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Original Research Article.

A Comparative Analysis of Different Phenotypic Methods Used in Detection of Methicillin Resistant *Staphylococcus Aureus*

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ABSTRACT

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) has been recognized as one of the major pathogen in both hospital and community settings, resulting in increased mortality and morbidity. Detection of *mec*A gene or its product by PCR is recognized as a gold standard for detection of MRSA. In resource limited settings, phenotypic method which is simple, rapid, accurate and cost effective is required. The aim of our 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 244 isolates of *S. aureus* were included in this study. Methicillin resistance was determined by oxacillin disc diffusion, cefoxitin disc diffusion, oxacillin screen agar test and MIC.

Results: Out of 244 isolates, 113 were found to be methicillin resistant by oxacillin disc diffusion test, 124 were resistant by oxacillin screen agar method, and 126 were resistant with cefoxitin disc diffusion. MIC for oxacillin showed MRSA in 126 isolates.

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

Keywords: *S. aureus,* MRSA, MIC, Cefoxitin Disc Diffusion, Oxacillin Screen Agar.

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INTRODUCTION

Staphylococcus aureus is an important etiological agent of hospital and community acquired infections. The organism has a differential ability to spread and cause outbreaks especially in hospitals.[1] Most serious aspects regarding treatment of *S. aureus* infections is resistance of the organism to methicillin and other beta-lactam group of antibiotics.[2] Methicillin was first introduced in 1959 to treat *S. aureus* infections resistant to penicillin. The first case of methicillin resistant *S. aureus* (MRSA) was reported in 1961. Methicillin resistance in *S. aureus* is due to the production of an additional penicillin binding protein, PBP2 or PBP2a, which is mediated by the mecA gene.[3] Strains that possess *mecA* gene are either heterogeneous or homogeneous in their expression of resistance. The heterogeneous expression sometimes results in minimal inhibitory concentrations that appear to be borderline and consequently the isolates may be interpreted as susceptible.[4] Due to increased rate of infections caused by MRSA, performance of reliable, accurate and rapid test for detection of MRSA is essential. Oxacillin disc and agar screening methods were used for MRSA detection. Clinical Laboratory Standards Institute (CLSI) recommended use of Cefoxitin 30 µg disc as standard marker for MRSA identification.[5] The aim of our study was to evaluate sensitivity, specificity, PPV, NPV and ease to perform different phenotypic methods i.e. Cefoxitin 30 µg disc, Oxacillin 1 µg disc and Oxacillin agar screening plate (6 µg/ml) for early and accurate identification of MRSA resistance by comparing with Minimum inhibitory concentration (MIC) determined by dilution methods. MIC methods now have been replaced by molecular methods which detect mecA gene as a gold standard for determining methicilin resistance in *S. aureus*.[6,7] However, the use of molecular methods for detection of MRSA is largely restricted to reference laboratories and is not done in our study.

MATERIALS AND METHODS

A total 244 strains of *Staphylococcus aureus* isolated were included in the study. This study was conducted at department of microbiology, Government medical college, Amritsar. The isolates were identified using conventional methods like Colony morphology, Gram staining, Catalase test, tube coagulase and

slide coagulase test and mannitol fermentation. MRSA was diagnosed using various phenotypic methods. All the isolates were tested for methicillin resistance by Oxacillin disk diffusion test, Oxacillin screen agar (OSA) method, Cefoxitin disc diffusion test and broth macrodilution method for knowing minimum inhibitory concentration (MIC).

1) Oxacillin disk diffusion test: Disk diffusion test was performed on all isolates of S. aureus with 1 µg of oxacillin disc on Mueller Hinton agar with 4% NaCl and incubated at 35ºC. The zone size was interpreted according to the CLSI that is susceptible ≥13 mm and resistant ≤ 10 mm.[8]

2) Oxacillin screen agar: Using a swab, the 0.5 McFarland suspension of the isolate was spotted on the MHA plate containing 6 µg/ml oxacillin and 4% NaCl in 10–15 mm area. Plates were observed carefully in transmitted light. Any visible growth after 24 h of incubation at 35°C was indicative of resistance.[9,10]

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.[8]

