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Detection of *mecA*-mediated resistance using reference and commercial testing methods in a collection of *Staphylococcus aureus* expressing borderline oxacillin MICs☆

Jana M. Swenson*, David Lonsway, Sigrid McAllister, Angela Thompson, Laura Jevitt, Wenming Zhu, Jean B. Patel

Epidemiology and Laboratory Branch, Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA

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Abstract

Phenotypic methods for detecting *mecA*-mediated resistance in *Staphylococcus aureus* include both oxacillin and cefoxitin susceptibility tests; many laboratories perform multiple tests. Conflicting oxacillin and cefoxitin susceptibility results are most likely to occur for isolates that either have reduced susceptibility to oxacillin by a non–*mecA*-mediated mechanism or are *mecA* positive but are very heteroresistant. To understand the performance of oxacillin and cefoxitin tests for such isolates, we tested 135 *S. aureus* isolates using either cefoxitin or oxacillin and compared the results with *mecA* polymerase chain reaction. These strains either expressed borderline oxacillin MICs (1–4 μg/mL) and had undetermined *mecA* status or were *mecA* positive but were not detected by oxacillin broth microdilution (BMD) or disk diffusion (DD) in original testing. For 24-h readings, performance of cefoxitin tests (sensitivity/specificity) were DD (99/100), Etest using ≤6 μg/mL as susceptible (99/98), and Phoenix MIC using ≤4 μg/mL as susceptible (98/100). Using 6 μg/mL of cefoxitin as a screen test in both BMD and agar dilution also worked well (98/98—100). Sensitivity/specificity of oxacillin methods were oxacillin agar screen (BBL: 80/86; Remel, Lenexa, KS: 85/50), DD (91/59), BMD (85/88), MicroScan (89/96), VITEK Legacy (82/93), VITEK 2 (91/73), and Phoenix, (67/96). These results suggest that a cefoxitin test can be used alone to predict *mecA*-mediated resistance in *S. aureus*.
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Keywords: Cefoxitin; *mecA*; *Staphylococcus aureus*; Oxacillin

1. Introduction

Oxacillin resistance in *Staphylococcus aureus* is most commonly mediated by PBP2a encoded by *mecA*. Some isolates with *mecA* are heteroresistant and may be phenotypically susceptible to oxacillin. However, even low-level *mecA*-mediated resistance is likely to be clinically relevant because exposure of such isolates to β-lactams can result in high-level resistance (Chambers et al., 1985). Conversely, non-*mecA* mechanism such as increased β-lactamase production or changes in native penicillin-binding proteins (PBPs) can cause low-level oxacillin resistance. The clinical significance of non–*mecA*-mediated oxacillin resistance remains unclear (Chambers, 1997).

There are many tests available to laboratories for detecting oxacillin resistance. These include oxacillin tests such as disk diffusion (DD), automated susceptibility testing systems, and oxacillin agar screen plate. In addition, the cefoxitin DD test was recently recommended by the Clinical and Laboratory Standards Institute (CLSI) for prediction of *mecA*-mediated resistance in *Staphylococcus* spp. because it performs as well as reference broth microdilution (BMD) for *S. aureus* but better for coagulase-negative staphylococci and is easier to read (Swenson et al., 2005). Finally, there are also *mecA*-specific tests such as *mecA* polymerase chain

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☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

* Corresponding author. Tel.: +1-404-639-0196; fax: +1-404-639-1381.

E-mail address: jswenson@cdc.gov (J.M. Swenson).

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reaction (PCR) and PBP2a latex agglutination test to detect resistance. Many laboratories are performing multiple tests. For example, a laboratory may test oxacillin susceptibility by an automated system and also perform the cefoxitin disk test. Conflicting laboratory results (e.g., oxacillin resistance and cefoxitin susceptibility) can create confusion about what should be reported to clinicians. The isolates most likely to produce conflicting results are heteroresistant *mecA*-positive isolates and isolates with non-*mecA* mechanisms that result in low-level oxacillin resistance.

To understand the performance characteristics of commonly used susceptibility testing methods for oxacillin borderline and heteroresistant isolates, we compared currently available phenotypic susceptibility methods with *mecA* PCR. A collection of 135 isolates was tested. These isolates either expressed borderline oxacillin MICs (1–4 μg/mL) and were selected without prior knowledge of *mecA* status or were *mecA* positive and missed by a CLSI reference method, that is, oxacillin BMD or DD. The methods evaluated included oxacillin susceptibility by BMD, DD, 5 commercial susceptibility testing systems, and oxacillin screen plate. In addition, we evaluated 5 additional cefoxitin susceptibility testing methods, including the cefoxitin disk test, BMD, Etest, an automated system, and a cefoxitin agar screen plate.

