American Journal of Infectious Diseases and Microbiology, 2018, Vol. 6, No. 1, 26-29
Available online at http://pubs.sciepub.com/ajidm/6/1/4

DOI:10.12691/ajidm-6-1-4

Validity of Cefoxitin Disc Diffusion Test for the Detection of Methicillin-resistant Staphylococcus Aureus

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Abstract Definite detection of MRSA is important for proper treatment. The purpose of the present study was to measure the validity of cefoxitin disc diffusion test for the detection of methicillin-resistant Staphylococcus aureus. This cross-sectional study was carried 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. S. aureus isolates were collected from different clinical samples including wound swab, pus, blood, urine, tracheal aspirate, throat swab, aural swab etc. Staphylococcus aureus (S.aureus) were isolated and confirmed by staining, biochemical tests. Routine antimicrobial susceptibility testing was performed cefoxitin discs diffusion test. PCR was performed for detection of the mecA gene for MRSA. Out of the 22 suspected MRSA isolates 19 were mecA positive by PCR and all of them 19(100.0%) were resistant to cefoxitin disc diffusion. The comparison of oxacillin and cefoxitin resistance and presence of mecA gene by PCR showed that out of 22 suspected MRSA isolates 19 were mecA positive by PCR and all the 19 (100.0%) showed resistance to cefoxitin disc diffusion. The sensitivity and specificity of cefoxitin disc diffusion were 100.0% and 100.0% respectively. Cefoxitin disc diffusion test has high sensitivity and specificity for the detection of MRSA.

Keywords: *validity test, cefoxitin disc diffusion test, methicillin-resistant, Staphylococcus aureus*

Cite This Article: Hafiza Sultana, Jogendra Nath Sarker, Md. Abdullah Yusuf, Md. Tofael Hossain Bhuiyan, Md. Mostaqimur Rahman, and Shirin Tarafder, "Validity of Cefoxitin Disc Diffusion Test for the Detection of Methicillin-resistant Staphylococcus Aureus." *American Journal of Infectious Diseases and Microbiology*, vol. 6, no. 1 (2018): 26-29. doi: 10.12691/ajidm-6-1-4.

1. Introduction

Antibiotic resistant pathogens constitute an important and growing threat to the public health1. Antibiotic resistance occurs when a microbe acquires a gene or alters its structural components, which allows the microbe to inactivate the antibiotic or nullify its antimicrobial activity2. This may occur as a spontaneous, genetic mutation or involve acquisition of a genetic element such as plasmid, transposon, integron or gene cassette3.

Emergence of MRSA poses threat to most of the available antibiotic classes and limits the therapeutic options4. Therefore, quick and reliable identification procedure is required to obtain information on the MRSA isolates5. This measure also allows faster implementation of appropriate control measures. Oxacillin disc diffusion test shows abnormalities for the detection of MRSA6-8. Therefore, the present study was undertaken to detect the MRSA by cefoxitin disc diffusion method and to compare with PCR for the detection of mecA gene for MRSA.

2. Methodology

This cross-sectional study was performed in the Department of Microbiology and Immunology at Bangabandhu Sheikh Mujib Medical University, Dhaka from January 2010 to December 2010 for a period of 12 months. Different clinical samples were collected from the patients at any age with both sexes like 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) and a private diagnostic center, Dhaka and inoculated into appropriate media, incubated aerobically at 370 C for 24 hours and colonies identified for Staphylococcus and were confirmed by Gram staining, colony morphology, haemolytic status, pigment production, mannitol fermentation test, motility test (MIU) and other relevant biochemical tests, catalase

test, coagulase test both slide and tube test as per standard methods9. In this study, screening for MRSA was done by oxacillin (Oxoid, Canada) and cefoxitin (Oxoid, Canada) disc diffusion methods6. Inhibition zone diameter of cefoxitin was ≤ 19 mm reported as resistant10. Conventional PCR was performed to detect mecA gene of 22 suspected S. aureus strains resistant to cefoxitin by disc diffusion 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 volts 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, de-staining was done in pure water for 20 minutes. The de-stained gel was then 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.

