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Comparison of the Vitek 2 yeast susceptibility system with CLSI microdilution for antifungal susceptibility testing of fluconazole and voriconazole against *Candida* spp., using new clinical breakpoints and epidemiological cutoff values

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ABSTRACT

A commercially available, fully automated yeast susceptibility test system (Vitek 2; bioMérieux, Marcy d'Etoile, France) was compared in 3 different laboratories with the Clinical and Laboratory Standards Institute (CLSI) reference microdilution (BMD) method by testing 2 quality control strains, 10 reproducibility strains, and 425 isolates of *Candida* spp. against fluconazole and voriconazole. Reference CLSI BMD MIC endpoints and Vitek 2 MIC endpoints were read after 24 hours and 9.1–27.1 hours incubation, respectively. Excellent essential agreement (within 2 dilutions) between the reference and Vitek 2 MICs was observed for fluconazole (97.9%) and voriconazole (96.7%). Categorical agreement (CA) between the 2 methods was assessed using the new species-specific clinical breakpoints (CBPs): susceptible (S) ≤2 μg/mL, susceptible dose-dependent (SDD) 4 μg/mL, and resistant (R) ≥8 μg/mL for fluconazole and *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* and ≤32 μg/mL (SDD), ≥64 μg/mL (R) for *Candida glabrata*; S ≤0.12 μg/mL, SDD 0.25–0.5 μg/mL, R ≥1 μg/mL for voriconazole and *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, and ≤0.5 μg/mL (S), 1 μg/mL (SDD), ≥2 μg/mL (R) for *Candida krusei*. The epidemiological cutoff value (ECV) of 0.5 μg/mL for voriconazole and *C. glabrata* was used to differentiate wild-type (WT; MIC ≤ ECV) from non-WT (MIC > ECV) strains of this species. Due to the lack of CBPs for the less common species, the ECVs for fluconazole and voriconazole, respectively, were used for *Candida lusitaniae* (2 μg/mL and 0.03 μg/mL), *Candida dubliniensis* (0.5 μg/mL and 0.03 μg/mL), *Candida guilliermondii* (8 μg/mL and 0.25 μg/mL), and *Candida pelliculosa* (4 μg/mL and 0.25 μg/mL) to categorize isolates of these species as WT and non-WT. CA between the 2 methods was 96.8% for fluconazole and 96.5% for voriconazole with less than 1% very major errors and 1.3–3.0% major errors. The Vitek 2 yeast susceptibility system remains comparable to the CLSI BMD reference method for testing the susceptibility of *Candida* spp. when using the new (lower) CBPs and ECVs.

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1. Introduction

The Clinical and Laboratory Standards Institute (CLSI) has standardized the broth microdilution (BMD) reference method for testing the triazoles, fluconazole, and voriconazole, against *Candida* spp. (CLSI, 2008a); has published quality control (QC) limits (CLSI, 2008b); and most recently, has validated 24-hour MIC readings (Pfaller et al, 2008; Pfaller et al, 2011a) and has developed new species-specific clinical breakpoints (CBPs) (Pfaller and Diekema, 2012; Pfaller et al, 2010; Pfaller et al, 2011b) and epidemiological cutoff values (ECVs) (Pfaller and Diekema, 2012; Pfaller et al, 2010; Pfaller et al, 2011c; Pfaller et al, 2011d) for these agents and several species of *Candida*. The new CBPs and ECVs replace the previously published non–species-specific CBPs of ≤8 μg/mL (susceptible [S]), 16–32 μg/mL (susceptible dose dependent [SDD]), and ≥64 μg/mL (resistant [R]) and ≤1 μg/mL (S), 2 μg/mL (SDD), and ≥4 μg/mL (R) for fluconazole and voriconazole, respectively (Pfaller et al, 2006a; Pfaller et al, 2006b), which were found to lack sensitivity in discriminating wild-type (WT) strains of *Candida* (lack acquired or mutational resistance mechanisms) from non-WT strains (possess efflux and/or mutational azole resistance mechanisms) (Pfaller and Diekema, 2010; Pfaller and Diekema, 2012; Pfaller et al, 2010; Pfaller et al, 2011b).

