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# Agreement of Direct Antifungal Susceptibility Testing from Positive Blood Culture Bottles with the Conventional Method for *Candida* Species

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Early availability of antifungal susceptibilities can ensure timely institution of targeted therapy in candidemia, which can improve patient outcomes. This study prospectively determines the agreement between the results of direct testing of antifungal susceptibilities from blood culture bottles by disk diffusion and Etest and the results of standardized susceptibility testing methods; direct testing would allow susceptibility results to be available 1 to 2 days earlier. A total of 104 blood cultures with different *Candida* species (28% *C. albicans*, 27% *C. parapsilosis*, 26% *C. tropicalis*, etc.) were evaluated between January 2012 and May 2013 for agreement of fluconazole, voriconazole, and amphotericin B susceptibility results by disk diffusion. Agreement in MICs obtained by Etest was determined for fluconazole (21 isolates), voriconazole (28 isolates), amphotericin (29 isolates), and caspofungin (29 isolates). The kappa scores for categorical agreement were highest for fluconazole by disk diffusion (0.902, standard error [SE] = 0.076) and Etest (1.00, SE = 0.218) and for amphotericin B by disk diffusion (1.00, SE = 0.098). The Pearson correlation (*r*) of zone diameters was strongest for fluconazole (0.69) and amphotericin (0.70) and moderate for voriconazole (0.60), and the Pearson correlation of MICs was strongest for fluconazole (0.94) and caspofungin (0.88). However, the moderate correlation of amphotericin MICs with zone diameters (−0.42) precludes the use of amphotericin B disk diffusion for susceptibility testing. There were no very major errors; however, there were 1 (1%) major and 5 (4.8%) minor errors with disk diffusion and 4 (13.3%) minor errors with Etest. Thus, antifungal disk diffusion directly from blood culture bottles is a rapid and easy method for fluconazole and voriconazole susceptibility testing for timely tailoring of candidemia therapy.

Invasive fungal infections caused by members of the genus *Candida* are important causes of morbidity and mortality in immunocompromised and hospitalized patients (1, 2). In hospitalized patients, *Candida* species are the fourth most common cause of bloodstream infections, with around 38% mortality (3, 4).

Globally, *Candida albicans* tends to be the most frequently (50 to 70%) reported species. In contrast, data from Pakistan report non-*albicans Candida* species, mainly *C. tropicalis*, as the most predominant species (5). With the emergence of non-*albicans Candida* species in many settings, resistance to fluconazole is a serious concern, as highlighted by recent surveillance data (6–8). Increased mortality has been reported for candidemia patients with delays in the initiation of appropriate antifungal therapy (9). Patients receiving antifungal treatment more than 12 h after having a positive blood culture sample drawn had a higher (33.1%) risk of hospital mortality than patients begun on antifungal treatment within 12 h (11.1%) (10). Hence, early and appropriate therapy is essential to prevent severe complications and eventual mortality.

The conventional method for determining fungal susceptibility requires subculturing of blood from bottles showing growth of yeasts on solid agar and incubation of those plates for 24 to 48 h to get growth of *Candida* species. Colonies are then used to prepare inocula for susceptibility testing, and final reporting takes another 24 h (11). This delay could lead to serious consequences if the species isolated is resistant to the empirical drug used for therapy. Therefore, in clinical practice, a prompt and cost-effective method is needed to perform antifungal susceptibility testing.

Direct susceptibility testing from positive bottles has been studied for bacterial pathogens and is now being used as standard

practice in clinical microbiology laboratories (12). This approach has reduced the time from positivity of blood culture to preliminary reporting of susceptibility results. This practice has also been evaluated for yeasts using Etest, which showed a 98% agreement rate between direct susceptibility testing and the conventional method (13, 14). A few other studies have evaluated direct susceptibility testing using Vitek antifungal cards, Sensititre YeastOne, and flow cytometry and have had various results (15–17). All of these techniques are expensive and may not be practical in all clinical laboratories.

The disk diffusion method is easy to perform in a clinical laboratory, the materials required are more cost-effective than the Etest, and clinical categorical interpretations of zone diameters of fluconazole and voriconazole are available for common *Candida* species (18). Thus, in this study, we evaluated direct disk diffusion testing as an alternative to the conventional method to detect antifungal susceptibilities. Direct determination of MICs with Etest was also performed on a limited number of isolates.

