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***Candida glabrata*: Multidrug Resistance and Increased Virulence in a Major Opportunistic Fungal Pathogen**

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Abstract

C. glabrata is widely acknowledged to be an important and potentially antifungal resistant cause of hematogenously disseminated candidiasis (HDC). In the United States (US) both the frequency of *C. glabrata* as a cause of HDC and in vitro resistance to fluconazole has increased steadily since 1992. Although this species is generally considered to be less virulent than *C. albicans*, recent findings suggest that gain of function (GOF) mutations in the transcriptional regulator CgPdr1p results not only in broad resistance to azole antifungals but also an increase in both fitness and virulence in animal models. Furthermore, case reports and case series suggest the emergence of multidrug resistance (MDR) in this species. Recent data from multicenter surveys conducted in the US have demonstrated the emergence of co-resistance to both azoles and echinocandins in clinical isolates of *C. glabrata*. These findings are highlighted in an effort to bring attention to this important development.

Keywords

C. glabrata ; multidrug resistance; virulence

BACKGROUND

Candida glabrata has emerged as an important and potentially antifungal-resistant pathogen [1–23]. Ever since the introduction of fluconazole in 1990 for the treatment of candidiasis, including hematogenously disseminated candidiasis (HDC), empirical antifungal therapy has been driven by fear of *C. glabrata* [21, 22, 24, 25]. Trick et al (26) have demonstrated that among the *Candida* species, *C. glabrata* alone has increased as a cause of bloodstream infection (BSI) in United States (US) intensive care units (ICU) since 1993. Most recently, the species-specific incidence of BSI due to *C. glabrata* in the Atlanta, GA, metropolitan area was shown to have increased from 1 infection per 100,000 population per year in 1992 to 1993 to 4.5 infections per 100,000 per year in 2008 to 2009 [23, 27]. The emergence of

C. glabrata as an agent of HDC may be associated with the increased use of fluconazole [2, 28] and the decreased susceptibility of this species to the triazole class of antifungal agents [29–32]. Suboptimal fluconazole dosing practices (low dose [< 6 mg/kg/day], short duration of treatment, and poor indications) may lead to an increased frequency of isolation of *C. glabrata* as an etiological agent of candidemia in hospitalized patients [2, 9, 33–37] and to increased fluconazole (and other azole) resistance secondary to the expression of CDR (*Candida* Drug Resistance) efflux pumps [38–43].

Among the newly introduced antifungal agents with activity against *Candida* spp., the echinocandin class (anidulafungin, caspofungin, micafungin) is notable for potent in vitro and clinical activity against *C. glabrata* [44–49]. Global surveys have documented the excellent in vitro susceptibility of *C. glabrata* to each of the echinocandins (MIC₉₀, 0.015 µg/ml [micafungin], 0.06 µg/ml [caspofungin], and 0.12 µg/ml [anidulafungin]), including fluconazole-resistant strains [50–52].

Although documentation of acquired resistance to echinocandins remains sporadic [20, 23, 52–56] several recent reports of acquired resistance in clinical isolates of *C. glabrata* focus concern on this species [3, 10, 20, 23, 31, 54, 57–63]. In *C. glabrata*, echinocandin resistance has been associated with point mutations in “hot-spot” (HS) regions of *FKS1* and *FKS2* genes encoding for the major and presumed catalytic subunit of 1,3-β-D-glucan synthase (GS) [23, 61, 64]. It is now clear that clinical isolates of *C. glabrata* with decreased susceptibility to one or more of the echinocandins harbor mutations in *FKS1* and/or *FKS2* and are also resistant clinically [3, 5, 20, 23, 55, 61, 65].

The excellent wild-type (WT) susceptibility of *C. glabrata* to the echinocandins coupled with broadening azole resistance has driven the use of echinocandins for treatment of infection due to *C. glabrata* and at the same time has generated selection pressure for resistant organisms [3, 5, 13, 20, 23, 25, 28, 31, 54, 58, 66]. The fact that echinocandins are recommended for use in the setting of prior azole exposure and specifically for the treatment of HDC due to *C. glabrata* raises the concern that acquired resistance to the echinocandins may emerge independently in fluconazole-resistant strains due to mutations in *FKS* [3, 13, 20, 23, 31]. This concern is underscored in a case-report from France where Chapeland-Leclerc et al (3) reported a patient with HDC due to *C. glabrata* in which the infecting strain acquired resistance to flucytosine, fluconazole, voriconazole, and caspofungin through successive independent events following prolonged exposure to each class of antifungal agent. The recovery of different isolates exhibiting clonality for microsatellite markers but genetic diversity for antifungal resistance markers (three unique resistance mechanisms) demonstrates the high propensity of *C. glabrata* to mutate in vivo in a single patient [3]. Two additional reports from France showed that an increasing use of caspofungin in two different hospitals was associated with increased proportions of *Candida* spp. having high caspofungin MICs, including *C. parapsilosis* and *C. glabrata* [7, 13]. Reports from medical centers in the US and Denmark provide further documentation of multidrug-resistant (MDR; resistant to two or more classes of antifungal agents) strains of *C. glabrata* [10, 20, 23, 31, 54, 60]. One potential explanation for the emergence of MDR in *C. glabrata* is that the haploid nature of the organism makes it especially adept at acquiring and expressing resistance mutations in response to drug pressure [3, 67–69].

These observations suggest that the emergence of MDR in *C. glabrata* is the next threat to the implementation of effective early empirical treatment of patients at risk for HDC. This potential threat comes at a time when consensus guidelines encourage the use of the echinocandins as first-line therapy for HDC and the prevailing attitude is that given the safety and potency of the echinocandins, any increase in non-*albicans* species (e.g. *C. glabrata* and *C. krusei*) and associated resistance to fluconazole is largely irrelevant [21, 22, 70].

One counterpoint that has been offered to mitigate concerns regarding emerging antifungal resistance in *Candida* spp. is that in many cases, antimicrobial resistance comes at a fitness cost to the organism, which is evident as reduced replication rate, virulence, or transmissibility [71–73]. Whereas resistance to echinocandins has been associated with decreased fitness and virulence in isolates of *C. glabrata* and *C. albicans* [74–76], it is interesting that gain of function (GOF) mutations in the transcription factor CgPdr1p of *C. glabrata*, as well as loss of mitochondrial functions, not only mediate antifungal (azole) resistance but also enhance virulence in animal models [77–79]. Thus, *C. glabrata* is proving to be both hypermutable and possibly hypervirulent [80] and certainly worthy of our continued respect as an invasive fungal pathogen.

The purpose of this review is to provide an overview of the mechanisms of resistance to antifungal agents that are operative in *C. glabrata*, to discuss the relationship between GOF mutations, virulence and antifungal resistance, and to highlight the recently recognized emergence of MDR in *C. glabrata* in the US and elsewhere.

Mechanisms of Resistance to Antifungal Agents in *C. glabrata*.

