

# Diagnostic Test Validity of Oxacillin Screen Agar for the Detection of Methicillin Resistant *Staphylococcus aureus*

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**How to cite this paper:** Sultana, H., Yusuf, Md.A., Sarker, J.N., Bhuiyan, Md.T.H. and Tarafder, S. (2018) Diagnostic Test Validity of Oxacillin Screen Agar for the Detection of Methicillin Resistant *Staphylococcus aureus*. *Open Journal of Medical Microbiology*, 8, 26-33.

<https://doi.org/10.4236/ojmm.2018.82003>

**Received:** February 7, 2018

**Accepted:** June 26, 2018

**Published:** June 29, 2018

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## Abstract

**Background:** Detection of Methicillin Resistant *Staphylococcus aureus* may vary in phenotypic and genotypic methods. **Objective:** The aim of this present study was to validate the oxacillin screen agar 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*) were performed by staining, culture and biochemical tests. Oxacillin screen agar test was performed to detect the methicillin-resistant *Staphylococcus aureus*. PCR was performed for detection of the *mecA* gene for MRSA. **Result:** Out of the 22 suspected MRSA isolates 19 were *mecA* positive by PCR. The sensitivity and specificity of oxacillin screen agar were 100.0% (95% CI 82.3 to 100.0%) and 33.3% (95% CI 24.0 to 90.6%) respectively. The positive predictive value and negative predictive value of oxacillin screen agar test were 90.5% (95% CI 81.0 to 95.5%) and 100.0% (95% CI 20.6 to 100.0%) respectively. The accuracy of the oxacillin screen agar test was 90.9% (95% CI 71.7 to 96.3%). **Conclusion:** In conclusion oxacillin screen agar test has high sensitivity with low specificity for the detection of MRSA.

## Keywords

Validity Test, Cefoxitin Disc Diffusion Test, Methicillin-Resistant,

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*Staphylococcus aureus*

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## 1. Introduction

The prevalence of MRSA among clinical isolates varies from country to country and from institution to institution [1]. In Bangladesh, a multi-centre study [2] shows the rate of isolation of MRSA from hospital patients ranged between 32 to 63%. Study by Zahan *et al.* [3] reported that, 15 (37.5%) out of 40 isolated *S. aureus* strains were MRSA by oxacillin disc diffusion and agar dilution method but by PCR 10 (25%) strains yielded *mecA* gene. These data show that screening of the suspected MRSA strain by oxacillin disc diffusion does not coincide with PCR closely which needs further study to find out other antibiotic or method which can be more reliable as a screening test.

The Clinical and Laboratory Standard Institute has recommended cefoxitin disc diffusion method for the detection of MRSA for primary screening [4]. Multiple reports on the use of cefoxitin as an accurate surrogate marker for detection of *mecA*-gene-mediated methicillin resistance have been published. Among them Anand *et al.* [5] reported a comparative result of 50 *S. aureus* isolates using oxacillin screening agar and PCR which shows 100% agreement between PCR while 96.8% with oxacillin agar screen. Similarly, Mathew *et al.* [6] published their report on 610 strains of *S. aureus*. As seen here, out of 228 isolates of MRSA, *mecA* gene was positive in only 20 (3.2%) cases which was confirmed by PCR. This discrepancy encourages a need for further study with oxacillin screen agar test to come to a conclusion. Batista *et al.* [7] reported another comparative study of 53 clinical isolates of *S. aureus* using VITEK-2 system, oxacillin screen agar test and PCR which shows oxacillin screen agar method fails to detect some MRSA population due to its heterogenous resistance. Therefore, this present study was undertaken to detect the MRSA by oxacillin screen agar method and to compare it with PCR for the detection of *mecA* gene for MRSA.

## 2. 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°C for 24 hours. Then colonies were identified for *Sta-*

*phylococcus* 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 [8]. In this study, screening for MRSA was done by oxacillin and oxacillin screening agar [6]. In case of oxacillin the diameter of zone of inhibition  $\leq 10$  mm was taken as resistant [9]. 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 (25 $\mu$ l) and running in thermo cycler. Primers used for detection of the *mecA* gene producing a 309-bp amplicon were as follows:

*mecA*1-F-5 'TGGCTATCGTGT CACAATCG 3' (positions 885 to 905) and

*mecA*2-R-5 'CTGGAACCTTGTTGAGCAGAG 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 (7.5  $\mu$ l) 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.

