Differentiation of Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus Aureus* and Coagulase-Negative *Staphylococci* using Cefoxitin

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**ABSTRACT**
Detection of the mecA gene or PBP2a is considered the gold standard for detecting mecA-mediated methicillin resistance in staphylococci. Among available phenotypic methods, the Clinical and Laboratory Standards Institute (CLSI) has introduced the cefoxitin disk diffusion (DD) test for predicting the presence of mecA in *S. aureus* and CoNS, which is preferred over the oxacillin DD test. Automated systems are widely used for species identification and susceptibility testing. The BD Phoenix system was compared to the cefoxitin disk diffusion test and oxacillin disk diffusion test for detection of methicillin resistance in 486 clinical specimens. Phoenix sensitivity and Specificity were 100%. The cefoxitin DD test also was much easier to read and did not require the use of transmitted light for detection of resistance.

**INTRODUCTION**
*Staphylococcus aureus* is a serious current health care concern. Both community-associated methicillin-resistant *S. aureus* (MRSA) and health care-associated MRSA are growing threats to the immunocompromised, as well as to the general public. Accurate detection of methicillin resistance in *S. aureus* is of the utmost importance to ensure effective treatment for the affected patient and to prevent further transmission.

The *mecA* gene confers resistance to methicillin in *S. aureus*. The gene is located on the staphylococcal chromosome cassette mec and encodes penicillin binding protein 2a (PBP2a). PBP2a is located in the bacterial cell wall and has a low binding affinity for β-lactams.

CLSI recommends usage of cefoxitin instead of oxacillin when using the disk diffusion method to determine resistance against methicillin for *S. aureus*. Cefoxitin results are easier to interpret and are thus more sensitive for the detection of *mecA*-mediated resistance than oxacillin results. The recommended resistance and susceptibility breakpoints for the 30-µg cefoxitin disk test used to detect *mecA*-mediated resistance in *S. aureus* were changed in January 2007 by CLSI from ≤19 mm and ≥20 mm to ≤21 mm and ≥22 mm, respectively. This study evaluated the new CLSI breakpoints for the cefoxitin disk test for determining *mecA*-mediated resistance in *S. aureus*.

**MATERIALS & METHODS**

The study was conducted at Al-Noor Specialist Hospital, Holy Makkah, KSA, a 500-bed tertiary care specialist hospital, and Saudi German Hospital Riyadh, KSA, a 300-Bed tertiary care specialist hospital. The study was performed on 486 *Staphylococcus* isolates collected during the 2010 routine clinical laboratory activity. Strains were isolated from clinical specimens of medical and surgical departments. Isolates obtained from consecutive cultures from the same patient were excluded. Species identification of the isolates was done by colony pigmentation, hemolysis and coagulase production.

(i) **Phoenix panels (BD).**
According to the manufacturer’s instructions of PMIC/ID gram-positive Phoenix panels (BD) the detection of isolates was based on both oxacillin and cefoxitin MICs, interpreted according to CLSI breakpoints (for oxacillin, susceptible with MICs of ≤2 µg/ml and resistant with MICs of ≥4 µg/ml; for cefoxitin, susceptible with MICs of ≤8 µg/ml and resistant with MICs of ≥8 µg/ml), in that if either oxacillin or cefoxitin MIC testing indicates that the isolate is resistant, the Phoenix final report is methicillin resistance.

(ii) **Disk Diffusion.**
Susceptibility to antimicrobial agents for isolates was evaluated by the CLSI disk diffusion method on Mueller-Hinton agar (oxoid, Germany). A direct colony suspension of each isolate was prepared to a 0.5 McFarland standard and plated on
Mueller-Hinton agar with 1 µg oxacillin and with 30 µg cefoxitin disks. The zones of inhibition were measured after 24 h of incubation at 35°C. The cefoxitin and oxacillin disks were read using transmitted light as the CLSI document recommends. The results were analyzed according to the breakpoints of the CLSI document.

(iii) meca and PBP2a status.

