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Clinical breakpoints for the echinocandins and *Candida* revisited: Integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria

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ABSTRACT

The CLSI established clinical breakpoints (CBPs) for caspofungin (CSF), micafungin (MCF) and anidulafungin (ANF) versus Candida. The same CBP (susceptible (S): MIC ≤ 2 mcg/ml; non-S: MIC > 2 mcg/ml) was applied to all echinocandins and species. More data now allow reassessment of these CBPs.

We examined cases of echinocandin failure where both MICs and fks mutations were assessed; wild type (WT) MICs and epidemiological cutoff values (ECVs) for a large Candida collection; molecular analysis of fks hotspots for Candida with known MICs; and pharmacokinetic and pharmacodynamic (PK/PD) data. We applied these findings to propose new species-specific CBPs for echinocandins and Candida.

Of 18 candidiasis cases refractory to echinocandins and with fks mutations, 28% (CSF), 58% (ANF) and 66% (MCF) had MICs in the S category using CBP of ≤2 mcg/ml, while 0–8% would be S using CBP of ≤0.25 mcg/ml. WT MIC distributions revealed ECV ranges of 0.03–0.25 mcg/ml for all major species except *C. parapsilosis* (1–4 mcg/ml) and *C. guilliermondii* (4–16 mcg/ml). Among Candida tested for fks mutations, only 15.7–45.1% of 51 mutants were detected using the CBP for NS of >2 mcg/ml. In contrast, a cutoff of >0.25 mcg/ml for *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis* detected 85.6% (MCF) to 95.2% (CSF) of 21 mutant strains. Likewise, a cutoff of >0.12 mcg/ml for ANF and CSF and of >0.06 mcg/ml for MCF detected 93% (ANF) to 97% (CSF, MCF) of 30 mutant strains of *C. glabrata*. These data, combined with PK/PD considerations, support CBPs of ≤0.25 mcg/ml (S), 0.5 mcg/ml (I), ≥1 (R) for CSF/MCF/ANF and *C. albicans*, *C. tropicalis* and *C. krusei* and ≤2 mcg/ml (S), 4 mcg/ml (I), and ≥8 mcg/ml (R) for these agents and *C. parapsilosis*. The CBPs for ANF and CSF and *C. glabrata* are ≤0.12 mcg/ml (S), 0.25 mcg/ml (I), and ≥0.5 mcg/ml (R), whereas those for MCF are ≤0.06 mcg/ml (S), 0.12 mcg/ml (I), and ≥0.25 mcg/ml (R).

New, species-specific CBPs for Candida and the echinocandins are more sensitive to detect emerging resistance associated with fks mutations, and better able to predict risk for clinical failure.

1. Introduction

The echinocandins (anidulafungin [ANF], caspofungin [CSF], and micafungin [MCF]) are lipopeptide antifungal agents that inhibit the synthesis of β-1, 3-D-glucan in the fungal cell wall and exhibit concentration-dependent fungicidal activity against most species of *Candida* (Cappelletty and Eiselstein-McKitrick, 2007; Chandrasekar and Sobel, 2006; Deresinski and Stevens, 2003; Dodds-Ashley et al., 2006; Messer et al., 2006a,b; Pfaller, 2004; Vazquez, 2005; Zaas and Alexander, 2005). All three agents have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of esophageal candidiasis and invasive candidiasis (IC), including candidemia (DeWet et al., 2004; Kuse et al., 2007; Mora-Duarte et al., 2002; Ostrosky-Zeichner et al., 2005; Pappas et al., 2007; Mycamine [MCF] package insert, 2005; Astellas Pharma US, Deerfield, IL; Cancidas [CSF] package insert, 2001, Merck and