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## MATERIALS AND METHODS

**Study background.** This prospective study was conducted in the clinical laboratory of the Aga Khan University Hospital (AKUH), Karachi, Pakistan, from January 2012 to May 2013. The laboratory has a national specimen collection network with more than 200 collection points in major cities and towns across the country.

**Specimens.** A total of 104 blood culture specimens submitted to the laboratory and smear positive for yeasts were included in this study. When the incubated BacTec 9420 aerobic and/or anaerobic blood culture bottles signaled positive, an aliquot was examined under the microscope for budding yeast cells. Blood cultures positive for yeast mixed with bacteria were excluded (data not recorded) from the study.

**Organism identification.** During the study period, *Candida* species were identified using the standard protocol, as follows: germ tube production, colony morphology on BiGGY agar (Becton Dickinson), urease test (Oxoid), cycloheximide sensitivity test (Becton Dickinson), and presence of pseudohyphae on corn meal agar with Tween 80. Isolated colonies were also evaluated for sugar assimilation on the biochemical test panel API 20C AUX (bioMérieux).

**Direct antifungal susceptibility testing.** The procedure for direct susceptibility testing from positive blood cultures was optimized as published previously (19). The concentration of *Candida* species in a positive blood culture bottle was not determined in this study, as a previous study has shown that *Candida* cell counts in positive blood culture bottles were in the range of  $10^5$  to  $10^8$  CFU/ml, with 87% of bottles having cell counts of  $10^6$  to  $10^7$  CFU/ml (exactly the count in 0.5 McFarland standards) (13). One hundred-microliter amounts of uncentrifuged broth from smear-positive blood culture bottles were used to make lawns on Mueller-Hinton agar supplemented with 2% dextrose and 0.5  $\mu$ g/ml methylene blue dye (MHA-MB). Neo-Sensitab (Rosco) disks with 25  $\mu$ g fluconazole, 25  $\mu$ g voriconazole, or 10  $\mu$ g amphotericin B were placed on the plates, and the plates were incubated for 20 to 24 h at  $35 \pm 2^\circ\text{C}$ . CLSI disk diffusion breakpoints using Rosco tablets have not been established and this product is not widely available; however, similar breakpoints have been used by other institutions using Rosco tablets (20). After 20 to 24 h, the zones of inhibition around each disk were measured using a millimeter scale and interpreted according to the manufacturer's recommendations (20). For voriconazole, for all species, the susceptible (S) inhibition zone diameter was  $\geq 17$  mm, the susceptible-dose-dependent (S-DD) inhibition zone diameter was 14 to 16 mm, and the resistant (R) inhibition zone diameter was  $\leq 13$  mm. For fluconazole,  $\geq 19$  mm was interpreted as S, 15 to 18 mm as S-DD, and  $\leq 14$  mm as R for *C. albicans*, *Candida parapsilosis*, and *C. tropicalis* and  $\geq 15$  mm was interpreted as S-DD and  $\leq 14$  mm as R for *Candida glabrata* (18). For amphotericin B, a  $\geq 15$ -mm zone of complete inhibition was interpreted as S, 10 to 14 mm as intermediate susceptibility, and  $< 10$  mm as R according to the manufacturer's recommendations (Neo-Sensitabs; Rosco), although there are no established interpretive criteria for amphotericin B (20).

MIC determinations using Etest (AB Biodisk, Solna, Sweden) for fluconazole, voriconazole, amphotericin B, and caspofungin were performed using RPMI agar as recommended by the manufacturer and read at 24 h as recommended by CLSI M27-A3 (21). Susceptibility cutoffs were determined using CLSI M27-S4 criteria (22). Although clinical breakpoints or epidemiological cutoff values (ECVs) for amphotericin have not been verified for MICs obtained by Etest, data suggest that broth microdilution cutoffs may be used (23). Hence, for amphotericin B, an ECV of 2  $\mu$ g/ml was used (24), and for those *Candida* species for which CLSI cutoffs were not available, ECVs were used according to the recommendations of Pfaller et al. (25).

