig2: showing cefoxitin sensitive strain

b) E- test MIC Oxacillin [7]

Muller Hinton Agar plate with 2% NaCl was prepared. The dried plates were lawn cultured with test strain using sterile non toxic cotton swab using standardized inoculum (0.5 McFarland). The Ezy MIC Oxacillin strips (EM-065, HiMedia, India) were applied on the inoculated plates as per manufacturer's instruction. The plates were incubated at 35ºC ± 2ºC for 24 hours and read when sufficient growth is seen and MIC is noted where the ellipse of zone of resistance intersected the MIC scale on the strip. The strains were considered to be MRSA when MIC of ≥ 4 µg/ml was observed and Methicillin sensitive *S.aureus* if MIC was ≤ 2.0 µg/ml. (Fig: 3 & 4)

Two standard strains, one methicillin sensitive *S. aureus* (MSSA) ATCC (29213) and one MRSA ATCC (43300) were included in each batch of testing by different method.



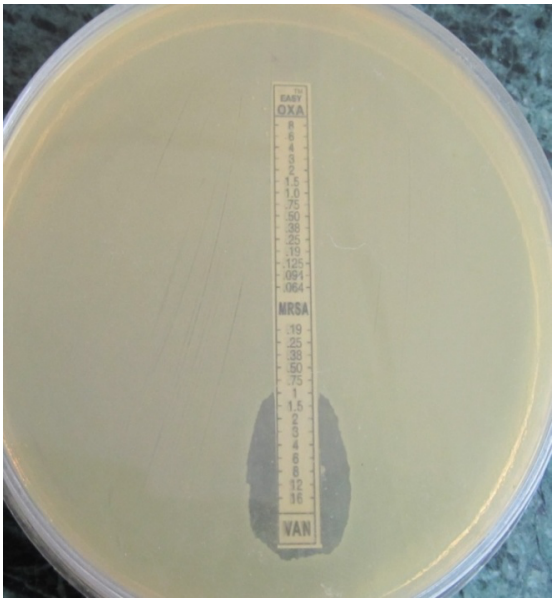


Figure 3: showing sensitivity to oxacillin in E- test Fig4: showing resistance to oxacillin in E- test

Result

Out of the 94 strains tested, 53 (56.38%) were resistant to oxacillin by E- test method and 51(54.26%) were resistant to cefoxitin.

Table 1: Strains(n=94) tested for Methicillin resistant and Methicillin sensitive strains by E- Test MIC(Oxacillin) and Cefoxitin disc diffusion test

Methods	MRSA	MSSA	Total
E- Test MIC (Oxacillin)	53	41	94
Cefoxitin disc diffusion test	51	43	94

Table 2: Showing percentage of Sensitivity and Specificity of E- Test MIC(Oxacillin) and Cefoxitin disc diffusion test

Methods	Sensitivity%	Specificity%
E- Test MIC (Oxacillin)	100	100
Cefoxitin disc diffusion test	96.23	100

Discussion

Testing of methicillin resistance in *S. aureus,* has been a challenge for clinical laboratories in recent years. Accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. Methods with high sensitivity and specificity are required and provide a major guideline for treatment of infections caused by this organism.

Detecting the *mec*A gene or its product by PCR is recognized as a gold standard for detection of MRSA [8]. In resource limited clinical settings, where difficulty in performing molecular methods, different phenotypic methods are used for the detection of MRSA. Studies by Sasirekha B. et al [9] and Karami S. et al [10] consider E-test MIC as a gold standard method for detection of MRSA as it approaches the accuracy of PCR for *mecA.*

Several studies have shown that cefoxitin disc diffusion method to be one of reliable method for detection of MRSA .In this study we evaluate and compare cefoxitin disc diffusion method with E- test MIC and find that cefoxitin has a 96% sensitivity and 100% specificity as seen in several studies. Studies like Jain A et al, Farahani et al , Rahbar M et al also suggested the same. [11,12,13]

In this study, Epidemiology of MRSA over different parts of India is not uniform, it varies from 30- 70%. In this study incidence of MRSA in our hospital was 56%. Study by Manjunath V. et al [14] also show higher resistance.

This study reveled that cefoxitin disc diffusion method had a high sensitivity and specificity for detection of MRSA. This method can be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, easy availability, media preparation and finally more cost-effective in comparison to other method.

Regular monitoring of antimicrobial susceptibility pattern of MRSA and formulation of a definite antimicrobial policy may be helpful for reducing the incidence of these infections.

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