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Echinocandin Resistance, Susceptibility Testing and Prophylaxis: Implications for Patient Management

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Abstract

This article addresses the emergence of echinocandin resistance among *Candida* species, mechanisms of resistance, factors that promote resistance and confounding issues surrounding standard susceptibility testing. Fungal infections remain a significant cause of global morbidity and mortality, especially among patients with underlying immunosupression. Antifungal therapy is a critical component of patient management for acute and chronic diseases. Yet, therapeutic choices are limited due to only a few drug classes available to treat systemic disease. Moreover, the problem is exacerbated by the emergence of antifungal resistance, which has resulted in difficult to manage multidrug resistant strains. Echinocandin drugs are now the preferred choice to treat a range of candidiasis. These drugs target and inhibit the fungal-specific enzyme glucan synthase, which is responsible for the biosynthesis of a key cell wall polymer. Therapeutic failures involving acquisition of resistance among susceptible organisms like *Candida albicans* is largely a rare event. However, in recent years, there is an alarming trend of increased resistance among strains of *Candida glabrata*, which in many cases are also resistant to azole drugs. Echinocandin resistance is always acquired during therapy and the mechanism of resistance is well established to involve amino acid changes in “hot-spot regions of the Fks subunits carrying the catalytic portion of glucan synthase. These changes significantly decrease the sensitivity of the enzyme to drug resulting in higher MIC values. A range of drug responses, from complete to partial refractory response, is observed depending on the nature of the amino acid substitution, and clinical responses are recapitulated in pharmacodynamic models of infection. The cellular processes promoting the formation of resistant Fks strains involve complex stress response pathways, which yield a variety of adaptive compensatory genetic responses. Stress-adapted cells become drug tolerant and can form stable drug resistant *FKS* mutations with continued drug exposure. A major concern for resistance detection is that classical broth microdilution techniques show significant variability among clinical microbiology laboratories for certain echinocandin drugs and *Candida* species. The consequence is that susceptible strains are misclassified according to established clinical breakpoints, and this has led to confusion in the field. Clinical factors that appear to promote echinocandin resistance include the expanding use of antifungal agents for empiric therapy and prophylaxis. Furthermore, host reservoirs such as biofilms in the gastrointestinal tract or intra-abdominal infections can seed development of resistant organisms during therapy. A fundamental understanding of the primary molecular resistance mechanism, along with cellular and clinical factors that promote resistance emergence, is critical to develop better diagnostic tools and therapeutic strategies to overcome and prevent echinocandin resistance.

1. Introduction

Fungal infections are increasingly recognized as a major global health problem. There are more than 300 million people afflicted by a serious fungal infection resulting in nearly 1.4 million deaths annually (www.gaffi.org) [1]. Fungal diseases cause life-threatening illnesses such as meningitis and pneumonias, chronic asthma, other respiratory distress syndromes, and recurrent diseases like oral and vaginal thrush. Serious fungal infections are a consequence of underlying health problems such as asthma, AIDS, cancer, organ transplantation and corticosteroid therapies with a majority of fungal deaths due to *Cryptococcus*, *Candida* and *Aspergillus species* [1]. The management of fungal diseases requires antifungal therapy. Yet, treatment options are limited, as the most prominent antifungal drugs target either the plasma membrane, nucleic acid biosynthesis or cell wall, and they comprise only a few chemical classes represented by polyenes, azoles, flucytosine, and echinocandins [2]. Azoles drugs, which include fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole inhibit the biosynthesis of the plasma membrane sterol ergosterol. The pore-forming polyene drug amphotericin B binds to ergosterol in the plasma membrane. Flucytosine (5-fluorocytosine) broadly inhibits pyrimidine metabolism and DNA synthesis, while the echinocandins drugs caspofungin, anidulafungin, and micafungin inhibit glucan synthase and are the first cell wall active agents. Echinocandins are recommended as first-line therapy for non-neutropenic patients with *Candida albicans*, *Candida glabrata* and suspected severe invasive candidiasis [3]. Recent CDC surveillance indicates that >60% of candidemia patients now receive an echinocandin [4]. It is the expanding application of echinocandins worldwide and emerging resistance among certain *Candida* species, which will be discussed in this review.

