JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2001, p. 4181–4183 Vol. 39, No. 11
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.11.4181–4183.2001

Trends in Antifungal Susceptibility among Swedish *Candida* Species Bloodstream Isolates from 1994 to 1998: Comparison of the E-test and the Sensititre YeastOne Colorimetric Antifungal Panel with the NCCLS M27-A Reference Method

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Received 16 April 2001/Returned for modification 30 July 2001/Accepted 30 August 2001

A comparative evaluation of the NCCLS macrodilution method, the E-test, and the Sensititre YeastOne Colorimetric Antifungal Panel for the susceptibility testing of fluconazole, itraconazole, amphotericin B, and flucytosine was conducted with 233 blood isolates of *Candida* species collected between 1994 and 1998 in Sweden. Antifungal susceptibility profiles of *Candida albicans* and non-*C. albicans Candida* species remained essentially unchanged within the 5-year study period. The overall agreement rates for the E-test and the NCCLS MICs and for the YeastOne and the NCCLS MICs were ≥86 and ≥87%, respectively, within ±1 dilution for fluconazole, amphotericin B, and flucytosine, and ≥66 and ≥57%, respectively, for itraconazole. The E-test and the YeastOne panels are equivalent, and both are convenient methods for routine use.

Non-*Candida albicans Candida* species are frequently isolated from bloodstream infections, although *C. albicans* remains the most common species (12). Susceptibility to antifungal drugs varies among different species of *Candida*, which highlights the importance of species identification and antifungal MIC determination (7, 11, 14). With the advent of the NCCLS reference method for antifungal susceptibility testing, it is now possible to compare and evaluate alternative, easier-to-perform methods (6). The commercially available E-test and Sensititre YeastOne antifungal panel have both demonstrated good agreement with the NCCLS method in previous studies (2, 3, 4, 16).

Here we present the first nationwide retrospective epidemiological survey of antifungal susceptibility patterns of *Candida* species isolated from blood cultures, initiated in 1998 by the Swedish Reference Group for Antimycotics—Methodology. Moreover, we compared the NCCLS procedure with the E-test and the YeastOne antifungal panel in order to evaluate these commercial methods for routine testing of antifungal agents.

Clinical isolates. *Candida* species blood isolates collected between 1994 and 1998 were requested from 15 Swedish microbiological laboratories. A total of 499 *Candida* species isolates (*C. albicans*, $n = 371$; non-*C. albicans Candida* spp., $n = 128$) were received, and 233 isolates were selected for the study. They comprised *C. albicans* ($n = 123$), *C. glabrata* ($n = 52$), *C. parapsilosis* ($n = 33$), *C. tropicalis* ($n = 11$), *C. krusei* ($n = 9$), and *C. lusitaniae* ($n = 5$). Isolates were identified by standard methods and stored frozen at −70°C until use. Prior to antifungal testing, each isolate was subcultured twice on Sabouraud's dextrose agar (Oxoid). *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as controls.

Susceptibility testing. Broth macrodilution testing was performed in accordance with the NCCLS M27-A guidelines (6). Antifungal agents were obtained from their respective manufacturers. The final drug concentration ranges were 0.125 to 64 μg/liter for fluconazole, 0.0313 to 64 μg/liter for flucytosine, 0.0313 to 16 μg/liter for itraconazole, and 0.125 to 4 mg/liter for amphotericin B. The E-tests (AB Biodisk, Stockholm, Sweden) were performed in accordance with the manufacturer's instructions. The MIC endpoints were determined after 48 h of incubation at 35°C. Sensititre YeastOne test panels (kindly supplied by AccuMed International Ltd., East Grinstead, United Kingdom) were processed in accordance with the manufacturer's instructions. The plates were incubated at 35°C, and the MICs were read after 24 h if the growth control well was red; otherwise, they were read after 48 h.

Data analysis. Both on-scale and off-scale MICs were included in the analysis. The low off-scale MICs were left unchanged, and the high off-scale MICs were converted to the next highest concentration.