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Co., Whitehouse Station, NJ; and Eraxis [ANF] package insert, 2006, Pfizer, Inc., New York, NY), and are now recognized as the preferred systemically active antifungal agents for the treatment of IC (Pappas et al., 2009). When used in the treatment of IC (e.g., bloodstream infections [BSI], deep tissue sites, other normally sterile site infections), ANF is administered as an initial intravenous loading dose of 200 mg followed by a daily dose of 100 mg; CSF is administered as a loading dose of 70 mg, followed by a daily dose of 50 mg; and MCF is administered as a daily dose of 100 mg without the requirement of a loading dose (Cappelletty and Eiselstein-McKitrick, 2007; Dodds-Ashley et al., 2006; Zaas and Alexander, 2005). Doses of ANF and MCF in excess of 300 mg/d have been shown to be well-tolerated (Chandrasekar and Sobel, 2006; Vazquez, 2005), and a recent multicenter, double-blind trial of CSF at a daily dose of 150 mg/d versus the standard dosing regimen found that the high-dose regimen was safe and efficacious in the treatment of IC (Betts et al., 2009).

There is now a broad clinical experience using the echinocandins to treat both mucosal and invasive forms of candidiasis (Bal, 2010; Glockner et al., 2009; Lichtenstein et al., 2008; Ortega et al., 2010; Sipsas et al., 2009a,b; Zaas et al., 2006). Despite the expanding use of these agents, clinical failures remain uncommon, although reports of echinocandin resistance among *Candida* spp. are becoming more prevalent (Baixench et al., 2007; Ghannoum et al., 2009; Kofteridis et al., 2010; Perlin, 2007; Perlin, 2009; Pfeiffer et al., 2010; Sipsas et al., 2009b; Sun and Singh, 2010). The application of in vitro susceptibility testing and the use of molecular methods have served to detect potentially resistant strains of *Candida* and to characterize the various mechanisms of resistance to the echinocandin class among clinical isolates of *Candida* spp. (Arendrup et al., 2010; Cleary et al., 2008; Garcia-Effron et al., 2009a,b; Perlin, 2007; Perlin, 2009; Pfaller et al., 2008a,b, 2010a,b; Wiederhold et al., 2008).

The Clinical and Laboratory Standards Institute (CLSI) Subcommittee for Antifungal Testing has developed and standardized broth microdilution (BMD) and disk diffusion methods for in vitro susceptibility testing of *Candida* spp. against the echinocandins (Clinical and Laboratory Standards Institute, 2008a; Clinical and Laboratory Standards Institute, 2008b; Clinical and Laboratory Standards Institute, 2008c). In addition to standardized testing methods, the CLSI Subcommittee has approved quality control (QC) limits for BMD testing of all three echinocandins and for disk diffusion testing of CSF and MCF (Clinical and Laboratory Standards Institute, 2008b; Clinical and Laboratory Standards Institute, 2007). These methods have been applied worldwide to generate a detailed and comprehensive understanding of the in vitro susceptibility profile of *Candida* spp. to ANF, CSF, and MCF (Arendrup et al., 2009a; Baixench et al., 2007; Espinel-Ingroff, 2003; Messer et al., 2006a; Ostrosky-Zeichner et al., 2003; Perlin, 2009; Pfaller et al., 2005, 2006, 2008a, 2010a; Pfaller and Diekema, 2007).

In 2007, the CLSI Subcommittee for Antifungal Testing used the accumulated clinical and microbiological data to propose clinical interpretive breakpoints (CBP) for MIC testing of the echinocandins against *Candida* spp. (Pfaller et al., 2008b). The CBPs, which were subsequently incorporated into CLSI documents M27-A3 and M27-S3 (Clinical and Laboratory Standards Institute, 2008a; Clinical and Laboratory Standards Institute, 2008b), were as follows: susceptible (S), MIC $\leq$ 2 mcg/ml for all three echinocandins and all species of *Candida*. Due to the lack of echinocandin resistance in the population of *Candida* isolates at that time, the Subcommittee decided not to define a resistant (R) breakpoint and recommended that isolates for which the MIC exceeded 2 mcg/ml be called non-susceptible (NS) and be referred to a reference laboratory for confirmation of species identification and susceptibility testing (Pfaller et al., 2008b). Recently, however, it has become apparent that clinically resistant *Candida* infections involving strains with mutations in *fks 1* and/or *fks 2* (encodes glucan synthase, the echinocandin target) do not necessarily have MICs above the CBP (Arendrup et al., 2009a; Arendrup et al., 2010; Baixench et al., 2007; Desnos-Ollivier et al., 2008; Garcia-Effron et al., 2008a,b; Garcia-Effron et al., 2009a,b, 2010; Laverdiere et al., 2006; Pfaller et al., 2010a,b; Thompson et al., 2008). Furthermore, kinetic studies of the glucan synthase (GS) enzyme complex suggest that a lower MIC cutoff of 0.25–0.5 mcg/ml may be more sensitive in detecting those strains with *fks1*/*fks2* mutations (Garcia-Effron et al., 2009a,b; Wiederhold et al., 2008). These observations call into question the ability of the current CBPs to reliably identify isolates with resistance mechanisms associated with treatment failure (Arendrup et al., 2010; Garcia-Effron et al., 2009a,b).

