Evaluation of Cefoxitin Disk Diffusion Test for Routine Detection of Methicillin-resistant Staphylococcus Aureus

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ABSTRACT

Background and Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community acquired infections. Detection of MRSA in laboratories is very important for treatment and appropriate infection control. The aim of this study was to evaluate cefoxitin disk diffusion method for detection of MRSA and comparison of this method with other conventional methods.

Methods: A total of 175 clinical isolates of S. aureus isolated from clinical specimens were studied. The isolates were identified by conventional laboratory methods. In this respect, E-test MIC, cefoxitin and oxacillin disk diffusion methods, and MAST ID Methicillin strips were used for detection of MRSA. All disk diffusion methods were performed as recommended by NCCL and manufacturers’ guidelines.

Results: Using E-test MIC, 53 out of 175 strains of S. aureus were resistant to methicillin. In addition, disk diffusion method using oxacillin disk showed that 52 strains are resistant to methicillin. In this respect, 8 strains had intermediate resistance to methicillin. For cefoxitin disk diffusion method, 52 strains were resistant to methicillin. This method had a good correlation with E-test MIC method. Meanwhile, MAST ID methicillin strips detected 47 strains that were resistant to methicillin. Sensitivity and specificity for both cefoxitin and oxacillin disk diffusion methods were 98% and 100% respectively. However cefoxitin was better than oxacillin for detecting intermediate resistant strains of S. aureus. Sensitivity and specificity for MAST ID methicillin strips were 91% and 100% respectively.

Conclusion: This study revealed that cefoxitin disk diffusion method is a good alternative for oxacillin disk diffusion method for detection of MRSA. This method is more reliable for identification of intermediate resistant strains of S. aureus.

Key words: Methicillin-resistant Staphylococcus aureus, Cefoxitin
**Introduction**

Staphylococcus aureus has been recognized as one of the major pathogens in humans in both community and hospitals (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causes of nosocomial infections. Methicillin as a semi-synthetic penicillinase-resistant penicillins was introduced in 1960 for the treatment of penicillinase producing strains of *S. aureus* and methicillin-resistant strains of *S. aureus* were identified in 1961(2). Treatment of infections caused by these strains has become problematic. Indiscriminate use of multiple antibiotics especially in developing countries, prolonged hospitalization, intravenous drug abuse, and transfer of MRSA through nose are few implicated risk factors for MRSA actuation (3).

Accurate and routine phenotypical detection of MRSA is difficult using standard disk diffusion, MIC determination, or agar breakpoint methods. This issue has been ascribed to the heterogeneous expression of methicillin resistance in many strains of *S. aureus*. Detection of mecA or PBP2a is therefore considered as the gold standard for exposing methicillin resistance in *S. aureus*. Few laboratories however have the technical and/or economical capacity to apply these tests to all isolates of *S. aureus* found at the microbiological laboratories. Disk diffusion methods using oxacillin is the most widely used method, but results are influenced by several factors including concentration of NaCl, temperature, inoculum, and test agent. Thus, there still remains a need for a reliable test for MRSA that can be performed easily in routine situations.

Recently, the cefoxitin disk diffusion method has been proposed as an alternative method for detecting MRSA (4). Cefoxitin is a cephomycin-type antibiotic and has been described as an inducer of methicillin resistance by producing the PBP2a. No specific incubation temperature is required and the test is less affected by hyperproduction of penicillinase (5). In this context, the use of cefoxitin rather than oxacillin for disk diffusion test has been advocated. Therefore, the present study compared the performance of disk diffusion tests for cefoxitin, oxacillin, and other two methods including methicillin strips and E-test for detection of MRSA.

**Materials and Methods**

The cross-sectional protocol of this study was carried out in Milad hospital (Tehran) from 15 April 2005 to 15 October 2005 to detect MRSA. A total of 175 strains of *S. aureus* isolated from varieties of clinical specimens including urine, blood, wound, and tracheal tube aspirates. All isolates identified by conventional microbiological methods including colony morphology, Gram stain, catalase test, slide coagulase test, tube coagulase tests, and DNAase test (6). Methicillin resistance susceptibility testing for detection of MRSA was determined using cefoxitin 30 microgram (Hi media, India) and oxacillin 1 microgram (Mast diagnostic group, UK) as recommended by NCCLS (7). We evaluated methicillin strips for detecting MRSA (methicillin strips are filter paper strips 75 mm by 6mm, printed methicillin). Each strip impregnated with 25 µg of methicillin. A clearly defined zone of inhibition around the strip of any size interpreted as sensitive and no or little zone of inhibition around strip interpreted as resistant as recommended by manufacturer. Susceptibility testing to the other antibiotics was performed by disk diffusion method as recommended by NCCLS (6).

Finally E-test method (AB Biodisk, Solna, Sweden) was used to determine MIC as recommended by manufacturer (Briefly, using Muller-Hinton agar with 2% NaCl and an inoculum density equivalent to 0.5 Mc Farland standards, application of inoculum with a swab and incubation at 35 °C for 24 hours). We used this method as gold standard and other methods were compared with E-test MIC. The other antibiotics used for susceptibility testing were penicillin (10 IU), erythromycin (15 µg), clindamycin (2 µg), linozolide (30 µg), mupirocin (5 µg), vancomycin (30 µg), and trimethoprim- sulfamethoxazole (1.25/23.75 µg). All antibiotic disks were provided by Mast diagnostic group (UK). Interpretive criteria (in mm) for oxacillin disk diffusion tests regarding *S. aureus* were ≥ 13 mm as susceptible, 11-12 mm as intermediate, and ≤10 mm as resistant. Interpretive criteria (in mm) for *S. aureus*...
using cefoxitin disk were ≥ 20 mm as susceptible
and ≤19 mm as resistant. *Staphylococcus aureus*
ATCC25923 and ATCC 29213 were used as quality
control strains.

