SUPPLEMENT ARTICLE

Echinocandin Resistance in *Candida*

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Invasive fungal infections are an important infection concern for patients with underlying immunosuppression. Antifungal therapy is a critical component of patient care, but therapeutic choices are limited due to few drug classes. Antifungal resistance, especially among *Candida* species, aggravates the problem. The echinocandin drugs (micafungin, anidulafungin, and caspofungin) are the preferred choice to treat a range of candidiasis. They target the fungal-specific enzyme glucan synthase, which is responsible for the biosynthesis of a major cell wall polymer. Therapeutic failure involves acquisition of resistance, although it is a rare event among most *Candida* species. However, in some settings, higher-level resistance has been reported among *Candida glabrata*, which is also frequently resistant to azole drugs, resulting in difficult-to-treat multidrug-resistant strains. The mechanism of echinocandin resistance involves amino acid changes in "hot spot" regions of *FKS*-encoded subunits of glucan synthase, which decreases the sensitivity of enzyme to drug, resulting in higher minimum inhibitory concentration values. The cellular processes promoting the formation of resistant *FKS* strains involve complex stress response pathways that yield a variety of adaptive compensatory genetic responses. Standardized broth microdilution techniques can be used to distinguish *FKS* mutant strains from wild type, but testing *C. glabrata* with caspofungin should be approached cautiously. Finally, clinical factors that promote echinocandin resistance include prophylaxis, host reservoirs including biofilms in the gastrointestinal tract, and intra-abdominal infections. An understanding of clinical and molecular factors that promote echinocandin resistance is critical to develop better diagnostic tools and therapeutic strategies to overcome resistance.

Keywords. echinocandin; *FKS*; *Candida*; micafungin; caspofungin.

Inavsive fungal infections are a consequence of underlying health problems often associated with immunosuppression [1]. They carry a high mortality, and clinical success depends upon response to antifungal therapy. Unfortunately, treatment options are restricted due to the availability of limited antifungal drug classes. For many patients, the echinocandins are recommended as primary therapy for invasive candidiasis [2], and 60% of patients with candidemia are reported to receive an echinocandin [3]. As echinocandin usage broadens, increasing clinical failures due to resistant organisms are a concern, especially among *Candida* species.

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EPIDEMIOLOGY OF ECHINOCANDIN RESISTANCE

Resistance to echinocandin-class drugs, which was first reported in 2005 [4], remains relatively low, at <3% with *Candida albicans* and most *Candida* species [5]. The exception is *Candida glabrata*, in which echinocandin resistance is rising and there is cause for alarm as many isolates show cross-resistance to azole antifungal agents [6–8]. Echinocandin resistance of 8.0%–9.3% was reported by the SENTRY Antimicrobial Surveillance Program for *C. glabrata* bloodstream isolates from 2006 to 2010 [9], while in a study at Duke hospital over a period of 10 years, echinocandin resistance rose from 2%–3% to >13% in 2009–2010 [6]. Resistance may vary with region, as a study of 1380 isolates of *C. glabrata* collected between 2008 and 2013 from 4 US cities (Atlanta, Georgia; Baltimore, Maryland; Knoxville, Tennessee; and Portland, Oregon) showed that 3.1%, 3.3%, and 3.6% of the isolates were resistant to anidulafungin [8]. Importantly, echinocandin resistance in *C. glabrata* is often associated with

cross-resistance to azole antifungals yielding multidrug-resistant strains. In a recent study, nearly 36% of echinocandin-resistant isolates were also resistant to fluconazole [8]. In many healthcare centers, the widespread use of echinocandin and azole prophylaxis has prompted an epidemiologic shift, with *C. glabrata* presenting as the dominant fungal bloodstream pathogen [10].

FKS MECHANISM OF RESISTANCE

Echinocandin resistance resulting in clinical failures is conferred by limited amino acid substitutions in the Fks subunits of glucan synthase [11]. The mechanism is highly restricted and quite apart from azole antifungals, which involve a wider array of mechanisms including drug transporters, target site mutations, and target overexpression [12]. *FKS* mutations conferring echinocandin resistance occur in 2 highly conserved "hot spot" regions of *FKS1* [13–15] encompassing residues Phe641-Pro649 and Arg1361 (*C. albicans* equivalent) [4] and in homologous regions of *FKS2* in *C. glabrata* [16]. The amino acid substitutions can decrease the sensitivity of glucan synthase by several log orders [13, 16], resulting in elevated minimum inhibitory concentration (MIC) values. For *C. albicans*, amino acid changes at Ser641 and Ser645 are the most frequent and cause the most pronounced resistance phenotype [11, 13, 16], whereas in *C. glabrata*, amino acid modifications at Ser663 in Fks2, Ser629 in Fks1, and Phe659 in Fks2 are the most prominent amino acid substitutions [16]. *FKS* mutant strains of *C. albicans* and *C. glabrata* show poor drug response in pharmacodynamic studies of murine infection models [17–20]. Less prominent *FKS* mutations confer resistance, but they respond to escalating doses in animal infection models [17]. Echinocandin resistance can vary with expression of *FKS* genes [16, 21]. *FKS2* expression in *C. glabrata* is calcineurin dependent [22], and *FKS2*-dependent resistance can be reversed following treatment with the calcineurin inhibitor FK506 [21]. Finally, a third hot-spot region defined by W695 of *Saccharomyces cerevisiae* Fks1 was defined from in vitro studies but has not been observed in clinical isolates [23].