### 3. Results

A total of 120 *staphylococcus aureus* (*S. aureus*) isolates were collected from 266 specimens from three different hospitals over a period of January, 2010 to December, 2010. Of which 80 (66.7%) *S. aureus* were from Bangabandhu Sheikh Mujib Medical University (BSMMU), 10 (8.3%) from Dhaka Medical College Hospital (DMCH) and rest 30 (25.0%) were collected from Popular Diagnostic Center (PDC), Dhaka. 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. Among the 22 *Staphylococcus aureus* isolates 21 (95.5%) isolates were oxacillin screen agar resistant indicating that these are MRSA and the rest 1 (4.5%) isolate was methicillin sensitive *Staphylococcus aureus* (MSSA). However, *mecA* gene was positive by PCR in 19 (100.0%) *Staphylococcus aureus* isolates out of 21 isolates ( $p = 0.01$ ) (Table 1).

**Table 1.** Comparison of oxacillin screen agar with PCR results on suspected MRSA isolates (n = 22).

Oxacillin screen agar	PCR Results		Total	P value
	Positive	Negative		
Resistant	19 (100.0%)	2 (66.7%)	21 (95.5%)	
Sensitive	0 (0.0%)	1 (33.3%)	1 (4.5%)	0.01
<b>Total</b>	19 (100.0%)	3 (100.0%)	22 (100.0%)	

The sensitivity and specificity of oxacillin screen agar were 100.0% (95% CI 82.3% to 100.0%) and 33.3% (95% CI 24.0% to 90.6%) respectively. The positive predictive value and negative predictive value of oxacillin screen agar test were 90.5% (95% CI 81.0% to 95.5%) and 100.0% (95% CI 20.6% to 100.0%) respectively. The accuracy of the oxacillin screen agar test was 90.9% (95% CI 71.7% to 96.3%) (Table 2).

The receiver operative characteristics (ROC) curve was calculated and had found that the value of area under the curve (AUC) was low which was 0.33 with a 95% CI 0.0001 to 0.727 ( $p = 0.363$ ) (Figure 1).

#### 4. Discussion

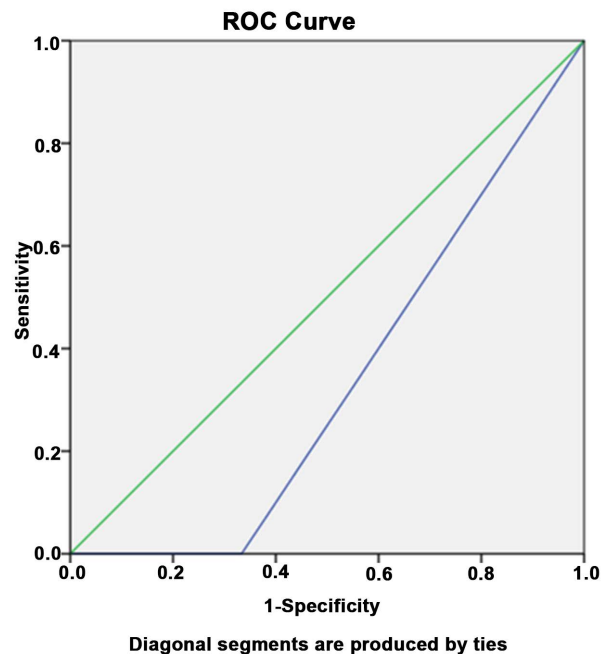
The methods that stand out among the most utilized or best capable in the identification of methicillin-resistant *Staphylococcus aureus* (MRSA) are agar dilution method, disk diffusion, screening on agar with methicillin, automated methods (Microscan, Vitek), latex agglutination and the molecular method using PCR for the detection of the gene *mecA* [8] The polymerase chain reaction in real time (RT-PCR) was described as a useful PCR-based diagnostic tool [9] [10].

Approximately 20.0% of healthy persons are persistent carriers of *S. aureus* and 60 percent are intermittent carriers. Colonization rates are increased in haemodialysis patients, illicit injecting drug users, surgical patients and patients with insulin dependence or poorly controlled diabetes [11]. Healthcare associated infection are leading causes of morbidity & mortality [12] and the management of all these condition has been critically compromised by the appearance & rapid spread of antimicrobial resistance among the organisms floating in the hospital. Therefore, the importance of MRSA detection especially for

**Table 2.** Diagnostic test validity of oxacillin screen agar in diagnosis of MRSA.

Variables	Values	95% CI
<b>Sensitivity</b>	100.0%	82.3% to 100.0%
<b>Specificity</b>	33.3%	24.0% to 90.6%
<b>PPV</b>	90.5%	81.0% to 95.5%
<b>NPV</b>	100.0%	20.6% to 100.0%
<b>Accuracy</b>	90.9%	71.7% to 96.3%

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



**Figure 1.** ROC curve with the value of area under the curve (value = 0.333, 95% CI 0.0001 to 0.727; p value = 0.363).

therapeutic & epidemiological purpose is an acute need. Hence, the methods used to detect MRSA in clinical samples should have high sensitivity and specificity and most importantly the result should be available within a short time [13].

