

# Detection of Oxacillin Resistance in *Staphylococcus aureus*: Comparison of Phoenix Oxacillin and Cefoxitin MICs, MicroScan Oxacillin MIC, Oxacillin and Cefoxitin Disk Diffusion, and *mecA* Gene Detection.

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## ABSTRACT

**BACKGROUND.** Phenotypic tests for detection of *mecA* containing staphylococci have been challenged. Cefoxitin (FOX) disk diffusion (DD) has been reported to more accurately predict *mecA* gene presence than oxacillin (OX) DD. The Phoenix instrument rapidly provides identification and susceptibility data and it is of interest to determine if an experimental panel containing OX and FOX could accurately detect OX resistance.

**METHODS.** *S. aureus* (n300) strains were tested by OX and FOX DD, MicroScan OX (range 0.25 – 2.0 µg/mL) MIC, and Phoenix investigational panels containing OX (0.25– 4.0 µg/mL) and FOX (1.0 – 64 µg/mL). Testing for *mecA* gene by real-time PCR was performed on 78 isolates, including all organisms for which any of the 5 phenotypic test results were not in agreement.

**RESULTS.** The results for 20/300 (6.7%) OX DD were intermediate or resistant and discordant from other phenotypic tests and *mecA* testing. Initially, the OX MIC category agreement was 294/300 between Phoenix and MicroScan. Repeat testing of the six discordant strains resulted in 3 MicroScan changes and 2 Phoenix changes resulting in category agreement with *mecA* results. Final agreement for OX MIC was 299/300 (99.7%). Results for one strain changed in both systems, so they remained discordant. This isolate was *mecA* positive. The OX DD was 6 mm and the FOX DD was 13 mm with discreet colonies within the zone. The Phoenix FOX MIC was 16 µg/mL and the Phoenix flagged this isolate as MRSA. Other than this isolate, there was 100% agreement with OX MIC  $\geq$  4.0 µg/mL and FOX MIC  $\geq$  16 µg/mL. Utilizing *mecA* as the gold standard, the category agreement for the 5 tests are:

Oxacillin disk	93.3%
Cefoxitin disk	99.7%
MicroScan oxacillin MIC	99.7%
Phoenix oxacillin MIC	99.7%
Phoenix Cefoxitin MIC	100.0%

**CONCLUSION.** The Phoenix OX or FOX MIC, the MicroScan OX MIC, and the FOX disk test are reliable for screening the *mecA* production in *S. aureus*. The OX disk test over called OX resistance.

## INTRODUCTION

*S. aureus* strains possessing *mecA* produce an altered penicillin binding protein 2 (PBP2) and this altered enzyme, with reduced affinity for beta lactam drugs, is referred to as PBP2a. Strains carrying the *mecA* gene may express it homogeneously with all cells in the population being resistant or other strains express it heterogeneously with only a few cells producing sufficient PBP2a to be resistant. The Clinical and Laboratory Standards Institute (CLSI) states that the oxacillin MIC and cefoxitin disk test are equivalent in sensitivity and specificity for detection of *mecA* mediated resistance in *Staphylococcus aureus* (1). This study evaluated the Phoenix Automated Microbiology System (BD Diagnostics, Sparks, MD) oxacillin and cefoxitin MIC for sensitivity and specificity in detection of oxacillin resistance among 300 recent clinical isolates of *S. aureus*. Results of the Phoenix oxacillin and cefoxitin MICs were compared to MicroScan oxacillin MIC measured after 24 hours incubation and oxacillin and cefoxitin disk diffusion tests. When results of all five phenotypic methods were not in agreement, *mecA* detection by PCR was performed and results of this methodology were considered the gold standard.

## MATERIALS AND METHODS

**Bacterial strains.** Isolates of *S. aureus* (n=300) collected from December, 2004 to March 2005 were tested in this study. No repeat isolates from the same patient were included. Isolates were collected from the specimens submitted from the eight hospitals comprising the Detroit Medical Center (DMC) and from Outreach specimens.

**Testing.** Disk testing was performed as recommended for staphylococci by CLSI (1). Strains were considered susceptible to oxacillin if the zone of inhibition around the 1 µg disks was ≥ 13 mm, intermediate if 11 – 12 mm, and resistant if the zone was ≤ 10 mm. Cefoxitin 30 µg disks were considered susceptible if the zone of inhibition was ≥ 20 mm, resistant if the zone was ≤ 19 mm (1). MIC testing was performed in Phoenix experimental panels containing oxacillin and cefoxitin. The susceptibility testing method is a broth based microdilution test that utilizes a redox indicator to enhance the detection of organism growth. Panels are read at 20 minute intervals by the instrument. The MicroScan panels were dried panels which were rehydrated with inoculums and incubated at 35°C for 24 hours before reading. Both systems were processed according to the manufacturers' instructions and oxacillin results interpreted as susceptible if the MIC was ≤ 2 µg/mL and resistant if the MIC was ≥ 4 µg/mL. The cefoxitin MIC breakpoints were determined by correlating MIC values with results from other testing results. Disk and MicroScan results were recorded after 24 hour incubation and Phoenix results were instrument read after 4 to 16 hours incubation.

