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Comparative Evaluation of a New Commercial Colorimetric Microdilution Assay (SensiQuattro *Candida* EU) with MIC Test Strip and EUCAST Broth Microdilution Methods for Susceptibility Testing of Invasive *Candida* Isolates

Hedda Luise Koehling,[a] Birgit Willinger,[b] Jan Buer,[a] Peter-Michael Rath,[a] Joerg Steinmann[a]

Institute of Medical Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany[a]; Department of Clinical Microbiology, Institute for Hygiene and Medical Microbiology, Medical University of Vienna, Vienna, Austria[b]

Candidemia is an important cause of morbidity and mortality in immunosuppressed patients. *Candida* isolates must be cultivated, identified, and tested for susceptibility. We compared the performance of a new colorimetric broth microdilution panel (SensiQuattro *Candida* EU) for antifungal susceptibility testing to that of Liofilchem's MIC test strip and the EUCAST reference broth microdilution protocol. We tested 187 blood culture isolates of 5 *Candida* spp. (120 *C. albicans*, 38 *C. glabrata*, 10 *C. parapsilosis*, 12 *C. tropicalis*, and 7 *C. krusei*) against seven antifungal agents (amphotericin B, fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin, and micafungin) and interpreted the MICs according to the EUCAST recommendations. If applicable, the overall essential agreement (EA) of the SensiQuattro panel with the reference broth microdilution was slightly higher for *C. albicans* (87%) than for other species (85.8%). We found that SensiQuattro performed best in testing amphotericin B (EA, 100%), voriconazole (EA, 93.7%), and posaconazole (EA, 94.8%) against *C. albicans*, but its error rate for this species was high (29.6%) because of mainly major errors (26.7%) in testing anidulafungin and micafungin. Compared to the SensiQuattro panel, the MIC test strip exhibited a higher level of agreement for most isolates. SensiQuattro assays are easy to perform, but they are currently not suitable for testing echinocandins against *Candida* spp.

Bloodstream infections caused by *Candida* spp. are the most common invasive fungal infections (1, 2). Patients at risk of candidemia are those who are immunocompromised, e.g., those with hematologic and solid-organ malignancies, those receiving immunosuppressive therapy, those with chronic renal failure, and those treated with antibiotics or invasive catheters (2, 3). In the population of the United States, the incidence of hospital-acquired candidemia is as high as 10 cases per 100,000 patients (4). The 2013 annual epidemiological report of the European Centre for Disease Prevention and Control (ECDC) stated that *Candida* spp. are the fifth most frequently isolated microorganism in intensive care unit (ICU)-acquired bloodstream infections in the European Union (5). Mortality rates due to *Candida* bloodstream infections vary from 45% to 53% depending on the population investigated (6, 7). Although *C. albicans* is still the most frequently isolated *Candida* species in candidemia, non-*albicans Candida* species are increasingly found to be the causative agents (8–12).

Of particular concern is emerging resistance to both the antifungal classes of azoles and the newer echinocandins, as recently reported in the World Health Organization's "Antimicrobial Resistance: Global Report on Surveillance 2014" (13). Resistance to antifungal drugs varies among the various species of *Candida* because of the intrinsically low susceptibility of *C. glabrata* to azole antifungals, such as fluconazole (FLC) (14), and because of multifactorial processes or mutations in the *fks1* and *fks2* genes (15, 16). For the initial targeted treatment of candidemia in adult patients, the guidelines of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommend echinocandins in addition to removal of medical devices (17). In general, *Candida* isolates must be tested for susceptibility so that the most appropriate antifungal drug can be selected; for such testing, the broth microdilution method (BMD) is well established in the recommendations of both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

A commercially prepared colorimetric microdilution panel, Sensititre YeastOne (TREK Diagnostic Systems, Cleveland, OH, USA), has been evaluated by various groups but is available only for CLSI recommendations (18–20). Recently, another colorimetric microdilution panel, SensiQuattro Candida EU (bestbion^{dx}, Cologne, Germany), which includes eight desiccated antimycotics, is available; this panel correlates with the antifungal clinical breakpoints set by EUCAST. The purpose of this study was to compare the performance of the SensiQuattro *Candida* EU system (SQ) to that of a EUCAST reference BMD protocol and that of the routinely used MIC test strip (Liofilchem srl, Roseto degli Abru-

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Address correspondence to Hedda Luise Koehling, Koehling.Hedda-Luise@uk-essen.de.