FKS POLYMORPHISMS AND REDUCED SUSCEPTIBILITY

Some *Candida* species have naturally occurring polymorphisms in *FKS* genes, which render them less susceptible to echinocandin drugs. Both the *Candida parapsilosis* family (*C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis*) and *Candida guilliermondii* have higher MIC values relative to other susceptible *Candida* species [24–26]. In the *C. parapsilosis* family, Pro660 in hot-spot 1 is present as alanine, whereas in *C. guilliermondii*, Leu633 and Thr334 are replaced by methionine and alanine, respectively. These changes decrease somewhat the sensitivity of glucan synthase for drug, resulting in elevated MIC values [27]. However, as glucan synthase is still inhibited at therapeutic levels, infecting strains can generally be treated successfully. Hence, echinocandins are largely effective in patients with *C. parapsilosis* family infections.

DRUG TOLERANCE

Inhibition of glucan synthase by echinocandin drugs weakens the fungal cell wall and creates significant cellular stress that induces a variety of adaptive protective mechanisms [28, 29]. These adaptive responses create a subpopulation of drug-tolerant persister cells with elevated in vitro MIC values to echinocandins. This behavior has been observed in murine pharmacodynamics studies, where exposure to therapeutic levels of all 3 echinocandins resulted in a stable subpopulation of *C. albicans* [19]. Cell wall stress is sensed by receptors (eg, Mtl2 and Wsc1) that induce a variety of stress adaptation pathways involving cell wall integrity, protein kinase C (PKC), calcineurin-Crz1, and HOG [30, 31]. Hsp90, another important stress response component, works through its client protein calcineurin and effector Crz1 [32]. Disruption of Hsp90 activity decreases the ability of *C. albicans* and *C. glabrata* to develop tolerance [32, 33]. Another important factor promoting drug tolerance is a compensatory increase in chitin synthesis to strengthen the cell wall. Chitin and glucans comprise the major structural components of the fungal cell wall, and both components show biosynthetic interdependence [34]. Cell wall mutant strains with elevated chitin content have been shown to be less susceptible to echinocandins in vitro [30, 31, 35] and in an animal model [36]. Changes in cell wall composition alter its thickness [37] and can have a pronounced host effect by enhancing immune recognition [38, 39]. Elevated chitin biosynthesis has also been linked with paradoxical growth observed at very high drug levels [40]. Recently, it was reported that sphingolipid biosynthesis can modulate, in a drug-dependent fashion, responses to caspofungin (less susceptible) and micafungin (more susceptible). This mixed susceptibility phenotype is linked to interactions of the aliphatic tail of echinocandins and membrane sphingolipids [41]. Overall, drug tolerance pathways are insufficient to account for clinical failures. Rather, they act to stabilize cells in the presence of drug. Even though these drug-tolerant cells do not induce therapeutic failure, they can develop higher-level resistance in time by forming stable *FKS* mutations. The underlying genetic basis is unclear, but it may involve defects in DNA repair. Genome plasticity observed in *C. albicans* and *C. glabrata* following azole exposure may be a factor for echinocandin drugs, as well [42–44].

STANDARDIZED TESTING FOR RESISTANCE

The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing

(EUCAST) have established standardized and microbroth dilution susceptibility tests for *Candida* and *Aspergillus* species for echinocandin susceptibility [36–38]. These methods demonstrate the potent activity of echinocandin drugs against most *Candida* species [45, 46]. The objective for susceptibility testing is to establish an in vitro assessment that differentiates infecting strains as either susceptible or likely to respond to therapy or as resistant with an enhanced probability to fail therapy. The CLSI used clinical and microbiological data to establish a preliminary clinical breakpoint (CBP) for all 3 echinocandins against *Candida* species [47]. However, the CBP misclassified a subset of resistant strains with *FKS* mutations [13, 48]. In response, the CLSI revised the CBP downward based on pharmacokinetic, microbiological, enzyme kinetic, and clinical data and established new species- and drug-specific breakpoints that better accounted for strains containing *FKS* mutations [49]. Similarly, EUCAST has established species-specific CBPs for micafungin against *C. albicans*, *C. glabrata*, and *C. parapsilosis* [50], as well as anidulafungin to accommodate use of these compounds in some clinical situations, especially when used as a surrogate for caspofungin [50, 51].