Although most of the effort to elucidate the mechanisms of antifungal resistance has been expended in studies of *C. albicans* [81, 82], the results of these studies have directed similar work in *C. glabrata* [3, 5, 10, 23, 31, 42, 43, 57, 58, 61, 64, 65, 67, 68, 77–79, 83–85].

Each of the antifungal classes utilizes a different means to kill or inhibit the growth of fungal pathogens [82, 86, 87]. Mechanisms of antifungal resistance are either primary or secondary, and are related to intrinsic or acquired characteristics of the fungal pathogen that interfere with the antifungal mechanism of the respective drug or drug class or that lower target drug levels within the cell. Resistance can also occur when environmental factors lead to colonization or replacement of a susceptible strain with a resistant one. The antifungal effects of both polyene and azole antifungals are due to actions on the fungal cell membrane, while echinocandins act by disrupting the fungal cell wall [41, 55, 87]. Flucytosine acts as an antimetabolite to interfere with DNA and RNA synthesis [68].

Many parallels exist between the well-studied antibacterial resistance mechanisms and antifungal resistance mechanisms [88]; however, there are no data to suggest that destruction or modification of antifungal agents is an important component of antifungal resistance. Likewise, it does not appear that fungi can employ the genetic exchange mechanisms that allow rapid transmission of antimicrobial resistance in bacteria [88]. On the other hand, it is apparent that multidrug efflux pumps, target alterations, and reduced access to targets are important mechanisms of resistance to antifungal agents, just as they are important in

antibacterial resistance [41, 81, 82, 86–88]. In contrast to the rapid emergence of high-level antimicrobial resistance that occurs among bacteria, antifungal resistance develops more slowly and involves the emergence of intrinsically resistant species (*C. krusei*, and to some extent *C. glabrata*, is intrinsically resistant to fluconazole) or stepwise alteration of cellular structures or functions that results in resistance to an agent to which there has been prior exposure [55, 81, 88]. Among the common species of *Candida*, *C. glabrata* appears to be unique in its ability to acquire and express drug resistance mutations in a relatively rapid time frame [3, 10, 23, 61, 67, 68, 77].

Amphotericin B.

Resistance to amphotericin B, both deoxycholate and lipid formulations, remains uncommon among *Candida* spp. despite extensive utilization for over 50 years. Primary resistance to amphotericin B among clinical isolates of *C. glabrata* has not been described, although it has been noted that *C. glabrata* may exhibit decreased susceptibility to amphotericin B compared with *C. albicans* [89, 90] and may require higher doses of amphotericin B for optimal treatment [25, 91]. Notably, a recent report from France [7] found that increased use of amphotericin B in an ICU setting was followed by increased amphotericin B MICs for *C. glabrata*.

Our understanding of the mechanism of resistance to amphotericin B in *C. glabrata* stems largely from characterization of rare clinical isolates from patients failing amphotericin B therapy [10, 67, 85]. The mechanism of amphotericin B resistance appears to be from a quantitative or qualitative alteration in the sterol content of the cells [67, 85]. Accordingly, mutants of *C. glabrata* resistant to amphotericin B have been shown to have reduced ergosterol content and replacement of amphotericin B-binding sterols by intermediates that bind polyenes less well [67, 85]. Studies of sterol profiles of clinical isolates indicate a block in the C-24 sterol methyltransferase step with depletion of ergosterol and accumulation of $\Delta 5,7$ -dienols [67, 85]. Vandeputte et al [67,85] described two different amphotericin B-resistant clinical isolates of *C. glabrata*, each of which contained a mutation in the *ERG6* gene encoding C-24 sterol methyltransferase. In each instance the inactivation of C-24 sterol methyltransferase resulted in the interruption of ergosterol biosynthesis and thus polyene resistance. The changes in the sterol composition of the plasma membrane was thought to result in disturbances in the fungal cell wall or plasma membrane due to impairment of protein targeting. Transmission electron microscopy revealed structural modifications (thinning) of the cell wall of both mutant strains and pseudohyphal growth in one strain with a missense mutation in *ERG6* [67, 85]. Both strains displayed a marked lack of fitness in vitro compared to WT strains suggesting that the *ERG6* mutations constitute a selective disadvantage in the absence of selection pressure. However, given that the *C. glabrata* genome is haploid [69] and thus there is a higher probability of expression of a mutated allele, such mutations may be clinically relevant in the current therapeutic context, which is dominated by prophylaxis and empirical antifungal therapy [67, 85].

Azoles.

The azoles act by inhibiting the fungal cytochrome P450-dependent enzyme lanosterol 14 α -demethylase, which is encoded by the gene *ERG11*. The enzyme converts lanosterol to

ergosterol, and its inhibition disrupts membrane synthesis in the fungal cell [92]. Resistance can arise from a modification in the quality or quantity of the target enzyme, reduced access of the drug to the target, or some combination of these mechanisms [41,81, 86, 87]. In the first instance, point mutations in *ERG11* lead to an altered target enzyme with decreased affinity for azoles. In addition to mutations in *ERG11*, overexpression of the gene results in the production of high concentrations of the target enzyme, creating the need for higher intracellular fluconazole (and other azoles) concentrations to inhibit the increased enzyme present in the cell [81, 82, 92]. The second major mechanism of azole resistance in *Candida* involves active efflux of fluconazole out of the cell through the activation of two types of multidrug efflux transporters: the major facilitators (encoded by MDR genes) and the ATP-binding cassette (ABC) superfamily (encoded by CDR genes) [81, 82].

It is now well-established that the primary mechanism of resistance to fluconazole in *C. glabrata* involves upregulation of the *CgCDR1* and *CgCDR2* genes resulting in resistance to multiple azoles [38, 42, 43, 93]. Borst et al [38] observed the rapid development of azole resistance (fluconazole, itraconazole, and voriconazole) following in vitro exposure to fluconazole among *C. glabrata* isolates that had never previously been exposed to azole antifungal agents. The resistance was stable and was associated with increased expression of *CgCDR1* and *CgCDR2*, but not *CgERG11*. Likewise, Sanguinetti et al [43] performed a careful analysis of azole resistance profiles of clinical isolates of *C. glabrata* and found that the majority of the isolates were resistant to multiple azoles (fluconazole, itraconazole, ketoconazole and voriconazole) and that the resistance phenotypes were strongly associated with upregulation of the ABC efflux transporters *CgCDR1*, *CgCDR2*, and *CgSNQ2*. Interestingly, they too found no alteration or overproduction of *CgERG11* among 20 different azole-resistant isolates. They concluded that cross-resistance is a very common feature in azole-resistant *C. glabrata* isolates, especially in those that are capable of expressing multiple mechanisms of resistance [43].

Although it has been suggested that alteration or overexpression of *CgERG11* is not involved in azole resistance of *C. glabrata* [43], both Marichal et al [40] and Redding et al [93] have reported an increase in lanosterol 14 α -demethylase in fluconazole-resistant *C. glabrata*. Marichal et al [40] found an increase in the amount of *ERG11* mRNA transcript that was secondary to a duplication of the entire chromosome containing the gene, whereas Redding et al [93] found upregulation of *CgERG11* along with that of *CgCDR1* and *CgCDR2* in a fluconazole-resistant strain of *C. glabrata* from a patient with oropharyngeal candidiasis (OPC) undergoing treatment with fluconazole. Taken together, the literature indicates that although efflux is a major mechanism of azole resistance in *C. glabrata*, the development of resistance in this species is a highly varied process involving multiple molecular mechanisms [93].

