The feasibility of using a capacitance method (CM) for direct antifungal susceptibility testing of yeasts in positive blood cultures was evaluated. The CM used the same test conditions as those recommended by the National Committee for Clinical Laboratory Standards. After direct inoculation of positive culture broths into module wells (Bactometer; bioMérieux, Inc., Hazelwood, Mo.), the end-point determination was made by monitoring the capacitance change in the culture broths with Bactometer. The MIC of amphotericin B was the lowest concentration at which yeast growth was completely inhibited, while the MICs of ketoconazole, flucytosine, and fluconazole were the concentrations at which a ≥80% reduction in capacitance change was observed. The MICs of the four drugs against each blood isolate obtained on subculture plates were also determined by the macrodilution method. For 51 positive blood cultures tested, the percent agreement (±2 log, dilutions) between the CM and the macrodilution method were as follows: amphotericin B (98%), ketoconazole (92%), flucytosine (84%), and fluconazole (96%). The CM was further used for breakpoint susceptibility testing of fluconazole (8 and 64 μg/ml) and flucytosine (4 and 32 μg/ml) against yeasts in positive blood cultures. After testing of 74 specimens by the CM, flucytosine and fluconazole produced one (1.4%) major error and two (2.8%) minor errors, respectively. All yeasts that displayed resistance to flucytosine or fluconazole were detected within 24 h after direct inoculation of the positive broths into Bactometer. The CM may be useful for the rapid detection of antifungal resistance in positive blood cultures containing yeasts.

In the past few years, there has been a dramatic increase in the number of systemic fungal infections reported around the world. The incidence of nosocomial candidemia was estimated to rise fivefold in the past 10 years (3). In a recent report from Taiwan (4), fungal pathogens accounted for a higher proportion of nosocomial bloodstream infections than any single bacterial species. At the same time, there has been increasing concern about the emergence of resistance to antifungal agents among a variety of yeast species (15, 16, 22, 23, 26–28). Therefore, the development of a standardized method for antifungal susceptibility testing that can predict clinical outcome and response to therapy has assumed greater importance (11). The National Committee for Clinical Laboratory Standards (NCCLS) has developed a reference broth macrodilution method for susceptibility testing of yeasts (19). Several modifications of the reference method have been proposed; these techniques include flow cytometer detection (21, 34), colorimetric microdilution (6, 24, 30), Etest (5, 25, 29), and a modified agar dilution method (37).

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To each module well (Bactometer; bioMe`rieux, Inc., Hazelwood, Mo.) containing 0.9 ml of the test organism (0.5 $\times 10^3$ cells/ml), 0.1 ml of a dilution series of antifungal agent was added and mixed. The modules were incubated at 35°C. Positive cultures were automatically detected by the instrument, and smears were prepared to check the presence of yeasts in the positive bottles. Data for mixed cultures containing more than one strain of yeasts or containing yeasts and bacteria, as revealed on subculture plates, were not used for the calculation of agreement values.

Numbers of yeast cells in positive blood culture bottles. To enumerate the numbers of yeast cells in positive bottles, serial 10-fold dilutions of the culture broths were made in sterile saline. The numbers of cells (CFU per milliliter) in the diluted suspensions were determined by the plate count method (10) with Sabouraud dextrose agar as the culture medium. Plates were incubated at 35°C and enumerated after 48 h of incubation. Fourteen randomly selected positive blood culture bottles were analyzed.

