

## Original Article

# Comparison of Cefoxitin and Oxacillin disc diffusion test for the detection of *mecA* mediated methicillin resistance in *Staphylococcus aureus*

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### Abstract

The study was designed to evaluate the efficacy of cefoxitin disc diffusion test to detect methicillin resistance in *Staphylococcus aureus* and compare it with oxacillin disc diffusion test and detection of *mecA* gene by PCR. A total 116 *S. aureus* were isolated from clinical samples, collected from SSMC&MH, BIRDEM and NMC hospital, from January 2011 to December 2011. It was isolated by culture and identified by standard laboratory procedure. Antibiotic susceptibility testing was performed by oxacillin (1µg) and cefoxitin (30 µg) discs. PCR for amplification of *mecA* gene was performed as a gold standard method. Out of 116 isolates, 28 were PCR positive, 33 and 31 were oxacillin and cefoxitin resistant respectively. The sensitivity and specificity for the detection of MRSA was 100% and 94.31% in oxacillin disc diffusion test, and 96.42% and 95.45% in cefoxitin disc diffusion test respectively. Specificity is higher (95.45%) in cefoxitin disc diffusion test than oxacillin disc diffusion test in the detection of MRSA. Use of disc diffusion tests for both oxacillin and cefoxitin can help in more accurate prediction of methicillin resistance than single test, especially in centers which are not equipped to carry out more sophisticated tests for the detection of MRSA.

**Key word:** MRSA, *mecA* gene, Cefoxitin, Polymerase chain reaction (PCR).

### Introduction:

In recent years, an increase in the number of methicillin resistant *Staphylococcus aureus* has become a serious clinical and epidemiological problem. These strains have spread worldwide, causing nosocomial and more recently, community based infections<sup>1</sup>. This has led to the overuse of antibiotics and to the emergence of vancomycin Intermediate *Staphylococcus aureus* (VISA) and vancomycin resistant *Staphylococcus aureus* (VRSA)<sup>2</sup>.

Methicillin resistance in *S. aureus* has been explained by the production of a characteristic penicillin binding protein (PBP), designated PBP' or PBP2a, that has decreased binding affinity for beta-lactam antibiotics<sup>3</sup>. PBP2a is encoded by the *mecA* gene, a component of a larger DNA fragment designated the *mec* region. Strains that possess *mecA* gene

are either heterogeneous or homogenous in their expression of resistance.

The Clinical and Laboratory Standards Institute (CLSI) recommends usage of cefoxitin instead of oxacillin when using the disc diffusion method to determine resistance against methicillin for *S. aureus*<sup>4</sup>. In the recent past, there have been multiple reports on the use of cefoxitin as a surrogate marker for the detection of *mecA* gene-mediated methicillin resistance<sup>5</sup> as cefoxitin is a potent inducer of the *mecA* regulatory system<sup>6</sup>.

The main objective of the study was to compare oxacillin and cefoxitin disc diffusion tests for detection of methicillin resistance in *S. aureus*, using PCR for *mecA* gene as the gold standard comparison assay.

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### Materials And Methods:

A total 116 strains of *S. aureus* were isolated from clinical samples of SSMC&MH, BIRDEM and NMC during period of January 2011 to December 2011. After isolation by culture on Blood agar media, confirmation of the organism was done by Gram stain and standard biochemical tests like catalase,

slide and tube coagulase test. Sensitivity of the isolated organism was done by using Kirby-Bauer's disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2007<sup>4</sup>. MRSA was confirmed by PCR for *mecA* gene. Reference strain of *S. aureus* 'MR-108' collected from Sapporo Medical University, Japan was used for oxacillin and cefoxitin disc diffusion test and PCR.

**Oxacillin Disc Diffusion Method :**

0.5 McFarland standard suspension of the isolated *S. aureus* was prepared. Mueller Hinton agar was inoculated with 0.5 McFarland suspension of the isolate which was streaked evenly by rotating the plate approximately 600 for three times to get a uniform distribution of inoculum. 1 microgram (µg) oxacillin per disc was placed and then incubated at 35°C for 24 hours. Resistance and sensitivity were measured as per recommendations of the National Committee for Clinical Laboratory Standards (NCCLS)<sup>7</sup>. An inhibition zone diameter of ≤13 mm was considered as resistant.

**Cefoxitin Disc Diffusion Method :**

0.5 McFarland standard suspension of the isolated *S. aureus* was prepared and then Mueller Hinton agar was inoculated with 0.5 McFarland suspension of the isolate. 30 microgram (µg) cefoxitin per disc was placed and then incubated at 37°C for 24 hours. An inhibition zone diameter of ≤21 mm was considered as resistant.