Among the commercially available systems for antifungal susceptibility testing of *Candida* is the highly automated Vitek 2 yeast susceptibility system (bioMérieux, Marcy l'Etoile, France). Several different laboratories have demonstrated excellent essential (EA; within 2 dilutions) and categorical agreement (CA; susceptibility results that fall within the same interpretive category) between Vitek 2 and the CLSI BMD method for testing fluconazole and voriconazole against *Candida* spp. (Borghi et al, 2010; Bourgeois et al, 2010;

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Cuenca-Estrella et al, 2010; Pfaller et al, 2007a; Pfaller et al, 2007b; Posteraro et al, 2009). Whereas most of these studies compared the Vitek 2 MIC results with CLSI MIC results determined after both 24 and 48 hours of incubation, all of the studies determined CA using the older non–species-specific CBPs. Furthermore, all of the studies except those of Pfaller et al (2007a; 2007b) were conducted in a single laboratory.

Previously, Pfaller et al (2007a, 2007b) performed a multicenter (3 laboratory) evaluation comparing the Vitek 2 yeast susceptibility system with the CLSI BMD method for testing fluconazole and voriconazole against a collection of *Candida* spp. This study demonstrated excellent reproducibility (98–100%) and an EA between Vitek 2 and 24-hour CLSI BMD MICs of 97.9% (fluconazole) and 96.7% (voriconazole). CA was assessed using the CBPs available at the time (≤8 μg/mL [S], 16–32 μg/mL [SDD], and ≥64 μg/mL [R] for fluconazole; ≤1 μg/mL [S], 2 μg/mL [SDD], and ≥4 μg/mL [R] for voriconazole) and ranged from 97.2% (fluconazole) to 98.6% (voriconazole). Given that the newly described species-specific CBPs for fluconazole and voriconazole are considerably lower than the initial CBPs for *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis* (S, ≤2 μg/mL; SDD, 4 μg/mL; R, ≥8 μg/mL for fluconazole and S, ≤0.12 μg/mL; SDD, 0.25–0.5 μg/mL; R, ≥1 μg/mL for voriconazole) and are also changed for *Candida glabrata* (SDD ≤32 μg/mL and R ≥64 μg/mL for fluconazole; WT ≤0.5 μg/mL and non-WT ≥1 μg/mL for voriconazole) and *Candida krusei* (S, ≤0.5 μg/mL; SDD, 1 μg/mL; and R, ≥2 μg/mL for voriconazole), we determined that the original data should be reanalyzed using these new CBPs to determine the CA between Vitek 2 and the 24-hour CLSI BMD method. This dataset also contained isolates of *Candida lusitaniae*, *Candida dubliniensis*, *Candida guilliermondii*, and *Candida pelliculosa* that have not been assigned CBPs due to lack of clinical outcomes data (Pfaller and Diekema, 2012; Pfaller et al, 2011d). For these species, the ECVs for fluconazole and voriconazole were used to categorize the isolates as WT (MIC ≤ ECV) or non-WT (MIC > ECV) in order to determine CA between the 2 methods.

2. Materials and methods

2.1. Study design

We compared the MIC results for fluconazole and voriconazole obtained with the Vitek 2 system to those obtained by the CLSI BMD (NCCLS, 2002) in 3 laboratories. Each laboratory tested at least 100 consecutive, fresh clinical isolates of *Candida* spp. (range 103–135 isolates) with the Vitek 2 system and the CLSI frozen reference BMD panel (a total of 346 clinical isolates). In addition, a challenge set of 80 well-characterized stock isolates (isolates with known on-scale MICs for both fluconazole and voriconazole) was tested by both methods in one of the laboratories. The intra- and interlaboratory reproducibilities were determined by testing a panel of 10 *Candida* spp. isolates in triplicate on 3 separate days in each of the participating laboratories. The MIC results obtained with the Vitek 2 system following 9.1 to 27.1 hours of incubation (depending on the organism growth rate) were compared with those obtained with the reference BMD panel read after 24 hours of incubation.

