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Rapid Detection (4 h) of Methicillin-Resistant *Staphylococcus aureus* by a Bioluminescence Method

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A 4-h bioluminescence method for methicillin susceptibility determination was compared with reference methods. Of the *Staphylococcus aureus* strains tested, 80 were methicillin resistant, 180 were methicillin susceptible, and 10 were borderline susceptible. There was 100% correlation between bioluminescence and reference methods for methicillin-susceptible and methicillin-resistant strains. All borderline-susceptible strains were identified as methicillin resistant by bioluminescence.

Rapid, accurate detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is important therapeutically, epidemiologically, and economically for health care institutions. Current methods include the use of screen plates containing 4% NaCl and 6 μl of oxacillin per ml (9), broth dilution using 2% NaCl in cation-supplemented Mueller-Hinton broth (7), and standard disk diffusion (6). Automated, commercial MIC methods are also being used, but their accuracy is questionable (1, 3, 4, 8). In addition, most of these methods require 18 to 24 h of incubation.

This study evaluated a 4-h bioluminescence bacterial ATP assay for the detection of MRSA and methicillin-susceptible *S. aureus* (MSSA). Of 270 *S. aureus* isolates included in this study, 180 were MSSA, 80 were MRSA, and 10 were borderline-susceptible *S. aureus* (BSSA) (5). The MRSA strains were provided by the Centers for Disease Control (Atlanta, Ga.), the Veterans Administration Medical Center (Baltimore, Md.), McGuire Veterans Administration Medical Center (Richmond, Va.), St. Vincent's Hospital (Toledo, Ohio), University of Iowa (Ames), Clinical Microbiology Services of the National Institutes of Health (Bethesda, Md.), Shady Grove Adventist Hospital (Gaithersburg, Md.), Austin Biological Laboratories (Austin, Tex.), Remel (Lenexa, Kans.), and the Fairfax Hospital (Falls Church, Va.). All of the BSSA and MSSA were isolated from clinical specimens at the Fairfax Hospital.

Screen plates (Remel) containing Mueller-Hinton agar with 4% NaCl and 6 μg of oxacillin per ml were inoculated with a suspension equivalent to a 0.5 McFarland standard. A swab was placed in the suspension and pressed against the side of the tube to express most of the fluid. It was then used to inoculate a spot on the screen plate. Plates were incubated at 35°C and observed for growth at 24 and 48 h (9).

The susceptibilities of all isolates were determined as follows. (i) Precept-methicillin single MIC trays (Austin Biological Laboratories) were used according to the instructions of the manufacturer. The trays were incubated at 35°C for 24 and 48 h. (ii) Bauer-Kirby agar diffusion was performed with a 1-μg oxacillin disk. Plates were incubated at 35°C for 24 h. (iii) Inoculum for the bioluminescence assay was harvested from an 18- to 24-h culture on blood agar to prepare a suspension in sterile distilled water equivalent to a 0.5 McFarland standard. Of this suspension, 50 μl was transferred into each of two tubes containing 3 ml of Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 2% NaCl and cations (magnesium and calcium). One tube was a test sample containing 8 μg of methicillin per ml prepared by adding two 12-μg methicillin disks (General Diagnostics, Div. Warner-Lambert Co., Morris Plains, N.J.) to the 3 ml of Todd-Hewitt broth; this was followed by a 30-min incubation at room temperature for elution. The other tube, a growth control, contained no antibiotic. Both tubes were incubated for 4 h at 30°C. Control and test tubes were assayed in a Biocounter M2010A (3M, St. Paul, Minn.). The resulting light emission was measured and expressed in relative light units (RLUs). A control RLU reading of >1,000 was necessary to ensure active growth. Organisms producing <1,000 RLUs in 4 h could not be assayed by this method. Susceptible strains were defined as those having a greater than 50% reduction in ATP activity compared with the control sample. This threshold was determined from previous experiments. The following formula was employed to determine percent reduction in ATP activity: [RLU (control) − RLU (test)]/RLU (control) × 100 = % reduction.

For all 180 MSSA isolates, methicillin MICs were ≤4 μg/ml and zone sizes were ≥13 mm, and the isolates did not grow on screen plates at 24 h. The range of reduction in ATP activity was 59 to 99% (Table 1). There was 100% correlation between the results of the bioluminescence method and those of the reference methods.