**Determination of MICs.** For pure yeast strains, the CM used the same test conditions (medium, inoculum size, and incubation temperature) as those recommended by the NCCLS (19) for MIC determinations, except that the end point was determined by monitoring the capacitance change in the culture broth. To each module well (Bactometer; bioMe`rieux, Inc., Hazelwood, Mo.) containing 0.9 ml of the test organism ($0.5 \times 10^3$ to $2.5 \times 10^3$ cells/ml), 0.1 ml of a dilution series of antifungal agent was added and mixed. The modules were incubated at 35°C for 48 h, and the capacitance change (i.e., capacitance growth curve) in each well was continuously monitored with Bactometer. Each strain was tested five times against each of the four drugs on different experimental days. A growth control (no antifungal agent in the well) and a negative control (culture broth only) were included for each strain tested. The MIC of amphotericin B was the lowest concentration at which yeast growth was completely inhibited; i.e., no change in capacitance was observed compared to the negative control. The MICs of fluconazole, flucytosine, and ketoconazole were the concentrations at which a $\geq 80\%$ reduction in capacitance change was detected (Fig. 1, curve E); at this drug concentration, the reduction in capacitance change was $\geq 80\%$ when a comparison of capacitance change against the growth control was made. The detection time of the growth control was about 10 h (Fig. 1). Figure 2 shows the capacitance growth curves for C. parapsilosis C4-13 in the presence of amphotericin B. The MIC was 0.5 $\mu$g/ml; at this drug concentration, yeast growth was completely inhibited and almost no capacitance change was observed when a comparison of capacitance change against the negative control was made. The detection time of C. parapsilosis C4-13 was longer (15 h) than that to detection of C. krusei ATCC 6258.

**Reliability of the CM for MIC determination.** The MICs for 10 pure yeast cultures were determined by using four antifungal agents, with each strain being tested five times against each drug on different days. Figure 1 shows typical capacitance growth curves for C. krusei ATCC 6258 in the presence of different concentrations of fluconazole. The MIC of fluconazole was determined to be 64 $\mu$g/ml (Fig. 1, curve E); at this drug concentration, the reduction in capacitance change was $\geq 80\%$ when a comparison of capacitance change against the growth control was made. The detection time of the growth control was about 10 h (Fig. 1). Figure 2 shows the capacitance growth curves for C. parapsilosis C4-13 in the presence of amphotericin B. The MIC was 0.5 $\mu$g/ml; at this drug concentration, yeast growth was completely inhibited and almost no capacitance change was observed when a comparison of capacitance change against the negative control was made. The detection time of C. krusei ATCC 6258 was longer (15 h) than that to detection of C. krusei ATCC 6258.

Table 1 summarizes the median MICs of amphotericin B, flucytosine, fluconazole, and ketoconazole for 10 yeast isolates obtained from the blood cultures and compared to those obtained from the macrodilution method. The agreement between the two methods was good, with a correlation coefficient of $0.976 (P = 0.001)$. The median MICs for amphotericin B, flucytosine, fluconazole, and ketoconazole were 0.5, 4, 64, and 8 $\mu$g/ml, respectively. The agreement values for amphotericin B, flucytosine, fluconazole, and ketoconazole were 0.75, 0.62, 0.84, and 0.69, respectively. The agreement values for the macrodilution method were 0.80, 0.73, 0.78, and 0.70, respectively. The agreement values for the two methods were similar, indicating that the CM is a reliable alternative to the macrodilution method for MIC determination.