2. Echinocandin class drugs

Echinocandin drugs are lipopeptides that inhibit glucan synthase, which is responsible for the biosynthesis of β-1,3-D-glucan, a major structural component of fungal cell walls [5]. The U.S. Food and Drug Administration approved them for the treatment of esophageal and invasive candidiasis, including candidemia, empirical therapy in febrile neutropenic patients and prophylaxis in patients undergoing hematopoietic stem cell transplantation (HSCT) [6, 7]. The first in-class drug, caspofungin, was also approved for salvage therapy for patients with invasive aspergillosis [8]. Maintenance of the fungal cell wall is essential for cell survival and echinocandin drugs often show *in vitro* fungicidal activity against susceptible *Candida spp.* [9, 10]. Echinocandins are fungistatic against molds where they can lyse the apical tips of expanding hyphae, alter morphology and modify cell wall composition and organization [11, 12]. However, they are largely inactive against invasive *Zygomycetes*, *Cryptococcus* spp., or *Fusarium* spp. The echinocandin drugs have a distinct mechanism of action, which enable them to be highly effective against yeasts with reduced susceptibility to azoles, such as *C. glabrata* and *C. krusei* [13, 14] [15], as well as some *Candida* biofilms [16–19]. The echinocandins have an excellent therapeutic index with a low potential for renal or hepatic toxicity or serious drug-drug interactions [20, 21]. All echinocandins have low oral bioavailability, and distribute well into tissues, but poorly into the CNS and eye. The echinocandin target, β-1,3-D-glucan synthase, is a fungal-specific multi-subunit enzyme complex comprised of Rho, a GTP-binding protein, which helps regulate the overall activity

of glucan synthase [22] and a catalytic subunit Fks encoded by three related genes, *FKS1*, *FKS2*, and *FKS3*. The *FKS1* gene is essential in *C. albicans* [23, 24] and other *Candida* spp., while in *C. glabrata*, *FKS1* and *FKS2* are functionally redundant [25]. The *FKS3* gene is expressed at a very low level relative to the other genes [26].

3. Epidemiology of Echinocandin Resistance

Most major *Candida* species are highly susceptible to echinocandin drugs [27, 28]. The notable exceptions are *C. parapsilosis* complex (*Candida parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. metapsilosis*) and *C. guilliermondii*, which display higher echinocandin antifungal MIC values relative to other highly susceptible *Candida* species [29–31] [32–34] and is reflected in higher breakpoints [35]. Intrinsic reduced susceptibility has an unclear clinical significance as patients are often successfully treated with echinocandin drugs [36–38] [39], although clinical efficacy may vary with patient population [40–42]. Since first reported in 2005, susceptible *Candida* spp. isolates resistant to echinocandin drugs are increasingly encountered [43, 44, 26, 45–51] [52, 53] [54], although the frequency remains relatively low (<2–3%) with *C. albicans* and most other *Candida* spp. [55–57] [58] [58]. The notable exception is *C. glabrata*, where resistance is growing more rapidly [59, 60]. In many healthcare centers, the growing use of echinocandins and azoles for prophylaxis has resulted in an epidemiologic shift with *C. glabrata* represented as the most dominant fungal bloodstream pathogen [61] [30]. Echinocandin resistance may occur after prolonged therapy [52] or it may be rapid, even shortly after initiation of therapy [62] [53]. Recently, the SENTRY Antimicrobial Surveillance Program from 2006–2010 reported echinocandin resistance of 8.0–9.3% among 1669 blood stream isolates (BSI) of *C. glabrata* [63]. Furthermore, in a ten year study involving 293 unique episodes of *C. glabrata* BSI, echinocandin resistance of *C. glabrata* rose from 2–3% during 2001–2006 to >13% in the years 2009–2010 [59]. Disturbingly, this rise in echinocandin resistance among *C. glabrata* paralleled a rise in azole resistance resulting in multidrug resistant strains (Fig 1). The generally excellent wild-type susceptibility of *C. glabrata* to the echinocandin drugs, even among azole resistant strains, has driven the widespread use of echinocandins for treatment of infections due to *C. glabrata.* Yet, at the same time, it has generated selection pressure for multidrug resistant organisms [59]. The underlying genetic basis for rapid emergence of resistance in *C. glabrata* is largely unknown, but it may stem from its haploid state and/or from its inherent genetic plasticity. In molds, echinocandin resistance has also been described in rare circumstances for *A. fumigatus* [64] and more readily for *A. lentulus* [65].