I studied the antifungal susceptibility patterns of 233 *Candida* sp. blood isolates cultured between 1994 and 1998 in Sweden. With the NCCLS method, yearly MICs for 50% of the *C. albicans* and non-*C. albicans Candida* species studied (MIC_{50}s) remained constant within the 5-year study period (data not shown). Equivalent MIC_{50}s and MIC_{90}s were obtained by all three methods for *C. albicans* isolates. Compared to those obtained by the NCCLS method, the flucytosine MIC_{90}s and itraconazole MIC_{50}s obtained by the E-test were higher for non-*C. albicans* isolates. On the other hand, the YeastOne method produced lower fluconazole, itraconazole, and flucytosine MIC_{50}s and MIC_{90}s for non-*C. albicans* isolates (Table 1).

The overall agreement between the MICs obtained by the E-test and NCCLS macrodilution methods was ≥86% within ±1 dilution for fluconazole, amphotericin B, and flucytosine and ≥66% for itraconazole (Table 2). The overall agreement between the MICs obtained by the YeastOne and NCCLS methods was ≥87% for fluconazole, amphotericin B, and flucytosine and ≥57% for itraconazole. The discrepancies be-

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TABLE 1. In vitro susceptibilities of *Candida* blood isolates cultured between 1994 and 1998 to fluconazole, itraconazole, flucytosine, and amphotericin B by the NCCLS macrodilution, E-test, and Sensititre YeastOne methods[a]

Species (no. of isolates) and antifungal drug	NCCLS method			E-test			YeastOne		
	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
C. albicans (n = 123)									
Fluconazole	≤0.125–1	0.25	0.5	0.032–0.5	0.25	0.25	<0.125–1	0.25	0.5
Itraconazole	≤0.0313–1	0.0625	0.125	0.008–0.5	0.125	0.25	<0.008–0.125	0.0313	0.0625
Amphotericin B	≤0.125–1	0.5	0.5	0.064–0.5	0.5	0.5	0.016–0.5	0.5	0.5
Flucytosine	≤0.0313–>32	0.0625	0.25	0.008–>32	0.064	0.25	<0.03–>64	0.0625	0.125
Non-*C. albicans Candida* spp. (n = 110)									
Fluconazole	≤0.125–>64	16	>64	0.25–>256	16	>256	<0.125–256	8	32
Itraconazole	≤0.0313–>16	0.5	>16	0.016–>32	2	>32	0.016–>16	0.125	1
Amphotericin B	0.25–1	0.5	1	0.125–1	0.5	1	0.0625–1	0.5	0.5
Flucytosine	0.0313–>32	0.0625	4	0.008–>32	0.032	>32	<0.03–64	<0.03	0.5

[a] All values are in micrograms per milliliter.

tween the YeastOne panel and the NCCLS macrodilution method results consisted mainly of lower itraconazole MICs, which were observed for 187 isolates. E-tests, on the other hand, gave higher itraconazole MICs for 119 isolates.

The categorization of *Candida* species within the established breakpoints of resistance for fluconazole (MIC, ≥64 mg/liter), itraconazole (MIC ≥1 mg/liter), and flucytosine (MIC ≥32 mg/liter), obtained by the three methods, is given in Table 3. Resistance to fluconazole, itraconazole, and flucytosine was almost entirely accounted for by *C. glabrata*, *C. krusei*, and, to a minor extent, *C. parapsilosis* isolates. Of 233 isolates, 15% were resistant to fluconazole by the NCCLS method, 12 were resistant by the E-test method, and 9% were resistant by the YeastOne method. Itraconazole resistance was found in 23% of the isolates by the NCCLS method, in 28% by the E-test method, and in 13% by the YeastOne method.

I found essentially unchanged antifungal susceptibility profiles of 233 Swedish *C. albicans* and non-*C. albicans Candida* sp. bloodstream isolates within the 5-year study period. Constant fluconazole susceptibility among *Candida* isolates other than *C. glabrata* and *C. krusei* was recently also reported in the United States (9). Conversely, Baran et al. found a trend toward slightly increasing fluconazole MICs (1). Our MIC_{50}s for *C. albicans* agree with those reported in North and South America (7, 10). Aside from *C. glabrata*, *C. krusei*, and, to a minor extent, *C. parapsilosis*, there were almost no fluconazole-, itraconazole-, and flucytosine-resistant isolates in Sweden. This has also been reported in other countries in Europe (8) and in North and Latin America (9, 10). Azole resistance among Swedish *C. glabrata* isolates was considerably more frequent than the 6.7% fluconazole and 32.8% itraconazole resistance recently reported in the United States (10), while no

