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Comparison of EUCAST and CLSI broth microdilution methods for the susceptibility testing of 10 Systemically active antifungal agents when tested against *Candida* spp.

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ABSTRACT

The antifungal broth microdilution (BMD) method of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) was compared with Clinical and Laboratory Standards Institute (CLSI) BMD method M27-A3 for amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole susceptibility testing of 357 isolates of *Candida*. The isolates were selected from global surveillance collections to represent both wild-type (WT) and non-WT MIC results for the azoles (12% of fluconazole and voriconazole results were non-WT) and the echinocandins (6% of anidulafungin and micafungin results were non-WT). The study collection included 114 isolates of *Candida albicans*, 73 of *C. glabrata*, 76 of *C. parapsilosis*, 60 of *C. tropicalis*, and 34 of *C. krusei*. The overall essential agreement (EA) between EUCAST and CLSI results ranged from 78.9% (posaconazole) to 99.6% (flucytosine). The categorical agreement (CA) between methods and species of *Candida* was assessed using previously determined CLSI epidemiological cutoff values. The overall CA between methods was 95.0% with 2.5% very major (VM) and major (M) discrepancies. The CA was >93% for all antifungal agents with the exception of caspofungin (84.6%), where 10% of the results were categorized as non-WT by the EUCAST method and WT by the CLSI method. Problem areas with low EA or CA include testing of amphotericin B, anidulafungin, and isavuconazole against *C. glabrata*, itraconazole, and posaconazole against most species, and caspofungin against *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. We confirm high level EA and CA (>90%) between the 2 methods for testing fluconazole, voriconazole, and micafungin against all 5 species. The results indicate that the EUCAST and CLSI methods produce comparable results for testing the systemically active antifungal agents against the 5 most common species of *Candida*; however, there are several areas where additional steps toward harmonization are warranted.

1. Introduction

The need for reproducible, clinically relevant antifungal susceptibility testing of *Candida* spp. has been prompted by the increasing number of infections, the expanding use of new and established antifungal agents, and the recognition of antifungal resistance as an important clinical problem (Arendrup et al., 2013; Cleveland et al., 2012; Kett et al., 2011; Ostrosky-Zeichner, 2013; Pakyz et al., 2011; Pfaller, 2012). The Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Antifungal Susceptibility Tests has standardized the broth microdilution (BMD) reference method for testing amphotericin B, flucytosine, the triazoles (including the investigational agent isavuconazole), and the echinocandins, against *Candida* spp. (CLSI, 2008b; 2012; Pfaller et al., 2012b, 2013b) and, most recently, has validated 24-h MIC readings for all agents (CLSI, 2012; Pfaller and Diekema, 2012; Pfaller et al., 2010a, 2011b, 2011c, 2012b, 2013b) and developed new species-specific clinical breakpoints (CBPs) (CLSI, 2012; Pfaller and Diekema, 2012; Pfaller et al., 2010a, 2011a, 2011d) and epidemiological cutoff values (ECVs) (Pfaller and Diekema, 2012; Pfaller et al., 2010b, 2010c, 2011b, 2012b) for these agents and several species of *Candida*. The new CBPs and ECVs replace the previously published non-species-specific CBPs for all of these agents (CLSI, 2008a), which were observed to lack sensitivity in discriminating wild-type (WT) strains of *Candida* (lack acquired or mutational resistance mechanisms) from non-WT strains (possess intrinsic or acquired resistance mutations) or where lack of clinical data precluded the establishment of CBPs (Pfaller, 2012; Pfaller and Diekema, 2012).

In addition to the CLSI BMD method, the only other international standard method for antifungal susceptibility testing of yeasts is that of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Arendrup et al., 2012). The similarities and differences (minor) between the 2 BMD methods have been discussed previously (Alastruey-Izquierdo and Cuenca-Estrella, 2012; Espinel-Ingroff et al., 2005; Espinel-Ingroff et al., 2013a; Rodriguez-Tudela et al., 2007). The 2 methods have been harmonized so that there is a close agreement

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between MIC results when testing fluconazole and voriconazole against *Candida* to the extent that there are common CBPs for the 2 methods for some species of *Candida* (Arendrup et al., 2012; Espinel-Ingroff et al., 2013a; Pfaller and Diekema, 2012). Due to the international importance of these 2 methods in clinical testing and surveillance of antifungal resistance, there is a need to continue the process of harmonization for the testing of other new and established antifungal agents.

In the present study, we examine the essential agreement (EA; MIC $\pm$ 2 $\log_2$ dilutions) between the 2 standardized methods for testing 10 antifungal agents (amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole, and voriconazole) against a collection of 357 clinical isolates of *Candida* selected to provide both WT and non-WT MIC phenotypes (using CLSI methods and ECVs) for most agents and species. We also provide an estimate of categorical agreement (CA; susceptibility results that fall within the same interpretive category) between the 2 methods by using the ECVs previously determined for each antifungal agent and species of *Candida* (Pfaller and Diekema, 2012) to categorize the isolates as WT (MIC, ≤ECV) or non-WT (MIC > ECV) as determined by each method.

