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Evaluation of Vitek GPS-SA Card for Testing of Oxacillin against Borderline-Susceptible Staphylococci That Lack *mec*

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Fifty-one *Staphylococcus aureus* strains lacking *mec* for which oxacillin MICs were 1 to 8 μg/ml were tested against oxacillin and the combination of oxacillin and clavulanic acid with the Vitek GPS-SA card, the reference broth microdilution method, and the oxacillin agar screen plate. Of the 51 strains, 44 (86%) did not grow on the oxacillin agar screen plate, broth microdilution MICs were 1 to 2 μg/ml, and GPS-SA card MICs were ≤2 μg/ml, with the exception of 3 strains that failed to grow in the card on repeated attempts. Another seven strains did grow on the oxacillin agar screen plate. For four of the latter group of strains, oxacillin broth microdilution MICs were >4 μg/ml and GPS-SA card MICs were ≥4 μg/ml; for the other 3 strains, corresponding MICs were 4 and ≤2 μg/ml, respectively. The GPS-SA card classified 86% of strains as oxacillin susceptible.

The distinction between oxacillin-susceptible and oxacillin-resistant staphylococci continues to pose a challenge for the clinical laboratory. Although homogeneously resistant isolates can generally be accurately classified by routine susceptibility testing procedures, heterogeneously resistant isolates belonging to the class 1 or 2 phenotype described by Tomasz et al. (11) remain problematical. Several studies have shown substantial false susceptibility rates by the disk diffusion test with phenotypic expression class 1 and 2 oxacillin-resistant staphylococci (2, 4, 5). False susceptibility was not, however, a serious problem when such isolates were tested by Knapp et al. (5) in the Vitek GPS-SA card.

One unresolved issue in the study by Knapp et al. (5) was the ability of the Vitek GPS-SA card to correctly classify staphylococci that lack *mec* and are borderline susceptible to oxacillin. Studies of such isolates by Gerberding et al. (3) and Unal et al. (12) have shown discrepancies among disk diffusion, microdilution, and oxacillin agar screening test results.

The purpose of this study was to evaluate the performance characteristics of the Vitek GPS-SA card with a well-characterized collection of clinical isolates of oxacillin borderline-susceptible *Staphylococcus aureus.*

MATERIALS AND METHODS

Clinical isolates. A total of 51 clinical isolates of *S. aureus* lacking *mec* were studied. Of the 51 isolates, 40 were from patients at the Cleveland Clinic Foundation and 11 were from the Centers for Disease Control and Prevention (kindly supplied by Carolyn Baker). The clinical isolates were originally selected on the basis of oxacillin MICs of 1 to 8 μg/ml.

Susceptibility tests. MICs were determined by the broth microdilution method as recommended by the National Committee for Clinical Laboratory Standards (8) using cation-adjusted Mueller-Hinton broth supplemented with 2% NaCl and the direct colony suspension method. The inoculum size was adjusted so as to deliver a final inoculum of approximately 5×10^5 CFU/ml. MICs were determined after a full 24 h of incubation. Oxacillin was tested at concentrations ranging on a $\log_2$ scale from 0.5 to 4 μg/ml. The combination of oxacillin and clavulanic acid was tested at concentrations ranging on a $\log_2$ scale from 0.25-0.12 to 4-2 μg/ml. Penicillin was tested in concentrations ranging on a $\log_2$ scale from 0.06 to 0.5 μg/ml. Each isolate was also inoculated on an oxacillin agar screen plate with Mueller-Hinton agar containing 6 μg of oxacillin per ml and 4% NaCl as recommended by the National Committee for Clinical Laboratory Standards (8). Finally, each isolate was inoculated into a GPS-SA card and processed according to the manufacturer's instructions.

***mec* probe.** All isolates were tested at the University of California at San Francisco with a *mec* probe according to the method described by Gerberding et al. (3). The *mec* probe was directed at the 1.1-kb internal *Bgl*I-*Xba*I region of the gene.

Statistical analysis. The microdilution test was used as the reference method with which to compare the GPS-SA card MIC results and was interpreted on the basis of established criteria (8). By using the broth microdilution method as the reference method, an essential agreement (EA) value was calculated by the following formula:

$$\mathrm{EA} = \frac{n - (\mathrm{VM} + \mathrm{M}) \times 100}{n}$$

where VM is the number of very major errors, M is the number of major errors, and n is the number of isolates tested.

RESULTS

All isolates lacked *mec*. Microdilution MICs of oxacillin for all ranged from 1 to 8 μg/ml. Of the 51 isolates, 44 demonstrated no growth on the oxacillin agar screen plate (Table 1). Three of the 44 agar screen-negative isolates failed to grow on repeated attempts in the GPS-SA card. Of the remaining 41 oxacillin agar screen-negative isolates, microdilution MICs for 40 were 1 to 2 μg/ml, and the oxacillin MIC for 1 was 4 μg/ml (Table 1). GPS-SA MICs for all 41 isolates were ≤2 μg/ml (Table 1). Of the seven oxacillin agar screen-positive isolates, oxacillin microdilution MICs were 8 μg/ml and GPS-SA MICs were ≥4 μg/ml for four; oxacillin microdilution MICs were 4 μg/ml and GPS-SA MICs were ≤2 μg/ml for another three isolates (Table 1). Only 8% of the isolates were susceptible to penicillin (i.e., MICs were ≤0.12 μg/ml). Oxacillin MICs in the presence of clavulanic acid remained unchanged for 13 isolates and decreased by 1 $\log_2$ dilution for 32 isolates, by 2 $\log_2$

