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Epidemiology of echinocandin resistance in *Candida*

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Abstract

Echinocandins are the newest antifungal agents approved for use in treating *Candida* infections in the US. They act by interfering with 1,3-β-D-glucan synthase and therefore disrupt cell wall production and lead to *Candida* cell death. There is no intrinsic resistance to echinocandins among *Candida* species, and isolates from historic collections archived before the release of the echinocandins show no resistance. Resistance to the echinocandins remains low among most *Candida* species and ranges overall from 0–1%. Among isolates of *Candida glabrata*, the proportion of resistant isolates is higher and has been reported to be as high as 13.5% in at least one hospital. Antifungal resistance is due to specific amino acid mutations in the Fksp subunit(s) of the 1,3-β-D-glucan synthase protein which are localized to one of two hotspots. These mutations are being recognized in isolates from patients who have failed echinocandin therapy, and often lead to a poor outcome. While the future looks bright for the echinocandins against most *Candida* species, *C. glabrata* remains a species of concern and resistance rates of *C. glabrata* to the echinocandins should be monitored closely.

Keywords

Echinocandin; antifungal; *Candida*; micafungin; anidulafungin; caspofungin; epidemiology

Introduction

In terms of therapy to treat infection, the antifungal drugs are the relative ‘new kid on the block’. The polyenes were introduced in the 1950s and were the lone weapon in the arsenal until the 1990s when the first azole antifungal was introduced into clinical practice. The first echinocandin, Echinocandin B, was isolated in 1974 and was soon discovered to have antifungal properties [1]. However, the hemolytic properties of natural echinocandins prevented them from being viable candidates for human use, and it wasn’t until the approval of the semisynthetic echinocandin, caspofungin, by the US Food and Drug Administration in 2001 that echinocandins were introduced into clinical practice.

Echinocandins, caspofungin, micafungin, and anidulafungin, act through noncompetitive inhibition of 1,3-β-D-glucan synthase, the enzyme responsible for the production of 1,3-β-D-glucan, a component of the cell wall in many species of fungus [2, 3]. The ability of an antifungal to target the fungal cell wall is unique to echinocandins. Unlike amphotericin B,

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which interacts with the sterols in both human and fungal cell membranes, use of echinocandins poses a much lower risk of side effects caused by incidental action of drug against the host's own cells. This capacity is particularly desirable because of the presence of cell walls in fungal but not in animal cells. Additionally, their distinct mechanism of action allows echinocandins to be used against isolates that have already developed resistance to cell membrane active antifungals. In *Candida* species, this disruption of the cell wall is fungicidal, leading to cell lysis via osmotic instability [3].

Echinocandins are only effective against fungal species with cell walls that contain abundant 1,3-β-D-glucan, which precludes their use against *Fusarium*, the Mucormycetes and *Trichosporon*. Although *Cryptococcus* species have 1,3-β-D-glucan, they remain resistant to the echinocandins [4]. In the United States and the European Union, this drug class is primarily approved for use against candidemia, esophageal candidiasis, and various forms of invasive candidiasis. Additionally, micafungin can be used as prophylaxis against *Candida* in hematopoietic stem cell transplant patients, and caspofungin is approved as treatment against invasive aspergillosis that is not responsive to other antifungals and for empiric treatment in febrile neutropenic patients.

Echinocandin Resistance

The primary mechanism of echinocandin resistance that has emerged since the introduction of the drug class into clinical practice is mutations in *FKS1*, and additionally in *C. glabrata, FKS2*, genes that encode subunits of 1,3-β-D-glucan synthase. These mutations, first described in *Candida* isolates in 2005 [5], occur in one of two areas, hot spot 1, located at amino acids 641-649 in *Candida albicans* [6], and hot spot 2, located at amino acids 1345-1365 [7]. Hot spot mutations have been identified in *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, C. dubliniensis,* and, *C. guilliermondii* [7, 8]. In two studies of *C. glabrata,* the presence of *FKS* mutations was significantly associated with treatment failure [9, 10]. The nature of the interaction between echinocandins and *FKS1* has not been elucidated, so the mechanisms by which these substitutions lead to reduced susceptibility are unclear. It has been demonstrated, however, that substitutions at different amino acids, even within the same hotspots, can have different impacts on resistance profiles in terms of which echinocandins are effected, and the extent of the decrease in susceptibility [11, 12]. Furthermore, more than one of these mutations can occur in the same isolate, producing a less susceptible phenotype than would result from either mutation singularly [13, 12].