4. Mechanism of Acquired Resistance

Clinical resistance resulting in therapeutic breakthrough infections involves modification of the catalytic Fsk subunit (Fks1 and Fks2) of glucan synthase. Unlike azole antifungal agents, echinocandins are not substrates for multidrug transporters [16, 15]. Echinocandin resistance is conferred by characteristic amino acid substitutions in Fks subunits [7], which induce elevated MIC values (10–100 fold) and reduce the sensitivity of glucan synthase (IC_{50}) to drug by 50- to 3000-fold [26, 45, 66]. Characteristic mutations in *FKS* genes are prominently associated with reduced clinical response [67, 68]. In a recent study of patients with invasive candidiasis, the presence of an *FKS* mutation was the only independent risk

factor associated with echinocandin failure and among *C. glabrata* isolates, and the presence of an *FKS* mutation was superior to MIC in predicting echinocandin therapeutic responses among patients [68]. In *C. albicans* and most other *Candida* spp., mutations occur in two highly conserved "hot-spot" regions of *FKS1* [45, 69, 70] encompassing residues Phe641-Pro649 and Arg1361 (Fig 2). Amino acid substitutions at Ser645 and Phe641 are the most abundant, nearly 80% in C. albicans (Fig 2), and cause the most pronounced resistance phenotypes [26, 45, 7, 71]. These *fks* mutants are effectively insensitive to drug and fail to respond in pharmacodynamic studies of murine models of infection [72–75]. In *C. glabrata*, resistance-associated mutations occur in homologous regions of *FKS1 and FKS2* [26, 66], although amino acid substitutions in Fks2 occur in clinical isolates at twice the frequency of Fks1 [26, 7, 71]. Alterations at Fks1 positions S629 and S663 and Fks2 position F659S confer the highest MIC values. Nonsense mutations in either *FKS1* or *FKS2* are also observed in *C. glabrata* [26, 66] [76]. The echinocandin resistance level conferred by hot spot mutations in *FKS1* or *FKS2* can also depend on the relative expression of their genes, which can vary more than 20-fold [26, 25]. *FKS2* expression is calcineurin dependent and down regulated by FK506 [77], and resistance conferred by *FKS2* can be reversed with FK506 [25]. Finally, mutations in *FKS1* for *Candida* species such as *C. tropicalis*, *C. krusei* and *C. kefyr* have been linked with increases in echinocandin MIC and clinical failures [78, 79, 62].

4. 1 FKS3, Virulence and Biofilms

A third highly conserved hot-spot region defined by W695 of *S. cerevisiae* Fks1 was recently identified [80], but it is not associated with clinical failures. Amino acid substitutions in Fks1 of *C. albicans* confer reduced fitness [26, 25] [81], since they can decrease the catalytic reaction rate maximum for glucan biosynthesis [26, 45] and alter cell wall morphology [81]. Echinocandin resistant strains compete poorly with their wild-type counterpart [81], which may explain why resistance is associated with acquired de novo resistance and horizontal transmission is not a factor.

Biofilms are an important complex communal structure of fungi contributing to antifungal drug resistance [82]. For echinocandin drugs, the extracellular matrix of the biofilm comprised mainly of β-glucan sequesters the drugs by decreasing their effective concentration at the surface of the fungal cell membrane [83]. The application of genetic or chemical means to decrease glucan production renders the biofilms more susceptible to antifungal agents [84]. Transcription factor Rlm, Smi1 and glucan synthase Fks1 are important factors that regulate glucan formation yielding drug-sequestering biofilms [84].

4.2 Serum, cellular stress and resistance emergence

The development of characteristic *FKS* mutations is an end stage event in the resistance process and there are a number of factors that condition cells and influence mutant selection. Firstly, the echinocandin drugs are highly serum protein bound, which reduces their relative efficacy and shifts MICs upward [85–87]. The nature of the shift depends on interactions with specific drugs; anidulafungin and micafungin show a larger relative shift than caspofungin. A consequence of this shift in efficacy is that serum alters the relative fungicidal properties of the drugs, often resulting in fungistatic behavior against certain

Candida species [88, 89]. This drug shift permits cellular responses, which promote survival. In particular, fungi possess a range of adaptive response mechanisms that help protect cells against environmental stresses [90, 91]. Secondly, yeast acutely sense cell wall stress. Inhibition of glucan biosynthesis by the echinocandins induces a variety of stress tolerance pathways including cell wall integrity, *PKC*, Ca^{2+}/calcineurin/Crz1, and *HOG* [92, 93]. Hsp90 induces tolerance to echinocandin drugs through its principal client protein calcineurin and the downstream effector crz1 [94, 95]. Finally, echinocandin action results in compensatory increases in chitin synthesis, which serves to maintain the structural integrity of the cell wall, as chitin replaces β-1,3 glucan [92], and cell wall mutants with enhanced chitin contents are less susceptible to echinocandins both in vitro [96, 92, 97, 93] and *in vivo* [98]. Elevated chitin and adaptive responses have been linked to paradoxical growth whereby susceptible cells show growth at very high levels of drug [99] [100] [101]. It has been proposed that sphingolipids can interact with echinocandins near their target and differentially decrease sensitivity to caspofungin while increasing sensitivity to micafungin [102, 103]. The collective adaptive cellular responses help stabilize cells in the presence of drug. Ultimately, they likely predispose cells and promote selection for *FKS* mediated resistance, even though by themselves they are insufficient to induce therapeutic failure.

