Comparative evaluation of MIC by E-test and Cefoxitin disc diffusion for detection of Methicillin Resistant Staphylococcus aureus (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. Cefoxitin disc diffusion is considered as surrogate marker for *mecA* gene, and could be considered as gold standard for MR isolates. The aim of this study was to do a comparative evaluation of E-test MIC against Cefoxitin disc diffusion for detection of Methicillin resistant *Staphylococcus aureus* (MRSA). A total of 174 *S. aureus* isolates were identified, which were subjected to both Cefoxitin disc diffusion and Oxacillin MIC by E-test. A total of 69 isolates were identified as MRSA by Cefoxitin disc diffusion test. In this study sensitivity and specificity of Cefoxitin is 100% while sensitivity and specificity of Oxacillin MIC by E-test comes out as 94.02% and 94.39%.

Keywords: MRSA(Methicillin resistant *Staphylococcus aureus*), E- test MIC

Introduction

Staphylococcus aureus is one of the most common bacteria encountered in the clinical practice. Despite the introduction of effective antimicrobial agents and improvements in hygiene, staphylococci have persisted as important hospital and community pathogens.[1,2,3]

As the incidence of MRSA is on rise in India from 6% to 80% in last two decades.[4,5] Increase in the number of bacterial strains that show resistance to methicillin (MRSA) has become a serious clinical and epidemiological problem. Methicillin resistance 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.[6]

MRSA infection is of concern because it is resistant to a number of widely used antibiotics. Treatment options for MRSA are limited and less effective, than options available for susceptible *S. aureus* infections leading to increased morbidity and mortality in hospitalized patients. Cost of treatment for MRSA isolates is another major problem found by patients in developing countries.[7]

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 and prevent their spread. This study was carried out for comparative evaluation of Minimum Inhibitory Concentration (MIC) for Oxacillin by E-test against Cefoxitin disc diffusion for detection of MRSA strains.

Material and Methods

This cross sectional prospective analytical study was carried out during November 2012 to April 2014 in the Department of Microbiology, People's College of Medical Sciences and Research Centre, Bhopal. A total of 174 *S. aureus* isolated from non-repetitive clinical samples from IPD and OPD of People's Hospital were included in study after Institutional Ethics Committee (IEC) approval.

All the samples were processed according to standard microbiological procedures available. The collected samples were plated onto nutrient agar, 5% sheep blood agar and MacConkey agar (MA). Urine samples were plated onto CLED and incubated at 37°C for 48 hours before being reported as negative. The isolates were confirmed as *S. aureus* by standard isolation & identification methods like colony morphology, Gram's stain, Catalase test, Slide and Tube coagulase tests, Mannitol fermentation and DNase test.

Tests for detection of MRSA
Cefoxitin Disc diffusion test[8]: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 35°C±2°C 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)

E-test MIC Oxacillin[9]: Muller Hinton Agar plate with 2% NaCl was prepared. The dried plates were lawn cultured with test strain using sterile 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 $35^oC\pm2^oC$ 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.

Fig. 1: Cefoxitin Sensitive Isolate

Fig. 2: Cefoxitin Resistant Isolate

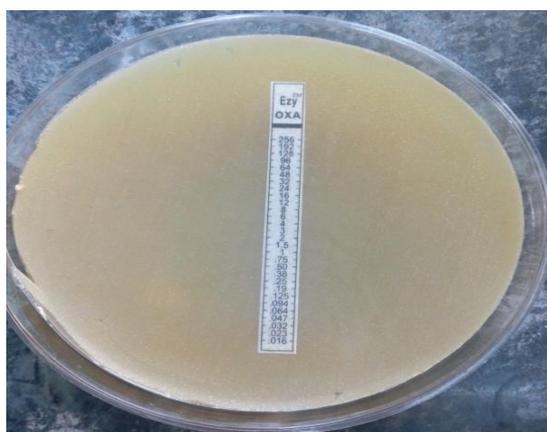**Fig. 3: E-test MIC Oxacillin Sensitive isolate**

Fig. 4: E-test MIC Oxacillin Resistant isolate

Results

A total of 174 *Staphylococcus aureus* strains isolated from the non-repetitive clinical samples were included and processed for MRSA identification. Out of 174 *S.aureus* isolates 69(39.65%) were found to be MRSA by Cefoxitin disc diffusion test.

A total of 63 (36.20%) isolates were found to be MRSA by Oxacillin MIC by E-test. When compared with Cefoxitin disc diffusion, Oxacillin MIC by E-test was found to be significant using Pearsons Chi-square test for significance with p value of <0.05. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 94.02%, 94.39%, 91.30% and 96.19% respectively.