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zzi, Italy) against a set of 187 blood culture isolates of five *Candida* species.

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MATERIALS AND METHODS

Study design. The study was designed to compare the MICs of amphotericin B (AMB), the azoles FLC, voriconazole (VRC), and posaconazole (POS), and the echinocandins caspofungin (CAS), anidulafungin (ANI), and micafungin (MICA) for the most common *Candida* spp. MICs were obtained by three different methods: the SQ system, the MIC test strip, and the EUCAST BMD protocol; the BMD results were used as a reference.

Test organisms. We tested 187 blood culture isolates of *Candida* spp. collected at two University hospitals (Essen, Germany, and Vienna, Austria) between 2008 and 2012. The collection included 120 isolates of *C. albicans*, 38 isolates of *C. glabrata*, 10 isolates of *C. tropicalis*, and 7 isolates of *C. krusei*. Species identification was performed by Vitek 2 and Vitek MS (both from bioMérieux, Marcy l'Etoile, France). Isolates were stored in the Protect Microorganism cryopreservation system (Technical Service Consultants Ltd., Lancashire, United Kingdom) at −80°C until further use. An aliquot was directly plated onto Sabouraud's dextrose agar (BD Diagnostics, Franklin Lakes, NJ, USA) and incubated at 36°C ± 1°C for 18 to 48 h before testing.

Quality control strains. The American Type Culture Collection (ATCC) strains *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, and *Candida krusei* ATCC 6258 served as quality controls, as recommended in the EUCAST definitive document (22).

SensiQuattro panels. SQ *Candida* EU is a 32-well commercial colorimetric microdilution panel containing 8 desiccated antimycotics at four doubling serial concentrations of AMB (0.5 to 2 mg/liter), FLC (1 to 8 mg/liter), VRC (0.06 to 0.5 mg/liter), POS (0.03 to 0.25 mg/liter), CAS (0.03 to 0.25 mg/liter), ANI (0.03 to 0.25 mg/liter), MICA (0.03 to 0.25 mg/liter), and flucytosine (4 to 32 mg/liter). Flucytosine was not investigated.

SQ preparations were established according to the manufacturer's instructions: yeast colonies harvested from Sabouraud's dextrose agar (BD) were suspended in physiological saline solution (0.5 McFarland standard) (Merck KgaA, Darmstadt, Germany). Each well of the dried SQ panels was rehydrated with 0.15 ml of the yeast suspension and incubated at 36°C ± 1°C for 24 ± 2 h. After incubation, the susceptibility of *Candida* spp. to antimycotic agents was assessed on the basis of growth or inhibition of the yeasts in the media containing antimycotic agents and a growth indicator. The well colors were interpreted as follows: a yellow/orange color indicated growth of yeast; a red color indicated inhibition of yeast growth.

Broth microdilution. EUCAST BMD testing was performed as outlined in the EUCAST definitive document, with Roswell Park Memorial Institute (RPMI) 1640-2% glucose medium (Sigma-Aldrich, St. Louis, MO, USA) and morpholinepropanesulfonic acid (MOPS) buffer (Merck KgaA, Darmstadt, Germany) (22). After five distinct yeast colonies had been harvested from Sabouraud's dextrose agar, a final yeast suspension with an inoculum of 1×10^5 to 5×10^5 CFU/ml was prepared. Reference powders of each agent were obtained from Sigma-Aldrich, St. Louis, MO, USA (amphotericin B and fluconazole), Merck KgaA, Darmstadt, Germany (posaconazole and caspofungin), Pfizer Inc., New York, NY, USA (voriconazole and anidulafungin), and Astellas Pharma Global Development, Inc., Northbrook, IL, USA (micafungin). After incubation at 35°C ± 2°C in ambient air for 24 ± 2 h without agitation, MIC values were determined spectrophotometrically at 530 nm as the lowest concentration of drug that resulted in at least 50% inhibition of yeast growth in relation to that of the growth control. Particularly for AMB, the MIC is defined as the lowest concentration leading to a growth inhibition of at least 90% that of the drug-free control, according to Arendrup (22).

