

# Echinocandin Resistance in *Candida*

David S. Perlin

Public Health Research Institute, New Jersey Medical School–Rutgers Biomedical and Health Sciences, Newark

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

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

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

## EPIDEMIOLOGY OF ECHINOCANDIN RESISTANCE

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

Correspondence: David S. Perlin, PhD, Public Health Research Institute, New Jersey Medical School–Rutgers Biomedical and Health Sciences, 225 Warren St, Newark, NJ 07103 (perlinds@njms.rutgers.edu).

**Clinical Infectious Diseases®** 2015;61(S6):S612–7

© The Author 2015. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.  
DOI: 10.1093/cid/civ791

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

## FKS MECHANISM OF RESISTANCE

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

## FKS POLYMORPHISMS AND REDUCED SUSCEPTIBILITY

Some *Candida* species have naturally occurring polymorphisms in *FKS* genes, which render them less susceptible to echinocandin drugs. Both the *Candida parapsilosis* family (*C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis*) and *Candida guilliermondii* have higher MIC values relative to other susceptible *Candida* species [24–26]. In the *C. parapsilosis* family, Pro660 in hot-spot 1 is present as alanine, whereas in *C. guilliermondii*, Leu633 and Thr334 are replaced by methionine and alanine, respectively. These changes decrease somewhat the sensitivity of glucan synthase for drug, resulting in elevated MIC

values [27]. However, as glucan synthase is still inhibited at therapeutic levels, infecting strains can generally be treated successfully. Hence, echinocandins are largely effective in patients with *C. parapsilosis* family infections.

## DRUG TOLERANCE

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

## STANDARDIZED TESTING FOR RESISTANCE

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

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

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

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

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

## RISK FACTORS FOR RESISTANCE EMERGENCE

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

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

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

## CONCLUSIONS

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

## Notes

**Financial support.** The author is supported by the National Institutes of Health (grant numbers AI069397 and AI109025) and Astellas Pharma.

**Supplement sponsorship.** This article appears as part of the supplement “Advances and New Directions for Echinocandins,” sponsored by Astellas Pharma Global Development, Inc.

**Potential conflict of interest.** The author serves on scientific advisory boards for Merck, Astellas, and Synexis; has received grant support from Astellas; and is an inventor in US patent 8 753 819, “Assays for Resistance to Echinocandin-Class Drugs.”

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

## References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med* **2012**; 4:165rv13.
2. 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**; 48:503–35.
3. Cleveland AA, Farley MM, Harrison LH, et al. Changes in incidence and antifungal drug resistance in candidemia: results from population-based laboratory surveillance in Atlanta and Baltimore, 2008–2011. *Clin Infect Dis* **2012**; 55:1352–61.
4. Park S, Kelly R, Kahn JN, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida* sp. isolates. *Antimicrob Agents Chemother* **2005**; 49:3264–73.
5. Castanheira M, Woosley LN, Diekema DJ, Messer SA, Jones RN, Pfaller MA. Low prevalence of fks1 hot spot 1 mutations in a worldwide collection of *Candida* strains. *Antimicrob Agents Chemother* **2010**; 54:2655–9.
6. Alexander BD, Johnson MD, Pfeiffer CD, et al. Increasing echinocandin resistance in *Candida glabrata*: clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin Infect Dis* **2013**; 56:1724–32.
7. 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**; 50:1199–203.
8. Pham CD, Iqbal N, Bolden CB, et al. Role of FKS mutations in *Candida glabrata*: MIC values, echinocandin resistance, and multidrug resistance. *Antimicrob Agents Chemother* **2014**; 58:4690–6.
9. 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*: results from the SENTRY Antimicrobial Surveillance Program (2006–2010) and the Centers for Disease Control and Prevention Population-Based Surveillance (2008–2010). *J Clin Microbiol* **2012**; 50:1199–203.
10. 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**; 55:532–8.
11. Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol* **2011**; 6:441–57.
12. Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of antifungal drug resistance. *Cold Spring Harb Perspect Med* **2014**; 5:pii:a019752.
13. Garcia-Effron G, Park S, Perlin DS. Correlating echinocandin MIC and kinetic inhibition of fks1 mutant glucan synthases for *Candida albicans*: implications for interpretive breakpoints. *Antimicrob Agents Chemother* **2009**; 53:112–22.
14. Johnson ME, Katiyar SK, Edlind TD. A new Fks hotspot for acquired echinocandin resistance in yeast, and its contribution to intrinsic resistance of *Scedosporium* species. *Antimicrob Agents Chemother* **2011**; 55:3774–81.
15. Katiyar SK, Edlind TD. Role for Fks1 in the intrinsic echinocandin resistance of *Fusarium solani* as evidenced by hybrid expression in *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* **2009**; 53:1772–8.
16. 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**; 53:3690–9.
17. 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**; 56:2435–42.
18. Howard SJ, Lestner JM, Sharp A, et al. Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: clinical implications for antifungal therapy. *J Infect Dis* **2011**; 203:1324–32.
19. Slater JL, Howard SJ, Sharp A, et al. Disseminated candidiasis caused by *Candida albicans* with amino acid substitutions in Fks1 at position Ser645 cannot be successfully treated with micafungin. *Antimicrob Agents Chemother* **2011**; 55:3075–83.
20. Wiederhold NP, Najvar LK, Bocanegra RA, Kirkpatrick WR, Patterson TF. Caspofungin dose escalation for invasive candidiasis due to resistant *Candida albicans*. *Antimicrob Agents Chemother* **2011**; 55:3254–60.
21. Katiyar SK, Alastruey-Izquierdo A, Healey KR, Johnson ME, Perlin DS, Edlind TD. Fks1 and Fks2 are functionally redundant but differentially regulated in *Candida glabrata*: implications for echinocandin resistance. *Antimicrob Agents Chemother* **2012**; 56:6304–9.
22. Eng WK, Faucette L, McLaughlin MM, et al. The yeast FKS1 gene encodes a novel membrane protein, mutations in which confer FK506 and cyclosporin A hypersensitivity and calcineurin-dependent growth. *Gene* **1994**; 151:61–71.
23. Johnson ME, Katiyar SK, Edlind TD. New Fks hot spot for acquired echinocandin resistance in *Saccharomyces cerevisiae* and its contribution to intrinsic resistance of *Scedosporium* species. *Antimicrob Agents Chemother* **2011**; 55:3774–81.
24. Pfaller MA, Boyken L, Hollis RJ, et al. Wild-type MIC distributions and epidemiological cutoff values for the echinocandins and *Candida* spp. *J Clin Microbiol* **2010**; 48:52–6.
25. Tortorano AM, Prigitano A, Lazzarini C, et al. A 1-year prospective survey of candidemia in Italy and changing epidemiology over one decade. *Infection* **2013**; 41:655–62.

