Research Article

COMPARISON OF CONVENTIONAL PHENOTYPIC METHODS FOR DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Background and Objectives: MRSA is probably the most challenging bacterial pathogen that currently affects patients in hospital and in the community. Hence Rapid and accurate detection of methicillin resistant *Staphylococcus aureus* (MRSA) is an important role of clinical microbiology laboratories to avoid treatment failure and to control the endemicity of MRSA. The aim of this study was to compare three conventional methods against the minimum inhibitory concentration (MIC) method to evaluate the best phenotypic method.

Materials and Methods: A total of 100 isolates of *S. aureus* were included in this study. Methicillin resistance was determined by oxacillin disc diffusion, cefoxitin disc diffusion the oxacillin screen agar test and MIC.

Results: Out of 100 isolates from our hospital, 29% and 36 % and 33% were identified as MRSA based on Cefoxitin disc diffusion, Oxacillin disc diffusion and Oxacillin screen agar test respectively. In all phenotypic methods, Cefoxitin disc diffusion test better correlates with gold standard method for detection of MRSA.

Conclusion: - Our study revealed that cefoxitin disk diffusion method had a high sensitivity and specificity comparative to other phenotypic methods for detection MRSA.

Keywords: *S. aureus* ,MRSA, MIC, Cefoxitin disc diffusion, oxacillin screen agar.

INTRODUCTION

Various types of infections caused by Staphylococcus aureus ranging from boils to life threatening endocarditis. Currently one of the most serious aspects about treatment of S. aureus infections is resistance of this organism to methicillin and other beta-lactam group of antibiotics [1]. Since first reported in 1961, methicillin resistant Staphylococcus aureus (MRSA) have become a major nosocomial pathogen worldwide [2]. Poor prognosis is seen in the patients, when infections caused by Methicillin resistant Staphylococcus aureus,.[3]

MRSA is defined as a strain of S. aureus that is resistant to a large group of antibiotics called β-lactams that includes penicillin's and cephalosporins.[4]

Meticillin resistance in S. aureus is associated with production of an altered penicillin-binding protein, a 78 kDa protein termed PBP2α, which has a low affinity for β-lactam antibiotics. These strains show resistance to a wide range of antibiotics, thus limiting the treatment options to few agents, such as teicoplanin and vancomycin. In contrast, methicillin-susceptible staphylococci are preferably treated with β-lactam antibiotics because these are more effective in treating such infections and other agents such as teicoplanin and vancomycin, are reserved for treating infections caused by oxacillin or methicillin-resistant isolates.[5] Therefore, it is clinically essential to rapidly determine whether S. aureus isolates are methicillin resistant or not because this determination is important to ensure correct antibiotic treatment in infected patients as well as control of MRSA isolates in hospital environments that is to avoid spreading of them.

There are many traditional and commercial systems for detection of MRSA in clinical microbiology laboratories include, oxacillin disc diffusion, oxacillin MIC and oxacillin screen agar, cefoxitin disc diffusion, latex agglutination have evolved for rapid detection of methicillin-resistant staphylococci, but the optimal method of detection remains controversial. Most of the methods are unable to detect methicillin resistance and species at the same time. Discrepancies in detection have lead to an adverse effect on patient management, thereby highlighting the importance of accuracy in detection. Most laboratories use disc diffusion methods for routine tests. Previously, before mecA based PCR method, gold standard method for antimicrobial susceptibility testing was MIC, determined by agar dilution method. however all laboratories do not have molecular biology techniques in their routine clinical practice mainly in developing countries and performing this test is costly.[6] In recent years, MIC methods have been replaced by molecular methods which detect the mecA gene, as a gold standard for determining classical methicilin resistance in S. aureus. However, the use of molecular methods for MRSA detection is largely restricted to reference laboratories and is not utilized in many microbiology laboratories as a routine test [7].

Hence, it is essential to evaluate the phenotypic method which is able to detect MRSA isolates in a rapid and accurate manner, in order to ensure correct antibiotic treatment and to avoid the spread of MRSA isolates in the hospital environment. Recently, the Clinical and Laboratory Standards Institute (CLSI) recommended the use of the cefoxitin disc diffusion method for MRSA detection.[8]

The aim of this study was to compare three phenotypic methods for detection of MRSA. Oxacillin MIC method was considered as gold standard method.

MATERIAL AND METHODS

A total 100 isolates of S. aureus from various clinical samples were used in this study. This study was conducted at department of microbiology, Bharati Vidyapeeth deemed university medical college and hospital, Sangli and Krishna institute of medical sciences, Karad. The isolates were identified using conventional methods like Colony morphology, Gram staining, Catalase test, tube coagulate and slide coagulase test, mannitol fermentation, heat stabile nucleases and DNase test. In the present study results read according to the CLSI guidelines and manufacturer's recommendations.