**Conventional antifungal susceptibility disk diffusion.** The conventional disk diffusion method was used as the gold standard for comparison (18). A lawn was made on MHA-MB using a suspension of *Candida* species equal to 0.5 McFarland turbidity standard. Neo-Sensitab fluconazole (25  $\mu$ g), voriconazole (25  $\mu$ g), and amphotericin B (10  $\mu$ g) disks were placed on the agar surface, and plates were incubated for 20 to 24 h at  $35 \pm$

TABLE 1 Description of *Candida* isolates included in the study

Organism(s)	No. of isolates	% of isolates	No. of isolates with indicated susceptibility <sup>a</sup> :	
			S-DD/I	R
<i>C. albicans</i>	29	27.9	0	0
<i>C. tropicalis</i>	27	25.9	0	0
<i>C. parapsilosis</i>	28	26.9	1 to VRC	1 to FLC
<i>C. glabrata</i>	8	7.7	7 to FLC, 3 to CAS	1 to FLC
Non- <i>albicans Candida</i>	4	3.8	0	1 to FLC
<i>C. lusitaniae</i>	4	3.8	0	1 to FLC
<i>C. pelliculosa</i>	2	1.9	0	0
<i>C. albicans</i> + <i>C. krusei</i> <sup>b</sup>	2	1.9	0	0
Total	104	100	9/104	4/104

<sup>a</sup> Isolates were categorized as S-DD and R to voriconazole and fluconazole when tested according to standard protocol and susceptibility criteria as described in reference 18. VRC, voriconazole; FLC, fluconazole; CAS, caspofungin; S-DD, susceptible-dose dependent; I, intermediate susceptibility; R, resistant.

<sup>b</sup> These isolates were categorized according to the standard susceptibilities of the most resistant of the two isolates (*C. krusei* in both cases).

$2^\circ\text{C}$ . After 20 to 24 h of incubation, the zones of inhibition around each disk were measured and interpreted similarly to the direct testing method described above. For fluconazole and voriconazole, the zone of inhibition showing from 50 to 80% drop in growth was measured, while for amphotericin B, the zone diameter from the point showing complete inhibition of growth was measured. Etests against fluconazole, voriconazole, amphotericin B, and caspofungin were performed (AB Biodisk, Solna, Sweden) using standard inocula from colonies on RPMI agar. For quality control, *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used with each new batch of medium prepared or new lot of antifungal disks or Etests used.

**Statistical analysis.** Descriptive statistics were calculated using Microsoft Excel. Statistical analysis was performed with Stata 12 statistical software. The frequencies of isolation of the different species and their rates of resistance were calculated. Kappa scores were generated for categorical agreement, and the Pearson correlation test was applied to assess whether the results of the direct disk diffusion and Etest methods of *Candida* susceptibility testing correlated with the results from the standard method. It was also applied to assess how well zone diameters correlated with MICs for amphotericin B. The following standards were used for the strength of agreement for the kappa coefficient: 0 to 0.0099, poor; 0.01 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; and 0.81 to 1, almost perfect.

A correlation coefficient of  $> 0.8$  was considered very strong correlation, 0.6 to 0.79 strong, 0.4 to 0.59 moderate, 0.2 to 0.39 weak, and  $< 0.19$  as very weak. The Pearson chi-square test was used to evaluate whether the total number of errors in categorizing a strain as S, S-DD, or R was significantly associated with any species. For all statistical tests, a *P* value of  $< 0.05$  was considered significant.

## RESULTS

One hundred six *Candida* species were isolated from 104 blood culture bottles. Two cases had polymicrobial candidemia, with *C. albicans* and *C. krusei*. Disk diffusion antifungal susceptibility testing using the direct method and the conventional method was performed on all 106 isolates, and Etest susceptibility testing was performed on 21 isolates for fluconazole, 28 for voriconazole, and 29 for amphotericin and caspofungin. A description of the isolates with their susceptibility patterns is shown in Table 1.

Comparison of the results of the conventional and the direct susceptibility testing methods by kappa score demonstrated excel-

**TABLE 2** Agreement rates and kappa scores of the results of susceptibility testing performed directly from blood culture bottles and following standard methodology

Antifungal	Test modality	No. of isolates tested	Categorical agreement (%)	Kappa score (SE)	Kappa <i>P</i> value
Voriconazole	Disk diffusion	104	97.12	-0.0130 (0.0923) <sup>a</sup>	0.5559
	Etest	28	100	— <sup>b</sup>	—
Fluconazole	Disk diffusion	104	97.12	0.9019 (0.0755)	<0.0001
	Etest	21	100	1.000 (0.2182)	<0.0001
Amphotericin B	Disk diffusion	104	100	1.000 (0.0981)	<0.0001
	Etest	29	100	— <sup>b</sup>	—
Caspofungin	Etest	29	86.21	0.4844 (0.1675)	0.0019

<sup>a</sup> The kappa score is negative because there was only one isolate in the nonsusceptible category and the expected agreement rate is higher than the detected agreement.