Echinocandins.

Echinocandins inhibit 1,3- β -D-glucan synthase (GS) and thereby disrupt biosynthesis of 1,3- β -D-glucan, a key component of the fungal cell wall. This causes the formation of a defective cell wall associated with cellular instability and lysis in yeasts such as *C. glabrata* [55, 61]. Mutations in the genes encoding elements of the GS complex have been associated

with *Candida* resistance to echinocandins. In particular, reduced susceptibility or resistance of *Candida* to echinocandins has been linked with point mutations in two “hot-spot” (HS) regions of *FKS1* (HS1 and HS2), the gene encoding for the major and presumed catalytic subunit of GS [55]. In *C. glabrata*, echinocandin resistance has also been associated with mutations in the *FKS2* gene [19, 23, 61]. Whereas resistance to azoles in isolates of *C. glabrata* has been documented to be due to the overexpression of *CDR* efflux pumps (see previous section above), there is little or no evidence that efflux pumps are involved in resistance to the echinocandins [55, 84, 94].

Mutations in FKS proteins 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 with corresponding enzymes from WT strains [61]. This pattern of decreased enzyme sensitivity to inhibition (increased IC₅₀) extends across all three of the echinocandins, conferring resistance across the entire class. The case study of Chapeland-Leclerc et al (3) (MDR in *C. glabrata*) demonstrated point mutations in *FKS2*, associated with caspofungin resistance, as well as overexpression of *CgCDR1* and *CgCDR2*, linked with fluconazole and voriconazole resistance.

Flucytosine.

The antifungal properties of flucytosine result from its conversion in the cytoplasm of fungal cells via the cytosine deaminase and the phosphoribosyltransferase into 5-fluorouridine triphosphate which incorporates into RNA and blocks protein synthesis or into 5-fluorodeoxyuridine monophosphate which inhibits the thymidylate synthase encoded by *CDC21*, a key enzyme of DNA synthesis [95]. Resistance mechanisms for flucytosine are well described among various species of *Candida* and include mutations in *FCY2*, *FCY1* and *FUR1* genes encoding for cytosine permease, cytosine deaminase, and uracil phosphoribosyltransferase, respectively. In studies of *C. glabrata* [68, 96], varying patterns of reduced susceptibility to flucytosine have been elucidated depending on the mutations present. Edlind and Katiyar (96) demonstrated that *C. glabrata* exhibited high-level flucytosine resistance (MIC, ≥ 32 $\mu\text{g/ml}$) associated with mutations in either *FCY1* or *FUR1* and moderately elevated MICs (MIC, 1 $\mu\text{g/ml}$) with mutations in *FCY2*. Vandeputte et al [68] conducted a study of laboratory mutants of *C. glabrata* obtained by exposure of a WT isolate to flucytosine. Mechanisms leading to flucytosine resistance were investigated in two mutant strains. One of the mutants exhibited a missense mutation in the *FCY1* gene, encoding the cytosine deaminase, and a two fold reduction in the expression of the *FUR1* gene, encoding the uracil phosphoribosyltransferase. The second mutant exhibited a missense mutation in *FCY2*, suggesting an altered function of the corresponding membrane transporter (cytosine permease), coupled with an overexpression of the *CDC21* gene, which encodes thymidylate synthase, a key enzyme of DNA synthesis. Thus, although mutations in the *FUR1* gene are the most common cause of resistance to flucytosine in *Candida* spp., other mechanisms may contribute to flucytosine resistance which may be easily acquired in *C. glabrata*, probably due to its haploid genome. Although the molecular events detected in the mutants described by Vandeputte et al [68] did not compromise their growth rate relative to the WT parent when assessed in independent cultures, they did represent a selective disadvantage in the absence of selection pressure since competitive growth experiments

(co-culture) revealed a decreased fitness when compared with the parent isolate. Thus, without selection pressure, mutations leading to flucytosine resistance may have limited clinical consequences.

Gain of Function (GOF) Mutations, Increased Virulence, and Antifungal Resistance.

Over the past two decades, there has been a significant increase in the appearance of antifungal drug resistance in *C. glabrata* as a result of the increased use of various classes of antifungal agents, combined with the exceptional ability of this haploid yeast species to develop antifungal resistance [1, 3, 5, 7, 10, 13, 16, 20, 23]. In bacteria, antimicrobial resistance is often associated with fitness costs and thus results in a competitive disadvantage against otherwise drug-susceptible organisms within the host [71, 72]. Extension of this reasoning to fungi such as *C. glabrata* would suggest that antifungal resistance may have a fitness cost in this fungal species and thus may result in a counter-selection against resistant strains without drug pressure. Indeed decreased fitness has been described for *C. glabrata* strains with mutational resistance to amphotericin B [67, 85], the echinocandins [74, 76], and flucytosine [68, 96]. In some instances, however, resistance confers no change in microbial fitness or may even increase fitness [72, 73, 77–79]. Furthermore, initial decreases in fitness due to resistance may be restored by still unknown compensatory mechanisms, which can stabilize the resistant populations and render them as fit as susceptible organisms [72, 97].

Resistance to azole antifungal agents in *C. glabrata* is known to be mediated almost exclusively by enhanced drug efflux and overexpression of the ABC transporters [81]. Several genes encoding these transporters have been identified including *CgCDR1*, *CgCDR2*, and *CgSNQ2* [38, 42, 43]. The expression of these genes is regulated by the zinc finger transcription factor CgPdr1p [98]. Single-point mutations in *CgPDR1* (encodes for CgPdr1p) are known to increase the expression of *CgCDR1* and *CgCDR2* and thus contribute to the azole-resistant phenotype of *C. glabrata* [77, 83]. Recently Ferrari et al [77,78] demonstrated that these point mutations were gain of function (GOF) mutations since they conferred hyperactivity to CgPdr1p revealed by constitutive high expression of *CgCDR1* and *CgCDR2*. These GOF mutations in *CgPDR1*, therefore, constitute the molecular basis of azole resistance in *C. glabrata* [77, 78,98]. Furthermore, Ferrari et al [77,78] found that resistant strains of *C. glabrata* were more virulent in mice and less susceptible to azoles in vivo as compared to WT strains, strongly suggesting a gain in fitness for the resistant isolates. The azole-resistant population effectively supplanted the WT population in the presence and absence of drug selection in vivo. The authors showed that it was the presence of GOF mutations rather than the presence of *CgPDR1* that accounted for increased virulence. Subsequently, these investigators found that only two genes were upregulated by all GOFs, *CgCDR1* and *PUP1* (*PDR1* *UP*regulated gene encoding a mitochondrial protein) [78]. Virulence and tissue burden studies demonstrated that these two *CgPDR1*-dependent and -upregulated genes contribute to the enhanced virulence of *C. glabrata* strains that acquired azole resistance [78].