In this review, we readdress the issue of echinocandin breakpoints for *Candida* spp. by using the available published molecular, microbiologic, pharmacodynamic (PD), and clinical data in an effort to optimize the ability of in vitro susceptibility testing to detect emerging echinocandin resistance and to ensure the safe and efficacious use of these agents in the treatment of IC. These analyses are summarized below.

2. Mechanisms of action and resistance to echinocandins in *Candida* spp.

Echinocandins inhibit β-1, 3-D-glucan synthase (GS), which catalyzes the biosynthesis of β-1, 3-D-glucan, the major glucan component of *Candida* cell walls (Douglas, 2001; Onishi et al., 2000). GS is an enzyme complex with at least two subunits, Fksp and Rho1p. The latter is a regulatory element involved in a number of cellular processes (Kondoh et al., 1997). Fksp, encoded by three related genes, *fks1*, *fks2*, and *fks3*, contains the active site, which catalyzes the transfer of sugar moieties from activated donor molecules to specific acceptor molecules forming glycosidic bonds (Kondoh et al., 1997; Sawistowska-Schroder et al., 1984; Tang and Parr, 1991). The inhibition of GS by echinocandin drugs disrupts the structure of the growing cell wall, resulting in osmotic instability and the death of susceptible yeast cells (Bowman et al., 2002; Kartsonis et al., 2003).

Echinocandin resistance in susceptible species such as *C. albicans*, *C. tropicalis*, and *C. krusei* is uncommon, but it has been associated with amino acid substitutions in Fks1p (Perlin, 2007). Likewise, it is now understood that amino acid substitutions in Fks1p and Fks2p are responsible for clinical echinocandin resistance in *C. glabrata* (Cleary et al., 2008; Garcia-Effron et al., 2009a; Garcia-Effron et al., 2010; Katiyar et al., 2006; Thompson et al., 2008). These mutations, which result in elevated MICs (4- to 30-fold MIC increases for CSF and 90- to 110-fold increases for ANF and MCF), reduce the sensitivity of GS to inhibition by drug by 30- to more than a thousand fold (Garcia-Effron et al., 2009a,b, 2010; Park et al., 2005) (Table 1). Among isolates of *C. albicans*, the most significant MIC increases have been shown to be related to amino acid changes at Ser 645 (S645P, S645F, and S645Y), whereas the other mutations account for smaller increases (Garcia-Effron et al., 2009b) (Table 1). As shown in Table 1, a relatively narrow spectrum of *fks1* mutations in strains of *C. albicans* confer reduced susceptibility across the entire class of echinocandin agents. Likewise, these mutations alter the GS enzyme kinetics resulting in significantly higher 50% inhibitory concentrations (IC50), as well as the kinetic inhibition constant (K_i), for the mutant enzymes when compared to corresponding enzymes from wild-type strains (Garcia-Effron et al., 2009b) (Table 1). Furthermore, this pattern of decreased enzyme sensitivity to inhibition (increased IC50) extends across all three of the echinocandins.

Similar to that seen with *C. albicans*, amino acid substitutions in both Fks1p and Fks2p of *C. glabrata* and in Fks1p of *C. tropicalis* and *C. krusei* have been linked with increases in echinocandin MIC and IC50 values, supporting the contention that changes in *fks* represent