2. Materials and methods

2.1. Organisms

A total of 357 clinical isolates of *Candida* spp. were selected from global surveillance collections (Pfaller et al., 2011f, 2013b, 2013c) to represent both WT and non-WT MIC results for the azoles (12.6% of fluconazole results were non-WT) and the echinocandins (6.4% of anidulafungin and micafungin results were non-WT). The study collection encompassed 5 species of *Candida*, including *Candida albicans* (114 isolates), *C. glabrata* (73 isolates), *C. parapsilosis* (76 isolates), *C. tropicalis* (60 isolates), and *C. krusei* (34 isolates). Species identification was established using Vitek (bioMerieux, Hazelwood, Missouri, USA), conventional reference methods (Howell and Hazen, 2011), and 28S and internal transcribed spacer (ITS) sequencing as described elsewhere (Pfaller et al., 2012c). The isolates were stored as water suspensions until used in the study. Prior to testing, each isolate was passaged at least twice onto potato dextrose agar (Remel) and CHROMagar *Candida* medium (Becton Dickinson and Company, Sparks, MD, USA) to ensure purity and viability.

2.2. Antifungal susceptibility testing

All isolates were tested for in vitro susceptibility to amphotericin B, flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, isavuconazole, itraconazole, posaconazole and voriconazole using the CLSI (CLSI, 2012) and EUCAST(EUCAST, 2013) BMD methods. Reference powders of each agent were obtained from their respective manufacturers. Personnel performing the in vitro susceptibility studies were blinded to the results of the CLSI method compared to the EUCAST method.

CLSI BMD testing was performed exactly as outlined in document M27-A3 (CLSI, 2008b) by using round-bottom trays and RPMI 1640 medium with 0.2% glucose, inocula of 0.5×10^3 to 2.5×10^3 cells/ml, and incubation at 35 °C. MIC values were determined visually after 24-h incubation as the lowest concentration of drug that caused complete inhibition (amphotericin B) or a significant diminution (≥50% inhibition; all other agents) of growth relative to that of the growth control.

EUCAST BMD testing was performed exactly as outlined in document EDef 7.2 (Arendrup et al., 2012) by using flat-bottom trays and RPMI1640 medium with 2.0% glucose, inocula of 0.5×10^5 to 2.5×10^5 cells/ml, and incubation at 35 °C. MIC values were determined spectrophotometrically (at 490 nm), after 24-h incubation, as the lowest concentration of drug that resulted in complete (100%, amphotericin B) or in ≥50% (all other agents) inhibition of growth relative to that of the growth control.

2.3. Quality control

Quality control was performed as recommended in CLSI document M27-A3 (CLSI, 2008b) using *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

2.4. Analysis of results

The MIC results for each triazole obtained with the EUCAST method were compared to those of the CLSI BMD method. High off-scale BMD MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies of more than 2 dilutions among MIC results were used to calculate the EA. The recently described CLSI ECVs for each agent and species (Table 1) were used to obtain CA percentages between the MIC values determined with the EUCAST method and those determined by the CLSI method. The ECVs were determined by the

Table 1
ECVs for systemically active antifungal agents and *Candida* spp. determined by 24-h CLSI broth microdilution methods.[a]

Species	Antifungal agent	No. of isolates	ECV (μg/mL)	
			WT	non-WT
C. albicans	Amphotericin B	9,252	≤2	>2
	Flucytosine	8,007	≤0.5	>0.5
	Anidulafungin	4,283	≤0.12	>0.12
	Caspofungin	4,283	≤0.12	>0.12
	Micafungin	4,283	≤0.03	>0.03
	Fluconazole	8,059	≤0.5	>0.5
	Itraconazole	14,716	≤0.12	>0.12
	Posaconazole	8,619	≤0.06	>0.06
	Voriconazole	8,619	≤0.03	>0.03
C. glabrata	Amphotericin B	3,117	≤2	>2
	Flucytosine	3,387	≤0.5	>0.5
	Anidulafungin	1,236	≤0.25	>0.25
	Caspofungin	1,236	≤0.12	>0.12
	Micafungin	1,236	≤0.03	>0.03
	Fluconazole	2,240	≤32	>32
	Itraconazole	5,769	≤2	>2
	Posaconazole	2,415	≤2	>2
	Voriconazole	2,415	≤0.5	>0.5
C. parapsilosis	Amphotericin B	3,107	≤2	>2
	Flucytosine	3,165	≤0.5	>0.5
	Anidulafungin	1,238	≤4	>4
	Caspofungin	1,238	≤1	>1
	Micafungin	1,238	≤4	>4
	Fluconazole	2,117	≤2	>2
	Itraconazole	4,894	≤0.5	>0.5
	Posaconazole	2,278	≤0.25	>0.25
	Voriconazole	2,279	≤0.12	>0.12
C. tropicalis	Amphotericin B	2,062	≤2	>2
	Flucytosine	2,046	≤0.5	>0.5
	Anidulafungin	996	≤0.12	>0.12
	Caspofungin	996	≤0.12	>0.12
	Micafungin	996	≤0.12	>0.12
	Fluconazole	1,771	≤2	>2
	Itraconazole	3,624	≤0.5	>0.5
	Posaconazole	1,895	≤0.12	>0.12
	Voriconazole	1,895	≤0.06	>0.06
C. krusei	Amphotericin B	577	≤2	>2
	Flucytosine	499	≤32	>32
	Anidulafungin	270	≤0.12	>0.12
	Caspofungin	270	≤0.25	>0.25
	Micafungin	270	≤0.12	>0.12
	Fluconazole	473	≤64	>64
	Itraconazole	809	≤1	>1
	Posaconazole	508	≤0.5	>0.5
	Voriconazole	507	≤0.5	>0.5

[a] Data compiled from refs (Pfaller et al. 2010a, 2011b, 2011d, 2012b).