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Original Research Article.

Evaluation of Oxacillin Disk Diffusion Test for the Phenotypic Detection of Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

Background: Different phenotypic detection method for the Methicillin Resistant *Staphylococcus aureus* is available.

Objective: The aim of this present study was to evaluate the oxacillin disk diffusion test for the detection of methicillin-resistant *Staphylococcus aureus* isolated from clinical specimens.

Methodology: This cross-sectional study was planned to carry out in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University, Dhaka from January, 2010 to December, 2010 for a period of one (01) year. Different clinical specimens were collected from the patients presented with infections at anybody sites. Isolation and identification of *Staphylococcus aureus (S. aureus)* was performed by staining, culture and biochemical tests. Oxacillin disk diffusion test was performed to detect the methicillin-resistant *Staphylococcus aureus*. PCR was performed for detection of the *mecA* gene for MRSA.

Results: Out of the 22 suspected MRSA isolates 19 were *mecA* positive by PCR. The sensitivity and specificity of oxacillin disc diffusion method for the detection of MRSA was 84.2% (95 CI 60.4 to 96.6%) and 66.2% (95% CI 9.4 to 99.2%) respectively. The positive predictive value and negative predictive value of oxacillin disc diffusion method were 94.1% (95% CI 76.1 to 98.8%) and 40.0% (95% CI 15.2 to 71.2%) respectively. The accuracy was 81.8% (95% CI 66.7 to 95.3%).

Conclusion: Oxacillin disk diffusion test shows high sensitivity with moderate specificity for the detection of MRSA from clinical specimens.

Keywords: Validity Test; Oxacillin Disc Diffusion Test; Methicillin-Resistant; *Staphylococcus aureus.*

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INTRODUCTION

Rapid identification of MRSA colonization is critical to the effectiveness of infection control.[1] Delays in detection results in late institution of infection control measures which causes the occult transmission of MRSA between patients.[2] Furthermore unnecessary contact precautions lead to increase hospital cost[3]. Methicillin resistance in *Staphylococcus aureus* is primarily mediated by the *mec*A gene, encoding penicillin-binding protein 2a (PBP2a) and this protein has reduced affinity for β-lactam antibiotics.[1] Because the *mec*A gene is heterogeneously expressed in vitro, selective media are necessary to facilitate recovery of MRSA in culture.[2] The time from culture inoculation to identification of MRSA is typically 48 hour, with some methods taking as long as 96 hours.[3] Furthermore, the sensitivity of any single selective medium method ranges between 65 and 100%.[4] Several techniques to shorten the time to identification of MRSA in the laboratory have been developed in the last decade, including slide latex agglutination assays to detect PBP2a, a colorimetric cycling probe assay to directly detect the mecA gene and real-time PCR methods to detect the mecA gene in conjunction with S. aureus-specific genome fragments.[5] While these assays are sensitive in detecting MRSA, these are unable to distinguish MRSA from *mec*A-positive strains of coagulase-

negative *Staphylococcus* species in mixed specimens, such as those obtained from the anterior nares and therefore still require initial culture and identification steps.[6] In this context the present study was undertaken to evaluate the oxacillin disk diffusion test for the detection of methicillin-resistant *Staphylococcus aureus* isolated from clinical specimens.

METHODOLOGY

This cross-sectional study was conducted in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University, Dhaka from January 2010 to December 2010 for a period of twelve (12) months.

Different clinical samples were collected from the patients at any age with both sexes which included wound swab, pus, blood, urine, tracheal aspirate, throat swab, sputum, aural swab, nasal swab, high vaginal swab, burn swab, drain fluid and fluid from pleural effusion. All specimens were collected aseptically from three hospitals, namely Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH), Dhaka and a private diagnostic center in Dhaka. The samples were inoculated into appropriate media and were incubated aerobically at 37^0 C for 24 hours. Then colonies were identified for *Staphylococcus* species and were confirmed by Gram staining, colony morphology, haemolytic status, pigment production, mannitol fermentation test, motility test (MIU) and other relevant biochemical tests like catalase test, coagulase test both slide and tube test as per standard procedure.[6] In this study, screening for MRSA was done by oxacillin and oxacillin screening agar. In case of oxacillin the diameter of zone of inhibition ≤10 mm was taken as resistant.[7] Conventional PCR was performed to detect *mecA* gene of 22 suspected *S. aureus* strains resistant to oxacillin by disc diffusion method at the Molecular Laboratory in the Department of Microbiology and National Forensic DNA Profiling Laboratory of Dhaka Medical College, Dhaka. Methicillin-resistant *S. aureus* (MRSA) strain [ATCC 43300] were used as positive control. PCR for *mecA* gene detection were performed by formation of bacterial pellet, DNA extraction, preparation of reaction mixture (25µl) and running in thermo cycler. Primers used for detection of the *mecA* gene producing a 309-bp amplicon were as follows:

