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Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* by Crystal MRSA ID System

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A commercially available method for the rapid detection of methicillin-resistant *Staphylococcus aureus* (BBL Crystal MRSA ID System) was evaluated and compared with conventional methods. All 52 isolates of methicillin-susceptible and 142 isolates of intrinsic methicillin-resistant *S. aureus* were correctly identified in 4 h by the test method, whereas correct identification took 11 to 24 h by conventional methods. The test is simple, rapid, and easy to perform and the results are easy to interpret.

The ability of methicillin-resistant *Staphylococcus aureus* (MRSA) to colonize and cause disease continues to be a major problem, especially in nosocomial infections which include pneumonias and infections of surgical wounds, urinary tracts, central venous catheters, and decubitus ulcers (1). In the community, the highest incidence of infection caused by MRSA occurs in intravenous drug abusers, with up to 30% of them harboring the organism in the nares or rectum (6). Colonized or infected patients as well as nursing home populations (4) are the most important reservoirs of this organism, and it is from members of these populations the bacterium could spread throughout a hospital or facility. Accurate recognition of MRSA is important because of the epidemiological and therapeutic problems the organism poses in health care facilities. Standard methods used for this purpose in diagnostic laboratories include the disk diffusion test, determination of MICs, or use of the agar screen plate containing methicillin (10 μg/ml) or oxacillin (6 μg/ml). By these methods, 24 h of incubation is required before the results are available. Some of the semiautomated or instrument-based methods require 8 to 12 h but are often associated with false positive susceptibility (1, 5, 7, 8). The need for the rapid identification of *S. aureus* isolates that are resistant to methicillin cannot be overemphasized. In 1992, Tokue et al. (12) described a sensitive and reliable procedure, using PCR and Southern blot analyses, for the rapid diagnosis of MRSA. However, the method remains an experimental one and would be of little use in the majority of diagnostic microbiology laboratories at this time. Recently, a commercial test that detects MRSA in 4 h was introduced by BBL. We evaluated this test using 194 recent clinical isolates of methicillin-susceptible *S. aureus* (MSSA) and MRSA. Our findings are presented here.

The strains of *S. aureus* used in the present study were recent clinical isolates cultured from patients at King Faisal Specialist Hospital & Research Centre, a 550-bed tertiary-care facility in Riyadh, Saudi Arabia. The organisms were identified by standard procedures by using the coagulase test, thermonuclease test, mannitol salt agar, and the BBL Staphyloslide test (Becton Dickinson, Cockeysville, Md.). Susceptibility to methicillin was determined by standard disk diffusion screening on agar plates containing 10 μg of methicillin per ml (3, 11), a broth microdilution method according to the recommendations of the National Committee for Clinical Laboratory Standards (9, 10), and by the Vitek system by using the gram-positive susceptibility flex panel (BioMerieux Vitek, Inc., Hazelwood, Mo.). The isolates were maintained on sheep blood agar and were used as coded unknowns in the evaluation of the BBL Crystal MRSA ID System (Becton Dickinson, Cockeysville, Md.). The test uses an oxygen-sensitive fluorescent indicator in the test panel which detects the respiration of the organism in three test wells. The fluorescence emission from the indicator, Tris-1,4-diphenyl-1,10-phenathroline ruthenium chloride pentahydrate in a silicone rubber base, is quenched in the presence of oxygen. However, in the presence of an actively metabolizing organism, oxygen is consumed, which allows the fluorescence to be observed under a UV light source. The test was performed according to the manufacturer's directions. Briefly, the test consists of adding 4 drops of a standardized broth suspension of the bacterium to three test wells. The panel lid contains prongs for each of the wells. The prong for the first well does not contain any antibiotic (positive control), but the prongs for the second and third wells contain dried oxacillin (test well) and vancomycin (negative control), respectively. When the lid is placed on the wells, the prongs enter the three wells; the concentration of oxacillin in the second well is 4 μg/ml and that of vancomycin in the third well is 16 μg/ml. The panel is incubated at 35°C and observed for fluorescence at 4 h with a long-wave UV illumination (365-nm) source. In order for the test to be valid, the first well should always be positive for fluorescence, the third well should always be negative for fluorescence, and the second well is positive if the organism is MRSA and negative if it is MSSA. A positive reaction gives 4+ (very bright) fluorescence. When reading the result, the test well (second well) is compared with the positive control (first well) and the negative control (third well). The quality control organisms used throughout the study were *S. aureus* ATCC 29213 (MSSA) and *S. aureus* ATCC 33592 (MRSA).

