Evaluation of a cefoxitin 30 µg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*

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**Objectives:** To evaluate the performance of a cefoxitin 30 µg disc on Iso-Sensitest agar, using a semi-confluent inoculum and overnight incubation at 35–36°C, for detection of methicillin-resistant *Staphylococcus aureus* (MRSA).

**Methods:** A total of 457 *S. aureus*, including 190 MRSA of several defined PFGE types and a number of low-level resistant isolates, were tested with a cefoxitin 30 µg disc on Iso-Sensitest agar, using a semi-confluent inoculum and overnight incubation at 35–36°C. This method was compared with the standard SRGA (Swedish Reference Group for Antibiotics) method (oxacillin 1 µg disc on Iso-Sensitest agar supplemented with 5% defibrinated horse blood, confluent growth and 24 h incubation in ambient air at 30°C).

**Results:** The cefoxitin method was excellent, with a sensitivity of 100% and a specificity of 99% using an interpretative zone diameter of S ≥ 29 mm and R < 29 mm. Its performance was much better than the SRGA method, which with this collection of difficult strains had a sensitivity of only 78% using the current breakpoint of S ≥ 12 mm.

**Conclusion:** We suggest that the cefoxitin method should replace that currently recommended by the SRGA for the detection of MRSA, and that it would fit well into BSAC methodology.

**Keywords:** susceptibility testing, disc diffusion, MRSA, Iso-Sensitest Agar

**Introduction**

Accurate, routine phenotypical detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is difficult using standard media with disc diffusion, MIC determination or agar breakpoint methods. This has been ascribed to the heterogeneous expression of methicillin resistance in many strains.1 These strains seem to be on the increase, both in number and in the level of heterogeneity, which is a challenge even with specialized phenotypical methods.2–6 Detection of mecA or PBP2a are therefore considered the gold standard for exposing methicillin resistance in staphylococci.1,2 Few laboratories, however, have the technical and/or economic capability to apply these tests on all *S. aureus* isolates found in the microbiological laboratory, and so disc diffusion remains the method of choice for routine screening for methicillin resistance. In order to enhance the sensitivity and/or specificity of phenotypical methods, the use of special media, a different sized inoculum and/or an altered incubation temperature, may be required.2–8 All this emphasizes the need for an improvement in the routine detection of MRSA.

Recently, two reports demonstrated the excellent performance of a cefoxitin 30 µg disc on Mueller–Hinton agar with standard inocula of 10⁶ (semi-confluent) and 10⁷ cfu/mL.4–9 We compared the method for detection of methicillin resistance recommended by the Swedish Reference Group for Antibiotics (SRGA), with a cefoxitin 30 µg disc on Iso-Sensitest agar, using a semi-confluent (10⁶ cfu/mL) inoculum and overnight incubation at 35–36°C, which are the SRGA standard conditions for susceptibility testing of other antibiotics.7 If accurate, this would eliminate the present need in the SRGA method for a separate test for MRSA. Furthermore, it would easily fit the BSAC recommendations for susceptibility testing.

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Table 1. Origin and meca status of the tested isolates

<table>
<thead>
<tr>
<th>Test performed</th>
<th>Origin of isolates</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total</td>
</tr>
<tr>
<td>Establishing of interpretive zone diameters</td>
<td>reference collection, SMI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>reference collection, SS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>184</td>
</tr>
<tr>
<td>Confirmatory testing</td>
<td>consecutive blood isolates, HH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>457</td>
</tr>
</tbody>
</table>

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Material and methods

Strains

A total of 457 *S. aureus*, all tested for the presence of the meca gene by the EVIGENE MRSA detection kit using the manufacturer’s instructions, were investigated. The isolates comprised 267 meca-positive and 190 meca-negative Danish and Swedish *S. aureus* isolates (Table 1). One hundred and eight of the isolates were consecutive, routine blood culture isolates from the Department of Clinical Microbiology, Hvidovre University Hospital, Copenhagen, and the rest represented modern MRSA collections from the Danish National Staphylococcal Reference Laboratory at Statens Serum Institut, Copenhagen, Denmark and the Swedish Institute for Infectious Disease Control, Solna, Sweden.

The Danish collection represented MRSA sent to the laboratory in 2001 (repeat samples excluded, n = 105), including low-level resistant variants, i.e. strains with oxacillin MIC ≤ 2 mg/L (Etest on Mueller–Hinton agar, supplemented with 2% NaCl), and 79 epidemiologically unrelated methicillin-susceptible *S. aureus* (MSSA). The Swedish collection contained 82 MRSA, including more than 22 different PFGE types (e.g. Berlin IV, Finland E7, France A and B, S German II, UK E1, E15 and E16, Spain E1), and 83 epidemiologically unrelated MSSA.

Susceptibility testing

All isolates were tested with a cefoxitin 30 μg disc (Oxoid, Basingstoke, UK), on Iso-Sensitest agar (ISA, Oxoid), using semi-confluent growth and overnight incubation in ambient air at 35–36°C, and with an oxacillin 1 μg disc (Oxoid) on ISA supplemented with 5% defibrinated horse blood, confluent growth and 24 h incubation in ambient air at 30°C (the method recommended by the SRGA). Using callipers, inhibition zone diameters were measured to the nearest millimetre at the inner zone edge. For cefoxitin, a sensitivity of 100% was the criterion for determining the interpretive zone diameter of resistance. For oxacillin, the SRGA recommendations were followed.