<sup>b</sup> —, the kappa statistic could not be generated due to too few categories (all isolates were susceptible with 100% agreement). The following standards for strength of agreement of the kappa coefficient were used: 0 to 0.0099, poor; 0.01 to 0.20, slight; 0.21 to 0.40, fair; 0.41 to 0.60, moderate; 0.61 to 0.80, substantial; 0.81 to 1, almost perfect.

lent agreement for fluconazole using both disk diffusion and Etest (Table 2) and for amphotericin using disk diffusion. Agreement for voriconazole using disk diffusion was low because there was only one nonsusceptible strain and the result was statistically insignificant ( $P = 0.56$ ). The Etest results for voriconazole and amphotericin B had 100% categorical agreement, but kappa scores could not be generated because there was only one category, i.e., susceptible. Although the caspofungin MICs only differed by one or two dilutions, they resulted in categorical disagreement and only moderate agreement by kappa score.

The mean zone diameters, MICs, and correlation data are shown in Table 3. The zone diameters from direct and standard testing correlated well: the results for fluconazole and amphotericin B correlated strongly, while the results for voriconazole correlated only moderately well. The Etest results were less consistent: the results for fluconazole and caspofungin showed very strong correlations and the results for voriconazole a moderate correlation, while the amphotericin B MICs using Etest correlated very weakly. The zone diameters of amphotericin B also correlated moderately well with the amphotericin B MICs using Etest. All correlation results were found to be statistically significant ( $P < 0.05$ ) except for the Etest results for amphotericin B, which appears to be more inoculum dependent than other agents.

Analysis of major and minor errors in disk diffusion results (Table 4) revealed 1 major error (for voriconazole against a *C.*

*parapsilosis* isolate) and 5 minor errors (for 2 *C. tropicalis* isolates, 2 *C. parapsilosis* isolates, and 1 *C. albicans* isolate) and no very major errors. These errors were not found to be significantly associated with species. There were no major or very major errors identified for the test method using Etest. However, four minor errors were identified, including 2 cases of *C. glabrata* and 2 cases of mixed *C. krusei* and *C. albicans*, showing that the probability of error in direct caspofungin MICs for these species was greater than by chance alone ( $P < 0.001$ ).

## DISCUSSION

We determined the agreement between the results of conventional and direct antifungal susceptibility disk diffusion testing of fluconazole, voriconazole, and amphotericin B for *Candida* species isolated from positive blood culture bottles. Additionally, the agreement between conventional and direct susceptibility testing using Etest was also assessed for fluconazole, voriconazole, amphotericin B, and caspofungin.

Using disks, excellent agreement was observed for fluconazole and amphotericin, confirmed by high kappa scores of 0.90 and 1.00, respectively. However, despite good reproducibility, the zone diameters of amphotericin B correlated moderately with the amphotericin B MICs, reconfirming that disk diffusion is not an acceptable method for amphotericin B susceptibility testing using either direct susceptibility or conventional disk diffusion testing.

**TABLE 3** Pearson correlation of zone diameters and Etest mean results and standard deviations

Antifungal	Test modality	No. of isolates tested	Mean value (SD) for disk diffusion (mm) or Etest ( $\mu\text{g/ml}$ ) using:		Pearson correlation ( <i>r</i> ) (strength <sup>a</sup> )	<i>P</i> value
			Direct method	Standard method		
Voriconazole	Disk diffusion	104	28.55 (5.45)	29.34 (5.64)	0.6040 (M)	<0.0001
	Etest	28	0.11 (0.12)	0.12 (0.16)	0.5808 (M)	0.0012
Fluconazole	Disk diffusion	104	26.49(6.12)	26.94 (6.38)	0.6907 (S)	<0.0001
	Etest	21	2.76 (5.59)	3.66 (10.37)	0.9476 (VS)	<0.0001
Amphotericin B	Disk diffusion	104	21.78 (3.55)	21.72 (3.23)	0.7017(S)	<0.0001
	Etest	29	0.24 (0.26)	0.14 (0.13)	0.1637 (VW)	0.3875
Caspofungin	Etest	29	0.32 (0.36)	0.24 (0.27)	0.8884 (VS)	<0.0001
Amphotericin B	Disk diffusion	29	23.37 (2.04)	0.14 (0.13)	-0.4216 (M) <sup>b</sup>	0.0203

<sup>a</sup> VW, very weak; M, moderate; S, strong; VS, very strong.