5. Susceptibility Testing and Its Foibles

The goal of susceptibility testing is to establish an *in vitro* marker to characterize infecting strains as either “susceptible” to a drug and likely to respond to therapy or “resistant” with a heightened probability to fail therapy. This probability is best described as the “90-60 rule” in which infections due to susceptible isolates respond to therapy approximately 90% of the time, whereas infections due to resistant isolates respond 60% of the time [104]. To address this need, the antifungal susceptibility testing subcommittees (AFST) of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) established independent, yet closely related standards for broth microdilution (BMD) antifungal susceptibility testing of echinocandins against *Candida* species, which generally yield comparable MIC results [105, 34, 106]. In 2007, the CLSI used clinical and microbiological data to establish a preliminary clinical breakpoint (CBP) for echinocandins against *Candida* spp. (Pfaller et al., 2008b) with an MIC ≤ 2 μg/ml considered susceptible for all three echinocandins and all species of *Candida.* However, it soon became apparent that resistant strains with acquired *FKS* mutations were often misclassified by this CBP [107, 45]. To address this issue, CLSI revised the CBP based on pharmacokinetic, microbiological and enzyme kinetic data, along with the clinical experience. New species and drug-specific breakpoints were established for CLSI BMD testing that accounted for strains containing FKS mutations (Pfaller et. 2011) (Table 1).

Yet the new lower CBPs posed a testing challenge, as BMD testing was not sufficiently robust to enable consistent interlaboratory testing without major errors encountered between groups [108–110]. Wide modal ranges were especially present with *C. glabrata* and caspofungin where numerous groups were unable to provide consistent testing with either CLSI or EUCAST methods [108–110] [111]. The underlying factor(s) contributing to this variability has not been ascertained. Since the use of a CLSI species-specific caspofungin CBP can lead to reporting an excessive number of wild-type isolates as either non-WT or

resistant isolates [109], it has been recommended that micafungin or anidulafungin be used as a surrogate class marker, since either drug behaves more predictably [110]. The problem with this approach is that many physicians who depend on caspofungin for therapy are uncomfortable with a drug surrogate, even among the same drug class. Epidemiological cutoff values (ECVs) have been established to define the upper limit of the "wild type" MIC distribution for each species with no acquired resistance mechanisms [112]. Species-specific ECVs aid in detecting non-WT isolates with reduced susceptibility to anidulafungin and micafungin due to *fks* mutations, and have been shown to classify 92.2% and 100% of the *fks* mutant strains, respectively [27] (Table 2). EUCAST has established breakpoints for anidulafungin (Table 1) and recommends anidulafungin MIC testing as a marker for the echinocandin class of drugs [113] [114]. However, due to the irregularities observed with testing between laboratories, EUCAST has not set caspofungin breakpoints and does not currently recommend caspofungin MIC testing for clinical decision-making involving echinocandin drugs [114].

5.1 Is it time for molecular testing?

The problem of conventional susceptibility testing to distinguish wild type susceptible isolates from echinocandin resistant isolates bearing *FKS* mutations raises the notion that molecular testing may be long overdue for this field. There is overwhelming data linking the presence of specific hot-spot mutations in FKS genes to reduced clinical efficacy, which is supported by extensive studies of pharmacodynamics, inhibition of glucan synthase, and MIC [35]. Several clinical studies have shown that the presence of an *FKS* mutation is the most important independent risk factor in predicting echinocandin therapeutic responses among patients with IC [68] [76, 67]. The downside of molecular testing is that not all *FKS* mutations harbor the same potential for high-level resistance [67] [7], which would require stratification of mutations. Nevertheless, only a few mutations account for the vast majority of therapeutic failures (Fig. 2), which would support a role for molecular testing [115]. Molecular testing requires knowledge of known resistance mechanisms, and any unknown mechanism would not be detected. Yet, this probability is sufficiently remote given the current body of data. Thus, it may be time to implement molecular testing to directly identify mutant strains containing *fks* mutations and end the susceptibility testing controversies, which prevent timely and proper assessment of resistance.

6. Prophylaxis: Benefits with a potential resistance cost

Antifungal prophylaxis is now a standard prevention in many settings involving patients at high risk for development of invasive fungal infections, especially patients undergoing transplantation or other conditions resulting in severe immune deficiency. For many years, fluconazole was the antifungal drug of choice for primary prophylaxis in HSCT recipients [116, 117]. However, fluconazole has a limited spectrum, even among prominent *Candida* spp., some of which are inherently less sensitive to azoles (e.g. *C. glabrata* and *C. krusei*). Furthermore, it is inactive against *Aspergillus* species and other molds, which led to a call for more potent antifungal drugs in prophylactic regimens [118].