However, the new lower CBPs present a clinical microbiology testing challenge, as standardized testing using either CLSI and EUCAST breakpoints failed to promote consistent interlaboratory test results without major errors between laboratory groups [52, 53].

In particular, there were wide modal ranges encountered with *C. glabrata* and caspofungin [52, 53]. In contrast, consistent results were obtained for micafungin and anidulafungin, and it was proposed that they could serve as testing surrogates for the class to assess resistance [54–56]. EUCAST in response has not set caspofungin breakpoints [50]. Epidemiological cutoff values, which define the upper limit of the wild-type MIC population in the absence of a known (*FKS*) resistance mechanism, have been defined for anidulafungin and micafungin against common *Candida* species [45, 57]. The epidemiological cutoff value, although not a CBP, does provide information for clinical assessment, especially when a CBP is not available.

The problem of susceptibility testing to distinguish wild-type isolates from *FKS* mutant (echinocandin resistant) isolates may be overcome by direct molecular evaluation of the *FKS* genotype by either direct DNA sequencing or real-time probing. Molecular testing is ideal for echinocandin resistance because the presence of an *FKS* mutation is a primary clinical indicator for diminished therapeutic responses [58], which is supported by numerous pharmacodynamics, MIC, and biochemical studies [59]. Molecular testing would eliminate the current controversy surrounding susceptibility testing, which interferes with an accurate determination of resistance.

RISK FACTORS FOR RESISTANCE EMERGENCE

The development of echinocandin resistance in *Candida* species generally requires prolonged and/or repeated drug exposure [60, 61], although it can emerge rapidly after initiation of therapy [62, 63]. There is no documented horizontal transmission of resistant strains, probably because the development of *FKS*-mediated echinocandin resistance carries a fitness cost. Fks hot-spot amino acid substitutions decrease the relative catalytic capacity of glucan synthase, yielding cells with thickened cell walls [37, 13]. Some *FKS* mutant strains are less virulent in animals and compete poorly with isogenic wild-type strains [16, 21, 37].

As host immunity changes, colonizing strains are the main source of infecting strains, and the gastrointestinal tract is a prominent reservoir for *Candida* colonization [64–68], with most cells present as part of a complex microbial biofilm [69]. Like most biofilms, drug penetration is irregular as the glucan matrix helps sequester drug [70]. Under these conditions, resistant mutants can emerge, where they can enter the systemic circulation and cause infection. Intra-abdominal candidiasis, another important source of resistant infections, occurs in 40% of patients following repeated gastrointestinal surgery, gastrointestinal perforation, or necrotizing pancreatitis [71]. This is largely because high microbial burden and poor drug penetration create strong selection pressure for resistance emergence.

As drug exposure is an important factor for resistance emergence, the expanding use of prophylaxis is another potential area of concern. Echinocandins are excellent prophylaxis agents against invasive candidiasis because they have favorable pharmacokinetics and safety profiles, and they are active against azole-resistant yeasts and molds. Micafungin is approved by the US Food and Drug Administration for prophylaxis of *Candida* infections in patients undergoing hematopoietic stem cell transplantation (HSCT) or expected to be neutropenic for at least 10 days [72], and the European Society of Clinical Microbiology and Infectious Diseases guidelines also recommend micafungin for prophylaxis against *Candida* infections in allogeneic HSCT adult and pediatric patients, and in pediatric patients with acute myeloid or recurrent leukemia. Both micafungin and caspofungin have been successfully used in adult and pediatric populations [73–75], and meta-analyses have shown that echinocandin prophylaxis can reduce the incidence of invasive fungal infections compared with azoles (fluconazole or itraconazole) [76]. The expanding use of echinocandins for prophylaxis has increased patient exposure to echinocandin drugs. This has implications for drug resistance as breakthrough infections have been reported [77]. It is not surprising that broadening patient exposure to echinocandin drugs may inadvertently promote the emergence of resistance.

CONCLUSIONS

Echinocandin resistance among *Candida* species is uncommon, except with *C. glabrata* where higher-level resistance is reported from some clinical sites and which is often associated with azole resistance. Acquired resistance occurs during therapy and involves amino acid changes in hot-spot regions of the Fks subunits of glucan synthase. Echinocandin action induces a variety of cellular stress response pathways creating drug-adapted persister states, which may ultimately break through and form *FKS*-resistant mutants. Host factors promoting resistance include biofilm formation within the gastrointestinal tract, intra-abdominal candidiasis, and the expanding use of echinocandin prophylaxis. New drug- and species-specific breakpoints pose challenges for standardized testing, which require either surrogate drugs for the class (eg, micafungin) or direct molecular testing for the presence of *FKS* mutations.

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