Mitochondrial dysfunction is another of the mechanisms by which azole resistance can occur in *C. glabrata* [79]. Cells with mitochondrial DNA deficiency (“petite mutants”)

upregulate the ABC transporter genes, *CgCDR1*, *CgCDR2*, and *CgSNQ2*, and thus display increased resistance to azoles. Notably, the upregulation of ABC transporters in mitochondrial mutants, while mediated by *CgPDR1*, is not dependent on GOF mutations in *CgPDR1* [77,79]. Surprisingly, even with an in vitro growth deficiency compared to a WT strain of *C. glabrata*, a mitochondrial mutant strain was more virulent (as judged by mortality and fungal tissue burden) in murine models of infection [79]. The increased virulence of the petite mutant correlated with a dramatic gain of fitness in mice compared to the parental (WT) isolate. The molecular basis for this property is unclear, and the factors responsible for enhanced virulence need to be identified. Ferrari et al [79] did report that in addition to increased expression of *CgPDR1* and other transcription factors in the petite mutant, some genes (*CgYPS1* to -11) required for cell wall integrity, adherence to mammalian cells, virulence and survival in macrophages [69] were also upregulated. Cell wall alterations as a cause of increased virulence in mitochondrial mutants of *C. glabrata* is an intriguing possibility that warrants further study.

These findings highlight the need for carefully monitoring drug resistance in *C. glabrata*, since increased virulence may have a negative impact on the outcome of disease in infected patients. The gain in fitness associated with ABC transporter upregulation may favor the emergence of azole-resistant *C. glabrata*. In addition to GOF mutations in *CgPDR1*, several genes involved in stress response, resistance to DNA damage, and cell wall structure are upregulated in azole-resistant strains of *C. glabrata*, all of which may contribute individually or in combination to modulate the virulence of *C. glabrata* [77].

Emergence of Multidrug Resistance (MDR) in *C. glabrata*.

On a global scale, the proportions of HDC caused by *C. glabrata* vary from 22 to 26% in North American to 4 to 6% in Latin America [31,89]. Within the US, the proportion of HDC due to *C. glabrata* has been shown to vary from 11% to 37% across the nine US Bureau of the Census Regions [16, 99, 100] and from <10% to >30% within single institutions over the course of several years [101–103]. In a US multicenter survey spanning the years 1992 to 2007 [16], *C. glabrata* was shown to have increased as a cause of HDC from 18% of all bloodstream infection (BSI) isolates in the time period 1992 to 2001 to 25% in 2001 to 2007 (Table 1).

In general, the incidence of antifungal resistance among the fungal pathogens of humans is moderate, especially when compared with the incidence of antibiotic resistance among bacterial pathogens [88, 104]. Among the common species of *Candida* (*C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*), *C. glabrata* is unique for its decreased susceptibility to fluconazole and cross-resistance to other azoles [16, 25, 52,89]. Resistance to fluconazole among isolates of *C. glabrata* may vary from a low of 13.0% in the Asia-Pacific region to a high of 19.5% in North America [18]. In the USA *C. glabrata* has not only increased in frequency as a cause of HDC, it has also shown a concomitant increase in resistance to fluconazole from 9% in 1992 to 2001 to 14% in 2001 to 2007 [16] (Table 1).

Given the distinct differences between the mechanisms of action and resistance for the azoles versus the polyenes, echinocandins, and flucytosine [81, 86, 88], the lack of cross-resistance among the four classes is not surprising and supports the current recommendation

that the echinocandins be used as first-line treatment of HDC, especially in patients with possible infection with *C. glabrata* [25]. The potent activity of the echinocandins against *C. glabrata* (and other species of *Candida*) has led some experts to suggest that if initial therapy is echinocandin-based, then any increase in non-*albicans* *Candida* species (e.g. *C. glabrata*) becomes largely irrelevant [21, 22]. This may be true given our current state of understanding; however, recent reports of acquired resistance to echinocandins, polyenes, and flucytosine in clinical isolates of fluconazole-resistant *C. glabrata* suggest that this species represents a major concern as a MDR pathogen [3, 5, 10, 13, 16, 18,19, 20, 23, 54, 57–60].

Data from global surveys demonstrate that the frequency of echinocandin resistance among clinical isolates of *C. glabrata* ranges from 1 to 3% and is higher among isolates from North America (3%) than among those from Europe (1%), Latin America (0.0%), or the Asia-Pacific region (0.0%) [105]. A recent US population-based survey conducted by the Centers for Disease Control and Prevention (CDC) found that approximately 3% of *C. glabrata* BSI isolates were resistant to one or more echinocandin [23]. In addition to geographic differences in echinocandin resistance among *C. glabrata* isolates, resistance may also vary according to the age and exposures of the infected patients. A study of 1,239 *Candida* BSI isolates from 79 medical centers conducted in 2008–2009 [17], found that resistance to both azoles and echinocandins was most prominent among isolates of *C. glabrata*, with the highest resistance rates to echinocandins (16.7%), fluconazole (16.7%), posaconazole (5.0%) and voriconazole (11.0%) among isolates from patients in the 20–39-year age group. This latter observation, coupled with case reports such as that of Chapeland-Leclerc et al [3], suggested that MDR may be an emerging threat in *C. glabrata*. These reports prompted us to review the experience of two large surveillance programs, the SENTRY Antimicrobial Surveillance Program for the years 2006 through 2010 and the CDC population-based surveillance conducted in 2008 to 2010 to assess the frequency of co-resistance to fluconazole and the echinocandins among clinical isolates of *C. glabrata* [19]. Among 1,669 BSI isolates of *C. glabrata*, 162 isolates (9.7%) were resistant to fluconazole (MIC, >32 µg/ml), of which 98.8% were nonsusceptible to voriconazole (MIC, >0.5 µg/ml), and 9.3%, 9.3%, and 8.0% were resistant to anidulafungin (MIC, >0.25 µg/ml), caspofungin (MIC, >0.25 µg/ml), and micafungin (MIC, >0.12 µg/ml), respectively. There were 18 fluconazole-resistant isolates that were resistant to one or more of the echinocandins (11.1% of all fluconazole-resistant isolates), all of which contained an acquired mutation in *FKS1* or *FKS2*. By comparison, there were no echinocandin-resistant strains detected among 110 fluconazole-resistant isolates of *C. glabrata* from the years 2001 to 2004 (Table 2).