<sup>b</sup> Zone diameter with MIC (both by standard method).

**TABLE 4** Distribution of major and minor errors among the *Candida* species isolates tested by disk diffusion and Etest directly from blood culture bottles compared to the results following the standard method<sup>a</sup>

Organism	No. (%) of isolates with <sup>b</sup> :			Total
	No errors	Minor errors	Major errors	
<b>Disk diffusion<sup>b</sup></b>				
<i>C. albicans</i>	28	1 (3.4)	0	29
<i>C. tropicalis</i>	25	2 (7.4)	0	27
<i>C. parapsilosis</i>	25	2 (7.1)	1 (3.6)	28
<i>C. glabrata</i>	8	0	0	8
Non- <i>albicans Candida</i>	4	0	0	4
<i>C. lusitaniae</i>	4	0	0	4
<i>C. pelliculosa</i>	2	0	0	2
<i>C. albicans</i> + <i>C. krusei</i> <sup>d</sup>	2	0	0	2
Total for disk diffusion	98 (94.2)	5 <sup>e</sup> (4.8)	1 <sup>f</sup> (1.0)	104
<b>Etest<sup>c</sup></b>				
<i>C. albicans</i>	7	0	0	7
<i>C. tropicalis</i>	5	0	0	5
<i>C. parapsilosis</i>	9	0	0	9
<i>C. glabrata</i>	2	2 (50)	0	4
Non- <i>albicans Candida</i>	1	0	0	1
<i>C. pelliculosa</i>	2	0	0	2
<i>C. albicans</i> + <i>C. krusei</i> <sup>d</sup>	0	2 (100)	0	2
Total for Etest	26 (86.7)	4 <sup>g</sup> (13.3)	0	30

<sup>a</sup> The antifungals tested by Etest were fluconazole, voriconazole, amphotericin B, and caspofungin. Caspofungin was not tested by the disk diffusion method.

<sup>b</sup> One hundred four strains were tested by disk diffusion. The Pearson chi-square value was 4.652, and the *P* value was 0.99 (errors were not significantly associated with species).

<sup>c</sup> Twenty strains were tested against all agents using the Etest, and 10 additional isolates against at least one agent. The Pearson chi-square value was 63.44, and the *P* value was <0.001 (errors were significantly associated with the species *C. glabrata* and the mixed species *C. krusei* and *C. albicans*).

<sup>d</sup> Direct susceptibilities were compared with standard susceptibilities of the most resistant strain of the two isolates (*C. krusei* in both cases).

<sup>e</sup> There were five minor errors in determining azole susceptibilities by disk diffusion, as follows: for voriconazole, 2 *C. tropicalis* isolates were reported as S-DD when they were S and one *C. parapsilosis* isolate was reported as S when it was S-DD, and for fluconazole, 1 *C. albicans* isolate was reported as S when it was S-DD and a *C. parapsilosis* isolate as R when it was S-DD.

<sup>f</sup> There was 1 major error for fluconazole by disk diffusion (one *C. parapsilosis* isolate was labeled R when it was S).

<sup>g</sup> All four cases of errors by Etest showed a minor discrepancy in caspofungin susceptibility results (2 *C. glabrata* isolates were reported as S when they were I and *vice versa*, and the 2 mixed cultures were reported as R and I while being I and S, respectively). Thus, there is perfect correlation by Etest if caspofungin is not tested directly.

<sup>h</sup> Minor errors included the reporting of S as S-DD or I or *vice versa*, and the major error was the reporting of S as R.

The Pearson correlation test also showed a moderate to strong correlation for azoles using standard and direct disk diffusion testing.

Similar results (100% agreement) were obtained for direct susceptibility testing of fluconazole, voriconazole, and amphotericin B using Etest. A slightly lower agreement (86%) with a moderate kappa score of 0.4844 was observed for caspofungin using Etest for direct susceptibility testing, in spite of very strong correlation, primarily due to minor errors (one dilution) at clinical breakpoints for mixed cultures and for *C. glabrata*.