Beyond *FKS* mutations, other mechanisms have been suggested to cause resistance to echinocandins. One of these is the increase of cell wall chitin content to compensate for the 1,3-β-D-glucan depletion caused by echinocandin treatment. *C. albicans* has been demonstrated to have higher chitin content following *in vitro* treatment with echinocandins, as has *C. tropicalis*, some isolates of *C. parapsilosis, C. guilliermondii*, and, more rarely, *C. krusei* [14]. Increased chitin content was also found in a lab strain of *C. glabrata* treated with caspofungin [15], but, in a separate study, it was not found in several clinical *C. glabrata* strains grown with caspofungin at each isolate's IC_{50} [14]. Multiple studies have correlated the induction of increased chitin content with decreased susceptibility to echinocandins *in*

vitro and *in vivo* [14, 16]. However, these cells with induced high chitin content have also been shown to be less virulent, and appear to be selected against in the absence of echinocandin [16], raising doubts as to their clinical viability. Nonetheless, there has been at least one report of a patient infected with a caspofungin-resistant *C. albicans* isolate with chitin levels four times greater than those of a sensitive reference strain, in addition to an *FKS1* hot spot 1 mutation [17]. While the extent to which the isolate's chitin levels contributed to its resistance is unclear, the case does demonstrate that it is possible for a strain with elevated chitin levels to cause a successful infection.

Interestingly, it has been suggested that increased chitin content may in fact precipitate *FKS* mutations. In a mouse model of infection, several isolates of a *C. albicans* strain that had been induced to possess increased chitin through growth with Ca^{2+} and calcofluor white were found to possess *FKS1* hot spot 1 mutations, even if the mice they infected had not been treated with echinocandins. In contrast, none of the normal-chitin parental strains injected into mice contained any hot spot mutations [16]. It is possible that induction of the pathway that is responsible for increasing chitin content is also involved in increasing the likelihood of mutations in *FKS1*. At present, it is unclear what cellular changes allow some *Candida* species or strains within species to have a higher chitin content in the presence of echinocandins than others.

Finally, the intracellular buildup of long-chain bases dihydrosphingosine and phytosphingosine, both intermediates in the sphingolipid biosynthesis pathway, has been proposed as the mechanism of a caspofungin reduced susceptibility, micafungin increased susceptibility ("CRS-MIS") profile observed in *C. glabrata* in both laboratory strains with induced caspofungin resistance and clinical strains [18, 19]. This phenotype was found to be induced by mutations to one of several enzymes involved in sphingolipid biosynthesis. The resulting excess of long-chain bases in the cell membrane has been hypothesized to alter the binding of echinocandins to Fks1p and Fks2p, weakening the interaction with caspofungin, while strengthening that with micafungin, based on the chemistry of the two echinocandins' tails [19]. While this "CRS-MIS" profile and its associated mutations have been observed in clinical isolates, there has been no research into whether the MIC effects are maintained *in vivo*. If they are, identifying isolates in which this phenomenon is active could inform patient treatment, preventing treatment escalation to amphotericin B in caspofungin-resistant isolates that would respond to micafungin.

Several species of *Candida* are of particular concern because of high in vitro MIC values to the echinocandins. *C. parapsilosis* has an intrinsic variation in its *FKS* sequence that coincides with a hot spot mutation [20], and the mechanisms of *C. lusitaniae* and *C. guilliermondii* may be similar [21, 22]. These appear to render them less susceptible to echinocandins *in vitro*, and the extent to which this affects the efficacy of clinical echinocandin treatment is not yet fully understood. While *C. glabrata* does not possess intrinsic reduced susceptibility to echinocandins, it is also a species of concern for the development of drug resistance. Data suggest that rates of echinocandin resistance are greater in *C. glabrata* than in most other intrinsically susceptible species of *Candida* [23, 12, 24–26], and it has been hypothesized that *C. glabrata's* haploidy and genomic plasticity allows it to develop and strongly express resistance mechanisms more quickly than other

Candida species [24, 12, 27]. This is particularly worrisome given that *C. glabrata* has reduced sensitivity to azoles, and now has echinocandins as its recommended first line treatment.