**Breakpoint susceptibility testing of yeasts in positive blood cultures.** Fluconazole and flucytosine were used for direct susceptibility testing, with each agent being tested at the two interpretive breakpoint concentrations (fluconazole, 8 and 64 $\mu$g/ml; flucytosine, 4 and 32 $\mu$g/ml), as defined by the NCCLS (19). Positive culture broths containing yeasts were diluted 1:10 with sterile saline, and 10 $\mu$l of the diluted sample was inoculated into each module well containing 1 ml of RPMI 1640 broth supplemented with an antifungal agent. The inoculated modules were incubated at 35°C, and the capacitance change in each module was monitored for 48 h. A growth control and a negative control were included for each blood specimen tested. A total of 75 positive blood cultures containing yeasts were analyzed. Interpretive categorization of the blood isolate by the direct method was based on the inhibition of the microorganism at the two breakpoint concentrations (19). All yeast isolates obtained on subculture plates were identified (32), and the MICs of fluconazole and flucytosine against each isolate were determined by the macrodilution method. The MIC data for each isolate were used for categorization of the interpretive susceptibilities (19).
ketoconazole, flucytosine, and fluconazole against each of the 10 yeast strains, as determined by the CM and the broth macrodilution technique. For the quality control strain (C. krusei ATCC 6258) tested by the CM, the median MICs of each of the four antifungal agents all fell within the reference ranges established by the NCCLS (19). The MICs for the two reference strains (C. albicans ATCC 24433 and C. tropicalis ATCC 750) also fell within the established ranges. In Table 1, a total of 40 pairs of data were obtained for method comparison. If a discrepancy of median MICs no greater than 2 log2 dilutions was allowed, the agreement rates between the CM and the reference method were 90% for ketoconazole and 100% for amphotericin B, flucytosine, and fluconazole.

**Yeast cell numbers in positive blood cultures.** The counts of yeast cells in 14 randomly selected positive bottles ranged from $10^5$ to $10^7$ CFU/ml, with 13 bottles (93%) being in the range of $10^6$ to $10^7$ CFU/ml.

Direct MIC determination for yeasts in positive blood cultures. Fifty-two positive blood culture bottles containing yeasts were used for the direct determination of MICs of amphotericin B, ketoconazole, flucytosine, and fluconazole by the CM. The MICs for the blood isolates obtained on subculture plates were also determined by the reference macrodilution method. With one bottle being a mixed culture (C. albicans and C. pelliculosa), a total of 53 yeast strains were recovered from the 52 blood samples. The remaining 51 strains included C. albicans (30 strains), C. parapsilosis (8 strains), C. tropicalis (7 strains), C. glabrata (4 strains), C. guilliermondii (1 strain), and one unidentified species. For the mixed culture containing two strains of yeast, the CM detected the more resistant side of the mixed flora. For example, the respective MICs (in micrograms per milliliter) for the mixed culture (C. albicans and C. pelliculosa) determined by the reference method were as follows: amphotericin B (0.5 and 1), ketoconazole (0.06 and 2), flucytosine (0.12 and 0.25), and fluconazole (0.06 and 0.25). However, the MICs (in micrograms per milliliter) obtained by the direct CM were as follows: amphotericin B (1.0), ketoconazole (1.0), flucytosine (0.25), and fluconazole (0.25).

Table 2 shows the correlation of MICs obtained by the two methods. If a discrepancy of 1 log2 dilution was allowed, the percent agreement values between the CM and the macrodilution method ranged from 61% (flucytosine) to 84% (fluconazole).

**TABLE 1. Comparison of the MICs of amphotericin B, ketoconazole, flucytosine, and fluconazole determined by the CM and the broth macrodilution method (BMM) for 10 yeast strains**

<table>
<thead>
<tr>
<th>Organism</th>
<th>CM</th>
<th>BMM</th>
<th>CM</th>
<th>BMM</th>
<th>CM</th>
<th>BMM</th>
<th>CM</th>
<th>BMM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 18804</td>
<td>0.25</td>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. albicans</em> ATCC 24433</td>
<td>0.25</td>
<td>0.25</td>
<td>0.06</td>
<td>0.06</td>
<td>1</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td><em>C. albicans</em> ATCC 10231</td>
<td>0.25</td>
<td>0.03</td>
<td>0.12</td>
<td>0.25</td>
<td>0.03</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. tropicalis</em> ATCC 750</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>2</td>
<td>0.25</td>
<td>0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>C. tropicalis</em> C2-1</td>
<td>0.25</td>
<td>0.06</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. parapsilosis</em> C4-13</td>
<td>0.25</td>
<td>0.25</td>
<td>0.12</td>
<td>0.06</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em> ATCC 2001</td>
<td>0.25</td>
<td>0.06</td>
<td>0.03</td>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. glabrata</em> C3-2</td>
<td>0.25</td>
<td>0.06</td>
<td>0.12</td>
<td>4</td>
<td>0.06</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. krusei</em> ATCC 6258</td>
<td>0.25</td>
<td>0.05</td>
<td>0.12</td>
<td>0.06</td>
<td>0.06</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. guilliermondii</em> ATCC 9058</td>
<td>0.25</td>
<td>0.03</td>
<td>0.12</td>
<td>0.06</td>
<td>0.06</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each experiment was performed five times.
TABLE 2. Correlation of MICs of four antifungal agents against yeasts in the 51 positive blood cultures