All isolates were screened using the Oxoid PBP2’ latex agglutination test kit. The test was carried out according to the manufacturer’s instructions on uninduced inocula for S. aureus or inocula induced with oxacillin disk for CoNS. Uninduced inocula of 5 µl and 10 µl from a 5% sheep blood agar (SBA) plate, as well as 5 µl and 10 µl of inoculum that had been grown overnight on a SBA plate containing a 1-µg oxacillin disk (Becton Dickinson, Sparks, MD) were used for PBP 2a detection. Colonies were taken directly from an SBA plate, and volumes were measured using 5 loopfuls of a 1-µl calibrated loop or a 10-µl calibrated loop. The meca result was used as the “gold standard” in all isolates. S. aureus ATCC 29213 (meca-negative) was included as a negative control, and S. aureus ATCC 43300 (meca-positive) was included as a positive control.

RESULTS

The isolation sites of the isolates collected were as follows: wound swab (305 isolates; 62.8%), respiratory tract (83 isolates; 17%), urine (49 isolates; 10.1%), bloodstream (19 isolates; 3.9%), and others (30 isolates; 6.2%) (Table-1).

A total of 486 culture samples were tested (from 162 patients), 174 of which grew a pure culture of S. aureus and 312 samples grew coagulase-negative staphylococci (CNS). The 312 CoNS studied included 124 S. epidermidis isolates, 98 S. haemolyticus isolates, 58 S. hominis isolates, 22 S. capitis isolates, 7 S. saprophyticus isolates, and 3 S. intermedius isolate (Table-2).

Among 174 S. aureus isolates, the cefoxitin DD test results and the Phoenix final reports were concordant for 82 methicillin-susceptible and 88 methicillin-resistant strains. As regarding the Phoenix oxacillin and cefoxitin MIC results, five S. aureus strains falsely identified as susceptible to oxacillin and 3 strains falsely identified as susceptible to cefoxitin were finally reported correctly by the Phoenix expert system, based on cefoxitin and oxacillin results, respectively, these strains were all identified as resistant by the cefoxitin DD test (Table – 4).
(Table – 4). Results of SA isolates

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<thead>
<tr>
<th></th>
<th>Phoenix</th>
<th>DD</th>
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<tr>
<td></td>
<td>ox/s</td>
<td>ox/r</td>
</tr>
<tr>
<td>cef/s</td>
<td>86 /S</td>
<td>3 /R</td>
</tr>
<tr>
<td>cef/r</td>
<td>5 /R</td>
<td>80 /R</td>
</tr>
</tbody>
</table>

ox/s: oxacillin susceptible, ox/r : oxacillin resistant, cef/s: cefoxitin susceptible, cef/r : cefoxitin resistant, S : reported as susceptible, R : reported as resistant

The sensitivity of the cefoxitin DD test (92.4%) was slightly lower than that of the oxacillin disk test (95.6%) for *S. aureus* isolates, whereas; the specificity of the cefoxitin disk test was 95.7%, and that of the oxacillin disk test was 92.6%. Both sensitivity and specificity of the combination of two disks (100%) were higher than that of the oxacillin disk alone (95.6%, 92.6%) and that of the cefoxitin disk alone (92.4%, 95.7%) respectively.

Unlike for *S. aureus*, oxacillin MIC alone is used by the Phoenix system to detect methicillin resistance in CoNS, interpreted according to CLSI breakpoints, with MICs for oxacillin between 0.5 and 2 µg/ml, as recommended by the CLSI. Among the 312, CoNS, results were concordant for 118 methicillin-susceptible and 190 methicillin-resistant strains. Discrepant results were obtained for 4 strains, all identified as susceptible by the cefoxitin DD test and resistant by the Phoenix oxacillin test. The latex test for PBP2a, performed after oxacillin induction, showed positive results for 1 strain, thus confirming it as methicillin resistant, and negative results for 3 strains. Thus, the Phoenix sensitivity and specificity were 100% and 97.5% respectively.

The sensitivity of the cefoxitin DD test (93.8%) was slightly lower than that of the oxacillin disk test (97.6%) for all CoNS species, whereas; the specificity of the cefoxitin disk test was (96.0%), and that of the oxacillin disk test was (98.5%).

**DISCUSSION**

Methicillin or oxacillin has been the agent of choice in selective media for *mecA*-positive *S. aureus* for over 20 years\(^{(11)}\). During the last several years, the CLSI-AST has attempted to improve the accuracy of detecting *mecA*-positive strains of both *S. aureus* and CoNS. Previous CLSI recommendations for detecting methicillin resistance in staphylococci\(^{(9)}\). The CLSI undertook several studies to investigate the utility of cefoxitin DD test originally proposed by\(^{(14)}\) and further investigated by\(^{(9)}\), as a potential alternative to *mecA* testing.\(^{(9)}\) used the BD Phoenix automated system (BD, Sparks, MD) to evaluate its performance in detection of methicillin resistance in comparison to the cefoxitin DD test.