MIC test strip. MIC test strip (Liofilchem) agar diffusion testing was performed with RPMI 1640-2% glucose agar medium and MOPS buffer (both Reactivos para Diagnóstico S.L., Barcelona, Spain). A final yeast suspension with an inoculum of 0.5×10^5 to 2.5×10^5 CFU/ml was prepared. The agar surface was inoculated with a swab dipped into the suspension. The yeast cells were spread homogeneously by unrolling the swab in three directions over the agar. One MIC test strip was applied to each inoculated agar plate. The plates were incubated at 36°C ± 1°C, and MICs were read after 24 h. The intersection between the zone of inhibition and the MIC test strip was considered the lowest inhibitory concentration of the antifungal agent. Microcolonies in the inhibition zone were not taken into account (23).

Antifungal susceptibility testing. EUCAST clinical breakpoints (CBPs) were used according to EUCAST antifungal clinical breakpoint table version 6.1 (24) for all tested organisms with the exception of caspofungin and with the exception of the agent-organism combinations FLC-*C. krusei*, VRC-*C. glabrata*, VRC-*C. krusei*, POS-*C. glabrata*, POS-*C. krusei*, MICA-*C. krusei*, and MICA-*C. tropicalis*, because the EUCAST CBPs for these agents have not yet been defined. In these cases, FLC-*C. krusei* breakpoints were obtained from CLSI (25); revised CAS CBPs and the CBPs for the agent/organism combinations mentioned above and breakpoints for MICA-*C. krusei*/*C. tropicalis* were obtained from Pfaller (23, 26–28).

Statistical analysis. The MIC results obtained by SQ and the MIC test strip after 24 h were compared with those of the reference BMD panels read after 24 h. As discussed in previous reports (29, 30), high off-scale MIC results were converted to the next highest concentration, and low off-scale MIC results were left unchanged. To calculate the essential agreement (EA) between SQ and BMD protocol results and between MIC test strip and BMD protocol results, we used discrepancies of more than 2 dilutions among MIC results. CBPs were used as outlined above to calculate categorical agreement (CA) between the MICs determined by SQ, the MIC test strip, and the BMD protocol (as the reference). A very major error (VME) was defined as a susceptible result for SQ or for the MIC test strip and a resistant result for the BMD reference method. A major error (ME) was a resistant result for SQ or for the MIC test strip and a susceptible result for the BMD protocol. Minor errors were defined as susceptible or resistant results for one method and an intermediate result for the other. For reliable results, the absolute CA between two methods should be at least 90%, and the VME rate should be no higher than 1.5% (31).

Problems occurred when the categorical agreement for SQ was classified for the agent-organism combinations FLC-*C. glabrata*, FLC-*C. krusei*, POS-*C. glabrata*, CAS-*C. albicans*, CAS-*C. glabrata*, CAS-*C. parapsilosis*, CAS-*C. tropicalis*, CAS-*C. krusei*, MICA-*C. parapsilosis*, MICA-*C. tropicalis*, and MICA-*C. krusei*. In these cases, it was impossible to place the MIC measurements obtained by SQ into the categories of susceptible, intermediate, or resistant; e.g., according to EUCAST, FLC MIC values of <0.002 mg/liter are considered susceptible, and values of >32 mg/liter are considered resistant for *C. glabrata*, but the SQ values were <1 mg/liter for susceptible and >8 mg/liter for resistant. Therefore, only EA was determined.

RESULTS

The MICs of 187 *Candida* species strains were assessed by the EUCAST reference BMD protocol; for SQ and MIC test strips, the strains were assessed according to the manufacturer's instructions. The *in vitro* susceptibilities as determined by the reference BMD protocol (considered the gold standard), MIC test strip, and SQ for the two sets of antifungal agents are summarized in Table 1 (AMB, FLC, VRC, and POS) and Table 2 (CAS, ANI, and MICA).

