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# Rapid Detection of Oxacillin-Resistant *Staphylococcus aureus* in Blood Cultures by an Impedance Method

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**The feasibility of using an impedance method for direct detection of oxacillin-resistant *Staphylococcus aureus* (ORSA) in blood cultures was evaluated. An aliquot (0.1 ml) of the positive blood culture, which showed growth of gram-positive cocci and demonstrated thermonuclease activity, was inoculated into the module well of a Bactometer incubator (bioMerieux Vitek, Hazelwood, Mo.) containing 0.6 ml of Mueller-Hinton agar supplemented with oxacillin (2 μg/ml). The modules were incubated at 37°C, and the change in impedance in each well was continuously monitored by the instrument at 6-min intervals for 24 h. ORSA strains from blood cultures could multiply in the oxacillin-containing medium, and a time point (detection time [DT]) at which an accelerating change of impedance occurred in the medium was obtained, with an average of 5.5 h. The growth of oxacillin-sensitive *S. aureus* (OSSA) strains was largely inhibited, and no DT was obtained for these strains within an incubation period of 24 h. For 96 positive blood cultures (38 ORSA and 58 OSSA) tested, 36 and 57 were found to be oxacillin resistant and oxacillin sensitive, respectively, by the impedance method. The impedance method had a sensitivity and specificity of 94.7 and 98.3%, respectively, for the detection of ORSA and had an agreement of 96.9% with the disc diffusion method. Comparable results were obtained by the testing of 235 clinical stock cultures of *S. aureus* (149 ORSA and 86 OSSA). The impedance test is simple for detecting ORSA in blood cultures and may allow proper antimicrobial treatment almost 36 h before the results of the conventional culture methods are available.**

Approximately 200,000 cases of septicemia occur annually in the United States, with a high rate of mortality (30). The incidence rates of bacteremia and fungemia were reported to be 3.4 to 28 per 1,000 hospital admissions and were estimated to average 10 per 1,000 admissions (1%) in the United States (31). The five most common isolates from blood cultures were *Escherichia coli Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* (5). *S. aureus*, when found in blood cultures, is usually representative of significant clinical disease (11), and prompt action is necessary.

Methicillin-resistant *S. aureus* (MRSA) strains were first discovered in 1961 in Britain (15). The first reported outbreak of MRSA infections in the United States occurred in 1967 (2). Since then, MRSA strains have become much more prevalent (10, 14). During 1995 and 1996, oxacillin-resistant *S. aureus* (ORSA) strains represented about 40% of the total *S. aureus* strains causing bloodstream infections at National Cheng Kung University Hospital, Tainan, Taiwan (unpublished data). Because of the high incidence of ORSA, a method for rapid identification of the microorganism in blood specimens is of clinical importance.

Positive blood cultures are normally streaked on the appropriate media, followed by species identification and antibiotic susceptibility testing; the whole procedure may take as long as 2 days. In recent years, there has been an emphasis in clinical microbiology laboratories on rapidly identifying and determining the antimicrobial susceptibility patterns of bacterial isolates from blood cultures (27). It is believed that if this information is provided more rapidly, it will result in more timely and efficient therapy. Several automated systems have been evaluated for early detection of MRSA. Among these systems, MicroScan-W/A (Baxter Diagnostics, Inc., West Sacramento, Calif.) and the Vitek Automicrobic system (bioMerieux Vitek, Hazelwood, Mo.) (20, 27, 28) have been the most popular. Although these instruments were good for susceptibility testing of some bacteria, a high percentage of false negatives was found for the detection of methicillin resistance among *S. aureus* strains (16, 29).