TABLE 2. Percent agreement of the E-test and YeastOne methods with the NCCLS reference macrodilution method

Species (no. of isolates) and antifungal drug	% Agreement	
	E-test vs NCCLS method	YeastOne vs NCCLS method
C. albicans (123)		
Fluconazole	85	93
Itraconazole	72	70
Amphotericin B	97	97
Flucytosine	89	94
Non-*C. albicans Candida* spp. (110)		
Fluconazole	77	78
Itraconazole	60	44
Amphotericin B	86	82
Flucytosine	84	75

TABLE 3. Categorization of *Candida* species within the established breakpoints for resistance to fluconazole, itraconazole, and flucytosine

Species (no. of isolates) and antifungal agent	% of isolates resistant by:		
	NCCLS method	E-test	YeastOne
C. albicans (123)			
Fluconazole	0	0	0
Itraconazole	1	0	0
Flucytosine	1	2	2
C. glabrata (52)			
Fluconazole	40	25	13
Itraconazole	92	98	58
Flucytosine	0	0	0
C. parapsilosis (33)			
Fluconazole	15	15	15
Itraconazole	3	15	0
Flucytosine	12	15	15
C. tropicalis (11)			
Fluconazole	0	0	0
Itraconazole	0	9	0
Flucytosine	9	9	9
C. krusei (9)			
Fluconazole	100[a]	100[a]	100[a]
Itraconazole	33	100	0
Flucytosine	0	100	0
C. lusitaniae (5)			
Fluconazole	0	0	0
Itraconazole	0	0	0
Flucytosine	0	0	0

[a] *C. krusei* isolates were considered to be intrinsically resistant to fluconazole regardless of the MIC (for six isolates, the MIC was 32 μg/ml, and for three isolates, the MIC was ≥64 μg/ml by the NCCLS method).

fluconazole resistance was found among Latin American and Canadian isolates (14).

The performance of both the E-test and the YeastOne panel was comparable to that of the NCCLS reference for *C. albicans* (2, 3, 5). In general, the E-test tended to give higher MIC_{50}s of flucytosine and itraconazole among non-*C. albicans Candida* isolates. Similar findings have previously been reported for *C. krusei* and *C. tropicalis* isolates, respectively (2, 5). The YeastOne method gave lower fluconazole, itraconazole, and flucytosine MICs, also observed for *C. glabrata*, *C. tropicalis* (3), and *C. albicans* (itraconazole) (4), than the NCCLS method. To et al. previously reported lower amphotericin B, fluconazole, and flucytosine MICs, when comparing the susceptibilities of some *Candida* species by the Alamar blue method with those obtained with the NCCLS macrodilution method (15), thereby supporting the findings reported here. The reasons for these species-specific discrepancies between the methods tested are not known.

Overall, the agreement between the E-test and NCCLS methods and between the YeastOne panel and the NCCLS method was good (2, 3, 4, 13). However, we found lower agreement for itraconazole, which was in accord with a recent report (5).

No amphotericin B-resistant isolates were identified, although the E-test is claimed to be superior for the detection of less-susceptible isolates (17). The major discrepancy between the non-NCCLS methods concerned the itraconazole resistance of *C. glabrata*, *C. krusei*, and *C. parapsilosis* isolates, which appeared to be resistant by the E-test method but susceptible by the YeastOne method. Both the E-test and YeastOne methods misclassified some *C. glabrata* isolates that were fluconazole resistant by the NCCLS method as susceptible. This is in contrast to one multicenter study of the E-test method, in which azole-susceptible isolates appeared to be resistant (18).

This is the first comparison of the NCCLS broth macrodilution, E-test, and YeastOne methods for susceptibility testing of *Candida* species. The E-test is equivalent to the YeastOne panels, and both are simple and convenient methods for routine use. However, because of inconsistency, the results of azole susceptibility testing of *C. glabrata*, *C. krusei*, and *C. parapsilosis* isolates should be confirmed by a reference method.

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