In general, the MIC results for all agents were typical for each *Candida* species, as previously described (28, 32). The overall EA of SQ was 87% for *C. albicans* isolates and 85.8% for non-*albicans* species isolates. The best result for *C. albicans* isolates was ob-

TABLE 1 *In vitro* susceptibilities of 187 isolates of *Candida* spp. to amphotericin B, fluconazole, voriconazole, and posaconazole[a]

Species (no. of isolates tested)	Antifungal agent	Test method	MIC (μg/ml)		% errors				
			Range	Mode	EA (%)	CA (%)	VME	ME	Minor errors
C. albicans (120)	Amphotericin B	BMD	0.25–2	1					
	Amphotericin B	MIC test strip	0.125–0.75	0.38	81.67	98.3	1.7	0	0
	Amphotericin B	SensiQuattro	<0.5–>2	0.5	100	97.4	1.7	0.9	0
	Fluconazole	BMD	0.12–>64	0.12					
	Fluconazole	MIC test strip	0.064–>256	0.38	86.67	91.7	3.3	2.5	2.5
	Fluconazole	SensiQuattro	<1–>8	1	45.7	94	3.4	2.6	0
	Voriconazole	BMD	0.016–>8	0.016					
	Voriconazole	MIC test strip	0.004–0.125	0.016	96.67	97.5	2.5	0	0
	Voriconazole	SensiQuattro	<0.06–>0.5	0.06	93.67	94.8	2.6	2.6	0
	Posaconazole	BMD	0.016–>8	0.06					
	Posaconazole	MIC test strip	0.016–3	0.064	95.8	91.7	3.3	5	0
	Posaconazole	SensiQuattro	<0.03–>0.25	0.03	94.83	93.2	3.4	3.4	0
C. glabrata (38)	Amphotericin B	BMD	0.25–2	1					
	Amphotericin B	MIC test strip	0.19–1	0.75	94.5	86.80	13.2	0	0
	Amphotericin B	SensiQuattro	<0.5–2	0.5	100	84.2	7.9	7.9	0
	Fluconazole	BMD	0.5–>64	4					
	Fluconazole	MIC test strip	2–256	8	97.4	97.40	0	0	2.6
	Fluconazole	SensiQuattro	<1–>8	8	65.8	ND	ND	ND	ND
	Voriconazole	BMD	0.016–4	0.25					
	Voriconazole	MIC test strip	0.032–32	0.094	89.5	94.70	0	5.3	0
	Voriconazole	SensiQuattro	0.06–>0.5	0.5	60.5	28.4	0	81.6	0
	Posaconazole	BMD	0.12–>8	0.5					
	Posaconazole	MIC test strip	0.125–12	0.5	92	92.10	0	7.9	0
	Posaconazole	SensiQuattro	>0.25	0.25	90	ND	ND	ND	ND
C. parapsilosis (10)	Amphotericin B	BMD	0.5–2	1					
	Amphotericin B	MIC test strip	0.094–0.5	0.25	60	90	10	0	0
	Amphotericin B	SensiQuattro	0.5	0.5	100	90	10	0	0
	Fluconazole	BMD	0.25–4	0.5					
	Fluconazole	MIC test strip	0.5–4	0.5	90	100	0	0	0
	Fluconazole	SensiQuattro	1	1	100	100	0	0	0
	Voriconazole	BMD	0.016–0.25	0.016					
	Voriconazole	MIC test strip	0.004–0.19	0.19	80	70	10	20	0
	Voriconazole	SensiQuattro	<0.06	0.06	100	90	10	0	0
	Posaconazole	BMD	0.03–0.12	0.06					
	Posaconazole	MIC test strip	0.023–0.25	0.094	100	80	0	20	0
	Posaconazole	SensiQuattro	<0.03	0.03	90	90	10	0	0
C. tropicalis (12)	Amphotericin B	BMD	0.5–1	1					
	Amphotericin B	MIC test strip	0.19–0.75	0.25	83.3	100	0	0	0
	Amphotericin B	SensiQuattro	0.5	0.5	100	100	0	0	0
	Fluconazole	BMD	0.12–4	0.5					
	Fluconazole	MIC test strip	0.25–4	0.38	100	100	0	0	0
	Fluconazole	SensiQuattro	<1–>8	1	50	58.3	0	41.7	0
	Voriconazole	BMD	0.016–0.03	0.016					
	Voriconazole	MIC test strip	0.012–0.047	0.016	100	66.7	0	33.3	0
	Voriconazole	SensiQuattro	<0.06–>0.5	0.06	66.7	66.7	0	33.3	0
	Posaconazole	BMD	0.016–0.25	0.06					
	Posaconazole	MIC test strip	0.008–0.125	0.064	100	66.7	0	33.3	0
	Posaconazole	SensiQuattro	<0.03–0.25	0.03	66.7	66.7	0	33.3	0
C. krusei (7)	Amphotericin B	BMD	1.0–2	2					
	Amphotericin B	MIC test strip	0.125–2	1	100	42.9	57.1	0	0
	Amphotericin B	SensiQuattro	0.5	0.5	100	28.6	71.4	0	0
	Fluconazole	BMD	16–>64	16					
	Fluconazole	MIC test strip	16–>256	32	100	100	0	0	0
	Fluconazole	SensiQuattro	<1–>8	8	85.7	ND	ND	ND	ND
	Voriconazole	BMD	0.25–2	0.25					
	Voriconazole	MIC test strip	0.038–3	0.19	85.7	85.7	14.3	0	0
	Voriconazole	SensiQuattro	<0.06–0.25	0.06	57.1	71.4	28.6	0	0
	Posaconazole	BMD	0.03–1	0.25					
	Posaconazole	MIC test strip	0.19–0.75	0.25	100	100	0	0	0
	Posaconazole	SensiQuattro	0.03	0.03	28.6	100	0	0	0