*S. aureus* ATCC 29213 was included on each occasion for quality control, and *S. aureus* ATCC 25923 was included in some of the test runs (Table 2). All values for the oxacillin 1 μg disc for *S. aureus* ATCC 29213 were within the published range.

Results

Of the 457 *S. aureus*, 190 were meca-positive (MRSA) and 267 were meca-negative (MSSA). Cefoxitin zone diameters for the MRSA were 6–28 mm and for the MSSA 24–38 mm (Figure 1). Using an interpretive zone diameter of R < 29 mm, a sensitivity of 100% and a specificity of 99% according to meca status, were obtained. The results for the quality control strains are shown in Table 2.

Oxacillin zone diameters for the MRSA were 6–20 mm and for the MSSA 6–30 mm (Figure 2). A substantial zone diameter overlap between MRSA and MSSA was recorded. Using the SRGA interpretive zone diameter of R < 12 mm, a sensitivity of 78% and a specificity of 99% were obtained.

Of the 457 *S. aureus* isolates, 108 were consecutive clinical blood isolates. All but three were meca-negative, with cefoxitin zone diameters between 29 and 38 mm. The meca-positive isolates exhibited zone diameters of 13, 15 and 18 mm, respectively.

We further investigated whether the addition of 5% defibrinated horse blood to the ISA medium or using a confluent inoculum (McFarland 0.5) affected the cefoxitin inhibition zone ranges for MRSA (n = 106) or MSSA (n = 182) isolates. Blood supplementation did not alter the inhibition zone ranges for MRSA or MSSA isolates. For the variant using ISA with blood and confluent growth, an interpretive zone diameter of R < 25 mm and S ≥ 26 mm resulted in a sensitivity of 100% and a specificity of 98% (data not shown).

Discussion

Staphylococci, especially *S. aureus*, are among the most common causes of nosocomial as well as community-acquired infections. Methicillin resistance (as a result of meca gene encoding the additional penicillin binding protein, PBP2a) renders *S. aureus* resistant to all β-lactam antibiotics, the most important group of antibiotics in the treatment of staphylococcal infections. Accurate and rapid detection of methicillin resistance in staphylococci is therefore important, not only for choosing appropriate antibiotic therapy for the individual patient, but also for control of the endemicity of MRSA. In areas where the spread of MRSA is still combated successfully, the speed and accuracy of detection has implications for hospital infection control.

The present study showed how well the cefoxitin 30 μg disc performed on ISA using standard incubation conditions, with a sensitivity of 100%, a specificity of 99% and an interpretive zone diameter of S ≥ 29 and R < 29 mm, thereby confirming the results obtained by Felten et al. on Mueller–Hinton agar. The small difference in the zone diameter ranges in the two studies could be related both to the difference in agar type and to the inclusion of many different (low-level resistant) isolates in this study. The latter is underlined by the fact that 22% of the MRSA went undetected by the screening method currently recommended by the SRGA. A further advantage of the
cefoxitin screening test is that it eliminates the need for separate inoculum, media, and incubation time and temperature. The cost and workload is thereby reduced.

Since our results were obtained on the medium and with the inoculum recommended by the BSAC Working Party on Antimicrobial Sensitivity Testing for *S. aureus* against other antibiotics, this method would fit the BSAC recommendations. The reliability of our findings and conclusions is strengthened both by the large number of MRSA PFGE types included in the study and by the use of very heterogeneous isolates. The results of testing consecutive blood culture isolates indicate that routine MSSA will rarely exhibit zones smaller than the proposed interpretive zone diameter of R < 29 mm. This is especially important in countries with a very low prevalence of MRSA, as is the case in Scandinavia, where it has been <1% for many years (national mandatory reporting systems).

One disadvantage of the cefoxitin method is that the zones reached are large, even for resistant isolates, and this could interfere with the inhibition zones of adjacent discs, at least when using the standard 9 cm agar plate. Furthermore, the gap between the inhibition zones of isolates with and without the mecA gene is very narrow (Figure 1).

We are therefore in the process of investigating discs with a lower cefoxitin content. This should establish whether these problems can be solved by reducing the zone sizes and by introducing a larger difference in zone diameter between MRSA and methicillin-susceptible isolates. These studies will also reveal whether the cefoxitin disc test can be used for coagulase-negative staphylococci.

In conclusion, a cefoxitin 30 µg disc on ISA, using a semi-confluent inoculum and overnight incubation at 35–36°C, performed significantly better than screening methods used hitherto for the detection of MRSA. In our hands, in two different laboratories, interpretive zone diameter breakpoints of S ≥ 29 mm and R < 29 mm resulted in a sensitivity of 100% and a specificity of 99%, despite the fact that difficult *S. aureus* strains were used in the study. We suggest that this method replaces that currently recommended by the SRGA for detection of methicillin resistance in *S. aureus*.

### References

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![Graph showing zone diameters of an oxacillin 1 µg disc against 457 S. aureus using the methodology recommended by the SRGA. A vertical line marks the present interpretive zone diameter for susceptibility. Black bars, mecA positive; white bars, mecA negative.]


