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Research Article

Evaluation of four phenotypic methods for the rapid identification of methicillin resistant *Staphylococcus aureus*

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA), a superbug has been recognized as one of the major pathogens in hospitals as well as community settings. The prevalence of MRSA is 30–70% and many studies have suggested an alarming rate of infections caused by this organism. In spite of modern diagnostic procedures and technological advancement, infections caused by MRSA still remain difficult to diagnose in developing countries like India. We tried to evaluate four phenotypic methods for the rapid identification of methicillin resistant *Staphylococcus aureus* (MRSA).
Methods: The present study was undertaken to evaluate the four phenotypic methods for the detection of MRSA by oxacillin disc diffusion, cefoxitin disc diffusion, HiCrome rapid MRSA agar and the latex agglutination test.
Results: Among 542 *Staphylococcus aureus* isolated, *304* were methicillin resistant *Staphylococcus aureus* (MRSA) and remaining 238 were methicillin sensitive *Staphylococcus aureus* (MSSA). Hence, the prevalence rate of MRSA in our study was 56.09%. Cefoxitin disc diffusion was found to be more specific and sensitive than oxacillin disc diffusion where as both HiCrome Rapid MRSA Agar and the latex agglutination tests showed similar specificity and sensitivity.
Conclusions: The cefoxitin disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI) was found to be a reliable method for MRSA detection but it should be supplemented with some other method like latex agglutination to enhance the isolation rate of MRSA. We recommend that along with cefoxitin disc diffusion with another reliable method, preferably latex agglutination should be routinely used in all microbiology diagnostic laboratories to detect MRSA which help for its control of spread.

Keywords: MRSA, Oxacillin disc diffusion, Cefoxitin disc diffusion, Latex agglutination, Hicrome MRSA agar

INTRODUCTION

Staphylococcus aureus is one of the most common causes of nosocomial or community acquired infections, leading to serious illnesses with high rates of morbidity and mortality. In recent years, the increase in the number of bacterial strains that show resistance to methicillin has become a serious clinical and epidemiological problem because this antibiotic is considered as the first option in the treatment of Staphylococcal infections and resistance to this antibiotic implies resistance to macrolides, lincosamides, aminoglycosides, glycopeptides and all beta-lactum agents.[1] Since the first case of MRSA was reported in 1961, the importance of MRSA as a nosocomial as well as community acquired pathogen was well documented.[2-9] Methicillin resistance

in *S. aureus* is based on the production of an additional penicillin binding protein, PBP2 or PBP2a, which is mediated by the mecA gene.[10] Considering the increasing rate of infections caused by MRSA, performance of reliable, accurate, rapid, cost effective and reproducible testing for detection of MRSA is essential for both antibiotic therapy and infection control measures.[11] The various phenotypic methods for the detection of MRSA include oxacillin disc diffusion, cefoxitin disc diffusion, oxacillin MIC by agar or broth dilution methods, cefoxitin MIC by agar or broth dilution methods, E-test, Chrome agar, and many automated systems.

Detection of *mecA* gene and PBP2a by PCR or latex agglutination respectively are the gold standard methods. However, the use of molecular methods for detection of MRSA is largely restricted to reference laboratories and is not utilized in many microbiology laboratories as a routine test because of its cost with poor resource settings.[12-15] Cefoxitin is a potent inducer of the *mecA* regulatory system. Hence, it is used as a surrogate marker in poor resource settings.[16] In the present study an attempt was made to evaluate oxacillin disc diffusion method, cefoxitin disc diffusion method, chrome agar and MRSA latex agglutination in relation to the detection of methicillin resistance and to compare and contrast their suitability as routine methods for detecting MRSA isolates in diagnostic microbiology laboratories.

METHODS

A total of 3680 specimens such as pus, sputum, throat, ear, nasal, vaginal swabs, pleural fluids, urine, blood were collected aseptically from various patients attending the outpatient and inpatient of various departments by 2 sterile swabs for a period of 1 year from June 2014 to May 2015 and processed. Gram's staining was done for all the samples for the likely organism by one swab and then the other swab was inoculated onto Nutrient agar, Blood agar, MacConkey agar & a selective medium (Mannitol salt agar) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai and incubated at 37^{o} C for 24 hours aerobically. The suspected colonies of *Staphylococci* were taken and Gram's Staining was done again, all the Gram–positive cocci in clusters were further confirmed using a battery of standard biochemical reactions including the production of bound and free coagulase enzymes using slide and tube coagulase tests based on standard testing methods for confirming *Staphylococcus aureus*.[17]

Staphylococcus aureus ATTC-25923 included as control. All isolates were tested with oxacillin (1 µg) and cefoxitin (30 µg) discs, using Mueller Hinton agar separately with a suspension equivalent to 0.5 McFarland standards of the *S. aureus* isolates. All plates were incubated at 37ºC for 24h. Zone of inhibition was measured and interpreted as per the guidelines recommended by CLSI.[18] The detection of MRSA on HiCrome Rapid MRSA agar, a suspension of 0.5 MacFarland was prepared and 10µL of bacterial suspension was streaked on above mentioned medium. All plates were incubated at 37ºC for 24h. Strains growing on HiCrome Rapid MRSA agar and yielding colonies with greenish yellow colour were considered MRSA as recommended by manufacture. Slidex MRSA Detection test by BioMérieux was done according to the instructions of the manufacturer which is a rapid and sensitive latex agglutination test detecting methicilin resistance in *Staphylococci* based on the production of low-affinity PBP2a, which is encoded by the *mec*A gene. Strains ATCC 43300 (MRSA) and ATCC 6538 (MSSA) were used as controls.