[a] As determined with the SensiQuattro *Candida* EU method (SQ), the EUCAST broth microdilution (BMD) protocol, and the MIC test strip. EA, essential agreement; CA, categorical agreement; VME, very major error; ME, major error. ND, nondeterminable: measurements of MIC cannot differentiate between susceptible, intermediate, or resistant.

TABLE 2 *In vitro* susceptibilities of 187 isolates of *Candida* spp. to caspofungin, anidulafungin, and micafungin[a]

Species (no. of isolates tested)	Antifungal agent	Test method	MIC (μg/ml)		EA (%)	CA (%)	% errors		
			Range	Mode			VME	ME	Minor errors
C. albicans (120)	Caspofungin	BMD	0.016–0.5	0.016					
	Caspofungin	MIC test strip	0.032–0.5	0.094	49.2	99.2	0	0	0.8
	Caspofungin	SensiQuattro	<0.03–>0.25	0.06	60.3	ND	ND	ND	ND
	Anidulafungin	BMD	0.016–4	0.016					
	Anidulafungin	MIC test strip	<0.002–>32	>32	0	95.8	2.5	0	1.7
	Anidulafungin	SensiQuattro	0.015–>0.12	0.12	32.8	14.7	0.8	82.8	1.7
	Micafungin	BMD	0.016–2	0.016					
	Micafungin	MIC test strip	0.002–0.094	0.012	92.5	68.3	24.2	7.5	0
	Micafungin	SensiQuattro	<0.03–>0.25	0.12	46.6	29.4	3.4	67.2	0
C. glabrata (38)	Caspofungin	BMD	0.016–0.12	0.03					
	Caspofungin	MIC test strip	0.064–0.25	0.19	85	42.1	0	0	55
	Caspofungin	SensiQuattro	<0.03–>0.25	0.12	68.4	ND	ND	ND	ND
	Anidulafungin	BMD	0.03–0.25	0.06					
	Anidulafungin	MIC test strip	<0.002–0.032	0.016	0	100	0	0	0
	Anidulafungin	SensiQuattro	<0.015–>0.12	>0.25	76.3	42.1	0	57.9	0
	Micafungin	BMD	0.016–0.03	0.016					
	Micafungin	MIC test strip	0.002–0.032	0.008	55	100	0	0	0
	Micafungin	SensiQuattro	<0.03–>0.25	>0.25	44.7	26.3	0	73.7	0
C. parapsilosis (10)	Caspofungin	BMD	0.25–1	0.5					
	Caspofungin	MIC test strip	0.19–2	0.38	100	40	2	0	4
	Caspofungin	SensiQuattro	<0.03–>0.25	>0.25	90	ND	ND	ND	ND
	Anidulafungin	BMD	2–4	2					
	Anidulafungin	MIC test strip	2–>32	>32	30	30	0	0	70
	Anidulafungin	SensiQuattro	<0.015–0.12	0.015	0	100	0	0	0
	Micafungin	BMD	1–4	1					
	Micafungin	MIC test strip	0.006–0.016	0.008	0	90	0	0	10
	Micafungin	SensiQuattro	<0.03–0.12	<0.03	0	ND	ND	ND	ND
C. tropicalis (12)	Caspofungin	BMD	0.016–0.12	0.12					
	Caspofungin	MIC test strip	0.064–0.5	0.19	75	91.7	0	0	8.3
	Caspofungin	SensiQuattro	<0.03–>0.25	>0.25	50	ND	ND	ND	ND
	Anidulafungin	BMD	0.016–0.12	0.03					
	Anidulafungin	MIC test strip	<0.002–0.002	<0.002	0	91.7	8.3	0	0
	Anidulafungin	SensiQuattro	0.03–>0.25	>0.12	41.6	25	0	75	0
	Micafungin	BMD	0.016–0.06	0.03					
	Micafungin	MIC test strip	0.003–>32	0.006	25	75	0	25	0
	Micafungin	SensiQuattro	0.03–>0.25	>0.25	50	ND	ND	ND	ND
C. krusei (7)	Caspofungin	BMD	0.016–8	0.06					
	Caspofungin	MIC test strip	0.19–>32	0.75	30	42.9	0	0	57.1
	Caspofungin	SensiQuattro	<0.03–>0.25	>0.25	44.4	ND	ND	ND	ND
	Anidulafungin	BMD	0.016–4	0.06					
	Anidulafungin	MIC test strip	<0.002–>32	<0.002	0	85.7	14.3	0	0
	Anidulafungin	SensiQuattro	<0.015–>0.12	>0.12	66.6	28.7	14.2	57.1	0
	Micafungin	BMD	0.03–>8	0.12					
	Micafungin	MIC test strip	0.004–3	3	0	0	10	80	10
	Micafungin	SensiQuattro	<0.03–>0.25	>0.25	22.2	ND	ND	ND	ND

