Reliability of Two Novel Methods, Alamar and E Test, for Detection of Methicillin-Resistant *Staphylococcus aureus*

S. M. NOVAK,* J. HINDLER, AND D. A. BRUCKNER

*Department of Pathology and Laboratory Medicine, Clinical Microbiology Section, UCLA Medical Center,
Los Angeles, California 90024-1713*

E test strips (AB Biodisk, Culver City, Calif.) tested on 2% NaCl-supplemented Mueller-Hinton agar and Alamar panels (Alamar, Sacramento, Calif.) correctly characterized 127 of 127 methicillin-resistant *Staphylococcus aureus* isolates and 100 of 100 methicillin-susceptible *S. aureus* isolates. These overnight antimicrobial susceptibility test systems reliably detect this clinically important resistance.

The importance of methicillin-resistant *Staphylococcus aureus* (MRSA) as a nosocomial pathogen is well documented (3, 4, 6). Because MRSA is often resistant to many antibacterial agents, infections with this organism are difficult to treat (7). In addition, since few cells in a population might actually express resistance, these heterogeneous strains can evade detection in standard susceptibility test systems (8, 13). Consequently, it is important to carefully evaluate new systems for their abilities to detect this clinically important resistance. This study describes the capabilities of two newer novel overnight methods, Alamar and E test, to detect MRSA. The results of Alamar and E test were compared with those of the National Committee for Clinical Laboratory Standards (NCCLS) disk diffusion and MRSA agar screen reference methods (9–11). Although both the Alamar and E test systems provide MIC results, the purpose of this study was to determine if these systems could accurately detect MRSA, as defined by an oxacillin MIC of >2 μg/ml.

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Two hundred and twenty-seven isolates were tested, which included 127 MRSA isolates and 100 methicillin-susceptible *S. aureus* (MSSA) isolates. An effort was made to test oxacillin-resistant isolates that were difficult to detect and suspected to have heterogeneous resistance, as evidenced by hazes (some very subtle) around an oxacillin disk. Genetic analysis was not performed to determine if the isolates contained the *mec* gene or were resistant by an alternative resistance mechanism.

Inocula were prepared by suspending colonies from growth on an 18- to 24-h Trypticase soy blood agar plate (BBL Microbiology Systems, Cockeysville, Md.) into saline to match the turbidity of a 0.5 McFarland standard. A single suspension was used for all tests.

For the Alamar test, the 0.5 McFarland standard suspension was further diluted by transferring 25 μl to 25 ml of Alamar Inoculum Broth (Alamar). Next, 100 μl was delivered to each well, which contained a disk impregnated with 2% NaCl in addition to oxacillin. Serial twofold dilutions of oxacillin at concentrations of 0.12 to 16 μg/ml were tested. The final number of organisms delivered to each well was approximately 1.5×10^4 CFU/100 μl. Trays were incubated

at 35°C in ambient air and examined at 20 and 24 h. The Alamar system employs an oxidation/reduction color indicator that measures metabolic reduction of the growth medium. When growth occurs, the indicator dye will change from blue to red. Any well with a color change noted when compared with a negative-growth control well (blue color) is considered positive for growth. By allowing light to shine through the back of the panel, the MIC is read as the first well showing no growth (no color change).

Agar plates used for the E test were inoculated by a procedure identical to that for disk diffusion testing (10). Each isolate was tested on Mueller-Hinton agar (MHA) (Remel, Lenexa, Kans.) and MHA with 2% NaCl (Remel). Following inoculation, E test strips containing a gradient of oxacillin concentrations ranging from 0.016 to 256 μg/ml were placed on the agar surface. Plates were then incubated at 35°C in ambient air and examined after 24 h of incubation. E test strips contain a continuous gradient of antimicrobial agent (discontinuous twofold dilutions) on the underside of the strip which diffuses out into the medium when the strip is placed on the surface of the agar plate. Inhibition of growth appears in the shape of an ellipse, and MICs are read where the zone edge or ellipse intersects the E test strip. Because of the transparent nature of MHA, using transmitted light and allowing it to shine through the underside of the plate allowed greater ease in interpretation of the MIC.

NCCLS disk diffusion and oxacillin agar screen tests were performed on all isolates, and results obtained with these reference methods were recorded as oxacillin susceptible or oxacillin resistant according to predefined criteria (9–11). *S. aureus* ATCC 29213 (sensitive strain), 33592 (resistant strain), and 43300 (resistant strain) were used for quality control and performed as expected for all tests.

In this study, Alamar correctly identified all isolates. For all 127 MRSA isolates the MIC was ≥16 μg/ml, and for the 100 MSSA isolates the MIC was ≤1 μg/ml. The Alamar procedure manual suggests that when staphylococci and oxacillin are tested, strains that appear susceptible at 20 h of incubation should be reincubated for an additional 4 h (total of 24 h); however, for isolates tested in this study 20 h of incubation was sufficient.

The E test using MHA with 2% NaCl identified all isolates. For 93 of the 127 MRSA isolates the MIC was >256 μg/ml. For the remaining MRSA isolates the MICs were within the range of 6 to 256 μg/ml. MICs for all MSSA isolates were ≤1 μg/ml.

Six of 127 MRSA isolates were not detected with the E

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* Corresponding author.

test using MHA without 2% NaCl. In order to enhance the expression of oxacillin resistance, salt must be present in the medium when the E test is performed.

The Alamar panel is easy to use, and the color change representing the MIC endpoint is obvious if the trays are examined properly. Although only oxacillin was evaluated in this study, Alamar manufactures a gram-positive MIC panel for testing staphylococci and enterococci which contains 20 antimicrobial agents, including oxacillin. Additionally, the manufacturer offers user-defined panels. The E test system is also easy to use and presents a practical alternative to conventional antimicrobial susceptibility test systems when only a few drugs require testing, when a special growth medium is required, or both. E test has been previously evaluated for testing the antimicrobial susceptibility of a variety of bacteria (1, 2, 5, 12). Currently, MHA with 2% NaCl can be used for reliable detection of oxacillin resistance, though other concentrations might also be effective. In this regard, our findings parallel those of other investigators (5). Incorporation of NaCl into the E test strip would alleviate the need for special media containing salt, and this is currently being investigated by the manufacturer. In summary, both Alamar and E test (using MHA with 2% NaCl) accurately detect MRSA.

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