**Results**

Using E-test MIC, it was found out that 53 of 175
strains of *S. aureus* were resistant to methicillin.
Disk diffusion method using oxacillin disk showed
52 strains resistant to methicillin. In this method, 8
strains had intermediate resistance to methicillin.
For disk diffusion method using cefoxitin, 52
strains were resistant to methicillin. This
method had a good correlation with E-test MIC
method. MAST ID methicillin strips showed
47 strains of *S. aureus* as resistant to methicillin.
Sensitivity and specificity for both cefoxitin and
oxacillin disk diffusion methods were 98% and
100% respectively. However, cefoxitin was better
than oxacillin to detect intermediate resistant
strains of *S. aureus*. Sensitivity and specificity for
MAST ID methicillin strips was 91% and 100%
respectively. All isolates were susceptible to
linozolid and vancomycin and 1.6% were resistant
to mupirocin and 28.5% of isolates were resistant
to trimethoprim-sulfamethoxazole.

<table>
<thead>
<tr>
<th>Method</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-test MIC</td>
<td>53/175</td>
<td>-</td>
<td>122/175</td>
</tr>
<tr>
<td>Oxacillin disk</td>
<td>52/175</td>
<td>8</td>
<td>115/175</td>
</tr>
<tr>
<td>Cefoxitin disk</td>
<td>52/175</td>
<td>-</td>
<td>123/175</td>
</tr>
<tr>
<td>Methicillin Strip</td>
<td>47/175</td>
<td>-</td>
<td>128/175</td>
</tr>
</tbody>
</table>

**Discussion**

Oxacillin resistance in *S. aureus* is caused
by expression of penicillin-binding protein 2a
(PBP2a) encoded by the *mecA* gene complex.
Laboratory methods have been developed to
enhance the expression of methicillin resistance
in staphylococci by modification of test condition,
including supplementation of media with NaCl
and prolonging the incubation period. Phenotypic
methods for detecting MRSA strains are not
usually correctly identified. Problems in detection
of MRSA may be caused by low-level expression
of oxacillin resistance in some strains of *S. aureus*
(8-10). Cefoxitin is considered to be a better
predicator than oxacillin for detecting oxacillin
heteroresistance because it is stronger for PBP2a
detection. In addition, it has high affinity for
staphylococcal PBP4 and previous experiments
have shown a relationship between PBP2, PBP4 and
methicillin resistance. Many studies have reported
that using cefoxitin disk has a high sensitivity and
specificity (11). In a study by Velsco et al, different
methods for detection of MRSA were evaluated. In
their study, cefoxitin disks had 100% sensitivity for
MRSA and showed negative and positive predictive
values of 100% and 98% respectively. They also
concluded that in the absence of availability of
molecular biology techniques, the cefoxitin disk
was the best predictor for methicillin resistance in
*S. aureus* among other available techniques (12).

**Table 1. Evaluation of different methods for
detection of MRSA**

**Table 2. Comparison of sensitivity and specificity of oxacillin and cefoxitin for detection of
MRSA in different studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Oxacillin Sensitivity(%)</th>
<th>Oxacillin Specificity(%)</th>
<th>Cefoxitin Sensitivity(%)</th>
<th>Cefoxitin Specificity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boubaker BB et al</td>
<td>90.4</td>
<td>99.1</td>
<td>96.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Felten e al</td>
<td>96.4%</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Skov et al</td>
<td>78</td>
<td>99</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Present study</td>
<td>98</td>
<td>100</td>
<td>98</td>
<td>100</td>
</tr>
</tbody>
</table>
Errors in determining oxacillin resistance may have serious adverse clinical consequences. False negative susceptibility results may lead to treatment failure and the spread of MRSA, especially if appropriate infection control measures are not applied. Conversely, false-resistance results may increase health care cost following unnecessary isolation precautions and may lead to overuse of glycopeptides such as vancomycin.

Detecting the mecA gene (or PBP2a) is recognized to be the most accurate and gold standard method for detection of MRSA. However, use of PCR assay is generally limited to referral laboratories especially in developing countries and neither method is used widely for routine methicillin susceptibility tests in diagnostic laboratories. This and other studies revealed that cefoxitin disk susceptibility test appear to be a useful procedure in that it is easy to perform in routine laboratories and has greater accuracy than oxacillin disk diffusion test. The cefoxitin disk diffusion test has the potential of wider use in diagnostic microbiology laboratories.

**Conclusion**

This study reveals that cefoxitin disk susceptibility test appear to be a useful procedure for detection of MRSA. This method is easy to perform in routine laboratories and has greater accuracy than oxacillin disk diffusion tests.

**References**

7. Performance standards for antimicrobial susceptibility for antimicrobial susceptibility testing. 14th information supplement M100-S14 national Committee for Clinical Laboratory Standards (NCCLS) 2004 Wayen. Pa