3. Results

A total of 120 Staphylococcus aureus (S. aureus) isolates were collected from 266 specimens from three different hospitals.

Table 1. Age distribution of the patients having S. aureus infection (n=120)

Age in year	Male	Female	Total
0 – 10	7 (58.3%)	5 (41.7%)	12 (10.0%)
11-20	9 (56.3%)	7 (43.7%)	16 (13.3%)
21-30	13 (43.3%)	17 (56.7%)	30 (25.0%)
31-40	10 (40.0%)	15 (60.0%)	25 (20.8%)
41-50	8 (61.5%)	5 (38.5%)	13 (10.8%)
51-60	2 (15.4%)	11 (84.6%)	13 (10.8%)
61-70	6 (60.0%)	4 (40.0%)	10 (8.3%)
71-80	1 (100.0%)	0 (0.0%)	1 (0.8%)
Total	**56 (46.7%)**	**64 (53.5%)**	**120 (100.0)**

Of total 120 S. aureus isolates 56(46.7%) isolates were belonged to male and 64 (53.3%) female patients. The data of the current study showed the highest percentage of S. aureus infection was in patients with age group 21 to 30 years next of which 31 to 40 years of age group. It also shows the age group distribution of patients having MRSA infection (Table 1).

Suspected MRSA isolates were detected by phenotypic and genotypic methods. Out of 22 suspected cases 17 (77.3%) were detected by oxacillin disc diffusion while it was 19(86.4%) by both cefoxitin disc diffusion and PCR for mecA gene (Table 2).

Table 2. Suspected MRSA isolates by phenotypic and genotypic methods (n=22)

Name of the methods	MRSA	MSSA
Oxacillin disc diffusion	17(77.3%)	5(22.7%)
Cefoxitin disc diffusion	19(86.4%)	3(13.6%)
PCR for mecA gene	19(86.4%)	3(13.6%)

MRSA=Methicillin-resistant Staphylococcus aureus; MSSA=Methicillin sensitive Staphylococcus aureus; Figure within parentheses indicates percentage.

The comparison of oxacillin and cefoxitin resistance and presence of mecA gene by PCR showed that out of 22 suspected MRSA isolates 19 were mecA positive by PCR which was ‘gold standard’ and all the 19 (100.0%) showed resistance to cefoxitin disc diffusion (Table 3).

Table 3. Comparison of Cefoxitin with PCR results on suspected MRSA isolates (n=22)

PCR Results	Fox disc diffusion		Total
	Resistant	Sensitive	
Positive	19(100.0%)	0(0.0%)	19
Negative	0(0.0%)	3(100.0%)	3
Total	**19(100.0%)**	**3(100.0%)**	**22**

Fox- cefoxitin; PCR- Polymerase chain reaction.

The sensitivity and specificity of cefoxitin disc diffusion were 100.0% and 100.0% respectively (Table 4).

Table 4. Validity of Cefoxitin disc diffusion in diagnosis of MRSA (n=22)

Values	Fox disc diffusion	PCR
Sensitivity	100.0	100.0
Specificity	100.0	100.0
Accuracy	100.0	100.0
PPV	100.0	100.0
NPV	100.0	100.0

PPV- positive predictive values; NPV- negative predictive values.

4. Discussion

Currently available phenotypic method for the detection of methicillin resistance in S. aureus is problematic because of the heterogeneous resistance displayed by many clinical isolates11. To overcome this problem many phenotypic and genotypic method have been used. Genotypic based methods for detecting MRSA are more accurate than susceptibility testing while PCR is considered as gold standard assay for detection of MRSA3,6. However, PCR is time consuming and expensive method12. Furthermore, it is not available in most of the routine laboratories; besides, its application, maintenance and reproducibility seem to be difficult

because of its complicated procedure and skill needed. Thus, the present study was undertaken to evaluate the efficacy of cefoxitin disc diffusion test to detect MRSA and detection of mecA gene by PCR.