*mec*A1-F- 5′ TGGCTATCGTGTCACAATCG 3′ (positions 885 to 905) and

*mec*A2-R- 5′ CTGGAACTTGTTGAGCAGAG 3′ (positions 1174 to 1194)

PCR reactions were performed in a thermocycler under the following conditions: initial denaturation for 10 minutes at 94°C followed by 30 cycles at 94°C for 1 minute, at 54°C for 1 minute, then at 72°C for 1 minute. Final extension was for 7 minutes at 72°C. Mixed the amplicon and ladder with dye (4-5:1 ratio). Then pipetting and dispensing were done onto the wells on gel made by comb. Start the gel electrophoresis at 100 volt for 60 minutes until the end of the reaction indicated by orange color advancement was over. Ethidium bromide (7.5µl) mixed with distilled water (100 ml). Gel was placed in this mixture for 30 minutes staining. Again destaining done in pure water for 20 minutes. The destained gel was placed on UV transilluminator and observed for the presence of DNA bands. Gels were visualized and photographed under ultraviolet illumination. Precautions were taken to prevent the samples from being contaminated by each other or by the skin of laboratory personnel.

Table 1: Comparison of Oxacillin disc diffusion with PCR results on suspected MRSA isolates (n=22)

Oxacillin Disc Diffusion	PCR Results		Total	P value
	Positive	Negative		
Resistant	16(84.2%)	1(33.3%)	17(77.3%)	0.051
Sensitive	3(15.8%)	2(66.7%)	5(22.7%)	
Total	19(100.0%)	3(100.0%)	22(100.0%)	

Table 2: Diagnostic Test validity of Oxacillin disc diffusion in diagnosis of MRSA

Variables	Values	95% CI
Sensitivity	84.2%	60.4 to 96.6%
Specificity	66.2%	9.4 to 99.2%
PPV	94.1%	76.1 to 98.8%
NPV	40.0%	15.2 to 71.2%
Accuracy	81.8%	66.7 to 95.3%

PPV- positive predictive values; NPV- negative predictive values

RESULTS

A total number of 22 *Staphylococus aureus* were isolated and identified. These isolates were subjected to antimicrobial susceptibility testing by oxacillin screen agar test for detection of phenotypical detection of MRSA and PCR for detection of the *mecA* gene. Out of 22 suspected MRSA isolates 19 were *mecA* positive by PCR and the rest 3 isolates were mecA gene negative. On the other hand out of 22 MRSA isolates 17(77.3%) isolates were detected as MRSA by oxacillin disc diffusion method and 5(22.7%) isolates were methicillin sensitive *Staphylococus aureus*. There for the true positive MRSA was 16(84.2%) isolates and true negative was 2(66.7%) isolates (p=0.051) (Table 1).

The sensitivity and specificity of oxacillin disc diffusion method for the detection of MRSA was 84.2% (95 CI 60.4 to 96.6%) and 66.2% (95% CI 9.4 to 99.2%) respectively. The positive predictive value and negative predictive value of oxacillin disc diffusion method were 94.1% (95% CI 76.1 to 98.8%) and 40.0% (95% CI 15.2 to 71.2%) respectively. The accuracy was 81.8% (95% CI 66.7 to 95.3%) (Table 2).

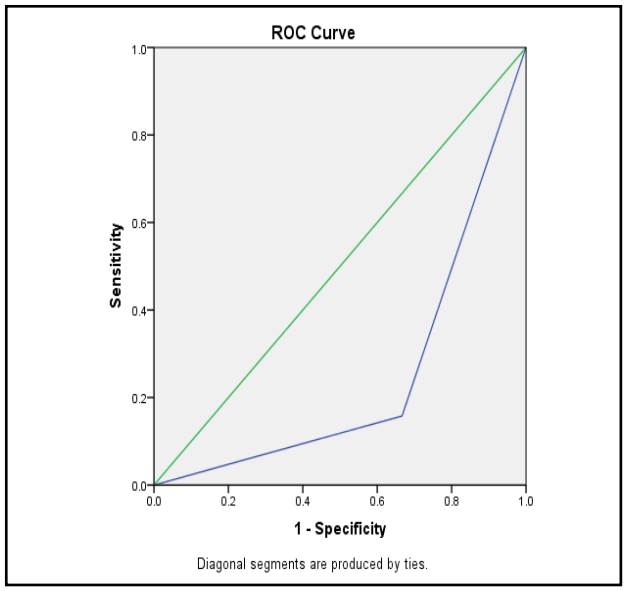

Fig 1: ROC curve with the value of Area Under the Curve (Value=0.246, 95% CI 0.0001 to 0.580; p=0.165)

The receiver operative characteristics (ROC) curve was calculated and had found that the value of area under the curve (AUC) was low which was 0.246 with a 95% CI 0.0001 to 0.580 (p=0.165) (Figure 1).