In this study, the number of suspected MRSA isolates by phenotypic methods was 22. Of these suspected cases 21 (95.5%) were detected MRSA by oxacillin screen agar while it was 19 (86.4%) by PCR for *mecA* gene. Identification of the *mecA* gene is the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular biology techniques in their routine clinical practice. For this reason, it is essential that phenotypic techniques able to detect MRSA isolates in a rapid and accurate manner are made available, in order to ensure correct antibiotic treatment and to avoid the spread of MRSA isolates in the hospital environment.

In the present study comparison of oxacillin screen agar 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, all were also resistant on oxacillin screen agar. The 3 strains of *S. aureus* negative by PCR for *mecA* gene, of which 2 (66.7%) were resistant on oxacillin screen agar. In the present study the sensitivity and specificity of oxacillin screen agar 100.0% and 33.3% respectively. Sensitivity and specificity of oxacillin screen agar which were reported by Mathew *et al* [6] were 97.5% and 75% respectively and by Anand *et al.* [5] were 96.8% and 100% respectively in their study in which sensitivity was almost similar to the present study. Baddour *et al.* [11] described several conventional methods to detect MRSA and were compared with polymerase chain reaction (PCR) for de-

tection of *mecA* gene-positive isolates. Oxacillin agar screen methods were found to be more sensitive than oxacillin disc-diffusion methods which were not with the similar statement with our study.

Various other studies conducted in Bangladesh indicate an increasing trend of MRSA all over the country. A multi-center study involving four divisional hospitals reported a MRSA rate range between 32% to 63.0% [2]. Study on patients of DMCH by Afroz [9], from NITOR (National institute of Traumatology and orthopedics Rehabilitation) and by Jahan *et al.* [4] reported a very high prevalence of MRSA which were 70.2% and 83.3% respectively. Such a high rate may be due to the specimen from post-operative wounds where the nosocomial infection is common. Other reasons may be small size of sample, severity of illness of the cases and the most important being that the above studies were based on susceptibility testing not confirmed by PCR. It seems that susceptibility testing for MRSA by oxacillin should only be treated as a screening test as Zahan *et al.* [3] reported 25% MRSA by PCR while it was 37.5% by oxacillin susceptibility testing from Mymensingh Medical College. Therefore, it is the time to take necessary measures to prevent the spread of MRSA by proper antibiotic policy, preventing nosocomial infection and proper detection and treatment of MRSA cases.

The present study indicates that the rate of MRSA among patients is moderate (15.8%) in nature. The laboratory technique that is evaluated in this study, the sensitivity and specificity of oxacillin screen agar varies from PCR which means it is highly sensitive but low specificity and may be helpful tool in the diagnosis of MRSA infection. In addition to that as MRSA were highly resistant to common antimicrobial agents accurate detection is very important [13]. 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.

AUC is an effective way to summarize the overall diagnostic accuracy of the test. It takes values from 0 to 1, where a value of 0 indicates a perfectly inaccurate test and a value of 1 reflects a perfectly accurate test. AUC can be computed using the trapezoidal rule [12]. In general, an AUC of 0.5 suggests no discrimination which indicates that the ability to diagnose patients with and without the disease or condition based on the test; 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered excellent, and more than 0.9 is considered outstanding. A value of 0.5 for AUC indicates that the ROC curve will fall on the diagonal that is 45-degree line and hence suggests that the diagnostic test has no discriminatory ability [13]. ROC curves above this diagonal line are considered to have reasonable discriminating ability to diagnose patients with and without the disease/condition. It is therefore natural to do a hypothesis test to evaluate whether the AUC differs significantly from 0.5.

## 5. Conclusion

In conclusion oxacillin screen agar test is highly sensitive for the detection of MRSA; however this test has a very low specificity in comparison to *mecA* gene

detection by PCR. Furthermore the area under the curve is low which indicates that the accuracy of oxacillin screen agar test is not a suitable test for the detection of MRSA from the clinical specimens.

### Contribution to Author

Hafiza Sultana, Jogendra Nath Sarker and Shirin Tarafder were involved from protocol preparation, data collection, analysis and report writing. Md. Abdullah Yusuf and Md. Tofael Hossain Bhuiyan were contributed in the manuscript preparation and revision.

### Conflict of Interest

There was no conflict of interest to any of the authors related to financial matter.

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