Echinocandin drugs are an attractive alternative for prophylaxis since they display favorable pharmacokinetics, have an excellent safety profile and are active against azole resistant

yeasts and molds. This is particularly true for micafungin, which minimizes the potential for drug interactions since it does not interact with compounds whose metabolism is mediated via cytochrome P450 [119]. In an early prospective trial of micafungin and fluconazole involving 899 patients, micafungin was shown to have a higher overall treatment success rate demonstrating its effectiveness as a prophylactic agent [120]. Similarly, caspofungin was evaluated as primary prophylaxis against invasive fungal infections in 123 stem cell transplant recipients who were poor candidates for triazole or polyene prophylaxis, and it was deemed an effective and well-tolerated option for primary antifungal prophylaxis for the highly immunosuppressed stem cell transplant patient population [121]. Caspofungin was equally effective as itraconazole in preventing invasive fungal infections in patients with hematologic malignancies and it was effective in both adult [122] and pediatric populations [123]. Micafungin was somewhat more effective than fluconazole for the prevention of all mold infections and invasive aspergillosis and reducing the need for empiric antifungal treatment [124]. In large meta analyses involving 17 studies covering 5122 patients [125] and 20 studies covering 4823 patients [124], respectively, echinocandin prophylaxis reduced the incidence of invasive fungal infections greater than fluconazole or itraconazole. Micafungin is now approved by the FDA for prophylaxis of *Candida* infections in patients undergoing hematopoietic SCT or expected to be neutropenic for at least 10 days [126]. The latest European Society of Clinical Microbiology and Infectious Diseases guidelines also recommend micafungin for prophylaxis against *Candida* infections in allogeneic HSCT adult and pediatric patients, as well as in pediatric patients with acute myeloid and recurrent leukemia. There is a recommendation for caspofungin prophylaxis to prevent invasive candidiasis/candidemia, as well as intra-abdominal *Candida* infection [127]. There is a marginal recommendation for prophylaxis of adult HSCT patients with caspofungin and no recommendation for the use of anidulafungin [128].

6.1 Prophylaxis as a resistance driver

The expanding use of echinocandins for prophylaxis and therapy, while beneficial in reducing the overall incidence of invasive disease in high-risk settings, raises a critical question about its role in inducing significantly higher rates of echinocandin drug resistance, especially among *C. glabrata*. In a recent report, involving a 25-year-old patient receiving micafungin prophylaxis, five *C. glabrata* isolates were obtained from blood cultures and were classified as multidrug-resistant isolates, since all exhibited high MICs for echinocandin and azole drugs [129]. The co-evolution of azole and echinocandin multidrug resistance among *C. glabrata* is an alarming trend [59]. Similarly, breakthrough infections involving *C. albicans* are also being reported in patients with graft-versus-host disease following a stem cell transplant who received micafungin prophylaxis [130]. Most recently, a disturbing report from a retrospective observational study involving echinocandin-based anti-*Aspergillus* prophylaxis for 152 patients with acute myeloid leukemia during remission-induction chemotherapy showed a higher risk of breakthrough IFI [131]. It is not surprising that broadening patient exposure to echinocandin drugs would promote development of resistance. Beyond anecdotal reports, there is firm data emerging that the *FKS* resistance mechanism is an important risk factor for therapeutic failure [68] and resistance emergence is directly linked to prior exposure [132]. There is a danger that broadening echinocandin prophylaxis may continue to fuel an increase in the frequency of isolates that are resistant to

multiple classes of antifungal drugs, which may reflect genomic plasticity among otherwise clonal organisms [133]. The trend toward echinocandin prophylaxis should be coupled with a renewed evaluation of drug dosing to ensure suitably high levels of drug are achieved. It may be time to reassess dosing strategy in the context of prophylactic regimens.

6.2 Reservoirs for resistance emergence?

The gastrointestinal (GI) tract is a normal commensal site for *Candida* species with the burden often exceeding 10^7 cfu/g of feces [134–141] and molecular genotyping has demonstrated that colonizing isolates often are the infecting strain for most patients with invasive candidiasis [142]. *Candida* colonization of the GI tract is often in the form of a mixed microbial biofilm [143]. A consequence of the biofilm microbial community is that there is varying levels of drug exposure to different parts of the biofilm, since there are induced mechanisms against azole drugs [144] and drug penetration into the glucan matrix is irregular [83]. Thus, there is a potential to select for resistant variants, which can desorb from the biofilm and cause systemic infections. The biofilm is difficult to eradicate and it can act as a reservoir that seeds resistant infections. Intra-abdominal candidiasis, including peritonitis and intra-abdominal abscesses, may occur in >40% of patients following repeat gastrointestinal surgery, GI perforation or necrotizing pancreatitis [145]. The high burden of *Candida* coupled with poor drug penetration into the biofilm creates a strong environment for selection of resistant variants.

