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Evaluation of MicroScan Rapid Gram-Positive Panels for Detection of Oxacillin-Resistant Staphylococci

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The ability of MicroScan rapid panels (Baxter MicroScan, West Sacramento, Calif.) to detect oxacillin resistance in 92 clinical isolates of *Staphylococcus aureus* and 103 coagulase-negative staphylococci was evaluated by comparing results with those of MicroScan 24-h MIC panels and, to resolve discrepancies, oxacillin agar screening. Both panels were interpreted by the MicroScan WalkAway-96 system. Rapid panels detected 96.7% of resistant *S. aureus* isolates and 72% of resistant coagulase-negative staphylococci, 22 of which did not grow in the panels.

Strains of *Staphylococcus aureus* resistant to oxacillin (methicillin) were first recognized in the United States in the mid-1970s and are now a significant problem in several institutions (1, 2). More recently, coagulase-negative staphylococci emerged as important nosocomial pathogens; consequently, detection of oxacillin resistance in these organisms also is a concern (8, 9). Most oxacillin-resistant (OR) staphylococci are heterogeneous, recognized only after specific manipulations of susceptibility test systems (3). One method is the oxacillin agar screen (Mueller-Hinton agar with 4% NaCl and 6 μg of oxacillin per ml incubated for 24 h at 35°C) (7). For disk diffusion and broth dilution testing, the following guidelines published by the National Committee for Clinical Laboratory Standards should be followed: (i) test oxacillin, (ii) prepare the inoculum directly from overnight growth on an agar plate, (iii) incubate at 35°C for 24 h, and (iv) for broth dilution, supplement the medium with 2% NaCl (4, 5).

Today, many clinical microbiology laboratories use automated systems rather than conventional methods for susceptibility testing. Moreover, in response to the current demand for rapid turnaround times, systems that require shorter incubation times have been developed. These automated systems designed for rapid testing must be able to reliably detect OR staphylococci.

MicroScan (Baxter MicroScan, West Sacramento, Calif.) 24-h MIC panels are reliable for the detection of OR *S. aureus* and coagulase-negative staphylococci (10). Recently, Baxter developed rapid MicroScan panels, the wells of which contain antimicrobial agents diluted in water containing fluorogenic compounds. The panels are incubated in the WalkAway system, and after 3.5 to 15 h, the instrument interprets the MIC by measuring the increase in fluorescence in the dilutions of antimicrobial agents and comparing it with the increase in fluorescence in growth control wells. For staphylococci, two control wells, one with and one without NaCl, are evaluated. The purpose of the study described here was to determine whether these rapid panels can reliably detect OR staphylococci.

Organisms. Fresh and frozen (stored at −70°C in Trypticase soy broth with 15% glycerol) clinical isolates (92 *S. aureus* and 103 coagulase-negative staphylococci) that were OR by testing with MicroScan conventional Pos MIC type 6 dry panels were tested. All organisms were subcultured twice on 5% sheep blood agar, and the second transfer was used to inoculate MicroScan rapid and 24-h MIC panels (the latter were retested to ensure consistency when the same inoculum was used). Isolates were stored in the refrigerator until completion of the study.

Antimicrobial susceptibility testing. MicroScan 24-h dry Pos MIC type 6 and rapid (dry Pos MIC type 1) panels were inoculated and incubated and the results were interpreted according to the manufacturer's directions. Both panels were incubated in and read with the MicroScan WalkAway-96 system. For 24-h MIC panels, results were interpreted after 24 h. Rapid panels were read at 3.5, 4.5, 5.5, 7, 8, 11, and 15 h; the result was reported when growth in the control wells was adequate. When the oxacillin susceptibility result reported by both panels agreed, that result was considered correct. If the two interpretations differed, both panels were repeat tested, and the result given by three of the four panels was considered correct. If this criterion was not met, oxacillin agar screening (medium was purchased from Remel Microbiology Products, Lenexa, Kans.), which was performed according to standard practice (7), was considered the reference method. Briefly, plates were spot inoculated with about 10^4 CFU and were then incubated for 24 h at 35°C. Isolates showing any growth were considered resistant. Control strains of OR and oxacillin-susceptible *S. aureus* also were tested.

Statistics. A *z* score (6) was calculated to determine whether the reliability of the rapid panels for detection of OR was significantly different from that of the 24-h MIC panels plus agar screening (as described above).

The results of the study are summarized in Table 1. On initial testing, all 92 *S. aureus* isolates were OR when tested with 24-h MIC panels. With rapid panels, most results of which were reported in 4.5 to 7 h, 89 isolates (96.7%) were resistant and 1 isolate was susceptible (MIC, <0.25 μg/ml), but 2 isolates did not grow. When the latter two isolates were retested, the oxacillin susceptibility results by testing with both panel types were resistance. The third isolate, which, again, was susceptible by the rapid panel method, was resistant by agar screening. For isolates of *S. aureus*, detection of OR with the rapid and 24-h MIC panels was not significantly different ($P = 0.08$).

When the 103 coagulase-negative staphylococci were tested

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TABLE 1. Reliability of MicroScan rapid panels for detection of OR staphylococci[a]

Organism	No. (%) of isolates with rapid panel result of:			Total no. of OX-R isolates tested[b]
	OX-R	OX-S	NG	
S. aureus	89 (96.7)	1 (1.1)	2 (2.2)	92
Coagulase-negative staphylococci	72 (72.0)	6 (6.0)	22 (22.0)	100

[a] OX, oxacillin; R, resistant; S, susceptible; NG, no growth.
[b] Total number of isolates found to be resistant after resolution of discrepancies.

initially, 100 were OR by 24-h MIC panels, 1 was susceptible, and 2 did not grow; by testing with the rapid panels, 72 were resistant, 9 were susceptible, and 22 did not grow. After resolving discrepancies by retesting and oxacillin agar screening, 100 coagulase-negative staphylococci were resistant and 3 were susceptible. MIC 24-h panels detected 97% of resistant strains initially; 3 with a resistance result on both testing occasions were susceptible by oxacillin agar screening. Rapid panels detected only 72 of the 100 (72%) resistant isolates on initial testing ($P < 0.001$) (92.3% of the resistant strains that grew) and gave no false-resistant results. When rapid panels were repeat tested, nine additional coagulase-negative staphylococci were OR (five that first had a result of susceptible and four that had not grown).

In summary, MicroScan rapid panels can provide antimicrobial susceptibility results in 3.5 to 15 h. If the test isolate is not inhibited by the concentration of antimicrobial agent in the well of the panel, the enzymes produced during its growth hydrolyze the fluorogenic compounds, thus increasing the fluorescence measured with the WalkAway system. The main advantage of these panels is their potential to decrease the time to reporting of results. Disadvantages include dependence on an instrument for interpretation and the requirement for refrigerated storage.

Current recommendations for the detection of OR staphylococci include incubation for at least 24 h. MicroScan rapid panels allow reliable detection of OR *S. aureus* in 3.5 to 15 h, and most within 4.5 to 7 h. Coagulase-negative staphylococci, however, often fail to grow in these panels or do not produce enzymes that hydrolyze the fluorogenic compounds. Therefore, until this problem is corrected, use of the rapid panels for susceptibility testing of coagulase-negative staphylococci is not recommended.

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