Only one study has previously evaluated the use of fluconazole

disks for direct susceptibility testing of *Candida* species; however, that study used CHROMagar, which is not a recommended susceptibility testing medium (26). Another approach has been the direct inoculation of Vitek antifungal cards for susceptibility testing. However, the results of that study were suboptimal, with a high number of errors (16). A recent study also evaluated direct inoculation of Sensititre YeastOne from blood cultures for a limited number of cases (15). Although the results were good, this approach is expensive and would not be practical in many resource-limited settings due to high shipping costs and shipment delays. MHA with methylene blue and 2% glucose is a much cheaper medium than RPMI broth for disc diffusion, and swabbing a lawn on a medium plate is easier and less technically demanding, resulting in less wastage than in broth-based susceptibility methods.

As previously reported for direct susceptibility testing using Etest (13), variations in the inoculum concentrations did not affect the susceptibility results using disks, as all results were interpretable after 24 h. This was also reflected by finding no significant differences between the mean zone diameters and mean MICs by conventional and direct method using either disks or Etest.

There were no very major errors in direct susceptibility testing using either disks or Etest. Major errors using disks were noted with only one strain of *C. parapsilosis*, where the result for fluconazole was reported as resistant. No major errors were noted in direct testing using Etest.

A high proportion of very major and major errors in direct sensitivity testing using Etest has not been reported previously for fluconazole, voriconazole, and caspofungin (14). Due to a high rate of very major (3%) and major errors (23%) reported in amphotericin B testing previously, direct susceptibility testing using Etest for amphotericin B has not been recommended (14). We noted no major errors in amphotericin B testing using disks and no errors using Etest for direct susceptibility. However, as there were no resistant isolates in our sample, the discriminatory ability of this technique to detect resistance could not be assessed.

Amphotericin B deoxycholate is the first-line agent in Pakistan for invasive candidiasis due to nonavailability of lipid preparations and echinocandins, and thus, amphotericin susceptibilities are relevant in this setting. There has also been an upsurge in less common *Candida* species in our region in the last 5 years (27), and therefore, it is important to document the susceptibility profiles of naive and treated *Candida* strains. The clinical benefit usually lies in whether the patient is currently on amphotericin therapy and whether the patient would benefit from a higher dose than 0.7 µg/ml.

Although a low percentage of *C. krusei* strains as agents of candidemia from Pakistan has been reported previously, the frequency in recent years has increased (27). Additionally, voriconazole treatment is also considered in patients at a high risk of concomitant invasive mold infection, for example, in patients with hematologic malignancies receiving chemotherapy or bone marrow transplant. As modification of therapy from voriconazole to fluconazole is difficult in such patient populations, the availability of voriconazole susceptibility results is relevant for these patients.

No errors were noted in direct susceptibility testing of *C. glabrata*, *C. albicans*, and *C. tropicalis*. Very major errors have been reported previously in direct testing of *C. glabrata* and *C. tropicalis* but were not found in our study (14). On the other hand, previous

studies have not reported such testing errors in *C. parapsilosis* as were noted in our study (14).

Antifungal susceptibility testing is not yet widely used for direct treatment of invasive candidiasis, despite the fact that antifungal resistance has emerged globally (28). A rapid, easy method of drug susceptibility testing can be expected to encourage laboratories with limited financial and technical resources to start monitoring antifungal susceptibilities with meaningful clinical implications, choosing at least those agents which are most commonly used in their center.

**Limitations.** There were only four fluconazole-resistant isolates and no amphotericin B- or caspofungin-resistant isolates in our collection. Due to the small sample size and rarity of resistance among *Candida* species in our study, we cannot say how the technique will perform in a setting with more resistant isolates. Therefore, we suggest that any unexpected results in terms of resistance must be confirmed using standard methodology until more data are available. Etest could not be performed for all isolates due to financial limitations. Another limitation of this study was that neither clinical outcomes nor tailoring of empirical antifungal therapy in accordance with the direct drug susceptibility testing results was recorded, as the study was designed as an *in vitro* study.

**Conclusion.** The use of Etest for direct susceptibility testing for *Candida* species has already been reported as a rapid antifungal susceptibility testing tool that could provide results in 24 to 48 h. The results of our study demonstrate that, as an alternative to Etest, antifungal disks could also be used for direct susceptibility testing for azoles. This approach will be very useful in settings with limited resources and expertise to allow early reporting of susceptibilities that will result in prompt administration of appropriate antifungal agents. However, the use of this technique for amphotericin B testing cannot be recommended.

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