A total of 120 S. aureus were isolated from 266 clinical specimens of which 80(66.7%) isolated bacteria were collected after prior confirmation from the department of Microbiology & Immunology laboratory of Bangabandhu Sheikh Mujib Medical University (BSMMU), 10(8.3%) isolates from Dhaka Medical College Hospital (DMCH) and rest 30(25.0%) isolates were collected from private diagnostic center, Dhaka to get a more representative picture. These isolates were subjected to antimicrobial susceptibility testing by oxacillin and cefoxitin and PCR for detection of the mecA gene. The rate of MRSA infection in different age group in comparison to MSSA infection found that, MRSA infection rate increased gradually with age. It was highest in age group 61-70 years (30%) and next of which 51-60 years (23.1%) where as in the age group 0 to 10 years and 11 to 20 years the MRSA rate was 0%, This result is in conformity with the reports of Khurram et al13 from Pakistan, Lepelletier et al14 from France and from Brazil Sadoyama and Filho15. They reported that MRSA infection was significantly higher in older patients, it may be because old people are more exposed to antimicrobial agents, which lead to selective antibiotic pressure and development of infection with resistant strains and in addition to that declining immunity in old age increases the risk of infection with MRSA strain which might be the reason for high incidences of MRSA infection in elderly.

In this study, suspected MRSA by phenotypic methods was 22 isolates. Of these suspected cases 17(77.3%) were detected by oxacillin disc diffusion while it was 19(86.4%) by both cefoxitin disc diffusion and PCR for mecA gene. Methicillin-resistant Staphylococci are heterogeneous in their expression of resistance to β-lactam agents and the test conditions have a major effect on the expression and therefore the detection of resistance16-20. Conflicting recommendations regarding the most reliable method for routine use are partly related to differences between strains and there may be a variable interaction between the factors affecting the expression of resistance, including the agent tested, the medium, the NaCl concentration, the inoculum, temperature and period of incubation and the reading of endpoints. Borderline resistant strains may have altered PBPs or be penicillinase hyper-producers, and these can be difficult to distinguish from resistant strains that carry the mecA gene16.

In the present study comparison of cefoxitin disc diffusion with oxacillin disc were evaluated for screening of MRSA. Of total 22 suspected isolates 19 were mecA positive by PCR which is gold standard. Out of these 19, 16 (84.2%) were resistant by oxacillin disc diffusion and 3(15.8%) were sensitive, on the cefoxitin disc diffusion all of them were resistant 19(100.0%) isolates. In the present study the sensitivity and specificity of cefoxitin disc diffusion is 100%. Sensitivity and specificity of cefoxitin disc diffusion were reported by Mathew et al17 were 100% and 100% and by Anand et al2 were 100% & 100% in their study which were almost like the present study. Baddour et al6 described several conventional methods to detect MRSA and were compared with polymerase chain reaction (PCR) for detection of mecA gene-positive isolates. Cefoxitin disc diffusion was found to be the most specific.

It seems that susceptibility testing for MRSA by oxacillin should only be treated as a screening test as Zahan et al18 reported 25% MRSA by PCR while it was 37.5% by oxacillin susceptibility testing. Currently introduced Cefoxitin in susceptibility testing looks promising which are studied by Mathew et al17; Anand et al2 showed almost no discrepancy between cefoxitin susceptibility testing and PCR results as that in this present study also. So, it is time to take measures to prevent the spread of MRSA by proper antibiotic policy, preventing nosocomial infection and proper detection and treatment of MRSA cases.

5. Conclusion

In conclusion the result of cefoxitin disc diffusion for the detection of MRSA has shown high sensitivity and specificity similar to mecA gene detection by PCR. Hence, it can be used as an alternative to the technically demanding PCR. During comparison of oxacillin and cefoxitin resistance and presence of mecA gene by PCR majority MRSA isolates are mecA positive by PCR and all are resistance to cefoxitin disc diffusion. Further large scale study should be carried out.

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