In this study, 100% sensitivity and specificity were found with Phoenix final reports of 174 *S. aureus* isolates, underlining that the optimal performance of this automated system relies on the fact that both oxacillin and cefoxitin MICs are determined in the panel.\(^{(10)}\) found that the Phoenix sensitivity and specificity were 100% and 99.86% respectively. These results are similar to those from a previous study when cefoxitin MIC testing was done to determine cefoxitin disk diffusion breakpoints\(^{(17)}\). In that 10-laboratory study using panels prepared with CAMHB of two manufacturers and the MIC breakpoints proposed here, the sensitivity was 98 to 99%, and specificity was 99 to 100%. Poor sensitivity with the Phoenix system for oxacillin has been observed in other studies\(^{(17)}\). As in this study, no false identification of susceptibility by the Phoenix system was reported by Fahr et al. among 54 MRSA isolates tested\(^{(9)}\).

The introduction of cefoxitin in the panels improved the performance of the Phoenix system in detecting MRSA. This system was introduced in 2005, with provisional breakpoints of ≤8µ g/ml for susceptibility and ≥16 µ g/ml for resistance, which decreased to ≤4 µ g/ml and ≥8µ g/ml, respectively, in 2006\(^{(11)}\). In a study with 135 borderline *S. aureus* isolates, the revised breakpoints improved sensitivity from 91.1% to 97.5% while specificity (100%) remained unchanged\(^{(18)}\). Similarly,\(^{(16)}\) reported that a MIC of ≤4 µ g/ml for cefoxitin was 100% predictive of methicillin resistance for *S. aureus*, and Votta et al. found that a cefoxitin breakpoint of ≥8 µ g/ml yielded 100% sensitivity and 99.2% specificity\(^{(20)}\).

Cefoxitin will detect only MRSA with a *mecA*-mediated resistance mechanism, there is a CLSI comment in the M100-S17 document warning of this limitation of cefoxitin as a substitute for oxacillin. However, non-*mecA*
mediated methicillin resistance in *S. aureus* is a rare occurrence\(^{10}\). Even with this limitation, cefoxitin disk 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\(^{15}\). The rate of false susceptibility associated with the oxacillin disk diffusion test has been noted to be as high as 4.4% in some studies\(^{16}\), well above the CLSI-recommended acceptability limit of \(\leq 1.5\)%. Oxacillin must also be read using transmitted light, unlike most other antimicrobials, including cefoxitin, to ensure correct interpretation\(^{19}\).

For CoNS, we found that cefoxitin MIC levels were sensitive but not specific in identifying the presence of *mecA* as stated by \(^{1}\) and \(^{11}\) who recommend that they not replace oxacillin MICs at this time. We found the cefoxitin disk diffusion test to be a valuable test due to its ease of reading and higher sensitivity. Our data emphasize the need for both oxacillin and cefoxitin results for MRSA detection, and validated the new CLSI recommendations for the 30-µg cefoxitin disk test to detect *mecA*-mediated resistance in *S. aureus*.

REFERENCES


تحديد خاصية المقاومة للجرثومة العقدية لعقار الميستيلين باستخدام عقار سيفوكستين

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يعتبر تحديد خاصية المقاومة للجرثومة العقدية لعقار الميستيلين من المتطلبات الضرورية لأى عدوى بكتيرية بالجرثومة العقدية وقد كان يتم ذلك من خلال عقار أوكسيسيلين، وفي توصية للمعهد القياسي للمعال والامراض السريرية باستخدام عقار سيفوكستين بدلاً من عقار أوكسيسيلين. احتوت هذه الدراسة على 486 عينة للجرثومة العقدية وتم اختبار مقاومة جميع العينات لعقار الميستيلين باستخدام كل من عقار أوكسيسيلين وعقار سيفوكستين وكانت نسبة اكتشاف مقاومة الجرثومة لعقار الميستيلين باستخدام كل من عقار أوكسيسيلين وعقار سيفوكستين مجتمعين أفضل من نسبة كل منهما على حدة.