7. New Therapy Trends: Large-daily infrequent doses

The echinocandins exhibit concentration-dependent effects on *Candida* species. Preclinical studies and pharmacokinetic and pharmacodynamic studies support the administration of large, infrequent doses and better outcomes were observed with higher maximum concentrations of drug in serum and large, infrequent doses [146]. Once-weekly micafungin therapy is as efficacious as daily therapy in a murine model of disseminated candidiasis [147]. This emerging therapeutic strategy may be appealing for certain forms of *Candidiasis*. Resistance emergence may be a concern as drug levels diminish. However, the development of resistance may actually be less likely to occur as the larger doses may place the drug exposure level within the mutant prevention concentration window that precludes development of single step resistance [148, 149].

8. CONCLUSIONS AND PERSPECTIVE

Echinocandin resistance is on the rise, especially among clinical isolates of *Candida glabrata*. This resistance trend is particularly alarming since such strains may also carry azole resistance leading to multidrug resistant strains resulting in difficult to manage infections. The Fks mechanism of resistance involving modification of the target enzyme glucan synthase is well established. But there is now emerging evidence that cellular stress pathways play a critical role in establishing drug adaptive states, which facilitate development of stable resistance with *FKS* genotypes. A current challenge and concern for clinical laboratories is the recent refinement of breakpoints by the CLSI to distinguish resistant strains containing *FKS* mutations from wild type susceptible strains using broth microdilution methodology. This has led to inter-laboratory variability resulting in the

misclassification of susceptible isolates as resistant. It has been suggested that either micafungin or anidulafungin, which show greater in vitro sensitivity under conventional MIC testing can serve as a surrogate for the class. However, as the objective of such testing is to identify strains with *fks* genotypes, it may be time for the field to move to direct sequence-based detection, as is done routinely for bacteria and viruses. Finally, the expanding use of echinocandin prophylaxis increases drug exposure in the host leading to resistance, as reservoirs of colonization and/or infections may have less than adequate drug exposure. It may be time to reassess prophylactic dosing regimens in patients at high risk for invasive fungal disease.

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Key Points

- Echinocandin drugs are important first line therapy for *Candida* species infections but drug resistance, especially among *C. glabrata*, is an emerging problem that impacts clinical outcome
- Mutations in *FKS* genes resulting in amino acid substitutions in the drug target glucan synthase confer higher MIC values, reduced enzyme sensitivity to drug, and diminished pharmacodynamics response.
- The emergence of *FKS*-mediated resistance requires drug adaptation involving a wide range of cellular responses to cell wall stress.
- Problems with susceptibility testing may necessitate the development of alternative methodologies, such as molecular profiling of *FKS* genes.
- Echinocandin prophylaxis is effective but may help fuel an increase in the frequency of isolates that are resistant to multiple classes of antifungal drugs