**PCR amplification of *mecA* gene**

A standard protocol for PCR was used. Forextraction of DNA, rapid lysis procedure was done, colonies were obtained from fresh subculture mixed with 300 µl of distilled water and bacterial suspension was heated at 95°C for 10 minutes and then quickly placed on ice. This suspension was then microcentrifuged at 14000 rpm for 10 minutes. Supernatant was used as template DNA.

Primer Sequences used in this study were:

Mec-A1 (+) 5' AAAATCGATGGTAAAGGTTGGC-3'

Mec-A2 (-) 5' AGTTCTGCAGTACCGGATTTGC-3'.

PCR were performed in a thermal cycler (Eppendorf) by using supermix (Promega, U.S.A.) according to the manufacturer's recommendations. 12.5 µl super mix and 2 µl template DNA were taken to each PCR reaction tube. 8.5 µl PCR water and 1.0 µl *mecA1*, 1.0 µl *mecA2* primer was added to each tube. So the final volume at each tube was 25 µl. A thermal step program that included the following parameters was used for DNA amplification: initial denaturation for 3 minute at 94°C, then final denaturation

continued for 40 cycles of 30s at 94°C, 45s at 60°C, annealing at 60°C for 1.5 minutes. Extension of primer at 72°C for 3.5 minutes with a total of 40 cycles.

The amplified PCR products were detected by 0.8% agarose gel with ethidium bromide dye under UV transilluminator. A 533-bp DNA fragment is detected and photographed under UV illumination.

**Results:**

Among 116 *S. aureus* isolates, 33 (28.44%) were oxacillin resistant in which *mecA* gene was found in 28 (84.84%) strains while *mecA* gene could not be detected in 5 (15.16%) isolates. *mecA* gene was not detected in 83 oxacillin sensitive strains. Out of 31 (26.73%) cefoxitin resistant isolates, 27 (87.00%) *S. aureus* had *mecA* gene while *mecA* gene was found in 1 (1.18%) cefoxitin sensitive strain which was oxacillin resistant (table-1). All 31 cefoxitin resistant strains were oxacillin resistant.

Cefoxitin disc diffusion test showed the higher specificity (95.45%) and accuracy (96.68%) than that of oxacillin disc diffusion test, where the specificity and accuracy were 94.31% and 95.68% respectively (Table-II). But the cefoxitin disc diffusion test was less sensitive (96.42%) than the oxacillin disc diffusion test (100%).

**Table -I:** Oxacillin and cefoxitin disc diffusion tests and their comparison with *mecA* gene ( n = 116 ).

Methods	Susceptibility	<i>mecA</i> gene	
		Present	Absent
Oxacillin disc diffusion test (1µg)	R 33 (28.44)	28 (84.84)	05 (15.16)
	S 83 (71.56)	00	83 (100)
Cefoxitin disc diffusion test (30µg)	R 31 (26.73)	27 (87.00)	04 (13.00)
	S 85 (73.27)	01 (1.18)	84 (98.82)

Figure within parentheses indicate percentage

R- Resistant, S- Sensitive

**Table - 2:** Comparison of oxacillin disc diffusion test with cefoxitin disc diffusion test for detection of MRSA

Test methods	Sensitivity (%)	Specificity (%)	Accuracy (%)
Oxacillin disc diffusion test (1µg)	100	94.31	95.68
Cefoxitin disc diffusion test (30µg)	96.42	95.45	96.68

**Discussion:**

Detection of *mecA* gene or its product, PBP2a, is considered as the gold standard for MRSA confirmation<sup>9</sup>. The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*. This study was attempted to evaluate two disc diffusion methods for detection of MRSA.

In the present study, it was found that the cefoxitin disc diffusion test was more specific (95.45%) and less sensitive (96.42%) than oxacillin disc diffusion test. The accuracy (96.68%) of cefoxitin disc diffusion test was better than that of oxacillin disc diffusion test (95.68%) for the detection of methicillin resistance in *S. aureus*. Cefoxitin disc diffusion test also does not require special testing conditions such as lower incubation temperature, and NaCl supplementation in the testing media, as required by the oxacillin disc diffusion test and according to previously reported studies, the sensitivity and specificity of cefoxitin disc diffusion test for *S. aureus* has been reported to be 95-100% and 98-100%<sup>5,10</sup> and for oxacillin disc diffusion test 90.4-98% and 83-99% respectively<sup>11</sup>.

In this study, out of 116 isolated *S. aureus*, 33 (28.44%) were oxacillin resistant, of which 28 were detected as MRSA by polymerase chain reaction (PCR). In 05 oxacillin resistant isolates, *mecA* gene was not detected. It might be possible that these strains were hyper beta-lactamase producer thereby they were accounting for *non-mecA* mediated oxacillin resistance or they had borderline resistance<sup>12</sup>. On the other hand these 5 isolates could represent false positive resistance to oxacillin because specificity of oxacillin disc diffusion test was 94.31%.