For all 80 MRSA strains, methicillin MICs were ≥8 μg/ml at 24 h and zone sizes were ≤9 mm, and the strains had good growth on screen plates at 24 h. The bioluminescence assay demonstrated a <50% reduction of ATP activity compared with that of the control tube. The range of reduction was between 0 and 41% (Table 1). Correlation between the bioluminescence and reference methods was again 100% when 50% reduction of ATP activity was used as the cutoff value.

For the 10 BSSA isolates, MICs varied from 2 to 8 μg/ml when isolates were incubated for 48 h; zone sizes ranged from 6 to 11 mm. Two isolates did not grow on screen plates, whereas others showed small colonies at 24 h which became larger at 48 h. The bioluminescence assay showed a <50% reduction in ATP activity, ranging from 0 to 49% (Table 1). This method could not distinguish BSSA from MRSA.

Previous semiautomated or automated methods were less accurate. In 1984, Jorgenson et al. (4) were able to recognize 94.7% of MRSA strains with the AutoMicrobic system (Vitek Systems, Inc., Hazelwood, Mo.) and 91.5% with the MS-2 system (Diagnostics Division, Abbott Laboratories,

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TABLE 1. Effect of methicillin (8 μg/ml) on ATP activity

No. of strains tested	MIC (μg/ml)	Reduction of ATP activity (%)
MSSA		
1	0.13	78
4	0.25	71–81
13	0.5	72–92
99	1.0	59–94
45	2.0	59–99
18	4.0	59–91
MRSA		
11	8.0	0–41
21	16.0	0–25
48	>16.0	0–38
BSSA		
1	2.0	49
6	4.0	0–42
3	8.0	0–32

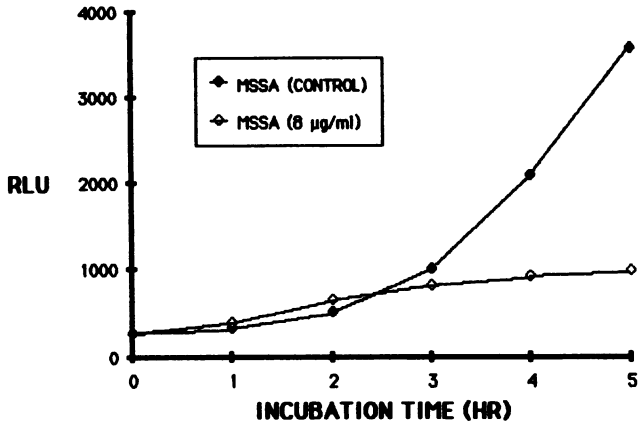

FIG. 1. Effect of methicillin (8 μg/ml) on ATP activity (RLU) versus incubation time.

Irving, Tex.). The following year, Putland and Guinness (8) detected only 86% of MRSA strains with the AutoMicrobic system. In 1987, Hansen and Walsh (3) correctly identified 100% of MRSA strains with a modified Sceptor panel (Johnston Laboratories, Inc., Towson, Md.) and AutoMicrobic systems. However, the latter resulted in 16.5% false resistance. These methods, with the exception of the AutoMicrobic system, require 18 to 24 h of incubation. In 1985, Hansen and Pope (2) evaluated a lyophilized broth screening system which required just 5 h of incubation, but only 96.9% of 129 MRSA strains were detected.

The minimum incubation time for the bioluminescence assay was 4 h (Fig. 1). All MRSA strains were correctly identified when 50% reduction of ATP activity was used as a cutoff point. The bioluminescence technique correlated very well with reference methods, but it was unable to distinguish between BSSA and MRSA. This is considered insignificant, since we follow the suggestion of McDougal and Thornsberry (5) and report BSSA as MRSA.

Todd-Hewitt broth with NaCl was used instead of Mueller-Hinton broth because it promoted better growth. Only one fastidious MSSA failed to grow (RLU, <1,000) in Todd-Hewitt broth in 4 h.

These data demonstrate that bioluminescence offers a relatively inexpensive test ($3 per isolate) for identifying MRSA and MSSA. This method provides rapid and accurate results when an urgent clinical decision is pending, such as the necessity for instituting patient isolation. However, it does not preclude the need for routine antimicrobial susceptibility testing. Although the cost per test is reasonable, the initial price of an instrument is several thousand dollars, which may be unjustifiable for testing only methicillin susceptibility. However, bioluminescence technology is versatile and can be used for rapid urine screening, rapidly determining susceptibility to other antimicrobial agents, and providing anaerobic susceptibility data in several hours (unpublished results).

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