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2. Materials and methods

2.1. Bacterial strains and study design

Isolates of *S. aureus* were selected from the Centers for Disease Control and Prevention culture collection if they met 1 or more of 3 criteria: i) strains for which the oxacillin MICs were 1 to 4 μg/mL without knowledge of *mecA* status, or ii) strains for which the oxacillin MICs were interpreted as susceptible but for which the oxacillin DD results were interpreted as intermediate or resistant, or iii) strains for which the oxacillin MICs were interpreted as susceptible but which carried *mecA*. A total of 135 strains met these criteria and were included in the study. The mechanism of resistance for some of the isolates tested was not determined before inclusion in the study. All strains were subcultured twice from frozen storage before testing. Multiple plates were prepared from a single colony pick of the initial subculture so that all testing could be done by several persons on the same day. Testing of all methods and systems was done from the 2nd plate streaked from the freezer.

2.2. Reference susceptibility test methods

All strains were tested by CLSI BMD (CLSI/National Committee for Clinical Laboratory Standards [NCCLS]) and DD (CLSI/NCCLS) methods using cation-adjusted Mueller–Hinton broth (CAMHB; Difco, BD, Sparks, MD) and Mueller–Hinton agar (BBL MH II, BD) and by the oxacillin salt agar screen (CLSI/NCCLS) using media purchased from 2 manufacturers (BD and Remel, Lenexa, KS) (CLSI/NCCLS, 2003a, 2003b). For BMD testing, plates were prepared in house using oxacillin and cefoxitin powder from Sigma-Aldrich, St. Louis, MO. For DD testing, oxacillin 1-μg and cefoxitin 30-μg disks were used. For cefoxitin susceptibility by BMD, plates contained CAMHB with and without 2% NaCl. For cefoxitin agar screen testing, plates were prepared by Remel using Mueller–Hinton agar with and without 4% NaCl supplementation. The oxacillin and cefoxitin agar screen plates were inoculated using both a 1-μL loop and a cotton-tipped swab. All tests were incubated at 35 °C and read after 18 and 24 h of incubation.

2.3. Commercial susceptibility test methods

Commercial methods (card or panel and agent tested) that were evaluated were Etest (cefoxitin; AB Biodisk, Solna, Sweden), MicroScan Walkaway (Pos MIC Type 20A, oxacillin; Dade Behring, West Sacramento, CA), Phoenix (PMIC/ID-25, oxacillin and cefoxitin; BD), VITEK Legacy (GPS 109, oxacillin; bioMérieux, Durham, NC), and VITEK 2 (AST-GP55 or AST-GP61, oxacillin; bioMérieux). All commercial methods were performed following the manufacturer's instructions. The inoculum for the MicroScan system was prepared using the BBL Prompt System (BD).

2.4. mecA PCR and sequence analysis

Cell extracts for *mecA* detection were prepared using the Beadbeater (Biospecs Products, Bartlesville, OK). Specifically, a 1.25-mL light suspension of the test isolate (cell paste picked up by a 1-μL plastic disposable loop) in modified 0.01 mol/L TE buffer, pH 8.0 (Tris–HCl 0.01 mol/L and ethylenediaminetetraacetic acid 0.0001 mol/L), and 1 mL of 0.1-mm glass beads was processed in the Beadbeater for 1 min. The extracts were centrifuged and the supernatant removed and stored at −20 °C before PCR testing. The *mecA* gene was detected by a real-time PCR as described by Killgore et al. (2000) with the following modifications: 2 μL of the cell extract was used in the reaction, and the reactions were performed in the Mx 3000P real-time PCR instrument (Stratagene, La Jolla, CA). The sequence of *mecA* from isolate BS-89 was determined as previously described (Bressler et al., 2005).

2.5. Resolution of discrepancies

Any strain for which there was a very major or major error (see definition hereinbelow) for oxacillin susceptibility by both BMD and more than 1 of the following when compared with the presence of *mecA* was retested: cefoxitin DD, MicroScan, Phoenix, VITEK Legacy, or VITEK 2. Strains with very major errors (n = 18), that is, *mecA*-positive strains reported as oxacillin susceptible, were retested using both uninduced growth and growth induced with cefoxitin (overnight growth from around a 30-μg