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Evaluation of BBL Crystal MRSA ID System

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The BBL Crystal system (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was evaluated for its accuracy in identifying oxacillin resistance in *Staphylococcus aureus* by testing of *mec*-specific-gene-positive and -negative isolates. Although the manufacturer makes no claim for use of the product for testing of staphylococci other than *S. aureus*, the product's potential utility in detecting oxacillin resistance in isolates of *mec* gene-positive and -negative *Staphylococcus epidermidis* was also explored. All *mec* gene-negative staphylococci yielded a negative MRSA ID test reaction. There was a close correlation between *mec* gene positivity and a positive reaction in the methicillin-resistant *S. aureus* identification system with 63 of 69 (91%) stock isolates of *S. aureus* yielding a positive result in 4 h, 66 of 69 (95%) yielding a positive result in 5 h, and 68 of 69 (99%) yielding a positive result in 6 h. The corresponding percentage agreements at 4, 5, and 6 h for *mec* gene-positive stock isolates of *S. epidermidis* were 87, 91, and 96%, respectively.

The accurate identification of methicillin resistance in staphylococci is of considerable clinical importance. Recently published data, however, have demonstrated the existence of significant problems in detecting methicillin resistance with the standard disk diffusion test (3–5), especially in heteroresistant isolates in the phenotypic expression class 1 or 2 (1, 4, 5). Although alternative methods, including microdilution and an automated system, as well as the oxacillin agar screen, have been shown to be accurate in detecting oxacillin-resistant staphylococci (3), many laboratories routinely use the disk diffusion method. A simple alternative method for the detection of methicillin resistance would, therefore, be desirable.

The BBL Crystal MRSA ID system is based on the use of a fluorescent indicator which is sensitive to the presence of dissolved oxygen in broth. In the presence of oxygen, fluorescence is quenched; however, in the presence of an actively growing microorganism, oxygen is consumed and fluorescence can be detected. By incorporating oxacillin in the broth, it is possible to distinguish between oxacillin-susceptible and -resistant isolates of *S. aureus* on the basis of inhibition or lack of inhibition of growth and the corresponding absence or presence of fluorescence.

One objective of this study was to examine the accuracy of the Crystal MRSA ID system with a previously well-characterized group of oxacillin-susceptible and -resistant isolates of *S. aureus* (4). Although the manufacturer makes no claim that the MRSA ID system may be used for testing *Staphylococcus epidermidis*, we investigated the potential utility of the system for identifying oxacillin resistance in previously well-characterized isolates of *S. epidermidis* (4).

All isolates used in this study were characterized as to the presence of the *mec*-specific gene (courtesy of H. de Lencastre and A. Tomasz, Rockefeller University, New York, N.Y., and H. F. Chambers, San Francisco General Hospital, San Francisco, Calif.) and by population analysis profile. Population analysis profiles were determined by the method of Hartman and Tomasz (2) and Tomasz et al. (7). Briefly, the isolates were categorized into their phenotypic expression classes by determining the ratio of CFU in overnight cultures of each isolate in the presence of methicillin to those cultured in methicillin-free medium. Isolates included in the study are listed in Table 1 according to species, the presence or absence of the *mec* gene, and phenotypic expression class. MICs for each isolate were determined by the reference microbroth dilution method according to procedures described by the National Committee for Clinical Laboratory Standards (6). Oxacillin agar screens were also performed with each isolate (3). Microbroth dilution trays and oxacillin agar screen plates were prepared in-house. Each stock isolate was subcultured twice on Trypticase soy agar containing 5% sheep blood prior to inoculum preparation and, in the cases of false-negative MRSA ID tests, was subcultured a third time before repeat testing.

The inoculum for the MRSA ID test was prepared by suspending several colonies of each isolate in saline and adjusting the density to 70% transmission on a Hach DR 100 colorimeter (Hach Systems for Analysis, Loveland, Colo.). A 0.5-ml sample of the adjusted suspension was transferred to a tube containing the MRSA ID broth. After mixing on a vortex shaker, a drop of the broth was transferred into each well of the test panel according to the manufacturer's instructions, and the panel was incubated at 35°C. Each panel was observed after 4, 5, and 6 h of incubation.

All *mec* gene-negative isolates of staphylococci were negative in the MRSA ID system after 4 to 6 h of observation. Table 1 lists the results of MRSA ID testing by species, by the presence or absence of the *mec* gene, and, for oxacillin-resistant isolates, by phenotypic expression class. All *mec* gene-positive isolates were resistant to oxacillin in both microbroth dilution and oxacillin agar screen tests. Of the 69 oxacillin-resistant isolates of *S. aureus*, 63 (91%) were positive in 4 h, 66 (95%) were positive in 5 h, and 68 (99%) were positive in 6 h (data not shown). After a third subculture of the false-negative isolates and repeat testing, 67 (97%) of the isolates were positive in 4 and 5 h and 68 (99%) were positive in 6 h (data not shown). Of 47 oxacillin-resistant isolates of *S. epidermidis*, 41 (87%) were positive in 4 h, 43 (91%) were positive in 5 h, and 45 (96%) were positive in 6 h (data not shown). On repeat testing after a third subculture of the false negatives, 46 (98%) isolates were positive in 4 h and 45 (96%) were positive in 5 and 6 h (data not shown).

There was a close correlation between the presence of the *mec* gene and a positive reaction in the MRSA ID test. There was, however no correlation between a false-negative MRSA

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TABLE 1. Crystal MRSA ID with *mec* gene-positive and -negative staphylococci

Organism	*mec* gene	Phenotypic expression class	No. of isolates	No. of isolates with positive MRSA ID reaction at indicated time (h)			No. of isolates with negative MRSA ID reaction
				4	5	6	
S. aureus	Present	1 or 2	9	7	1	1	
		3 or 4	60	56	2	1	1
	Absent		27	0	0	0	27
S. epidermidis	Present	1 or 2	9	7	1		1
		3 or 4	38	34	1	2	1
	Absent		11	0	0	0	11

ID test result and the phenotypic expression class of heteroresistance (Table 1). In other words, there was no greater possibility of a false-negative MRSA ID test result with class 1 or 2 heteroresistant isolates than with class 3 or 4 heteroresistant isolates.

The optimal time for interpretation of the MRSA ID system in our laboratory with stock isolates of staphylococci was 6 h. Whether this time interval would be shorter with tests of fresh, rather than stock, clinical isolates is not known; however, our data suggest that isolates of *S. aureus* yielding a negative test result in 4 h should be observed for an additional 2 h before being reported as negative.

The ultimate utility of the MRSA ID system will depend on a comparison of its accuracy with those of other existing methodologies, on test turnaround times of this system, and on relative cost. Since there are documented problems with the disk diffusion test (1, 3, 4), laboratories using the disk diffusion method might be well advised to consider an alternative or supplemental method for confirming that staphylococcal isolates that appear to be susceptible to oxacillin are not in fact resistant. The MRSA ID system might serve as a supplementary test in such instances. On the other hand, automated approaches, such as the Vitek system with updated software programs, have been shown to identify methicillin resistance accurately and provide additional susceptibility data within approximately the same time frame (4).

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