These data document the broad emergence of co-resistance over time to both azoles and echinocandins in clinical isolates of *C. glabrata*. Whereas resistance to azoles in isolates of *C. glabrata* has been documented to be due to overexpression of CDR efflux pumps [42, 43], there is little or no evidence that efflux pumps are involved in resistance to the echinocandins [84, 94]. The documentation of *FKS* mutations in isolates of *C. glabrata* showing in vitro resistance to both azoles and echinocandins suggests the sequential accumulation of acquired resistance mechanisms as demonstrated by Chapeland-Leclerc et al [3]. The lack of co-resistance in *C. glabrata* in the 2001–2004 time period is not surprising given that among the echinocandins only caspofungin was available, having

been just approved in the US and Europe in 2001. Since that time, the overall use of echinocandins in the US has increased significantly, from 7.7 ± 5.3 days of therapy (DOT) per 1,000 patient-days in 2004 to 13.1 ± 8.6 DOT per 1,000 patient-days in 2008 (mean $\pm$ standard deviation) ($P < 0.001$) [28]. During the same time period (2004 to 2008), the use of azoles increased as well, from 67.6 ± 29 to 72 ± 33 DOT per 1,000 patient-days, but this change was not significant ($P = 0.1520$) [28].

Thus persistent and expanding antifungal drug pressure may predispose to both azole and echinocandin resistance in *C. glabrata*. These findings emphasize the importance of both rapid identification to the species level as well as antifungal susceptibility testing in patients with recurrent *Candida* isolation who are receiving or were previously exposed to antifungal treatment [21, 22, 25, 56].

SUMMARY AND CONCLUSIONS

C. glabrata is arguably the most important of the non-*albicans* species of *Candida*. This haploid yeast has proven to be remarkably “plastic” in its ability to acquire and express resistance mutations to several different classes of antifungal agents. In contrast to conventional wisdom these mutations do not necessarily result in decreased fitness or virulence in this species. Recently it was shown that GOF mutations in the transcription factor CgPdr1p not only result in resistance to multiple azoles but also contribute to enhanced in vivo fitness and virulence in resistant strains. Thus *C. glabrata* may be considered to be both hypermutable and hypervirulent. Recent data indicate that in the US *C. glabrata* is not only increasing in frequency as a cause of HDC but that it is also becoming more broadly resistant to the azole class of antifungal agents. Against this background of increasing azole-resistance, and possibly increased virulence, relentless exposure to both azole and echinocandin antifungal agents appears to be encouraging the emergence of clinical isolates of *C. glabrata* with resistance to both classes of agents. Although the vast majority of *C. glabrata* isolates remain highly susceptible to the echinocandin class of antifungal agents, the increase in MDR *C. glabrata* strains is a serious concern and argues for increased application of standardized antifungal susceptibility testing to monitor the situation and to adjust therapy as needed. Whereas prior exposure to azoles is often considered to be important in predicting infection with *C. glabrata*, physicians must be cautioned that a new episode of sepsis after a recent prescription of any antifungals may be due to isolates with decreased susceptibility to the prescribed agent, including echinocandins [13].

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REFERENCES

1. Alexander BD, Schell WA, Miller JL, Long GD, Perfect JR. *Candida glabrata* fungemia in transplant patients receiving voriconazole after fluconazole. Transplantation. 2005; 80:868–871. [PubMed: 16210978]
2. Arendrup MC, Bruun B, Christensen JJ, et al. National surveillance of fungemia in Denmark (2004 to 2009). J Clin Microbiol. 2011; 49:325–334. [PubMed: 20980569]
- *3. Chapeland-Leclerc F, Hennequin C, Papon N, et al. Acquisition of flucytosine, azole, and caspofungin resistance in *Candida glabrata* bloodstream isolates serially obtained from a hematopoietic stem cell transplant recipient. Antimicrob Agents Chemother. 2010; 54:1360–1362. [PubMed: 20038613] A remarkable case report that illustrates the ability of *C. glabrata* to assimilate multiple resistance mutations to antifungal agents.
4. Cohen Y, Karoubi P, Adrie C, et al. Early prediction of *Candida glabrata* fungemia in nonneutropenic critically ill patients. Crit Care Med. 2010; 38:826–830. [PubMed: 20042858]
- *5. Dannaoui E, Desnos-Ollivier M, Garcia-Hermoso D, et al. *Candida* spp. with acquired echinocandin resistance, France, 2004–2010. Emerg Infect Dis. 2012; 18:86–90. [PubMed: 22257484] An important report concerning the emergence of echinocandin resistance in France.
6. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans *Candida* spp among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis. 2010; 14:e954–e966. [PubMed: 20797887]
7. Fournier P, Schwebel C, Maubon D, et al. Antifungal use influences *Candida* species distribution and susceptibility in the intensive care unit. J Antimicrob Chemother. 2011; 66:2880–2886. [PubMed: 21980066]
8. Klevay MJ, Ernst EJ, Hollanbaugh JL, Miller JG, Pfaller MA, Diekema DJ. Therapy and outcome of *Candida glabrata* versus *Candida albicans* bloodstream infection. Diagn Microbiol Infect Dis. 2008; 60:273–277. [PubMed: 18024053]
9. Klevay MJ, Horn DL, Neofytos D, Pfaller MA, Diekema DJ. Initial treatment and outcome of *Candida glabrata* versus *Candida albicans* bloodstream infection. Diagn Microbiol Infect Dis. 2009; 64:152–157. [PubMed: 19376667]
10. Krogh-Madsen M, Arendrup MC, Heslet L, Knudsen JD. Amphotericin B and caspofungin resistance in *Candida glabrata* isolates recovered from a critically ill patient. Clin Infect Dis. 2006; 42:938–944. [PubMed: 16511756]
11. Lee I, Morales KH, Zaoutis TE, Fishman NO, Nachamkin I, Lautenbach E. Clinical and economic outcomes of decreased fluconazole susceptibility in patients with *Candida glabrata* bloodstream infections. Am J Infect Control. 2010; 38:740–745. [PubMed: 20542354]
12. Lee I, Zaoutis TE, Fishman NO, Morales KH, Nachamkin I, Lautenbach E. Risk factors for fluconazole resistance in patients with *Candida glabrata* bloodstream infection: potential impact of control group selection on characterizing the association between previous fluconazole use and fluconazole resistance. Am J Infect Control. 2010; 38:456–460. [PubMed: 20371135]
- *13. Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F. Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. Antimicrob Agents Chemother. 2011; 55:532–538. [PubMed: 21078946] Another report from France that illustrates the fact that prior exposure to both fluconazole and caspofungin affects both species distribution and antifungal susceptibility.
14. Malani AN, Psarros G, Malani PN, Kauffman CA. Is age a risk factor for *Candida glabrata* colonisation? Mycoses. 2011; 54:531–537. [PubMed: 20707848]
15. Moran C, Grussemeyer CA, Spalding JR, Benjamin DK Jr., Reed SD. Comparison of costs, length of stay, and mortality associated with *Candida glabrata* and *Candida albicans* bloodstream infections. Am J Infect Control. 2010; 38:78–80. [PubMed: 19836856]
16. Pfaller MA, Messer SA, Hollis RJ, et al. Variation in susceptibility of bloodstream isolates of *Candida glabrata* to fluconazole according to patient age and geographic location in the United States in 2001 to 2007. J Clin Microbiol. 2009; 47:3185–3190. [PubMed: 19656983]
17. Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. Variation in *Candida* spp. distribution and antifungal resistance rates among bloodstream infection isolates by patient age: Report from