[a] As determined with the SensiQuattro Candida EU (SQ), the EUCAST broth microdilution (BMD) protocol, and the MIC test strip. EA, essential agreement; CA, categorical agreement; VME, very major error; ME, major error. ND, nondeterminable: measurements of MIC cannot differentiate between susceptible, intermediate, or resistant.

tained with SQ and AMB (EA, 100%). When all tested *Candida* species were taken into account, SQ obtained the best EA result for *C. parapsilosis* isolates (97.5%). In contrast, the overall EA of SQ for testing echinocandins was poor against *C. albicans* isolates (46.5%) and non-*albicans* species isolates (53.1%). The MIC test strip was better for *C. albicans* isolates (overall EA, 92.5%) than for non-*albicans* species isolates (overall EA, 90.2%). Within the non-*albicans* species isolates, the overall EA for the MIC test strip and for the BMD protocol was at least 90% in every case except for *C. parapsilosis* (EA, 82.5%).

SQ could not classify the CA for 11 agent/organism combinations, as described in Materials and Methods. Therefore, only EA was calculated in these cases. Anidulafungin could be evaluated at all times.

For *C. albicans*, the overall CA of the SQ and the MIC test strip, taken together, was 82%. The error rate corresponding to the ap-

plied methods was significantly higher for SQ (29.6%) than for the MIC test strip (8.2%); this difference was caused by a higher rate of MEs in SQ evaluations (26.7%). The VME rate of the two methods was similar (2.6% for SQ and 5.4% for the MIC test strip). Most of the MEs in SQ measurements occurred with ANI and MICA. In general, SQ performed better in testing *C. albicans* against AMB and the azoles than in testing against the echinocandins (error rate for azoles and AMB, 5.2%; error rate for echinocandins, 38%). For *C. albicans*, a good CA was detected for all agents except for MICA and ANI testing with SQ. The VME rates in testing AMB, the azoles, and ANF were less than 5% but were 24.2% in testing MICA; in these tests, most of the VMEs occurred with the MIC test strip (29 of 120 results).

For SQ and MIC test strip, taken together, the overall CA was small for non-*albicans* species (73.3%), and the error rate of the two methods for these species was considerably higher than that for *C. albicans* (SQ, 42.8%; MIC test strip, 16.8%). However, the non-*albicans* species VME rate of the two methods was equal to that for *C. albicans* (4.1%), with a simultaneous decrease of VME caused by MIC test strip (VME, 3.6%) and increase of ME (17.3%) and minor errors (5.4%). The increase of the latter is especially due to false resistant measurements of CAS in *C. glabrata* with the MIC test strip (55% of the 40 minor errors).