RESULTS

A total of 3680 specimens such as pus, sputum, throat, ear, nasal, vaginal swabs, pleural fluids, urine, blood were collected aseptically from various patients attending the outpatient and inpatient of various departments by 2 sterile swabs for a period of 1 year from June 2014 to May 2015 and processed. Gram's staining was done for all the samples for the likely organism by one swab and then the other swab was inoculated onto Nutrient agar, Blood agar, MacConkey agar & a selective medium (Mannitol salt agar) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai and incubated at 37^{o} C for 24 hours aerobically.

The suspected colonies of *Staphylococci* were taken and Gram's Staining was done again, all the Gram–positive cocci in clusters were further confirmed using a battery of standard biochemical reactions including the production of bound and free coagulase enzymes using slide and tube coagulase tests based on standard testing methods[17] for confirming *Staphylococcus aureus*. *Staphylococcus aureus* ATTC-25923 included as control. All isolates were tested with oxacillin (1 µg) and cefoxitin (30 µg) discs, using Mueller Hinton agar separately with a suspension equivalent to 0.5 McFarland standards of the *S. aureus* isolates. All plates were incubated at 35ºC for 24h. Zone of inhibition was measured and interpreted as per the guidelines recommended by CLSI.[18] The detection of MRSA on HiCrome Rapid MRSA Agar, a suspension of 0.5 MacFarland was prepared and 10µL of bacterial suspension was streaked on above mentioned medium.

All plates were incubated at 37ºC for 24h. Strains growing on HiCrome Rapid MRSA Agar and yielding colonies with greenish yellow colour were considered MRSA as recommended by manufacture. Slidex MRSA Detection test by BioMérieux was done according to the instructions of the manufacturer which is a rapid and sensitive latex agglutination test detecting methicilin resistance in *Staphylococci* based on the production of low-affinity PBP2a, which is encoded by the *mec*A gene. Strains ATCC 43300 (MRSA) and ATCC 6538 (MSSA) were used as controls.

Table 1: Comparison of results by different phenotypic testing methods for confirmation of MRSA.

Oxacillin disc diffusion (1µg)		Cefoxitin disc diffusion (30µg)		HiCrome Agar		Slidex MRSA	
Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
290	14	296	8	300	4	300	4

Table 2: Sensitivity and Specificity of different phenotypic testing methods for confirmation of MRSA.

Oxacillin disc diffusion (1µg)		Cefoxitin disc diffusion (30µg)		HiCrome Agar		Slidex MRSA	
Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
95.39	94.96	97.37	97.48	98.68	98.74	98.68	98.74

DISCUSSION

Early and accurate diagnosis of methicillin resistance is very important in the management of infections caused by *Staphylococcus aureus*. Although many phenotypic detection methods have been developed to achieve this goal, the lacunae in the specificity and sensitivity of these tests may not ensure proper treatment of patients with MRSA infections. The gold standard for MRSA detection is identification of the *mecA* gene.

The use of molecular methods for detection of mecA gene may not be possible in routine clinical practice in a normal microbiological laboratory. Therefore, it is desirable to identify a sensitive, specific, reliable, accurate, rapid, cost effective and reproducible phenotypic method for the detection of MRSA.[19] In present study, the disc diffusion methods for detection of MRSA, oxacillin disc had sensitivity and Specificity 95.39% and 94.96% whereas cefoxitin disc had a sensitivity and Specificity of 97.37% and 97.48%. Similar findings were observed by various workers who showed that the cefoxitin disc method has better sensitivity than the oxacillin disc method for MRSA detection.[20-22]

This higher sensitivity to cefoxitin can be explained by the increased expression of the *mecA* encoded protein PBP2a, cefoxitin being an inducer of the *mecA* gene.[22] Our study also coincides with these studies by showing cefoxitin is superior to oxacillin for the detection of methicillin resistance. In our present study HiCrome Rapid MRSA agar showed 98.68% sensitivity and 98.74% specificity. This sensitivity could be increased to 100% by increasing the incubation period and reporting of MRSA from 24 to 48 hours.[23]

Present study found that the Slidex latex agglutination test had 98.68% sensitivity and 98.74% specificity for the detection of MRSA. Many recent studies have reported the sensitivity of the latex agglutination test to be ≥97%.[24-26] Latex agglutination has the advantages of being rapid, giving results on the same day, and easy to perform with very good sensitivity and specificity. This method could detect even low levels of PBP2a that are usually missed in routine disc diffusion methods by Oxacillin or Cefoxitin. A study was showed that the sensitivity of the latex agglutination test can be improved (93.5 to 100%) by induction with cefoxitin using growth from the edge of the inhibition zone of cefoxitin to perform the test.[27] As per our study that the cefoxitin disc diffusion method recommended by the CLSI is a rapid, cost effective, reproducible method for MRSA detection but it should be supplemented with some other method so that no MRSA is missed. It is always advisable to combine two methods, one with high sensitivity and the other with high specificity. According to our results, the best combination is the cefoxitin disc diffusion method and the latex agglutination test. Since the latex agglutination test is expensive, its use can be minimised by testing those *Staphylococcus aureus* which are showing zone diameters more than 21mm need to be confirmed by latex agglutination.

CONCLUSION

Diagnostic microbiology laboratories associated with health-care systems should combine screening with the cefoxitin disc diffusion along with another method that is feasible, sensitive and specific as well as cost effective for that institute to reliably detect MRSA to stop its spread. In our observation, cefoxitin disc diffusion with latex agglutination will be a reliable combination, even though chromogenic media is also a good combination with cefoxitin disc diffusion, but one should wait for 48 hrs for its reliable result.

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