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.抗真菌药的使用受到限制，由于可用的抗真菌药物种类有限。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.

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 $FKS_1$ [13–15] encompassing residues Phe641-Pro649 and Arg1361 (C. albicans equivalent) [4] and in homologous regions of $FKS_2$ 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 echinocandins. 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 pharmacokinetics, 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 persistate states, which may ultimately break through and form FKS-resistant mutants. Host factors promoting resistance include biofilm formation within the gastrointestinal tract, intrabdominal 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.

Notes

**Financial support.** The author is supported by the National Institutes of Health (grant numbers AI069397 and AI109025) and Astellas Pharma. This article appears as part of the supplement “Advances and New Directions for Echinocandins,” sponsored by Astellas Pharma Global Development, Inc.

**Potential conflict of interest.** The author serves on scientific advisory boards for Merck, Astellas, and Syneixs; has received grant support from Astellas; and is an inventor in US patent 8,753,819, “Assays for Resistance to Echinocandin-Class Drugs.” The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


Echinocandin Resistance • CID 2015:61 (Suppl 6) • S615