Phenotypic methods for detection of MRSA

1)   Oxacillin disk diffusion test

Disk diffusion test was performed on all isolates of S. aureus with 1 ug of oxacillin per disc on Mueller‑Hinton agar with 4% NaCl. Incubated at 35°C. The zone size was interpreted according to the CLSI that is susceptible ≥13 mm and resistant ≤ 10 mm[8].

2)   Oxacillin screen agar

Muller-Hinton agar plates containing 4% NaCl and 6 μg/ml of oxacillin were prepared. The Oxacillin screen agar (OSA) test was performed on the same isolates, following CLSI guidelines by using direct colony suspension and adjusted to match 0.5 McFarland turbidity standards. The suspension of the isolate was deposited as a spot on the agar surface inoculated on oxacillin screen agar (OSA). Plates were incubated at 35^0C. The plates were observed carefully in transmitted light for any growth. Growth of any number of colonies after 24 hours was interpreted as oxacillin resistance.

3)   Cefoxitin disc diffusion test

Cefoxitin disc diffusion test was carried out using a 30 μg disc of cefoxitin on Muller Hinton agar plate on all isolates of S. aureus. Lawn culture of the bacterial suspension standardised to 0.5 Mc Farland standards was done on the agar plates. The plates were incubated at 37°C for 18 to 24 hrs and zone diameters were measured. Zone diameters ≤19mm was reported as methicllin resistant and zone diameters ≥22mm was considered as methicillin sensitive. Colonies that grew within the zones were tested again and the zone of inhibition reported.

4)   Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration to Oxacillin was done using agar dilution method. The bacterial suspension was prepared by emulsifying portions of 4-5 discrete colonies into 4-5 ml of nutrient broth, opacity adjusted by McFarland standard 0.5. Gradient plates of Muller- Hinton agar (MHA) containing 4% Nacl were prepared with doubling dilutions from 0.25 to 256μg/ml of oxacillin. The plates was inoculated as spot of about 5-8mm in diameter using sterile cotton swab stick and incubated at 35^0C for 24hours. MIC of oxacillin was ≤ 2μg/ml indicated that strain was susceptible

and MIC ≥ 4µg/ml indicates methicillin resistance (NCCLS 2003). NCCLS has not made recommendations for using Cefoxitin to define methicillin resistance using agar dilution tests.

RESULT

Out of 100 S. aureus isolates 30 (30%) isolates were detected as MRSA based on MIC method which was considered as gold standard method for detection of MRSA. By oxacillin disc diffusion method 36 isolates detected as MRSA, 33% strains were identified as MRSA by oxacillin screen agar method and by cefoxitin disc diffusion method 29 isolates were detected as MRSA.

Sensitivity of all these three methods was 100% but specificity and positive predictive values were different. Performance characteristics of the all these phonotypic methods are shown in Table-1.

Fig 1 showed that the isolated strain was resistant to oxacillin but sensitive to cefoxitin by disc diffusion test. Fig- 2 showed that the strain No- 3 was not grown on oxacillin screen agar medium containing 6ug/ml oxacillin powder and strain No. 1, 2 grown on this medium suggestive for MRSA.

Staphylococcus aureus has long been recognized as important pathogen in hospitalized patients as well as in community and has severe consequences, despite antibiotic therapy.

MRSA is probably the most challenging bacterial pathogen that currently affects patients in hospital and in the community [9]. MRSA are being recognized as highly virulent and important human pathogens causing significant morbidity and mortality in hospitals as well as in community and are difficult to eradicate because they are becoming multidrug resistant. Rapid and accurate detection of MRSA is an important role of clinical microbiology laboratories to avoid treatment failure. Methicillin resistance renders S. aureus resistant to all beta lactam antibiotics, which is the most important group of antibiotics in the treatment of staphylococcal infections. Accurate and rapid detection of methicillin resistance in staphylococci is therefore important, not only for choosing appropriate antibiotic therapy for the individual patient, but also for control of the endemicity of MRSA [10].

Table 1: Comparison of three laboratory methods for the detection of methicillin resistant *Staphylococcus aureus*.

	Phenotypic Methods		
	OxDD	**OSA**	**CxDD**
Sensitivity	100%	100%	100%
Specificity	91.42%	95.71%	98.59%
PPV	83.33%	90.90%	96.66%
NPV	100%	100%	100%

PPV-Positive predictive value, NPV- Negative predictive value, Ox DD- Oxacilline disc diffusion, CxDD-Cefoxitin disc diffusion, OSA- Oxacillin screen agar.

S. aureus strain is resistat to oxacillin but sensitive to cefoxitin

Figure 1: CxDD and OxDD test for MRSA

S. aureus strain No 1 and 2 sensitive to oxacillin and 3 resistant to oxacillin by oxacillin screen agar method.
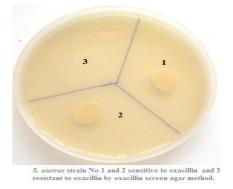
Figure 2: O.S.A. for MRSA

DISCUSSION

A number of methods, as recommended by CLSI, are being used for the detection of MRSA. These methods except for PCR and Latex agglutination methods are prone to errors due to heterogeneous nature of methicillin resistance and dependence on environmental conditions.