- the SENTRY Antimicrobial Surveillance Program (2008–2009). *Diagn Microbiol Infect Dis*. 2010; 68:278–283. [PubMed: 20846808]
18. Pfaller MA, Diekema DJ, Gibbs DL, et al. Geographic variation in the frequency of isolation and fluconazole and voriconazole susceptibilities of *Candida glabrata*: An assessment from the ARTEMIS DISK Global Antifungal Surveillance Program. *Diagn Microbiol Infect Dis*. 2010; 67:162–171. [PubMed: 20338711]
 - *19. Pfaller MA, Castanheira M, Lockhart SR, Ahlquist AM, Messer SA, Jones RN. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *J Clin Microbiol*. 2012; 50:1199–1203. [PubMed: 22278842] The first report of the emergence of co-resistance to fluconazole and the echinocandins in *C. glabrata* isolates from the US.
 20. Pfeiffer CD, Garcia-Effron G, Zaas AK, Perfect JR, Perlin DS, Alexander BD. Breakthrough invasive candidiasis in patients on micafungin. *J Clin Microbiol*. 2010; 48:2373–2380. [PubMed: 20421445]
 21. Sobel JD. Changing epidemiology of invasive candidiasis in intensive care units--much ado about nothing? *Crit Care Med*. 2008; 36:2188–2189. [PubMed: 18594223]
 22. Sobel JD. Changing trends in the epidemiology of *Candida* blood stream infections: A matter for concern? *Crit Care Med*. 2010; 38:990–992. [PubMed: 20168159]
 - *23. Zimbeck AJ, Iqbal N, Ahlquist AM, et al. *FKS* mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother*. 2010; 54:5042–2047. [PubMed: 20837754] A report concerning echinocandin resistance in *C. glabrata* isolates from a US population-based survey.
 24. Pappas PG, Rex JH, Sobel JD, et al. Guidelines for treatment of candidiasis. *Clin Infect Dis*. 2004; 38:161–189. [PubMed: 14699449]
 25. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2009; 48:503–535. [PubMed: 19191635]
 26. Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989–1999. *Clin Infect Dis*. 2002; 35:627–630. [PubMed: 12173140]
 27. Ahlquist A, Farley MM, Harrison LH, et al. Epidemiology of candidemia in metropolitan Atlanta and Baltimore City and County: Preliminary results of population-based active, laboratory surveillance - 2008–2009. In: 49th ICAAC, September 12–15, 2009. San Francisco, California, USA: Antimicrob. Agents Chemother., 2009: Abstr. M-1241.
 28. Pakyz AL, Gurgle HE, Oinonen MJ. Antifungal use in hospitalized adults in U.S. academic health centers. *Am J Health Syst Pharm*. 2011; 68:415–418. [PubMed: 21330683]
 29. Hachem R, Hanna H, Kontoyiannis D, Jiang Y, Raad I. The changing epidemiology of invasive candidiasis: *Candida glabrata* and *Candida krusei* as the leading causes of candidemia in hematologic malignancy. *Cancer*. 2008; 112:2493–2499. [PubMed: 18412153]
 30. Hajjeh RA, Sofair AN, Harrison LH, et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *J Clin Microbiol*. 2004; 42:1519–1527. [PubMed: 15070998]
 31. Pfaller MA, Diekema DJ. The epidemiology of invasive candidiasis. Calderone RA and Clancy CJ (ed), *Candida and Candidiasis*, 2nd ed. Washington, D.C. USA: American Society for Microbiology 2012; 449–480.
 32. Sobel JD. The emergence of non-*albicans* *Candida* species as causes of invasive candidiasis and candidemia. *Curr Infect Dis Rep*. 2006; 8:427–433. [PubMed: 17064635]
 33. Chow JK, Golan Y, Ruthazer R, et al. Factors associated with candidemia caused by non-*albicans* *Candida* species versus *Candida albicans* in the intensive care unit. *Clin Infect Dis*. 2008; 46:1206–1213. [PubMed: 18444857]
 34. Garey KW, Pai MP, Suda KJ, et al. Inadequacy of fluconazole dosing in patients with candidemia based on Infectious Diseases Society of America (IDSA) guidelines. *Pharmacoepidemiol Drug Saf*. 2007; 16:919–927. [PubMed: 17286303]

35. Horn DL, Neofytos D, Anaissie EJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. *Clin Infect Dis*. 2009; 48:1695–1703. [PubMed: 19441981]
36. Pasqualotto AC, Zimerman RA, Alves SH, et al. Take control over your fluconazole prescriptions: the growing importance of *Candida glabrata* as an agent of candidemia in Brazil. *Infect Control Hosp Epidemiol*. 2008; 29:898–899. [PubMed: 18785850]
37. Sendid B, Cotteau A, Francois N, et al. Candidaemia and antifungal therapy in a French University Hospital: Rough trends over a decade and possible links. *BMC Infect Dis*. 2006; 6:80. [PubMed: 16670011]
38. Borst A, Raimer MT, Warnock DW, Morrison CJ, Arthington-Skaggs BA. Rapid acquisition of stable azole resistance by *Candida glabrata* isolates obtained before the clinical introduction of fluconazole. *Antimicrob Agents Chemother*. 2005; 49:783–787. [PubMed: 15673768]
39. Dery M, Hasbun R. Fluconazole-resistant *Candida*: Mechanisms and risk factor identification. *Curr Fungal Infect Rep*. 2011; 5:23–28.
40. Marichal P, Vanden Bossche H, Odds FC, et al. Molecular biological characterization of an azole-resistant *Candida glabrata* isolate. *Antimicrob Agents Chemother*. 1997; 41:2229–2237. [PubMed: 9333053]
41. Pfaller MA, Diekema DJ. Azole antifungal drug cross-resistance: Mechanisms epidemiology, and clinical significance. *J Invasive Fungal Infect*. 2007; 1:74–92.
42. Sanglard D, Ischer F, Calabrese D, Majcherczyk PA, Bille J. The ATP binding cassette transporter gene *CgCDR1* from *Candida glabrata* is involved in the resistance of clinical isolates to azole antifungal agents. *Antimicrob Agents Chemother*. 1999; 43:2753–2765. [PubMed: 10543759]
43. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrob Agents Chemother*. 2005; 49:668–679. [PubMed: 15673750]
44. Betts RF, Nucci M, Talwar D, et al. A Multicenter, double-blind trial of a high-dose caspofungin treatment regimen versus a standard caspofungin treatment regimen for adult patients with invasive candidiasis. *Clin Infect Dis*. 2009; 48:1676–1684. [PubMed: 19419331]
45. Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: A phase III randomised double-blind trial. *Lancet*. 2007; 369:1519–1527. [PubMed: 17482982]
46. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med*. 2002; 347:2020–2029. [PubMed: 12490683]
47. Pappas PG, Rotstein CM, Betts RF, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. *Clin Infect Dis*. 2007; 45:883–893. [PubMed: 17806055]
48. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med*. 2007; 356:2472–2482. [PubMed: 17568028]
49. Shorr AF, Wu C, Kothari S. Outcomes with micafungin in patients with candidaemia or invasive candidiasis due to *Candida glabrata* and *Candida krusei*. *J Antimicrob Chemother*. 2011; 66:375–380. [PubMed: 21147825]
50. Messer SA, Jones RN, Fritsche TR. International surveillance of *Candida* spp. and *Aspergillus* spp.: report from the SENTRY Antimicrobial Surveillance Program (2003). *J Clin Microbiol*. 2006; 44:1782–1787. [PubMed: 16672407]
51. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. In vitro activities of anidulafungin against more than 2,500 clinical isolates of *Candida* spp., including 315 isolates resistant to fluconazole. *J Clin Microbiol*. 2005; 43:5425–5427. [PubMed: 16272464]
52. Pfaller M, Boyken L, Hollis R, et al. Use of epidemiological cutoff values to examine 9-year trends in susceptibility of *Candida* species to anidulafungin, caspofungin, and micafungin. *J Clin Microbiol*. 2011; 49:624–629. [PubMed: 21147948]
53. Castanheira M, Woosley LN, Pfaller MA, Diekema DJ, Messer SA, Jones RN. Low prevalence of *fkp1* hotspot 1 mutations in a worldwide collection of *Candida* spp. *Antimicrob Agents Chemother*. 2010; 54:2655–2659. [PubMed: 20368396]