4) Minimum inhibitory concentration (MIC): Oxacillin MIC – Serial dilutions ranging from 0.25 to 256 µg/ml of oxacillin were prepared in Mueller Hinton Broth (MHB) (Hi Media Mumbai) containing 2% NaCl. The inoculum was prepared by diluting 0.5 McFarland suspension to the concentration of 10^5 CFU/ml. The tubes were inoculated and incubated at 35°C for 24 h. The lowest concentration at which there was no visible growth was taken as the MIC. The strains for which MIC was > 2 µg/ml were considered resistant.[11]

Table 1: Oxacillin MIC range of MRSA isolates

Oxacillin MIC value (µg/ml)	No. of strains	Percentage(%)
4	16	6.55
8	41	16.80
16	29	11.88
32	20	8.19
64	12	4.91
128	8	3.27

Table 2: Comparison of phenotypic methods for detection of MRSA

Test method	Detected as MRSA	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Oxacillin disc diffusion	113	89.68%	100%	100%	90.07%
Oxacillin screen agar	124	98.41%	100%	100%	98.33%
Cefoxitin disc diffusion	126	100%	100%	100%	100%
MIC Oxacillin broth dilution	126	100%	100%	100%	100%

RESULTS

Out of 244 *S.aureus* strains, 113(46.32%) isolates were detected as MRSA by oxacillin disc diffusion method, 124(50.82%) strains were identified as MRSA by oxacillin screen agar method and by cefoxitin disc diffusion method 126 (51.64%) isolates were detected as MRSA. MIC for oxacillin was performed on all 244 strains and 126 (51.64%) strains were resistant to oxacillin. Majority of MRSA isolates had MIC in the range of 8-64 µg/ml.(Table 1) Specificity of all these three methods was 100% but sensitivity and negative predictive values were different. Performance characteristics of all these phenotypic methods is shown in Table-2.

DISCUSSION

Methicillin resistant *Staphylococcus aureus* is a highly virulent pathogen, causing significant morbidity and mortality and difficult to eradicate as they are multidrug resistant. Accurate and rapid detection of methicillin resistance in *Staphylococcus* is therefore important, for choosing appropriate antibiotic therapy and for control of the endemicity of MRSA.[12] A number of methods are being used for the detection of MRSA. These methods, except for PCR are prone to errors due to heterogeneous nature of methicillin resistance and dependence on environmental conditions. However, genotypic tests involving mecA gene detection by PCR, is not practical for routine use in microbiology laboratories. In the present study, we evaluated different phenotypic methods for the detection of MRSA. The MIC method approaches the accuracy of PCR for mecA gene. We used oxacillin MIC as a gold standard method for detection of MRSA.[13,14] The sensitivity and specificity for cefoxitin disc diffusion method was 100% and comparable to MIC method. There are multiple published reports suggesting the use of cefoxitin as surrogate marker for the detection of MRSA.[15,16] Disc diffusion method is an easy method for detection of MRSA in microbiology laboratories. The oxacillin screen agar test showed 98.41% sensitivity and 100% specificity for MRSA detection in our study. Difficulty in MRSA detection by oxacillin screen agar base occur if the organism have their MIC near break points i.e. (borderline resistance strain) and also where hetero resistant strains were included in study group, as it is subjected to many environmental conditions such as temperature, salt concentration, incubation time.[17-19] The sensitivity and specificity of oxacillin disc was 89.68% and 100% respectively. The sensitivity and specificity value of phenotypic methods used for identification of MRSA vary depending on the media used for incubation, the concentration of NaCl used in medium, the incubation time and temperature.[19]

Cefoxitin is a better inducer of mecA expression which explains 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.[20] In a study done by Anand et al. cefoxitin disc diffusion tests correlate better with the presence of mecA than do the results of disc diffusion tests using oxacillin.[18] Similar results were also shown by other studies.[21,22] In a laboratory where it is not possible to carry out molecular method as a routine, cefoxitin disk diffusion test is a good surrogate marker for detecting methicillin resistance. It is superior to most of the currently recommended phenotypic method like oxacillin disc diffusion and oxacillin screen agar method. No special medium or incubation temperature is required for cefoxitin as is required for oxacillin and results are easy to read in both transmitted and reflected light. It is now an acceptable method for detection of MRSA by many reference groups including CLSI.

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 as 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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