# Agar dilution and agar screen with cefoxitin and oxacillin: what is known and what is unknown in detection of meticillin-resistant *Staphylococcus aureus*

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In this study we evaluated the performance of the oxacillin agar screen test, and agar dilution tests using cefoxitin and oxacillin antimicrobials, to detect meticillin resistance in *Staphylococcus aureus* isolates. The presence of the *mecA* gene, detected by PCR, was used as the standard to which agar screen and agar dilution tests were compared. The best performance was obtained using the agar dilution test (99.4 % accuracy) with breakpoints of 4  $\mu$ g ml<sup>-1</sup> for oxacillin and 8  $\mu$ g ml<sup>-1</sup> for cefoxitin, and using the oxacillin agar screen test. Also, a strong correlation between MIC values of cefoxitin and oxacillin permits the use of either drug for detection of meticillin resistance.

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# INTRODUCTION

Meticillin-resistant *Staphylococcus aureus* (MRSA) remains the leading causative agent of hospital-acquired infections. In Brazil, about 40–60% of hospital staphylococcal infections (respiratory tract, urinary tract, blood, surgical wound, invasive infections and others) are caused by MRSA (Teixeira *et al.*, 1995).

Meticillin-resistance is attributable to the *mecA* gene, encoding penicillin-binding protein (PBP) 2a, which presents low affinity for  $\beta$ -lactam antimicrobials (Fuda *et al.*, 2004). Heterogeneous resistance to meticillin occurs among *S. aureus* isolates due to variations in the expression of the *mecA* gene, or alteration of constitutive PBPs (Chambers, 2001). The Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) criteria have recently been modified to better define meticillin resistance (CLSI, 2006b).

The detection of the meticillin resistance represents a real challenge for the routine clinical microbiology laboratories since molecular methods, the gold standard, are not available for most medical institutions in Brazil. Thus, phenotypic methods for characterization of the resistance to meticillin are frequently evaluated (Skov *et al.*, 2003; Cauwelier *et al.*, 2004; Fernandes *et al.*, 2005; Pottumarthy

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MRSA, meticillin-resistant *S. aureus*; PBP, penicillin-binding protein.

*et al.*, 2005; Sharp *et al.*, 2005; Swenson & Tenover, 2005; Velasco *et al.*, 2005).

This study was designed to evaluate the performance of the oxacillin agar screen and the MIC values of oxacillin and cefoxitin antimicrobials as determined by agar dilution test, to predict resistance among *S. aureus* harbouring the *mecA* gene.

### METHODS

**Bacterial isolates.** From August to December 2002, 167 *S. aureus* isolates were obtained from blood cultures of patients hospitalized at three hospitals in Porto Alegre, RS, Brazil. Only one isolate from each patient was included in the study.

**Oxacillin agar screen.** This test was carried out according to the CLSI guidelines (CLSI, 2006b). A McFarland 0.5 suspension was spotted onto Mueller–Hinton agar (Becton Dickinson) containing 4% (w/v) NaCl and 6 µg oxacillin ml<sup>-1</sup>, and incubated at 35 °C for 24 h.

**Determination of MIC.** Susceptibility to oxacillin and cefoxitin was evaluated by the agar dilution method according to CLSI guidelines (CLSI, 2006a). The oxacillin and cefoxitin concentrations used ranged from 0.5 to 256  $\mu$ g ml<sup>-1</sup>. Mueller–Hinton agar plates without antimicrobial were used as controls of bacterial growth.

**Detection of the mecA gene by PCR.** PCR was used to confirm the presence of the *mecA* gene. Primers  $mecA_1$  (5'-TGG CTA TCG TGT CAC AAT CG) and  $mecA_2$  (5'-CTG GAA CTT GTT GAG CAG AG)

were used to amplify a 310 bp segment of the gene; these were electrophoresed in a 1.5% agarose gel and visualized under UV light by the addition of ethidium bromide (0.5  $\mu$ g ml<sup>-1</sup>) (Vannuffel *et al.*, 1998).

**Control strains.** Meticillin-susceptible *S. aureus* (MSSA) ATCC 29213 and MRSA ATCC 33591 strains were used as controls in susceptibility tests and PCRs.

**Statistical parameters.** The concordance between MIC values of oxacillin and cefoxitin was calculated by Pearson's correlation coefficient (r). The following value and correlation criteria were adopted: 0, null; 0–0.3, weak; 0.3–0.6, regular; 0.6–0.9, strong; 0.9–1, very strong; 1, full or absolute.