The present study showed that, 31 *S. aureus* (26.73%) were found to be cefoxitin resistant, in which *mecA* genes were detected in 27 strains. Cefoxitin falsely identified one isolate as susceptible that was oxacillin resistant and *mecA* positive. Additional 4 resistant isolates were resistant to oxacillin and was found to be negative for *mecA* gene.

From clinical perspective, it is important to differentiate *mecA* positive resistant isolates from the isolates having borderline resistance because it may affect therapy. Strains that possess *mecA* classic resistance are either homogenous or heterogenous in their expression of resistance. Oxacillin disc diffusion test does not detect heterogenous resistance accurately due to its low expression. Moreover the test result may be affected by various components of Mueller Hinton agar media, temperature and duration of incubation<sup>13</sup>.

Cefoxitin is considered to be a better predictor than oxacillin for the detection of heteroresistant MRSA because it is a strong inducer of PBP2a<sup>14</sup> but cefoxitin limitedly can detect only MRSA with *mecA* mediated resistance mechanism<sup>6</sup>. Even with this limitation, cefoxitin disc diffusion zones are much easier to read than those of oxacillin due to the frequent hazy oxacillin zones, which are commonly misinterpreted as evidence of oxacillin susceptibility. This rate of false susceptibility associated with oxacillin disc diffusion test has been noted to be as high as 4.4% in some studies<sup>15</sup>.

The present study provides an evidence that cefoxitin can be used as an accurate surrogate marker in disc diffusion test for the detection of MRSA as the result of cefoxitin disc diffusion test is in concordance with PCR for *mecA* gene. Thus the test can be an alternative to PCR for detection of MRSA in resource constrained settings.

**Reference:**

1. Chambers HF. The changing epidemiology of *Staphylococcus aureus*. *Emerg Infect Dis* 2001;7:178-182.
2. Centers for Disease Control and Prevention (CDC). *Staphylococcus aureus* resistant to vancomycin- United States, 2002. *Morb Mortal Wkly Rep* 2002;51:565-567.
3. Skov R, Smith R, Clausen M, *et al*. Evaluation of a cefoxitin 30g disc on Iso-Sensitest agar for detection of methicillin resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 2003; 52: 204-207.
4. CLSI. Performance standards for antimicrobial susceptibility testing; 17th Informational supplement. 2007. CLSI M100-S17. CLSI Wayne, PA.
5. Swenson JM and Tenoyer FC. The Cefoxitin Disk Study Group. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *staphylococcus* spp. *J Clin Microbiol* 2005 ; 43:3:3818-3823.
6. Swenson JM, Lonsway D, McAllister S, *et al*. Detection of *mecA* - mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing borderline oxacillin MICs. *Diagn Microbiol Infect Dis* 2007; 58: 33-39.
7. Villanova PA. Performance standards for antimicrobial disc susceptibility tests. In: National Committee for Clinical Laboratory Standards. Approved standard. 7th edn. National Committee for Clinical Laboratory Standards: 2000. M2-A7.

8. Ercis S, Sancak B, Hascelik G. A comparison of PCR detection of *mecA* with oxacillin disc susceptibility testing in different media and sceptor automated system for both *Staphylococcus aureus* and coagulase negative *Staphylococci* isolates. *Indian J. Med Microbial* 2008; 26(1):21-24.
9. Skov R, Smyth R, Larsen AR, *et al*. Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disc diffusion testing and e-test on Mueller-Hinton agar. *J Clinical Microbial* 2006.; 44:4395-4399.
10. Flayhart D, Hindler JF, Bruckner DA, *et al*. Multicenter evaluation of BBL CHROMagar MRSA medium for direct detection of methicillin resistant *Staphylococcus aureus* from surveillance cultures of the anterior nares. *J Clin Microbiol* 2005; 43: 5536-5540.
11. Amita J, Astha A and Rajkumar V. Cefoxitin disc diffusion test for detection of methicillin-resistant staphylococci. *J Med Microbiol* 2008; 57: 957-961.
12. Nicole MB, Tam TV, Timothy AM., Steven AM and David MW. Comparison of cefoxitin and oxacillin disc diffusion methods for detection of *mecA*- mediated resistance in *Staphylococcus aureus* in a large - scale study. *J Clin Microbial* 2009; 47 (1): 217-219.
13. Fernandes CJ, Fernandes LA, Collignon P: Australian Group on Antimicrobial Resistance. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother.* 2005 ; 55 : 506-510.
14. Felten A, Grandry B, Lagrange PH and Casin I. Evaluation of three techniques for detection of low level methicillin resistant *Staphylococcus aureus* (MRSA): a disc diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. *J Clinical Microbial* 2002;40: 2766-2771.
15. Pottumarthy STR, Fritsche and Jones RN. Evaluation of alternative disc diffusion methods for detecting *mecA* - mediated oxacillin resistance in an international collection of *Staphylococci*: validation report from the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2005; 51:57-62.