Epidemiology

Changes in the rates of echinocandin resistance are difficult both to ascertain and to interpret. One major problem in tracking the levels of echinocandin resistance and the temporal and geographic changes is that the available published data have been sorted according to several different sets of clinical breakpoints, including the current, species-specific Clinical and Laboratory Standards Institute (CLSI) breakpoints [28], the 2008 CLSI breakpoints [29], and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [30], none of which are directly comparable to one another. While CLSI and EUCAST antifungal susceptibility testing methods have generally shown high categorical agreement between wild type and non-wild type isolates as judged by epidemiological cutoff values for anidulafungin and micafungin when applied to the major *Candida* species [31], it is unclear whether the same is true of susceptibility according to their respective breakpoints. Additionally, the fact that many publications describing rates of echinocandin resistance in the early to mid-2000s defined resistance according to the original, non-species-specific CLSI breakpoints has rendered much of this early data difficult to compare to current numbers, making it harder to determine how resistance to echinocandins has emerged since their introduction into practice. It should also be noted that while there are always questions as to the consistency of antifungal susceptibility results between testing sites, caspofungin MICs are notoriously variable, and there is some doubt as to whether they can be relied upon at all [32].

Beyond the question of the consistency of antifungal susceptibility testing results and definitions from laboratory to laboratory and study to study, it is currently unclear how *in vitro* echinocandin resistance should be understood as an indicator of clinical resistance and treatment failure. Alexander and coworkers [25] found that every *C. glabrata* isolate with an echinocandin-resistant MIC that caused a treatment failure also contained an *FKS* mutation. They concluded that the question lies in whether *in vitro* resistance “serve[s] as a sensitive but nonspecific phenotypic screen for the presence of clinically significant *FKS* mutations,” or if elevated echinocandin MICs are independently predictive of an FKS mutation. The former theory was supported in one study where the only independent risk factor for echinocandin treatment failure against invasive *C. glabrata* was the presence of an *FKS* mutation [33]. However, another study of *C. glabrata* candidemia found that the caspofungin MIC was effective in predicting which *FKS* mutants would cause treatment failure, reaffirming the usefulness of *in vitro* susceptibility data as more than a proxy [34]. The same study identified prior echinocandin exposure, but neither MIC nor *FKS* mutations, as an independent risk factor for treatment failure, suggesting that there may indeed be other clinically relevant mechanisms of resistance that such exposure could be selecting for beyond just *FKS* mutations.

Rates of echinocandin resistance as defined by MIC value in *Candida* vary by species, region, institution, and patient population. Among *C. albicans*, resistance tends to be very

low. SENTRY, a surveillance study including invasive *Candida* from North America, Europe, Latin America and Asia-Pacific in 2010 and 2011, found resistant and intermediate rates in this species to be 0.0% and 0.4% to anidulafungin, 0.2% and 0.4% to caspofungin, and 0.1% and 0.3% to micafungin, respectively [26]. In the United States, a study of candidemia from hospitals in Atlanta and Baltimore found 0.3% resistance to anidulafungin, 0.5% resistance to caspofungin, and 0.3% resistance to micafungin [35]. *C. parapsilosis* also shows generally low levels of echinocandin resistance, with SENTRY capturing resistance rates of 0.5%, 0.0%, and 0.0%, and Atlanta and Baltimore-based surveillance finding 0.0%, 0.3%, and 0.0% to anidulafungin, caspofungin, and micafungin, respectively.