The change in electrical properties (impedance, conductance, and capacitance) due to the growth of microorganisms in the culture media has been utilized for the rapid estimation of total bacterial counts (23), coliforms (13), yeasts (1), *Salmonella* spp. (6, 25), and residual antibiotics (9) in a variety of samples. The principle of the impedance measurement is based on the phenomenon that at a time point (i.e., detection time [DT]) at which bacteria have grown to a population of approximately $10^7$ CFU/ml or higher, an accelerating change in impedance (or conductance) will occur in the growth media. The decrease in impedance (or increase in conductance) is due to the breakdown of the substrate molecules in the media to smaller molecules (e.g., acids), which have more charges than the substrate itself. The purpose of this study was to test the feasibility of using the impedimetric measurement to directly detect ORSA in positive blood cultures which showed growth of gram-positive cocci and demonstrated thermonuclease activities.

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## MATERIALS AND METHODS

**Bacterial strains, media, and reagents.** Among the 238 stock cultures of *S. aureus* tested by the impedance method, ATCC 29213 (oxacillin-sensitive *S. aureus* [OSSA]), 33591, and 33592 (both ORSA) were obtained from the Amer-

ican Type Culture Collection, Rockville, Md. The remaining 235 strains (149 ORSA and 86 OSSA) were isolated at the National Cheng Kung University Hospital from various clinical specimens. Oxacillin susceptibility of the clinical isolates was determined by the standardized disc diffusion method (21). Oxacillin was obtained from Sigma Chemical Co., St. Louis, Mo. Mueller-Hinton agar, tryptic soy agar (TSA), tryptic soy broth (TSB), and other media were purchased from Difco Laboratories, Detroit, Mich.

**Effect of oxacillin concentration on the impedance curves.** Mueller-Hinton agar containing different concentrations of oxacillin (0 to 128 μg) was prepared at two-fold serial dilutions, and 0.6 ml of the medium was dispensed into the module wells of a Bactometer M-128 incubator (bioMerieux Vitek). The three *S. aureus* strains from the American Type Culture Collection were used to observe the effect of oxacillin concentration on the impedance curves during bacterial growth. These strains were subcultured on TSA for 18 to 24 h at 37°C. One single colony on TSA was inoculated into 10 ml of TSB, incubated at 37°C for several hours, and diluted with 0.1% peptone water to the turbidity of a 0.5 McFarland standard. An aliquot (0.1 ml) of the bacterial suspensions was inoculated into the module well containing Mueller-Hinton agar supplemented with oxacillin. The inoculated modules (each module contains 16 wells) were inserted into the Bactometer incubator, which was set at a temperature of 37°C. The impedance change in each well was continuously monitored and recorded by the instrument at 6-min intervals for 24 h, and results were obtained graphically as impedance growth curves. DT in hours for each well was determined automatically by the instrument software when three consecutive readings of impedance change exceeded the default value in the instrument or was determined manually by locating the inflection point (where an accelerating change of impedance was evident) on the impedance curve.

**Oxacillin susceptibility tests of clinical *S. aureus* isolates by the impedance method.** Susceptibility tests of the 235 clinical isolates of *S. aureus* were performed in a similar way to that used for the American Type Culture Collection strains, except that only one concentration (2 μg/ml) of oxacillin in Mueller-Hinton agar was used. A negative control (Mueller-Hinton agar without oxacillin) was included for each strain tested. A strain was designated as OSSA if, in the presence of the antibiotic, there was no DT obtained within an incubation period of 24 h. A strain was designated as ORSA if, in the presence of the antibiotic, the DT was not influenced or the delay in DT was less than 3 h compared to that with the negative control. *S. aureus* ATCC 29213 (OSSA) and ATCC 33592 (ORSA) were run at the same time as the control strains. Strains showing discrepant results were reconfirmed with oxacillin screen agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.).