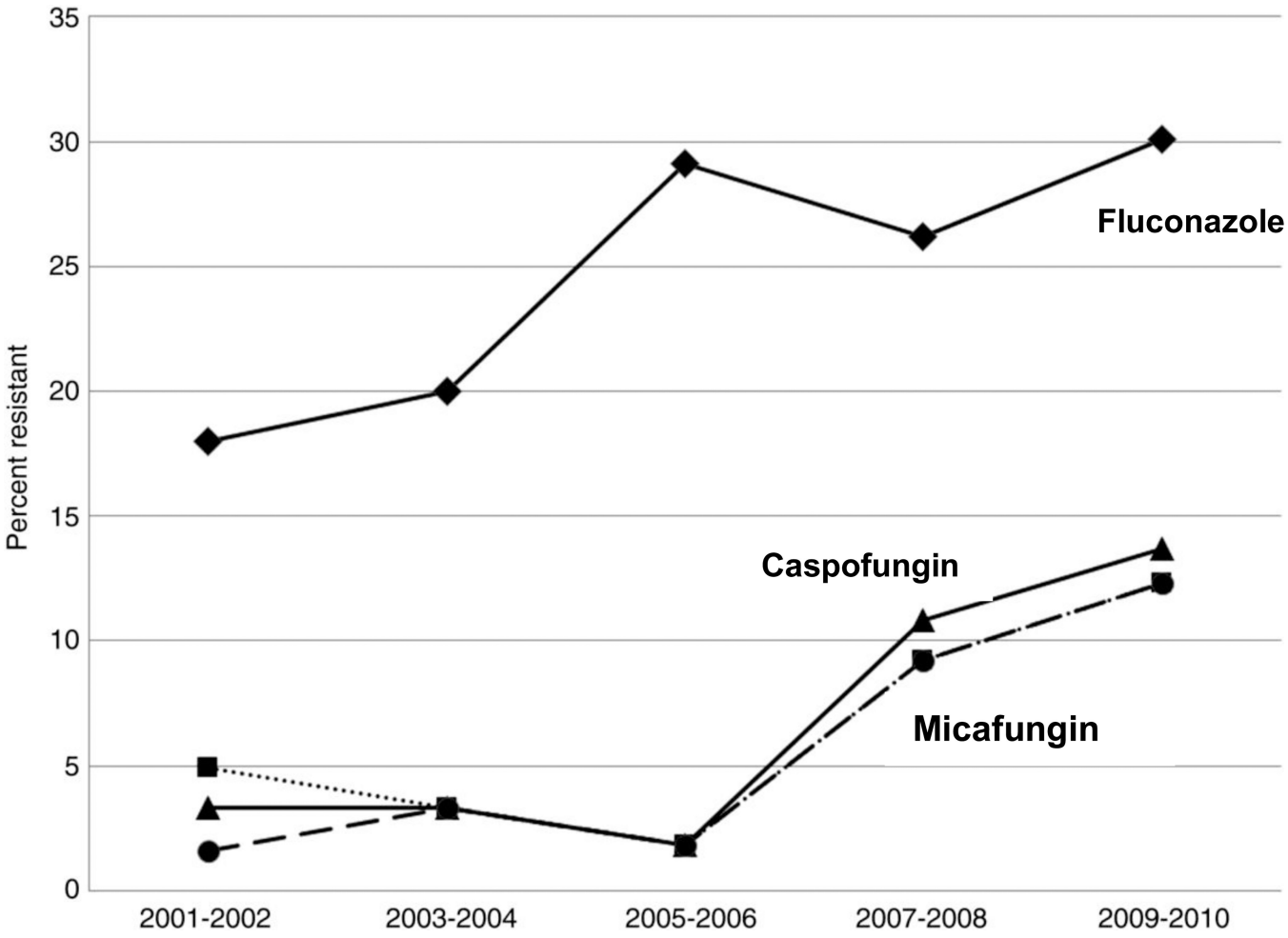

Fig 1.

Temporal trends in antifungal resistance of *Candida glabrata* isolates to fluconazole, anidulafungin, caspofungin, and micafungin. Adapted from Alexander et al. [59]

A

Organism	Fks1 Hot Spot1	Fks1 Hot Spot 2	Fks2 Hot Spot 1	Fks2 Hot Spot 2	Fks3 Hot Spot 1
C. albicans	F_{641}LTLSLRDP	DWIRRYTL	NO	NO	NO
C. krusei	F_{655}LILSIRDP	DWIRRYTL	NO	NO	NO
C. glabrata	F_{625}LILSIRDP	DWIRRYTL	F_{659}LILSLRDP	DWIRRYTL	NO
C. guilliermondii	F_{632}MALSIRDP	DWIRRYTL	NO	NO	NO
C. tropicalis	F___LTLSIRDP	DWIRRYTL	NO	NO	NO
*C. parapsilosis**	F_{652}LTLSIRDA	DWIRRYTL	NO	NO	NO
C. orthopsilosis	F___LTLSIRDA	DWVRRYTL	NO	NO	NO
C. metapsilosis	F___LTLSIRDA	DWIRRYTL	NO	NO	NO