Correct identification of MRSA using conventional methods is complex and some strains are difficult to classify, a strain can appear susceptible by one method and borderline or resistant by another method [11,12]. For these reasons, several molecular methods have been developed to detect the mecA gene in MRSA clinical isolates [13]. However, genotypic tests involving mecA gene detection by PCR, which is considered to be the reference, are not practical for routine use in microbiology laboratories. Currently surveillance data for MRSA are difficult to interpret, because there is no uniform testing method for detection of MRSA, and laboratories vary in their Standard operating procedure and interpretation of breakpoint values [14].

Various phenotypic methods are available but the optimal method of detection remains controversial. In recent years there are multiple published report suggest the use of cefoxitin as surrogate marker for the detection of MRSA. Isolates which harvest any one of this should be reported oxacillin resistant as very rare mechanism other than mecA cause oxacillin resistant [15]. Same time CLSI guidelines recommended cefoxitin to be used to identify MRSA. According to CLSI recommendation a 30ug of cefoxitin disc is used and a zone of less than 19 mm or equal is considered as resistant strain [16].

Several studies have been showed that detection of mecA gene is gold standard method for diagnosis of MRSA in clinical microbiology laboratories[7]. However, most laboratories especially in developing countries are not in position to perform molecular methods. In the present study, we evaluated different phenotypic methods for the detection of MRSA.

The MIC method has the advantages of being easy to perform as a disc diffusion test and approaches the accuracy of PCR for mecA. There are many studies comparing MIC with broth dilution and PCR methods with generally has been yielded satisfactory results. We used oxacillin MIC as a gold standard method for detection of MRSA. [17,18] The sensitivity and specificity for cefoxitin disc diffusion method was 100%

and 98.59%, respectively. Disc diffusion method is an easy method for performance of MRSA in microbiology laboratories. The oxacillin screen agar test showed 100% sensitivity and 95.71% specificity for MRSA detection in our study. Swenson et al. noted that sensitivity decreased when heterogeneous resistant strains were tested and specificity decreased with strains having borderline MIC. In addition, the sensitivity and specificity of the oxacillin disc was determined to be 100% and 91.42% respectively. The lower specificity in the present study could be because of differences in the manufacturer's disc. As already reported, the oxacillin disc diffusion test was the least reliable test for detection of MRSA [19].

All methods were considered satisfactory in detecting MRSA and showed similar sensitivity. Although the specificity and positive predictive value of cefoxitin disc diffusion method is more as compared to Oxacillin disc diffusion method and Oxacillin screen agar methods.

The sensitivity and specificity value of phenotypic methods used for identification of MRSA are known to vary depending on the media used for incubation, the concentration of NaCl used in medium, the incubation time and temperature and the experience of personnel's which carry out the tests. [7]

Discrepant results among conventional assays for detection of methicillin resistance were reported to be mainly due to the heterogeneous expression of resistance [20].Other factors also influence the phenotypic expression of resistance such as addition of sodium chloride in the culture medium, incubation at 30^0C or passage in the presence of beta-lactam antibiotics enhances the expression of resistance. These factors also necessitate the requirement for a simple, rapid, accurate and sensitive method for the detection of MRSA in routine diagnostic laboratories.

The presence of resistance in S. aureus isolate on an oxacillin screen agar plate generally means that the isolates mecA positive. Occasionally, however heteroresitant mecA-positive strains is not detected due to low expression of resistance. Oxacillin screen agar 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 [21]. Study done by Swenson, a high correlation between MICs of cefoxitin and presence of mecA

in staphylococcus spp. [14] Several studies including the current one have reported that the results of the cefoxitin disc diffusion test correlate better with oxacillin MIC method compared with those of the oxacillin disc diffusion test [22]. Cefoxitin is a better inducer of mecA expression; this could explain why heterogeneous MRSA populations variably expressing the mecA are better detected by disc diffusion with cefoxitin than with oxacillin, which is a weak inducer of PBP2a production. This is considered to be the underlying mechanism for the higher sensitivity of cefoxitin than oxacillin. Regarding cefoxitin disc diffusion, Anand et al. and many other studied reported that the results of cefoxitin disc diffusion tests correlate better with the presence of mecA than do the results of disc diffusion tests using oxacillin[23,24]. The oxacillin disc diffusion method was found to be less sensitive for the detection of MRSA.

CONCLUSION

Cefoxitin is a more potent inducer of the mecA regulatory system and an accurate surrogate marker for the detection of MRSA in the routine susceptibility testing. This method can be preferred in clinical microbiology laboratories because it is easy to perform, do not require special technique, incubation temperature, media preparation and more cost-effective in comparison to other methods. Our study revealed that cefoxitin disc diffusion method had a high sensitivity and specificity comparative to other routinely used methods for detection MRSA.

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