**Detection of ORSA in blood cultures by the impedance method.** Blood specimens were collected at the National Cheng Kung University Hospital during a period in 1996. The BACTEC NR6A and NR7A vials (Becton Dickinson Microbiology Systems) were normally inoculated with 3 to 5 ml of blood from the patients, inserted into a BACTEC NR660 instrument (Becton Dickinson Microbiology Systems), and incubated at 37°C. A small aliquot (0.1 to 0.2 ml) of the culture broth was drawn from the positive vials showing growth of gram-positive cocci (as determined by Gram staining), heated in a boiling water bath for 15 min, and tested for thermonuclease activities on slides coated with toluidine blue-DNA agar (3). A positive thermonuclease reaction was the development of a pink halo extending at least 1 mm from the periphery of the reaction well. An aliquot (0.1 ml) of the positive blood cultures demonstrating thermonuclease activities was inoculated into the Bactometer module wells containing Mueller-Hinton agar (the negative control) or the same agar supplemented with oxacillin (2 μg/ml). The modules were incubated at 37°C, and DTs of the wells were automatically determined by the instrument or by manual inspection of the impedance curves. A vial was designated as ORSA or OSSA positive according to the same parameters set for pure stock cultures. The presence of *S. aureus* in the blood culture vials was determined by the conventional culture and identification methods, while oxacillin susceptibility of the isolates was analyzed by the agar disc diffusion method (21). Positive blood culture vials showing growth of mixed cultures, as revealed by Gram staining, were not included in this study.

**Sensitivity and specificity.** Sensitivity and specificity were determined as described by McClure (18).

## RESULTS

**Effect of oxacillin concentration on impedance curves.** The impedance curves of ***S. aureus*** ATCC 29213 (OSSA) grown under different concentrations (0 to 0.25 μg/ml) of oxacillin are shown in Fig. 1. In the absence of oxacillin, the test strain had a DT of 4 h (Fig. 1, curve a), and there was a small increase (0.1 h) in DTs at oxacillin concentrations of 0.063 and 0.125 μg/ml (Fig. 1, curves b and c, respectively). However, when the oxacillin concentration increased to 0.25 μg/ml, the growth of *S. aureus* ATCC 29213 was almost completely inhibited and no DT was obtained (Fig. 1, curve d). It was noted that a large decrease in the final values of impedance change occurred for the three impedance curves (Fig. 1, curves b, c, and d) in the presence of oxacillin.

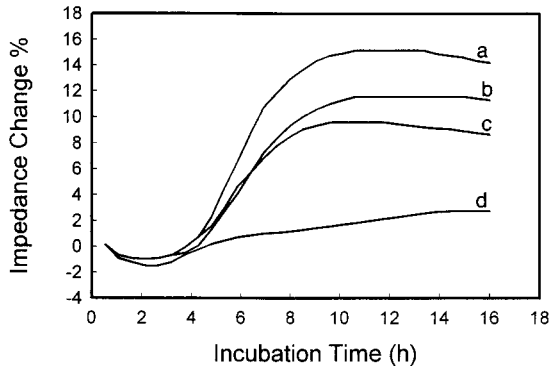


FIG. 1. Impedance curves of OSSA ATCC 29213 grown on Mueller-Hinton agar containing 0 (curve a), 0.063 (curve b), 0.125 (curve c), and 0.25 (curve d) μg of oxacillin per ml. The DTs for curves a, b, and c were 4.0, 4.1, and 4.1 h, respectively. No DT was obtained for curve d.

In contrast, the growth of *S. aureus* ATCC 33592 (Fig. 2A) and 33591 (Fig. 2B), both of which are oxacillin resistant, was only slightly inhibited at an oxacillin concentration as high as 64 μg/ml (Fig. 2A and B, curves c). For ATCC 33592, the DTs increased from 5.3 h in the absence of the antibiotic to 6.3, 7.0, and 9.3 h, respectively, in the presence of 32, 64, and 128 μg of oxacillin per ml of the test medium. There was a similar trend