2.2. Test organisms

The test organisms included 2 American Type Culture Collection (ATCC) strains that have been established as QC strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) by CLSI (CLSI, 2008b; NCCLS, 2002). A challenge set of 80 isolates of *Candida* spp. selected to provide on-scale MIC results and to represent both clinically important species and resistance mechanisms were tested in 1 of the laboratories. The challenge set included 32 isolates of *C. albicans*, 6 of *C. dubliniensis*, 14 of *C. glabrata*, 3 of *C. guilliermondii*, 5 of *C. krusei*, 5 of *C. lusitaniae*, 1 of *Candida norvegensis*, 7 of *C. parapsilosis*, 2 of *C. pelliculosa*, and 5 of *C. tropicalis*. An additional 346 recent clinical isolates of *Candida* spp. were also tested. The clinical isolates included 166 isolates of *C. albicans*, 2 of *C. dubliniensis*, 69 of *C. glabrata*, 46 of *C. krusei*, 4 of *C. lusitaniae*, 36 of *C. parapsilosis*, and 23 of *C. tropicalis*. Reproducibility within and among laboratories was assessed by using a panel of 10 *Candida* isolates: *C. glabrata* strain 304201, *C. glabrata* strain 304927, *Candida haemulonii* strain 304848, *C. krusei* strain 304204, *C. krusei* strain 304845, *C. krusei* strain 304850, and *Candida lipolytica* strain 204856, *C. lusitaniae* strain 304205, *C. norvegensis* strain 304852, and *C. pelliculosa* strain 304847. The reproducibility isolates were tested in triplicate on 3 different days in each of the 3 laboratories (a total of 270 results for each antifungal agent). All isolates were identified by standard methods (Hazen and Howell, 2003). Before the tests were performed, each isolate was passaged at least twice on Sabouraud dextrose agar (Remel, Lenexa, KS) to ensure its purity and viability.

2.3. Antifungal agents and microdilution panels

The Vitek 2 cards containing serial 2-fold dilutions of fluconazole (range, 1–64 μg/mL) and of voriconazole (range, 0.12–8 μg/mL) were provided by the manufacturer. The frozen BMD reference panels containing serial 2-fold dilutions of fluconazole (range, 0.12–128 μg/mL) and of voriconazole (range, 0.03–16 μg/mL) were provided by Trek Diagnostic Systems (Cleveland, OH). The Vitek 2 cards were shipped in sealed packages and stored at 2 to 8 °C until testing was performed. The BMD panels were shipped frozen in sealed packages and were stored at −70 °C until the day of the test.

2.4. Inoculum preparation

Stock inoculum suspensions of *Candida* spp. were obtained from 24-hour cultures on Sabouraud dextrose agar at 35 °C. The inoculum suspensions for the Vitek 2 were prepared in sterile saline to a turbidity equal to a 2.0 McFarland Standard by using the bioMérieux DensiChek instrument. The inoculum suspensions for the reference BMD were prepared by diluting a portion of the 2.0 McFarland suspension prepared for the Vitek 2 to match the turbidity of a 0.5 McFarland standard.

2.5. CLSI BMD method

Reference BMD testing was performed exactly as outlined in CLSI document M27-A2 (NCCLS, 2002) with a final inoculum concentration of $1.5 \times 10^3 \pm 1.0 \times 10^3$ cells/mL and RPMI 1640 medium with 0.2% glucose and buffered to pH 7.0 with 0.165 mol/L morpholinepropanesulfonic acid buffer. The panels were incubated in air at 35 °C and observed for the presence or absence of growth at 24 hours of incubation. The fluconazole and voriconazole MICs were read visually as the lowest concentration that produced a prominent decrease in turbidity (ca. 50% reduction in growth) relative to the drug-free control (NCCLS, 2002).

2.6. Vitek 2 yeast susceptibility test

The standardized 2.0 McFarland inoculum suspension was placed into a Vitek 2 cassette along with a sterile polystyrene test tube and a yeast susceptibility test card for each organism. The loaded cassettes were then placed into the Vitek 2 instrument, and the respective yeast suspensions were diluted appropriately as defined by the manufacturer, after which the cards were filled, incubated, and read automatically. The time of incubation ranged from 9.1 to 27.1 hours, based on the rate of growth in the drug-free control well, and the results were expressed as MICs in μg/mL.

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