DISCUSSION

The aim of the present study was to compare the performance of the SQ microdilution assay with that of the MIC test strip and the reference BMD protocol in antifungal susceptibility testing of 187 *Candida* blood culture isolates.

The standardized BMD method of antifungal susceptibility testing is regarded as the reference method, but it is time-consuming and cumbersome for routine use in the clinical laboratory. Because SQ does not require complex handling, we evaluated this method as an alternative method of testing antifungal agents *in vitro*.

SQ has the advantage of being a commercially prepared colorimetric microdilution panel that is ready to use and easy to perform. The agreement between SQ and BMD depends on the antifungal agent and the *Candida* species tested. In general, testing *C. albicans* isolates with SQ resulted in higher overall EAs than testing non-*albicans* species isolates. The performance of SQ for the echinocandins was not satisfactory, with low CAs when CA could be determined at all. The calculated CA showed a high range of variation, and only the combination of ANI-*C. parapsilosis* achieved a CA value higher than 90% (CA = 100%).

Problems occurred in some cases in the interpretation of SQ MIC results: the positive control remained red, but with repetition the control turned yellow or orange so that the test could be assessed as valid. In general, the transition between red (no microbial growth) and orange (good microbial growth) was often smooth, and clear-cut endpoints were missing. Therefore, visual assessment of MICs was difficult, and this difficulty can lead to misinterpretation. No problems with poor growth of the *Candida* strains were observed. Moreover, the CA for a total of 11 agent-organism combinations could not be assessed with SQ, because the established SQ MIC was not related to an exact valuation area. This problem affected primarily CAS (5 cases) and MICA (3 cases). For CAS, the established MICs of at least 0.25 μg/ml indicate intermediate or resistant strains, and the susceptible breakpoint for *C. albicans*, for example, is not higher than 0.25 μg/ml. Additional dilutions of every antifungal agent, at both lower and higher concentrations, are needed for SQ, because only in this way can resistant isolates be identified. Using additional dilutions allowed us to obtain a better harmonization of the dilution ranges and the EUCAST breakpoints.

Nevertheless, antifungal susceptibility testing with echinocandins is generally difficult. According to the newest EUCAST antifungal clinical breakpoint table, "EUCAST breakpoints have not yet been established for caspofungin, due to significant interlaboratory variation in MIC ranges for caspofungin" (33). However, Pfaller and colleagues recommend testing anidulafungin and micafungin for predicting caspofungin MIC (26, 34). Sensititre YeastOne (SYO; TREK Diagnostic Systems), a well-described colorimetric antifungal panel that includes itraconazole in addition to the 8 antifungal agents included in SQ, is already equipped with 11 dilution steps, e.g., for CAS, 0.008 to 8 μg/ml. Comparisons of the performance of SQ and SYO have not yet been reported and should be determined. However, several studies have reported that the SYO method achieves excellent results in terms of reproducibility and CAs with both the EUCAST BMD protocol and the CLSI broth microdilution reference method (19, 20, 32, 34–36).

To assess the reliability of the agar gradient diffusion method, generally known as Etest, we used the MIC test strip (Liofilchem) as a cost-effective alternative. With the exception of the carrier material, this product is a strip on which antifungal agents are impregnated with a predefined concentration gradient in 15 2-fold dilutions; therefore, it is similar to Etest (bioMérieux, Marcy l'Etoile, France). No other systematic comparison of methods for antifungal susceptibility testing with the use of MIC test strips has been reported to date. In our study, the agreement between the results achieved with the MIC test strip and the BMD protocol was lower than the agreement reported by Pfaller and colleagues, who used a different gradient strip (37). Additional comparative studies with MIC test strips and the BMD protocol are warranted.

Potential shortcomings of the present study are the limited number of non-*albicans* species strains included and the absence of echinocandin-resistant *Candida* species isolates. However, as described here, the SQ in its present format is not suitable for testing echinocandins against *Candida* species. To the best of our knowledge, this is the first study to investigate the performance of the SensiQuattro *Candida* EU as a new commercially available colorimetric assay for antifungal susceptibility testing of yeasts. We conclude that the SQ method of antifungal susceptibility testing is easy to perform and has strengths in testing azoles and AMB. However, further improvements are necessary for higher antifungal dilutions and more precise readability of MICs. Currently, the SQ method is not suitable for testing echinocandins against *Candida* spp.

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