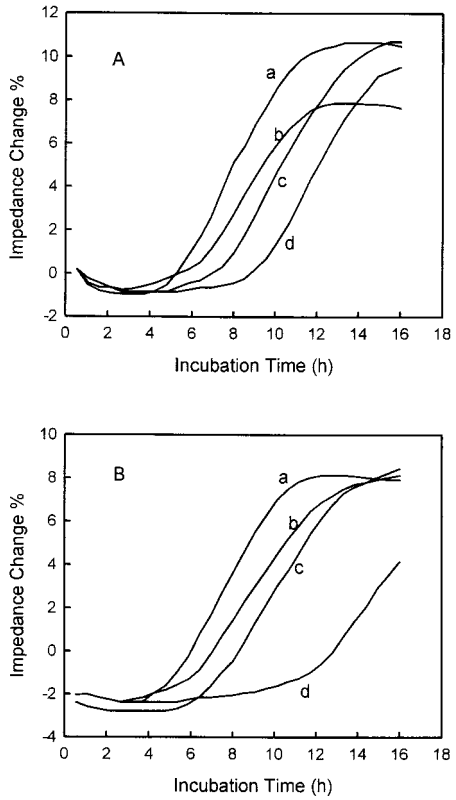


FIG. 2. Impedance curves of ORSA strains grown on Mueller-Hinton agar containing 0 (curve a), 32 (curve b), 64 (curve c), and 128 (curve d) μg of oxacillin per ml. (A) *S. aureus* ATCC 33592. The DTs for curves a, b, c, and d were 5.3, 6.3, 7.0, and 9.3 h, respectively. (B) *S. aureus* ATCC 33591. The DTs for curves a, b, c, and d were 5.3, 6.2, 6.8, and 12.1 h, respectively.

TABLE 1. Results of oxacillin susceptibility testing and the DTs of clinical *S. aureus* isolates obtained by the impedance method

| Bacterium | No. of strains | | DT in h (range) on Mueller-Hinton agar[a] | |
|---|---|---|---|---|
| | Tested | Correctly identified | Without oxacillin | With oxacillin (2 μg/ml) |
| OSSA | 86 | 84 | 4.5 ± 0.4 (4.1–6.0)[b] | —[c] |
| ORSA | 149 | 141 | 5.7 ± 0.6 (4.4–6.7) | 6.0 ± 0.7 (4.7–9.1) |

[a] Values are means ± standard deviations.
[b] Significant difference from the other value in the column by the unpaired *t* test at $P < 0.0001$.
[c] —, DT was not found within an incubation period of 24 h.

in the delay of DTs of ATCC 33591 in the presence of the antibiotic (Fig. 2B). It seemed that *S. aureus* ATCC 33592 was more resistant than ATCC 33591 at a high concentration of oxacillin. At an oxacillin concentration of 128 μg/ml, ATCC 33592 (Fig. 2A, curve d) still had a typical impedance curve having a high value of impedance change and showing a drastic change in the slope of the impedance curve at the DT point. However, at the same concentration (128 μg/ml) of oxacillin, ATCC 33591 displayed an impedance curve with a relatively lower value of impedance change and had a DT of 12.1 h (Fig. 2B, curve d).

The growth of the two oxacillin-resistant strains (ATCC 33592 and 33591) was not inhibited to a large extent even at an oxacillin concentration as high as 128 μg/ml; this was in strong contrast to the oxacillin-sensitive strain (ATCC 29213), whose growth was almost completely inhibited at an oxacillin concentration of 0.25 μg/ml. For this reason, it seemed possible to discern between ORSA and OSSA strains by the impedance method.

**Susceptibility tests of clinical *S. aureus* isolates by the impedance method.** Since an *S. aureus* strain for which the MIC was ≤2 μg was defined as oxacillin sensitive by the MIC method (22, 32), this concentration was used in the impedance method to screen a battery of 235 clinical isolates of *S. aureus* (86 OSSA and 149 ORSA).

Of the 86 strains of OSSA tested, 84 had no DT within an incubation period of 24 h and were considered oxacillin sensitive by the impedimetric technique (Table 1). Two OSSA strains (no. 64 and 73) had DTs similar or equal to the DTs of the negative controls (no oxacillin in the Mueller-Hinton agar) and were recognized as ORSA by the impedance method. The resistance of the two strains to oxacillin was reconfirmed with the oxacillin screen agar. The 84 strains of OSSA had an average DT of 4.5 ± 0.4 h (range, 4.1 to 6.0 h) (Table 1) when grown in the absence of oxacillin. Of the 86 strains of OSSA tested, the specificity of the impedance method was 97.7% (84 of 86).