**PCR procedure.** The *Staphylococcus aureus* isolates were grown on blood agar plates for 18 hours. A single colony from each plate was used to inoculate 5 mL of trypticase soy broth. After 18 hours of incubation at 37°C, 1.8 mL of each liquid culture was used for preparation of genomic DNA. A Gram stain was performed to check the purity of liquid culture. The isolation of nucleic acids from liquid culture was performed using UltraClean Microbial DNA Isolation Kit (Mo BIO laboratories, Inc.), based on manufacturers' recommendation. The nucleic acids from each isolate were eluted in 50 µL of elution buffer and kept at -20°C until tested. Primers and probes for the real-time *mecA* gene PCR were purchased from TIB Mol Biol. The LightCycler Fast Start DNA Master Hybridization Kit was purchased from Roche and used in PCR assay as recommended by the manufacturer. The PCR was performed using the Roche LightCycler with a total reaction volume of 20 µL. The *Staphylococcus aureus* ATCC™ 43300 was used as positive control for the *mecA* gene in the real-time PCR assay.

## RESULTS

The 300 *S. aureus* isolates included in the study had initial MICs of ≤ 0.25 to >2.0 mcg/mL as shown in Table 1. Susceptible strains comprised 64.7% of the strains (n=194) with 24% (n=72) having an MIC of 0.5 to 2.0 mcg/mL.

Results of the 5 phenotypic tests were considered concordant if CLSI recommended category interpretations were in agreement. Strains for which all 5 tests did not agree had a *mecA* test performed and this genetic test was considered correct.

Of the 300 strains tested, 274 were concordant in all five phenotypic tests with CLSI category interpretations. The oxacillin disk diffusion results from twenty strains were discordant with the other tests. For these 20 strains, the disk interpretation was intermediate or resistant but the other tests were interpreted as susceptible, including being *mecA* negative. The initial testing resulted in 98% (294/300) agreement between the Phoenix and MicroScan oxacillin MIC interpretations. The six isolates were re-tested in each system with three MicroScan values changing so that interpretations agreed with Phoenix results. Likewise, two isolates had different Phoenix results which now agreed with the MicroScan results. The final repeat results for all five isolates were in agreement with the *mecA* presence. Repeat testing on the sixth isolate resulted in a category change in both systems so they still disagreed. This isolate was *mecA* positive by PCR. The 300 cefoxitin disk tests correctly identified oxacillin interpretations.

A selection of 78 isolates, including all those for which the phenotypic tests were not in agreement, were tested for *mecA*. As shown in Table 2, there is a direct correlation of presence of *mecA* and cefoxitin MIC ≥ 16 mcg/mL. All strains with an MIC of 4 mcg/ml were *mecA* negative.

## REFERENCES

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Table 1. Distribution of oxacillin MICs of the 300 *S. aureus*.

MIC (mcg/mL)	Number of <i>S. aureus</i>
0.25	122
0.50	56
1.00	15
2.00	1
>2.00	106

Table 2. Correlation of detection of *mecA* gene and cefoxitin MIC for 78 *S. aureus*.

Cefoxitin MIC	No. of isolates	No. of <i>mecA</i> positive	No. <i>mecA</i> Negative
4	71	0	71
8	0	0	0
16	1	1	0
32	2	2	0
64	0	0	0
>64	4	4	0

## DISCUSSION

Poor expression of *mecA* gene in some *S. aureus* presents a challenge to the clinical microbiology laboratory since these strains may appear susceptible in routine antimicrobial testing. Several testing modifications have been introduced to increase the *mecA* gene expression such as testing at lower temperature and increasing the salt concentration. It has been appreciated for some time that the oxacillin disk diffusion has a poor sensitivity. Furthermore, the cephamycin, cefoxitin, has been found to have greater sensitivity in the disk diffusion test than oxacillin and to correlate to oxacillin MIC for *S. aureus*.

Our results confirm the superiority of cefoxitin for screening for *mecA* presence. In this report, cefoxitin disk diffusion correctly

detected all oxacillin resistance and was also 100% specific. The major goal of this study was to investigate if cefoxitin MIC results could also accurately measure oxacillin resistance or susceptibility. By using  $\geq 16$  mcg/mL, the cefoxitin MIC also was 100% sensitive and specific in detecting oxacillin resistance in the 300 strains included in this study. Although the oxacillin MIC was correct on initial testing 297/300 (99%), the cefoxitin MIC at 16 mcg/mL breakpoint was 100%.

In conclusion, cefoxitin disk diffusion and MIC ( $\geq 16$  mcg/mL) were 100% sensitive and specific in interpretation of oxacillin antimicrobial interpretation. The oxacillin MicroScan and Phoenix MIC were 99% correct while the oxacillin disk was 94%.

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