**Table 2: Comparison of E-test with Cefoxitin Disc Diffusion (n=174)**

Test applied		Cefoxitin test		Total
		Positive	Negative	
E-test	Positive	63	4	67
	Negative	6	101	107
Total		69	105	174

Among MRSA isolates 73.91% resistance was as observed to Ampicillin, 71.01% to Erythromycin, 62.31% to Gentamicin, 57.97% to Amoxycillin

clavulanic acid, 57.97% to Clindamycin, 53.62% to Azithromycin, 52.17% to Ciprofloxacin, 39.13% to Pristinomycin, 27.53% to Netilmicin, 24.63% to

Doxycycline, 5.79% to Linezolid. There is no resistance for Teicoplanin & Vancomycin. (Graph 1)

Graph 1: Antibiotic Sensitivity Pattern in MRSA isolates
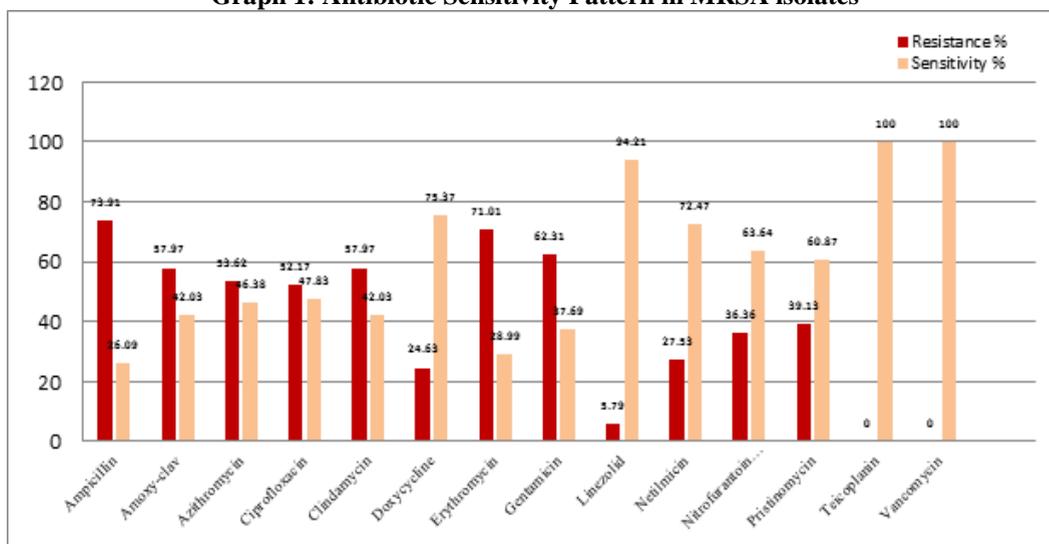
Discussion

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

MRSA are being isolated with increasing frequency from clinical specimens and clinical problems posed by their multidrug resistance in recent years have led to the interest in the present study. Several studies have been showed that detection of *mecA* gene is a gold standard method for diagnosis of MRSA in clinical microbiology laboratories.[10] However, most laboratories especially in developing countries are not in position to perform molecular methods.

In various study results of Cefoxitin disc diffusion test are in concordance with the PCR for *mec*A gene. Thus, the test can be an alternative to PCR for detection of MRSA in resource constraint settings.[11,12] Cefoxitin disc diffusion is considered as surrogate marker for *mecA* gene, and could be considered as gold standard for MR isolates.[13]

During the study, a total of 174 *S. aureus* were isolated from various clinical samples by conventional method. Out of that 69 isolates were MRSA by Cefoxitin disc diffusion test which is considered as gold standard. In the present study, we evaluated and compared Cefoxitin disc diffusion and MIC for Oxacillin by E-test for the detection of MRSA which was found to be significant for determination of MRSA with p value of <0.05 and high Sensitivity & specificity.

Studies by B. Sasirekha[14] and S. Karami et al[15] considered E-test MIC as a gold standard method for detection of MRSA. The E-test method has the advantages of being easy to perform as a disc diffusion test and approaches the accuracy of PCR for *mecA*. Despite of its high sensitivity and specificity this test is expensive, and in our experience Oxacillin MIC strip is sensitive to temperature change and affects the results by losing its potency.

Cefoxitin disc diffusion test should be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, media preparation and finally more cost-effective in comparison to E-test MIC. So Cefoxitin disc diffusion can be used in routine settings. Studies like A. Jain et al[13], M. Rahbar et al[16]also suggested the same.

In this study there is high resistance for Ampicillin, Erythomycin, Gentamicin, Amoxy- clav, Clindamycin, Azithromycin and Ciprofloxacin because of their frequent use in the wards. While Netilmicin and Doxycycline show less resistance as compared to other studies because in this geographical area, these drugs are not commonly prescribed by the clinicians. So it might be a good alternative for MRSA in this area.

To conclude, MIC determination by E-testing provides a good alternative for Cefoxitin disc diffusion test with high sensitivity and specificity so also for better confirmation using two or more methods for diagnosis of MRSA.

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