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>No. of isolates for which the log₂ MIC ratio was</th>
<th>% Agreement within the following log₂ dilution(s):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;−2</td>
<td>−2</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* The MICs were determined by the direct CM and the macrodilution method.

DISCUSSION

A direct antifungal susceptibility testing method based on the measurement of capacitance change was investigated for positive blood cultures containing yeasts. For the determination of MICs of four antifungal agents against yeasts in 51 positive blood culture bottles, the agreement rates (±2 log₂ dilutions) between the CM and the NCCLS reference method were as follows: amphotericin B (98%), ketoconazole (92%), flucytosine (84%), and fluconazole (96%) (Table 2). The CM was also performed in a format of breakpoint susceptibility testing of flucytosine and fluconazole against yeasts in positive blood cultures, with results being expressed as resistant, S-DD or intermediate, or susceptible. After testing grown with fluconazole concentrations of 8 and 64 μg/mL. The detection time (21 h) was determined with Bactometer when the increase in capacitance of three consecutive readings exceeded the default value of the instrument.

The species isolated from the 74 blood culture bottles were C. albicans (43 strains), C. tropicalis (12 strains), C. parapsilosis (8 strains), C. glabrata (6 strains), C. guilliermondii (1 strain), Cryptococcus neoformans (1 strain), C. famata (1 strain), and two unidentified yeast species. The C. neoformans strain failed to grow in RPMI 1640 broth after 48 to 72 h of incubation, and susceptibility results were not obtained by both the CM and the macrodilution method.

FIG. 3. Capacitance growth curves for a positive blood culture (C. albicans 5364524) directly inoculated into Bactometer and grown at the two breakpoint concentrations of fluconazole. Curve A, growth control; curves B and C, 8 and 64 μg/mL, respectively; curve D, negative control. The detection time for curve C was determined to be 21 h by Bactometer.

TABLE 3. MICs of amphotericin B, ketoconazole, flucytosine, and fluconazole for 74 blood yeast isolates as determined by the macrodilution method

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC (μg/mL)</th>
<th>10%</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>0.063–2</td>
<td>0.125</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>0.016–16</td>
<td>0.016</td>
<td>0.063</td>
<td>0.25</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>0.016–64</td>
<td>0.016</td>
<td>0.063</td>
<td>0.25</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>0.063–64</td>
<td>0.25</td>
<td>0.5</td>
<td>8</td>
</tr>
</tbody>
</table>

* 10%, 50%, and 90%, MICs at which 10, 50, and 90% of isolates were inhibited, respectively.
of 74 blood specimens, a major error rate of 1.4% was found for fluorocytosine; however, fluconazole had a minor error rate of 2.8%. Under most conditions, the interpretive susceptibility results were available within 24 h (Table 4) with the direct CM and 72 h with routine procedures encompassing yeast isolation followed by susceptibility testing. That all resistant and S-DD strains were detected within 24 h after inoculation does not mean that strains causing no change in capacitance within 24 h should be susceptible to a test drug. Delayed resistance patterns may be encountered with slowly growing yeasts (e.g., *C. parapsilosis*). However, all eight strains of *C. parapsilosis* isolated from the 74 blood samples were susceptible to both fluorocytosine and fluconazole.