DISCUSSION

Staphylococcus aureus is one of the most significant human pathogens, causing both nosocomial and community-acquired infections.[8] Its main habitats are the nasal membranes and the skin of humans and warm-blooded animals. *S. aureus* can cause a range of infectious diseases from mild conditions, such as skin and soft tissue infections, to severe, life-threatening debilitation.[9]

Strains of methicillin-resistant *S. aureus* (MRSA) were first detected in the early 1960s, shortly after methicillin came into clinical use.[10] Resistance to methicillin is mediated by the presence of penicillin-binding protein 2a (PBP-2a), encoded by the *mecA* gene. No available β-lactam binds effectively to PBP-2a, and staphylococci resistant to methicillin or oxacillin should be generally regarded as resistant to all β-lactams.[11] Since the end of the 1970s, the occurrence of MRSA has increased steadily. Molecular epidemiological studies have shown that a limited number of MRSA strains have spread by clonal dissemination between different hospitals, cities, countries, and even continents and are now the cause of hospital infections worldwide.[12] MRSA strains are usually introduced into an institution by an infected or colonized patient or by a colonized health care worker. Thus, epidemiological surveys and control measures are particularly important for MRSA. Rapid screening followed by accurate and timely identification of MRSA becomes an elemental procedure in preventive measures.

MRSA is now one of the most important nosocomial pathogens worldwide.[13] The prevalence of MRSA, however, varies markedly by country. The prevalence of MRSA in northern European countries is low; this is assumed to be due at least in part to the prompt implementation of aggressive infection control measures. Screening high-risk patients and health care workers for MRSA is one of the control measures. Several studies have found that such screening programs are cost-effective.[14] The conventional culture methods are time- and labor-consuming, and the diagnostic values are not as good as those of the new MRSA screening method.[15] Especially because the number of samples for MRSA screening has increased dramatically in current years, a more efficient method is needed to meet the clinical requirements.

The emergence of methicillin resistance in *S. aureus* is of great concern, as MRSA strains are often multidrug resistant. Infections with MRSA are known to be associated with considerable morbidity and mortality.[16] Many studies have shown that effective control measures, including the systematic screening of persons exposed to MRSA, can confine or even eliminate the nosocomial spread of MRSA.

The sensitivity and specificity of oxacillin disc diffusion method for the detection of MRSA was 84.2% (95 CI 60.4 to 96.6%) and 66.2% (95% CI 9.4 to 99.2%) respectively. The positive predictive value and negative predictive value of oxacillin disc diffusion method were 94.1% (95% CI 76.1 to 98.8%) and 40.0% (95% CI 15.2 to 71.2%) respectively. The accuracy was 81.8% (95% CI 66.7 to 95.3%). Sensitivity and specificity of oxacillin disc diffusion which were reported by Mathew et al[8] were 100% and 56% and by Anand et al[6] were 87.5% and 100% in their study which were almost similar to the present study. Baddour et al[13] described several conventional methods to detect MRSA and were compared with polymerase chain reaction (PCR) for detection of *mecA* gene-positive isolates. The sensitivity and specificity of oxacillin disc diffusion varies with PCR which means it is highly sensitive and specific and can be a helpful tool in accurate diagnosis of MRSA infection. Therefore, it is necessary that antimicrobial susceptibility test on all MRSA isolates should be done to promote the rationale use of drugs and combat spread of MRSA.

There are some limitations of the study. As a majority of strains were collected from Laboratory isolates the history of the patients could not ascertained to find out the rate of MRSA among admitted patients of different wards. The *S. aureus* strains that were collected for this study were from Dhaka city where the intensity of use of drugs is very high. If the samples could be collected from low intensity of drug using area then the picture of the susceptibility pattern of isolates would be different.

CONCLUSION

In conclusion oxacillin disc diffusion shows high sensitivity with moderate specificity for the detection of MRSA from the clinical specimens. However, the value of the area under the curve is very low which indicates that the test method is not effective for the MRSA isolates. Regular monitoring of antimicrobial sensitivity pattern of MRSA and formulation of definite antibiotic policy for a hospital will be helpful in reducing incidence of MRSA infection.

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