While still fairly low, the rates of resistance to *C. glabrata* are noticeably higher than those of the other *Candida* species. SENTRY surveillance in 2010 & 2011 found that rates of resistant and intermediate isolates were 1.8% and 4.6%, 1.6% and 2.5%, and 1.2% and 0.9% for anidulafungin, caspofungin, and micafungin, respectively. These rates reveal considerable geographic variation, with no resistance in Latin America, equivalent resistance rates in Europe and North America (anidulafungin, 1.7% and 1.6%, caspofungin, 1.7% and 1.6%, and micafungin, 1.1% and 1.3% in Europe and North America, respectively), and higher rates in Asia-Pacific (anidulafungin: 3.8%, caspofungin: 1.9%, and micafungin 1.9%) [26]. The 2008–2011 candidemia surveillance in Atlanta and Baltimore captured 2.7%, 2.4% and 2.7% resistance to anidulafungin, caspofungin, and micafungin, respectively, with rates slightly higher in Atlanta than Baltimore (2.5–3.1% versus 2.3–2.5% resistance to the three drugs). It should be noted that *C. glabrata* resistance rates in some individual institutions have been reported to be much higher, as with one eastern US hospital, which reported 12.5% resistance to anidulafungin and micafungin, and 13.5% resistance to caspofungin in 2009 and 2010 [25].

There is considerable evidence that exposure to echinocandins predisposes patients to developing echinocandin-resistant *Candida*. This has been identified as an independent predictor of the presence of an *FKS* mutation and echinocandin treatment failure in *C. glabrata* candidemia cases [34, 25], and exposure to caspofungin within 30 days has been found to be an independent risk factor for blood stream infection with a *Candida* isolate with reduced caspofungin susceptibility in multiple studies [36, 37]. Epidemiological studies of drug resistance tend to include just the incident isolate of *Candida* infection. Many studies will not capture the isolates that develop echinocandin resistance over the course of treatment [35, 38], isolates which have been described in multiple publications [39, 40]. It is therefore possible that current estimates of echinocandin resistance rates do not entirely encompass the population of patients affected by these reduced-susceptibility isolates.

There is evidence that rates of echinocandin resistance are rising, though the extent to which this is true seems to vary greatly by location. Several surveillance studies have compared past and recent rates of resistance, frequently revealing an upward drift over many years. This may be seen very slightly with *C. albicans*, as in one global surveillance study that found rates of non-susceptibility (resistant + intermediate) increase from <0.1%, 0% and 0% in 2003–2007 to 0.4%, 0.6% and 0.4% in 2010–2011 for anidulafungin, caspofungin, and micafungin, respectively [26]. However, it is found far more dramatically in *C. glabrata*, the non-susceptibility rates of which climbed from 2.8%, 1.5% and 0.9% to 6.4%, 4.1% and

2.1% for the same drugs over the same time period [26]. A particularly extreme example of this is the study from the eastern US hospital cited above, which monitored *C. glabrata* echinocandin resistance from 2001 to 2010. During the first six years of the study period, resistance to each echinocandin ranged from ~1.7% to ~5.0% during each two year period, after which it veered sharply upwards to ~9.5%-10.5 for each drug in 2007–2008, and them ~12.5%–13.5% in 2009–2010 [25]. Of course, there are also institutions and areas that do not seem to be experiencing the emergence of echinocandin resistance, as with a Spanish institution which found no echinocandin resistance in *C. glabrata* or *C. parapsilosis* from 2007 through 2013 (according to EUCAST antifungal testing methods and breakpoints) [41], or the Latin American laboratories contributing isolates to the global SENTRY surveillance program, which identified no *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis*, or *C. krusei* isolates resistant to anidulafungin or micafungin in 2008 and 2009 (isolates were not tested for susceptibility to caspofungin in this time range) [24], and no isolates of these species resistant to any of the echinocandins, with the exception of a single anidulafungin-resistant *C. parapsilosis* isolate among the 104 collected [26].

The other factors contributing to the overall rise of echinocandin resistance among *Candida* isolates are the downward trend in relative *C. albicans* prevalence, and corresponding upward trend in *C. glabrata* prevalence. This rise in *C. glabrata* has been noted by many studies [35, 42, 43, 38, 44], and has been attributed to the widespread use of fluconazole, to which *C. glabrata* has intrinsically reduced susceptibility. Given that *C. glabrata* has shown itself more likely to develop echinocandin resistance, its increasing prevalence in the patient population increases the likelihood of a person with candidemia having a strain with reduced susceptibility.