Among the 149 strains of ORSA tested, 141 had DTs equal to the DTs of the negative controls or the delay in DTs was less than 3 h and were considered oxacillin resistant by the impedimetric technique (Table 1). Among the eight false-negative strains (no. 43, 69, 127, 161, 169, 173, 177, and 181), seven had no DT within an incubation period of 24 h, with the remaining strain (no. 127) having a DT of 22.7 h that was 17 h behind that of the negative control (DT, 5.7 h). The oxacillin susceptibility of these eight strains was reconfirmed with the oxacillin screen agar. The 141 ORSA strains had an average DT of 5.7 ± 0.6 h (range, 4.4 to 6.7 h) (Table 1) in the absence of oxacillin. The average DT increased to 6.0 ± 0.7 h (range, 4.7 to 9.1 h) in the presence of oxacillin (2 μg/ml). After 149 strains of ORSA had been tested, a sensitivity of 94.6% (141 of 149) was obtained by the impedance method. The overall agreement of the method with the conventional disc diffusion method was 95.7% (225 of 235).

It is interesting that the average DT (5.7 h) of the ORSA strains in the absence of the antibiotic was significantly ($P < 0.0001$) higher than the average DT (4.5 h) of the OSSA strains, as determined by the unpaired *t* test (Table 1). This indicates that the growth of most OSSA strains was faster than that of ORSA strains in Mueller-Hinton agar.

**Direct detection of ORSA in blood cultures.** The blood culture vials showing growth of gram-positive cocci and demonstrating thermonuclease activities indicated that there were *S. aureus* cells in these vials (17). A total of 96 such blood cultures were used for direct detection of ORSA by the impedance method. Among the 38 vials containing ORSA as determined by the conventional methods, 36 displayed typical impedance curves of ORSA with an average DT of 5.5 h. Among the 58 blood cultures containing OSSA, 57 exhibited typical impedance curves having no DTs within an incubation period of 24 h. Therefore, the impedance method had a sensitivity of 94.7% (36 of 38) and a specificity of 98.3% (57 of 58) for the detection of ORSA in blood cultures and had an agreement of 96.9% (93 of 96) with the conventional culture techniques comprising strain isolation and the subsequent antibiotic susceptibility tests.

## DISCUSSION

An impedance method for direct detection of ORSA in positive blood cultures was developed in this study. The results were available within an average of 10 h (2- to 4-h test of thermonuclease and about 6-h test of impedance) by the direct method versus an average of 48 h by the routine techniques. This allowed the detection of ORSA to be completed on the same day that the bacteremia was detected in the clinical laboratory. The method is simple; only a single step of transfer of 0.1 ml of the positive blood culture to the module well containing Mueller-Hinton agar supplemented with oxacillin is required. Since only one concentration of oxacillin (2 μg/ml) was used in the susceptibility testing medium, the impedance method can be considered as a breakpoint assay to differentiate between ORSA and OSSA.

In this study, only positive blood cultures showing growth of gram-positive cocci and exhibiting thermonuclease activities were tested by the impedimetric technique; this was to avoid the testing of other non-*S. aureus* cocci. Thermonuclease is a specific enzyme of *S. aureus* and has been used as a marker for detecting *S. aureus* in blood cultures (8, 17). The enzyme assay normally takes 2 to 4 h to obtain clear results from positive blood cultures (17, 24). Although some coagulase-negative staphylococci (e.g., *S. intermedius*, *S. hyicus*, and *S. delphini*) (8) were found to produce thermonuclease, these species are rarely found in blood cultures (unpublished data, National Cheng Kung University Hospital) and were not misidentified as *S. aureus* in this study. A more simple latex agglutination test also can be used to rapidly identify *S. aureus* in blood cultures (11); however, the bacterial cells in the culture broth need to be concentrated by centrifugation before the latex test can be performed. A 2-h tube coagulase test also can be used to rapidly identify *S. aureus* from blood culture bottles (19).