The ability of the BBL Crystal MRSA ID System to distinguish between MSSA and MRSA was compared with the abilities of four other methods to distinguish between these isolates. Of the 194 isolates tested, 52 were MSSA and 142 were MRSA. All MSSA isolates were correctly recognized by all the methods, including the BBL Crystal MRSA ID System in 4 h. Of the 142 isolates with intrinsic methicillin resistance, as determined by methicillin agar plate screening, and for which the oxacillin MIC was ≥4 μg/ml, 139 were correctly recognized as MRSA by disk diffusion with a 1-μg oxacillin disk, 141 were correctly identified by the Vitek system (oxacillin MIC, >8 μg/ml), and 142 were correctly identified by the

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TABLE 1. Accuracy and comparative aspects of intrinsic MRSA detection by different methods[a]

Method	No. of strains positive	Incubation time (h)	Special equipment required
Disk diffusion	139	24	None
Methicillin agar plate screen	142	24	None
Broth microdilution	142	24	None
Vitek system	141	11	Yes
Crystal MRSA ID System	142	4	None

[a] A total of 142 isolates were tested by each method.

Crystal MRSA ID System (Table 1). The results of the standard disk diffusion, methicillin agar plate screening, and broth microdilution methods were read at 24 h, the results of the Vitek system were read at 7 and 11 h, and the results of the Crystal MRSA ID System were read at 4 h. Two strains of *S. aureus* for which the oxacillin MIC was 2.0 μg/ml by both the broth microdilution method and the Vitek system were recognized by disk diffusion as MRSA and MSSA by methicillin agar plate screening and the Crystal MRSA ID System. We believe these to be methicillin-intermediate *S. aureus* strains with reduced levels of susceptibility to methicillin (methicillin MICs, 4 μg/ml; oxacillin MICs, 1 to 2 μg/ml). Tomasz et al. (13) coined the term MODSA for these organisms and reported that they produce PBPs 1 and 2 of normal molecular size but have a low affinity for beta-lactam antibiotics. The specificity, sensitivity, and predictive value of the Crystal MRSA ID System compared with those of the MIC determinations obtained by the broth microdilution method, the "gold standard" (and methicillin agar plate screening, for which the results were exactly the same as those of the broth microdilution method), were as follows: 142 isolates (MRSA) were positive by both the broth microdilution method and the Crystal MRSA ID System, and 52 isolates (MSSA) were negative by the broth microdilution method and the Crystal MRSA ID System. All 142 strains of MRSA and 52 strains of MSSA (including 2 strains of MODSA) were correctly identified by the Crystal MRSA ID System.

Since the first report of MRSA in 1961 in Europe, the isolations of MRSA from clinical specimens have continued to rise. Haley et al. (6) reported that in the United States the incidence of MRSA is mainly concentrated in tertiary-care teaching hospitals, where its incidence has been reported to vary between 8 and 45% (2, 8). In the United States in 1991, MRSA constituted 15, 20, and 38% of *S. aureus* isolates in hospitals with fewer than 200 beds, 200 to 499 beds, and 500 or more beds, respectively (4). Since colonization and infection with MRSA can be extremely difficult to treat, the quick detection and rapid identification of MRSA are highly desirable for the control and prevention of their spread. Standard methods such as disk diffusion, MIC determinations, and methicillin and oxacillin agar plate screenings require 24 h of incubation. Semiautomated instruments can generate results within 7 to 11 h, but many of them are available only in relatively large medical facilities and their procurement remains beyond the financial means of the majority of hospitals. Recently described rapid methods that use molecular biology techniques (12) are in the experimental stage and will probably be restricted to use in a few large medical centers when they become commercially available. All of the conventional methods are dependent on the growth of microorganisms, and hence require prolonged incubation periods. On the other hand, the BBL Crystal MRSA ID System is growth independent and is designed to detect the active respiration of bacteria in the presence and absence of oxacillin. This makes the reading of results possible in 4 h.

During the present evaluation, we found that the BBL Crystal MRSA ID System correctly detected all 52 isolates of MSSA and 142 isolates of MRSA in 4 h. We found it to be a rapid, reliable, and useful method that is comparable to the more time-consuming conventional methods used for the detection of MRSA. It does not require any special equipment and is easy to perform, and the results are easy to interpret. When used in conjunction with the Staphyloslide test or the coagulase test, or both, it provides the laboratory with the capability of identifying MRSA 18 to 20 h earlier than is allowed by conventional methods. Although molecular biological and semiautomated methods for the rapid identification of MRSA are being developed or described, the BBL Crystal MRSA ID System does not require special equipment or expertise and can be performed in any laboratory. We believe that use of the test for the rapid screening of MRSA will reduce the delays in their rapid recognition, thus providing physicians and infection control practitioners with early relevant information for better patient management and the ability to implement appropriate infection control measures. Large medical centers, especially those with a high incidence of MRSA, may find oxacillin or methicillin agar screening plates more economical. The cost of vancomycin, even as empirical therapy for 1 day, and the implementation of patient isolation practices, if warranted, are other factors a potential user of the system may have to evaluate.

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