## **RESULTS AND DISCUSSION**

Presence of the mecA gene was observed in 41.1 % (69/167) of the S. aureus isolates in this study. Since 2004, the CLSI guidelines have recognized the use of cefoxitin, a cephamycin, as a surrogate marker of meticillin resistance. However, the agar dilution method is only standardized for oxacillin; S. aureus isolates are characterized as resistant and susceptible with MIC  $\ge 4 \ \mu g \ ml^{-1}$  and  $\le 2 \ \mu g \ ml^{-1}$ , respectively (CLSI, 2006b). Using these breakpoints, one isolate was mischaracterized as resistant (mecA-negative with an MIC of  $4 \mu g$  oxacillin ml<sup>-1</sup>) (Table 1). For cefoxitin, the best results were obtained with breakpoints of  $\leq 4 \ \mu g \ ml^{-1}$  and  $> 4 \ \mu g \ ml^{-1}$  for susceptible and resistant, respectively (only one false-resistant isolate), with 99.4 % accuracy. Skov et al. (2006) used a highly diverse collection of S. aureus isolates to perform a comprehensive investigation on test conditions to accurately define MRSA, and suggested interpretative criteria of  $\leq 4 \ \mu g$  cefoxitin ml<sup>-1</sup> and >4  $\mu$ g cefoxitin ml<sup>-1</sup> for susceptible and resistant, respectively. However, Skov et al. (2006) used the Etest to determine MIC, and the breakpoint of  $\leq 4 \ \mu g \ ml^{-1}$  and  $>4 \ \mu g \ ml^{-1}$  resulted in one *mecA*-positive isolate being misclassified as susceptible. The breakpoint established in the CLSI guidelines for susceptibility testing using cefoxitin  $(\leq 8 \ \mu g \ ml^{-1}, 16 \ \mu g \ ml^{-1} \text{ and } \geq 32 \ \mu g \ ml^{-1} \text{ for suscept-}$ ible, intermediate and resistant, respectively) presented low sensitivity for mecA gene detection and must not be utilized for this purpose (Table 1); this was also evident in the study by Skov et al. (2006).

The oxacillin agar screen test showed 98.5%(68/69), 100%(98/98) and 99.4%(166/167) sensitivity, specificity and accuracy, respectively; it is therefore highly reliable in discriminating isolates harbouring the *mecA* gene (only one false-susceptible result was obtained).

A potential limitation of this study lies in the fact that the genetic background of the isolates is not clearly defined. In Brazil, a specific clone predominates among MRSA (Amaral *et al.*, 2005), and at least one additional distinct clone was observed when nine randomly selected isolates were submitted to PFGE analysis after restriction with *SmaI* (data not shown). The significance of our findings may therefore be limited by possible over-representation of a few MRSA clones.

 Table 1. Detection of meticillin resistance among S. aureus

 isolates by presence of the mecA gene, and MIC of cefoxitin

 and oxacillin

Breakpoint (μg ml <sup>-1</sup> )*	Cefoxitin		Oxacillin	
	mecA- negative† (n=98)	mecA- positive† (n=69)	mecA- negative† (n=98)	mecA- positive† (n=69)
≤0.25	0	0	15	0
0.5	1	0	12	0
1	23	0	36	0
2	68	0	34	0
4	5	0	1	0
8	1	6	0	7
16	0	7	0	2
32	0	3	0	1
64	0	3	0	22
128	0	34	0	19
≥256	0	16	0	18

\*Cefoxitin and oxacillin MICs were determined by an agar dilution method (CLSI, 2006a).

†Presence of mecA was determined by PCR.

The Pearson's correlation coefficient (Fig. 1) shows that there is a strong association between cefoxitin and oxacillin MIC values (r=0.73). This means that the expression of resistance or susceptibility is comparable for the two antimicrobial agents when the same technique and conditions are adopted. Either drug may be used as a surrogate for testing meticillin resistance when an adequate methodology is used.

Finally, our results showed that the best method for predicting resistance mediated by the *mecA* gene was obtained by the agar dilution test using oxacillin (breakpoint of 4  $\mu$ g ml<sup>-1</sup>), cefoxitin (breakpoint of 8  $\mu$ g ml<sup>-1</sup>) or the oxacillin agar screen (99.4 % accuracy).



**Fig. 1.** Correlation between cefoxitin and oxacillin MIC values of *S. aureus*. MIC values were obtained by agar dilution testing of *S. aureus* strains as described by CLSI (2006a). Pearson's correlation coefficient was used to determine concordance between these two methods.

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