54. Kofteridis DP, Lewis RE, Kontoyiannis DP. Caspofungin-non-susceptible *Candida* isolates in cancer patients. *J Antimicrob Chemother.* 2010; 65:293–295. [PubMed: 20016020]
- *55. Perlin DS. Echinocandin-resistant *Candida*: Molecular methods and phenotypes. *Curr Fungal Infect Reports.* 2011;5:113–119. A very well written review of aspects of echinocandin resistance in *Candida*.
56. Sanguinetti M, Posteraro P, Posteraro B. Echinocandin antifungal drug resistance in *Candida* species: a cause for concern? *Curr Infect Dis Rep.* 2010; 12:437–443. [PubMed: 21308552]
57. Cleary JD, Garcia-Effron G, Chapman SW, Perlin DS. Reduced *Candida glabrata* susceptibility secondary to an FKS1 mutation developed during candidemia treatment. *Antimicrob Agents Chemother.* 2008; 52:2263–2265. [PubMed: 18378714]
58. Costa-de-Oliveira S, Marcos Miranda I, Silva RM, et al. FKS2 mutations associated with decreased echinocandin susceptibility of *Candida glabrata* following anidulafungin therapy. *Antimicrob Agents Chemother.* 2011; 55:1312–1314. [PubMed: 21149621]
59. Daneman N, Chan AK, Poutanen SM, Rennie R, Sand C, Porter S. The emergence of caspofungin resistance during treatment of recurrent *Candida glabrata* candidaemia. In: 16th ESCMID, April 1–4, 2006. Nice, France, 2006: Abstr. P1204.
60. Dodgson KJ, Dodgson AR, Pujol D, Messer SA, Soll DR, Pfaller MA. Caspofungin resistant *C. glabrata*. *Clin Microbiol Infect.* 2005; 11:364.
61. Garcia-Effron G, Lee S, Park S, Cleary JD, Perlin DS. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrob Agents Chemother.* 2009; 53:3690–3699. [PubMed: 19546367]
62. Garcia-Effron G, Chua DJ, Tomada JR, et al. Novel FKS mutations associated with echinocandin resistance in *Candida* species. *Antimicrob Agents Chemother.* 2010; 54:2225–2227. [PubMed: 20145084]
63. Sun HY, Singh N. Characterization of breakthrough invasive mycoses in echinocandin recipients: an evidence-based review. *Int J Antimicrob Agents.* 2010; 35:211–218. [PubMed: 20036518]
64. Katiyar S, Pfaller M, Edlind T. *Candida albicans* and *Candida glabrata* clinical isolates exhibiting reduced echinocandin susceptibility. *Antimicrob Agents Chemother.* 2006; 50:2892–2894. [PubMed: 16870797]
65. Thompson GR III, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS Jr., Patterson TF. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother.* 2008; 52:3783–3785. [PubMed: 18676885]
66. Chandwani S, Wentworth C, Burke TA, Patterson TF. Utilization and dosage pattern of echinocandins for treatment of fungal infections in US hospital practice. *Curr Med Res Opin.* 2009; 25:385–393. [PubMed: 19192983]
67. Vandeputte P, Tronchin G, Larcher G, et al. A nonsense mutation in the *ERG6* gene leads to reduced susceptibility to polyenes in a clinical isolate of *Candida glabrata*. *Antimicrob Agents Chemother.* 2008; 52:3701–3709. [PubMed: 18694952]
68. Vandeputte P, Pineau L, Larcher G, et al. Molecular mechanisms of resistance to 5-fluorocytosine in laboratory mutants of *Candida glabrata*. *Mycopathologia.* 2011; 171:11–21. [PubMed: 20617462]
69. Kaur R, Domergue R, Zupancic ML, Cormack BP. A yeast by any other name: *Candida glabrata* and its interaction with the host. *Curr Opin Microbiol.* 2005; 8:378–384. [PubMed: 15996895]
70. Pappas PG. Candidemia in the intensive care unit: miles to go before we sleep. *Crit Care Med.* 2011; 39:884–885. [PubMed: 21613835]
71. Anderson JB. Evolution of antifungal-drug resistance: mechanisms and pathogen fitness. *Nat Rev Microbiol.* 2005; 3:547–556. [PubMed: 15953931]
72. Andersson DI. The biological cost of mutational antibiotic resistance: any practical conclusions? *Curr Opin Microbiol.* 2006; 9:461–465. [PubMed: 16890008]
73. Clancy CJ, Nguyen MH. At what cost echinocandin resistance? *J Infect Dis.* 2011; 204:499–501. [PubMed: 21791650]

74. Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W. Differential in vivo activity of anidulafungin, caspofungin and micafungin against *C. glabrata* with and without *FKS* resistance mutations. *Antimicrob Agents Chemother.* 2012;in press.
- *75. Ben-Ami R, Garcia-Effron G, Lewis RE, et al. Fitness and Virulence Costs of *Candida albicans* FKS1 Hot Spot Mutations Associated With Echinocandin Resistance. *J Infect Dis.* 2011; 204:626–635. [PubMed: 21791665] A novel and important use of an in vivo fly model to assess fitness and virulence costs of FKS mutations in *C. albicans*.
76. Zhao Y, Paak S, Delmas G, Gamarra MS, Perlin DS. Fitness costs of *Candida glabrata* FKS mutations associated with echinocandin resistance. In: 51st ICAAC, September 12–15, 2011. Chicago, IL, USA, 2011: Abstr. M-394.
77. Ferrari S, Ischer F, Calabrese D, et al. Gain of function mutations in *CgPDR1* of *Candida glabrata* not only mediate antifungal resistance but also enhance virulence. *PLoS Pathog.* 2009; 5:e1000268.
- *78. Ferrari S, Sanguinetti M, Torelli R, Posteraro B, Sanglard D. Contribution of *CgPDR1*-regulated genes in enhanced virulence of azole-resistant *Candida glabrata*. *PLoS One.* 2011; 6:e17589. An important illustration of the role of GOF mutations in virulence and azole resistance in *C. glabrata*.
79. Ferrari S, Sanguinetti M, De Bernardis F, et al. Loss of mitochondrial functions associated with azole resistance in *Candida glabrata* results in enhanced virulence in mice. *Antimicrob Agents Chemother.* 2011; 55:1852–1860. [PubMed: 21321146]
80. Edlind T, Shiffrin E, Vermitsky J, Katiyar S. Evidence for a hypervirulent, hypermutable *Candida glabrata* genotype. In: 51st ICAAC, September 12–15, 2011. Chicago, IL, USA, 2011: Abstr. M-295.
81. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis.* 2002; 2:73–85. [PubMed: 11901654]
82. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev.* 1998; 11:382–402. [PubMed: 9564569]
83. Berila N, Borecka S, Dzugasova V, Bojnansky J, Subik J. Mutations in the *CgPDR1* and *CgERG11* genes in azole-resistant *Candida glabrata* clinical isolates from Slovakia. *Int J Antimicrob Agents.* 2009; 33:574–578. [PubMed: 19196495]
84. Posteraro B, Sanguinetti M, Fiori B, et al. Caspofungin activity against clinical isolates of azole cross-resistant *Candida glabrata* overexpressing efflux pump genes. *J Antimicrob Chemother.* 2006; 58:458–461. [PubMed: 16757500]
85. Vandeputte P, Tronchin G, Berges T, Hennequin C, Chabasse D, Bouchara JP. Reduced susceptibility to polyenes associated with a missense mutation in the *ERG6* gene in a clinical isolate of *Candida glabrata* with pseudohyphal growth. *Antimicrob Agents Chemother.* 2007; 51:982–990. [PubMed: 17158937]
86. Kanafani ZA, Perfect JR. Antimicrobial resistance: resistance to antifungal agents: mechanisms and clinical impact. *Clin Infect Dis.* 2008; 46:120–128. [PubMed: 18171227]
87. Peman J, Canton E, Espinel-Ingroff A. Antifungal drug resistance mechanisms. *Expert Rev Anti Infect Ther.* 2009; 7:453–460. [PubMed: 19400764]
88. Ghannoum MA, Rice LB. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *Clin Microbiol Rev.* 1999; 12:501–517. [PubMed: 10515900]
89. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. *Clin Microbiol Rev.* 2007; 20:133–163. [PubMed: 17223626]
90. Pfaller MA, Messer SA, Bolmstrom A. Evaluation of Etest for determining in vitro susceptibility of yeast isolates to amphotericin B. *Diagn Microbiol Infect Dis.* 1998; 32:223–227. [PubMed: 9884840]
91. Rex JH, Pfaller MA. Has antifungal susceptibility testing come of age? *Clin Infect Dis.* 2002; 35:982–989. [PubMed: 12355386]
92. Sheehan DJ, Hitchcock CA, Sibley CM. Current and emerging azole antifungal agents. *Clin Microbiol Rev.* 1999; 12:40–79. [PubMed: 9880474]

93. Redding SW, Kirkpatrick WR, Saville S, et al. Multiple patterns of resistance to fluconazole in *Candida glabrata* isolates from a patient with oropharyngeal candidiasis receiving head and neck radiation. *J Clin Microbiol.* 2003; 41:619–622. [PubMed: 12574256]
94. Niimi K, Maki K, Ikeda F, et al. Overexpression of *Candida albicans* *CDR1*, *CDR2*, or *MDR1* does not produce significant changes in echinocandin susceptibility. *Antimicrob Agents Chemother.* 2006; 50:1148–1155. [PubMed: 16569823]
95. Vermes A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother.* 2000; 46:171–179. [PubMed: 10933638]
96. Edlind TD, Katiyar SK. Mutational analysis of flucytosine resistance in *Candida glabrata*. *Antimicrob Agents Chemother.* 2010; 54:4733–4738. [PubMed: 20823283]
97. Cowen LE, Kohn LM, Anderson JB. Divergence in fitness and evolution of drug resistance in experimental populations of *Candida albicans*. *J Bacteriol.* 2001; 183:2971–2978. [PubMed: 11325923]
98. Vermitsky JP, Edlind TD. Azole resistance in *Candida glabrata*: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor. *Antimicrob Agents Chemother.* 2004; 48:3773–3781. [PubMed: 15388433]
99. Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Variation in susceptibility of bloodstream isolates of *Candida glabrata* to fluconazole according to patient age and geographic location. *J Clin Microbiol.* 2003; 41:2176–2179. [PubMed: 12734273]
100. Pfaller MA, Diekema DJ. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. *Clin Microbiol Infect.* 2004; 10 Suppl 1:11–23. [PubMed: 14748799]
101. Baddley JW, Smith AM, Moser SA, Pappas PG. Trends in frequency and susceptibilities of *Candida glabrata* bloodstream isolates at a university hospital. *Diagn Microbiol Infect Dis.* 2001; 39:199–201. [PubMed: 11337190]
102. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M. The changing epidemiology of healthcare-associated candidemia over three decades. *Diagn Microbiol Infect Dis.* 2012; in press.
103. Malani A, Hmoud J, Chiu L, Carver PL, Bielaczyc A, Kauffman CA. *Candida glabrata* fungemia: Experience in a tertiary care center. *Clin Infect Dis.* 2005; 41:975–981. [PubMed: 16142662]
- *104. Sanglard D. Diagnosis of antifungal drug resistance mechanisms in fungal pathogens: Transcriptional gene regulation. *Curr Fungal Infect Rep.* 2011; 5:157–167. A very clear discussion of the role of transcriptional gene regulation in antifungal resistance.
105. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). *J Clin Microbiol* 2011;49:396–399. [PubMed: 21068282]

Table 1.*C. glabrata* Emergence USA Hospitals

	1992–2001 (N=3683)		2001–2007 (N=2536)	P
N (%) <i>C. glabrata</i>	559 (18%)	→	642 (25%)	<0.001
% Fluconazole resistance among <i>C. glabrata</i>	9%	→	14%	0.006

Data compiled from refs 16 and 99..

Table 2.Emergence of Echinocandin Resistance Among Fluconazole-Resistant *C. glabrata*^a

Time Period	Antifungal agent	No. tested	% ^b		
			S	I	R
2001–2004	Anidulafungin	110	98.2	1.8	0.0
	Caspofungin	110	96.4	3.6	0.0
	Micafungin	110	100.0	0.0	0.0
2006–2010	Anidulafungin	162	87.0	3.7	9.3
	Caspofungin	162	87.0	3.7	9.3
	Micafungin	162	87.1	4.9	8.0

^aData compiled from ref 19.^b% of isolates in each susceptibility category: S, susceptible; I, intermediate; R, resistant.