There are several patient characteristics that have been found to predispose infecting *Candida* isolates to reduced echinocandin susceptibility. Not surprisingly, limited global surveillance has found nosocomial *C. albicans* and *C. glabrata* candidemia isolates to possess more echinocandin resistance than community-acquired isolates, though *C. albicans* resistance rates were still very low in nosocomial cases [45]. Resistance also seems to be associated with younger age, as global surveillance found the highest rates of *C. glabrata* echinocandin resistance in patients ages 20–39 (16.7% to each echinocandin), followed by those ages 40–59 (7.0–4.2%, by drug), with little to no resistance in the older age groups [46]. In this study, there were only 5 *C. glabrata* isolates from patients 0–19 years old, among which there was no resistance, but another study of Paris-area hospitals found age less than 15 years to be an independent risk factor for candidemia with reduced echinocandin susceptibility [37].

Gastrointestinal (GI) disorders and recent GI surgery have both been significantly associated with infection with an isolate containing an *FKS* mutation. Each has also been associated with echinocandin treatment failure, the latter in univariate analysis only, and the former in multivariate analysis [34, 33]. *FKS* mutations have also been found more frequently in solid organ transplant patients with *C. glabrata* candidemia [25]. Additionally, a French study found that patients with hematological malignancy were significantly more likely to have recent exposure to caspofungin, suggesting that they might also be at elevated risk for developing echinocandin-resistant infections [37]. This conclusion is supported by the fact

that half of *FKS* mutants collected by the French National Reference Center for Mycoses and Antifungals were from patients with hematological malignancy [40]. It is important to note that there is very little data from broad surveillance studies on the medical conditions that predispose patients to developing echinocandin-resistant infections, so much of this data comes from research specific to single institutions or regions. Knowing how widely antifungal prescribing practices can vary, it is unknown whether these findings are generalizable to a larger patient population.

Given that *C. glabrata* demonstrates an intrinsically reduced susceptibility to fluconazole, in addition to an apparent predisposition for the development of echinocandin resistance, there is a growing concern that multidrug resistant *C. glabrata* will emerge as a considerable clinical problem. A study combining 2006–2010 SENTRY global surveillance and 2008–2010 CDC Atlanta and Baltimore-based surveillance from found that 9.7% of *C. glabrata* isolates were resistant to fluconazole. Of these, 11.1% (accounting for 1.1% of all *C. glabrata*) were resistant to at least one echinocandin and all contained an *FKS* mutation [24]. When comparing this data to echinocandin resistance among fluconazole-resistant *C. glabrata* collected from 2001–2004, the authors saw a sharp increase, with resistance to each echinocandin rising from 0% in the first time period to 8.0–9.3% in the second time period. A study of *C. glabrata* candidemia isolates from four US cities found that 14.8% of fluconazole-resistant isolates were resistant to at least one echinocandin, accounting for 1.5% of all isolates [12]. In a study of *C. glabrata* candidemia isolates from 2001–2010, 14.1% of fluconazole-resistant isolates were resistant to at least one echinocandin (3.5% of all isolates), and 10.3% were resistant to all three (2.5% of all isolates) [25]. Interestingly, in this study, prior patient exposure to echinocandin therapy predicted not just echinocandin resistance, but also fluconazole resistance, most likely because echinocandin treatment was more frequently selected for patients who had already been exposed to fluconazole or whose infective agent was suspected to have reduced susceptibility to this drug. Multidrug resistant profiles have also been found in other species of *Candida*, but these incidents are far less frequent, as with the single echinocandin and fluconazole-resistant *C. albicans* found in Baltimore between 2008 and 2011, accounting for 0.26% of all *C. albicans* bloodstream isolates [35].

Conclusions

There are limited antifungal agents available to treat fungal infections. The development of resistance to echinocandins in *Candida* is a serious threat to our ability to manage these infections. Echinocandins are the empiric treatment of choice for candidemia, but it is essential that triazoles be used as soon as species or appropriate susceptibility of the infective agent can be determined. This step down therapy is one important step in appropriate stewardship of the echinocandins. Although MDR *Candida* infections are still relatively uncommon, the emergence of these infections demonstrates the need for action now to improve stewardship and to continue to monitor for the development of resistance, as well as to study acquisition and transmission of resistance. It is imperative that we focus on antifungal resistance now before losing the ability to use these important drugs.

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