It is generally assumed that the results of microbiological analyses should be made available to the clinician in a relevant time frame. In some instances, the clinical utility of information provided by the microbiology laboratory is largely diminished by the length of time it takes to generate that information. Instruments that can automatically identify bacteria and

determine susceptibilities to antimicrobial agents represent a major technical advance in clinical microbiology. However, such automated systems have yielded spurious susceptibility results when certain drug-organism combinations were tested.

Direct identification and antimicrobial susceptibility testing of bacterial blood culture isolates were attempted by Trenholme et al. (27). Starting from a 10-ml portion of a positive blood culture, they used several centrifugation steps to remove blood cells and to concentrate the bacterial cells for the inoculation of the Vitek Automicrobic system (bioMerieux Vitek). However, they found a major portion (44%) of the nonidentifiable strains were *S. aureus*. In a recent study, Knapp et al. (16) found that the Vitek systems had 15% (10 of 67) false negatives for ORSA detection.

MicroScan (Baxter Diagnostics, Inc.) has been used recently for direct susceptibility testing from positive blood cultures. A centrifugation step with a serum separator was first used to concentrate a big volume (9.5 ml) of blood specimens for inoculation, and some very major and major errors were found to be caused by ORSA (29). The detection principle of these automatic identification-susceptibility systems is based on the change of turbidity or fluorescence, and the systems are primarily designed for the testing of pure cultures. Because of the deep red color of blood, the systems mentioned above may not be suitable for direct inoculation of blood samples into these machines. However, the method described in this study is based on the change of impedance and is basically not influenced by the color of the test samples.

A portion (5.3% [2 of 38]) of the ORSA strains in blood cultures were not correctly detected by the present impedance method. It has been noticed for a long time that some strains of MRSA are heteroresistant to methicillin; i.e., only a fraction of the daughter cells derived from a single colony are phenotypically resistant to methicillin, whereas the majority are sensitive to methicillin. Supplementation of media with NaCl (2 to 5%) is recommended when *S. aureus* strains are tested for methicillin resistance (4, 7, 21, 26, 32). It was postulated that NaCl may promote the growth of a slow-growing methicillin-resistant subpopulation of *S. aureus*. However, the medium (Mueller-Hinton agar) used in this study for susceptibility testing was not supplemented with NaCl, because high ionic strength has an adverse effect on impedimetric (or conductimetric) measurement (12). This might account for the 5.3% false negatives during the detection of ORSA in blood cultures by the present method. Another factor causing the several false negatives during ORSA detection might be the carryovers of residual antibiotic(s) present in the culture vials to the module wells for the impedance test which inhibited the growth of *S. aureus.*

The false-positive rate (1.7% [1 of 58]) for the detection of ORSA in blood cultures by the impedance technique was low. In the beginning of this study, multiple blood samples from healthy donors were inoculated into the module wells containing Mueller-Hinton agar (without oxacillin in it), the wells were monitored for 24 h, and no impedance change was observed. For this reason, the only false positive result might not be caused by the metabolic activities of blood cells transferred to the module well.

In view of the high rate of isolation of ORSA from bacteremic episodes, a rapid detection method for this bacterium is useful. The common practice used in hospitals is to subculture blood specimens at the time when Gram stain is positive or microbial growth is apparent (e.g., by turbidity, gas production, or hemolysis). The subculture and susceptibility testing steps normally take 48 h. The average time required for the impedance method to detect ORSA in positive blood cultures is less than 10 h, and multiple samples can be automatically analyzed at the same time. In conclusion, the impedance method is proposed as a supplementary test for early detection of ORSA; the method is simple and may allow proper antimicrobial treatment almost 36 h before the results of other commonly used systems are available.

## ACKNOWLEDGMENTS

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