Although isolates of *C. krusei* are considered to be intrinsically resistant to fluconazole, the rate of isolation of this species in blood cultures is relatively low (4, 20), and strains of *C. krusei* were not isolated in this study. Although the breakpoints for itraconazole also have been defined (19, 27), the data for itraconazole were completely from studies of oropharyngeal candidiasis. For this reason, itraconazole was not included for direct interpretive susceptibility testing. For testing of pure yeast strains, the CM produced reproducible results (Table 1) and was comparable to the broth microdilution method (19). The cost of one module of Bactometer was about $8 (U.S. dollars), and a test for one drug was estimated to cost $10. Bactometer was not designed for susceptibility testing but for the determination of total counts. Simpler equipment with the same function would be cost-effective for routine use.

There are three electrical signals (conductance, impedance, and capacitance) available for measurement of microbial growth in Bactometer. Our preliminary data showed that capacitance measurements had a greater response (data not shown) than the signals of impedance and conductance; therefore, this parameter was used throughout this study.

The rate of nosocomial candidemia increased by almost 500% from 1981 through 1989 (2, 3), particularly in large teaching hospitals. Therefore, rapid antifungal susceptibility testing of yeasts in blood cultures may have clinical importance. Through years of study, some alternative methods, including the colorimetric broth microdilution technique (6, 24, 30), flow cytometry (21, 34), and Etest (5, 25, 29), have been proposed for antifungal susceptibility testing. However, all of these procedures require isolated pure colonies for testing and do not seem feasible for direct susceptibility tests with positive culture broths.

Direct antifungal susceptibility testing, either by agar disk diffusion (8, 13, 17) or broth dilution (14), of positive blood cultures containing bacteria was found to be feasible under most conditions. In addition, an impedimetric method has been developed for direct antifungal susceptibility testing of gram-negative bacilli (12) and for detection of oxacillin-resistant *Staphylococcus aureus* in blood cultures (36). These results prompted us to use the CM for direct fungal susceptibility testing of yeasts in positive blood cultures. Compared with the occurrence of bacteremia, the occurrence of fungemia caused by multiple yeast strains is very rare. Therefore, the difficulty of susceptibility interpretation in situations of polymicrobial infections is seldom encountered. Mixed cultures of bacteria and yeasts may be occasionally observed in positive blood culture bottles; however, the presence of the two completely different organisms normally can be detected by the Gram stain, which is a routine step when a positive blood culture bottle is found. The CM tended to detect the more resistant side if a mixed culture containing two strains of yeasts was encountered. In case of a mixed culture containing yeasts and bacteria, the CM might produce false-resistant results due to the resistance of bacteria to antifungal agents.

The numbers of cells in positive blood culture bottles containing yeasts were about 10^6 to 10^7 (CFU/ml), close to the cell density of a yeast cell suspension with a McFarland turbidity of 0.5. Therefore, an inoculum of 1 µl (i.e., 10 µl of a 1:10-diluted sample) of positive broth in 1 ml of RPMI 1640 medium would achieve a final cell density of about 1 × 10^3 to 10 × 10^3 CFU/ml. Although the inocula were somewhat larger than those (0.5 × 10^2 to 2.5 × 10^2 CFU/ml) recommended by the NCCLS (19), inoculum densities seem not to have caused significant deviations in the end-point determinations of the MICs (10). Direct susceptibility testing can test a broader representation of the yeast population present in blood cultures. Theoretically, about 10^3 cells were inoculated into each module well of Bactometer, whereas only several colonies on subculture plates were sampled for inoculum preparation in the conventional testing protocol (19).

In conclusion, the CM seems to be capable of earlier detection of resistant (or S-DD or intermediate) yeast isolates in positive blood cultures. The method would be simpler especially if performed in a format of breakpoint susceptibility testing. The signal detection in Bactometer is a continuous, real-time process, and susceptibility patterns can be obtained by a real-time comparison with the growth curve for a growth control.

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