B

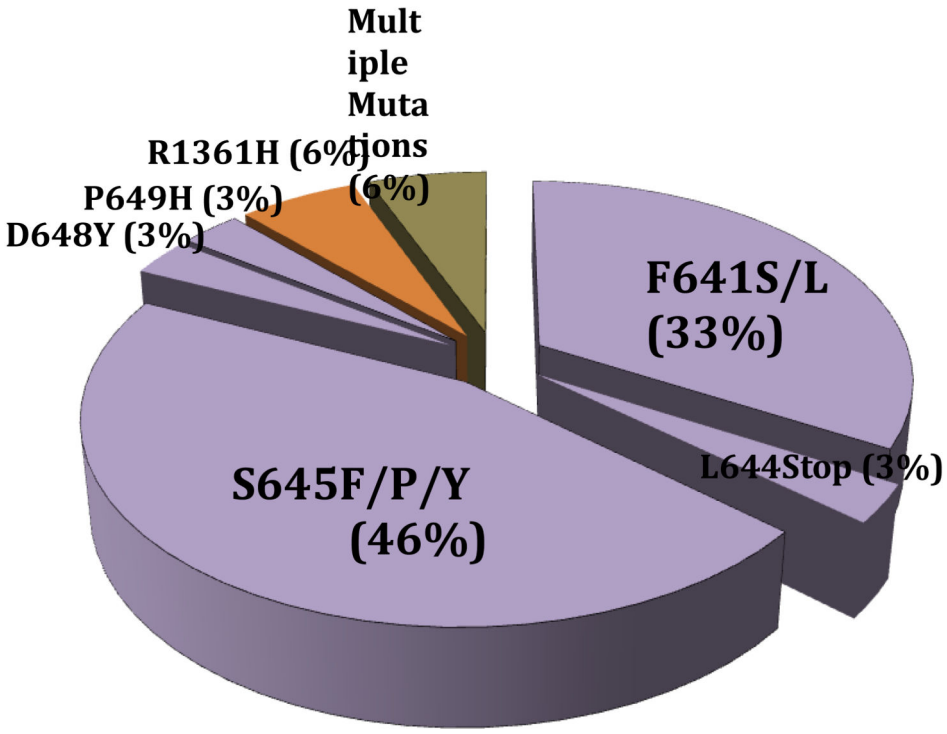

Fig 2.

A. Amino acid sequences of Fks “hot-spot” sequences for major *Candida* species and positions associated with prominent resistance (red), weaker resistance (yellow) and naturally-occurring polymorphisms that cause reduced susceptibility (green). B. Relative frequency of Fks amino acid substitutions in *C. albicans* causing echinocandin resistance from Perlin Lab echinocandin reference center.

Table 1

EUCAST and CLSI antifungal breakpoint comparison for major *Candida* species[1]

Antifungal agent	MIC breakpoint (mg/L)									
	Candida albicans		*Candida glabrata*		*Candida krusei*		*Candida parapsilosis*		*Candida tropicalis*	
	S≤	R>	S≤	R>	S≤	R>	S≤	R>	S≤	R>
Anidulafungin										
EUCAST	0.03	0.03	0.06	0.06	0.06	0.06	0.002	4	0.06	0.06
CLSI	0.25	0.5	0.12	0.25	0.25	0.5	2	4	0.25	0.5
Caspofungin										
EUCAST	ND[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND
CLSI	0.25	0.5	0.12	0.25	0.25	0.5	2	4	0.25	0.5
Micafungin										
EUCAST	0.016	0.016	0.03	0.03	IE[3]	IE	0.002	2	IE	IE
CLSI	0.25	0.5	0.06	0.125	0.25	0.5	2	4	0.25	0.5

[1] Adapted from Arendrup et al. [114]

[2] ND- Not determined due to significant inter-laboratory variation in MIC ranges

[3] IE- Insufficient evidence (IE) due to small number of cases

TABLE 2

Anidulafungin and micafungin ECVs for eight species of Candida[1]

Antifungal agent	*Species*	No. of isolates	MIC (μg/ml)		ECV (μg/ml)[2]		
			Range	Mode	≥95%	≥97.5%	≥99%
Anidulafungin	*C. albicans*	8,210	0.008–2	0.03	0.06	0.12	0.12
	C. glabrata	2,680	0.008–4	0.06	0.12	0.12	0.25
	C. parapsilosis	3,976	0.008–8	2	4	8	8
	C. tropicalis	2,042	0.008–2	0.03	0.12	0.12	0.12
	C. krusei	322	0.008–2	0.06	0.12	0.25	0.25
	C. lusitaniae	234	0.008–1	0.25	1	1	1
	C. guilliermondii	222	0.03–4	1	4	8	8
	C. dubliniensis	131	0.015–4	0.03	0.12	0.12	0.12
Micafungin	*C. albicans*	7,874	0.008–4	0.015	0.03	0.03	0.03
	C. glabrata	3,102	0.008–4	0.015	0.03	0.03	0.03
	C. parapsilosis	3,484	0.015–4	1	2	4	4
	C. tropicalis	1,605	0.008–8	0.015	0.06	0.06	0.12
	C. krusei	617	0.015–1	0.06	0.25	0.25	0.25
	C. lusitaniae	258	0.008–≥16	0.25	0.5	0.5	1
	C. guilliermondii	234	0.015–8	0.5	2	2	4
	C. dubliniensis	117	0.008–8	0.06	0.12	0.12	0.12

[1] Adapted from Pfaller et al. 2014 [27]

[2] Calculated ECVs comprising ≥95%, ≥97.5%, or ≥99% of the statistically modeled MIC population.