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TABLE 1. Comparison of oxacillin MICs of borderline-susceptible strains of *S. aureus* lacking *mec* classified according to oxacillin agar screen results

Result on oxacillin agar screen	No. of strains	MIC[a] (no. of strains[b])	
		Microdilution	GPS-SA
No growth	41[c]	1–2 (40) 4 (1)	≤2 (41)
Growth	7	8 (4) 4 (3)	≥4 (4) ≤2 (3)

[a] Expressed in micrograms per milliliter.
[b] Numbers in parentheses represent number of strains for which the MIC was as specified.
[c] An additional three strains failed to grow in the GPS-SA card.

dilutions for a single isolate, and by 4 $\log_2$ dilutions for 5 isolates. The oxacillin MICs obtained by microdilution were 2 μg/ml for four of the latter five isolates and 4 μg/ml for the fifth isolate, and all the oxacillin MICs decreased to 0.5 μg/ml in the presence of clavulanic acid. The oxacillin MICs were ≤2 μg/ml for all five of these isolates in the GPS-SA card. Only one of these isolates grew on the oxacillin agar screen plate.

Compared with microdilution, there was 94% essential agreement with the oxacillin agar screen results and 92% essential agreement with the GPS-SA results.

DISCUSSION

Borderline susceptibility of *S. aureus* to oxacillin has been ascribed to hyperproduction of β-lactamase or decreased affinity of normal penicillin-binding proteins for oxacillin or both (1, 7, 9, 10, 12, 13). Hyperproduction of β-lactamase appeared to be necessary for the expression of borderline susceptibility within certain phage group 94/96 strains studied by Barg et al. (1); however, the production of comparable amounts of β-lactamase by transformants belonging to other phage groups did not confer borderline susceptibility to oxacillin. Thus, mechanisms other than hyperproduction of β-lactamase appear to play a role in the expression of borderline susceptibility. Regardless of the mechanism(s) leading to reduced oxacillin susceptibility of *S. aureus*, there appears to be sufficient clinical evidence to suggest that such isolates can be considered susceptible to oxacillin (1, 13). It is therefore important to distinguish between borderline-susceptible isolates and those isolates of *S. aureus* that are heterogeneously resistant to oxacillin in the clinical laboratory.

Until practical and inexpensive tests become available for the detection of the *mecA* gene or PBP 2a, the clinical laboratory must rely on conventional susceptibility tests for the identification of borderline-susceptible isolates of *S. aureus*. In contrast to our experience, a high rate of false resistance to oxacillin was encountered in a commercially available microdilution system by Ünal et al. (12). The majority of falsely resistant isolates in their study were hyperproducers of β-lactamase lacking *mecA*, and a fourfold or greater decrease in oxacillin MICs to concentrations of ≤1 μg/ml occurred in the presence of sulbactam. There was a fourfold decrease in oxacillin MICs in the presence of clavulanic acid for only five of our isolates. Disk diffusion testing and the oxacillin agar screen (supplemented with 4% NaCl) correctly identified the susceptibilities of the 13 isolates lacking *mecA* studied by Unal et al. (12).

Gerberding et al. (3) encountered discrepancies between disk diffusion, microdilution, and oxacillin agar screen results in a study of borderline-susceptible strains of *S. aureus* that had been classified genotypically as lacking *mec*. They also studied the oxacillin agar screen with and without 4% NaCl supplementation and inocula of 10^4 and 10^7 CFUs. Maximal sensitivity and specificity for correctly classification of borderline-susceptible strains were obtained with the lower inoculum on unsupplemented agar. We used the procedure recommended by the National Committee for Clinical Laboratory Standards (8) in which the inoculum from a cotton-tipped swab dipped into a suspension equivalent in density to a 0.5 McFarland standard is applied to NaCl-supplemented agar. Under these test conditions 7 (13.7%) of our 51 isolates were misclassified as oxacillin resistant. We did not study the NaCl-unsupplemented oxacillin agar screen.

The GPS-SA card correctly classified as susceptible all but 4 of the 48 isolates that grew in the card (3 isolates failed to grow on repeated attempts). These same four isolates lacking *mec* were resistant in the microdilution test and were oxacillin agar screen positive.

The major challenge for the clinical laboratory is the differentiation between borderline oxacillin-susceptible and phenotypic expression class 1 or 2 oxacillin-resistant staphylococci lacking *mec*. Such isolates are often falsely susceptible by the disk diffusion test (2, 5, 6); however, these isolates usually grow on salt-supplemented oxacillin agar screen plates, although growth may not be evident for 48 h (5). We do not have data on the effects of a lower inoculum of these heterogeneously resistant isolates on salt-unsupplemented oxacillin agar screen plates.

In conclusion, the GPS-SA card correctly classified more than 90% of oxacillin borderline-susceptible isolates of *S. aureus*. Definitive differentiation between such isolates and oxacillin-heteroresistant isolates, however, requires testing for the presence of the *mec* gene.

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