26. Pfaller MA, Boyken L, Hollis RJ, et al. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. *J Clin Microbiol* **2008**; 46:150–6.
27. Garcia-Effron G, Katiyar SK, Park S, Edlind TD, Perlin DS. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* accounts for reduced echinocandin susceptibility. *Antimicrob Agents Chemother* **2008**; 52:2305–12.
28. Perlin DS. Resistance to echinocandin-class antifungal drugs. *Drug Resist Updat* **2007**; 10:121–30.
29. Walker LA, Gow NA, Munro CA. Fungal echinocandin resistance. *Fungal Genet Biol* **2010**; 47:117–26.
30. Munro CA, Selvaggini S, de Bruijn I, et al. The PKC, HOG and Ca<sup>2+</sup> signalling pathways co-ordinately regulate chitin synthesis in *Candida albicans*. *Mol Microbiol* **2007**; 63:1399–413.
31. Walker LA, Munro CA, de Bruijn I, Lenardon MD, McKinnon A, Gow NA. Stimulation of chitin synthesis rescues *Candida albicans* from echinocandins. *PLoS Pathog* **2008**; 4:e1000040.
32. Singh SD, Robbins N, Zaas AK, Schell WA, Perfect JR, Cowen LE. Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. *PLoS Pathog* **2009**; 5:e1000532.
33. Singh-Babak SD, Babak T, Diezmann S, et al. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. *PLoS Pathog* **2012**; 8:e1002718.
34. Firon A, Lesage G, Bussey H. Integrative studies put cell wall synthesis on the yeast functional map. *Curr Opin Microbiol* **2004**; 7:617–23.
35. Plaine A, Walker L, Da Costa G, et al. Functional analysis of *Candida albicans* GPI-anchored proteins: roles in cell wall integrity and caspofungin sensitivity. *Fungal Genet Biol* **2008**; 45:1404–14.
36. Lee KK, Maccallum DM, Jacobsen MD, et al. Elevated cell wall chitin in *Candida albicans* confers echinocandin resistance in vivo. *Antimicrob Agents Chemother* **2012**; 56:208–17.
37. Ben-Ami R, Garcia-Effron G, Lewis RE, et al. The fitness and virulence cost of *fks1* mutations causing echinocandin-resistance in *Candida albicans*. *J Infect Dis* **2011**; 204:626–35.
38. Wheeler RT, Fink GR. A drug-sensitive genetic network masks fungi from the immune system. *PLoS Pathog* **2006**; 2:e35.
39. Hohl TM, Feldmesser M, Perlin DS, Pamer EG. Caspofungin modulates inflammatory responses to *Aspergillus fumigatus* through stage-specific effects on fungal beta-glucan exposure. *J Infect Dis* **2008**; 198:176–85.
40. Stevens DA, Ichinomiya M, Koshi Y, Horiuchi H. Escape of *Candida* from caspofungin inhibition at concentrations above the MIC (paradoxical effect) accomplished by increased cell wall chitin; evidence for beta-1,6-glucan synthesis inhibition by caspofungin. *Antimicrob Agents Chemother* **2006**; 50:3160–1.
41. Healey KR, Katiyar SK, Castanheira M, Pfaller MA, Edlind TD. *Candida glabrata* mutants demonstrating paradoxical reduced caspofungin susceptibility but increased micafungin susceptibility. *Antimicrob Agents Chemother* **2011**; 55:3947–9.
42. Coste A, Selmecki A, Forche A, et al. Genotypic evolution of azole resistance mechanisms in sequential *Candida albicans* isolates. *Eukaryot Cell* **2007**; 6:1889–904.
43. Selmecki A, Forche A, Berman J. Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science* **2006**; 313:367–70.
44. Shin JH, Chae MJ, Song JW, et al. Changes in karyotype and azole susceptibility of sequential bloodstream isolates from patients with *Candida glabrata* candidemia. *J Clin Microbiol* **2007**; 45:2385–91.
45. Pfaller MA, Espinel-Ingroff A, Bustamante B, et al. Multicenter study of anidulafungin and micafungin MIC distributions and epidemiological cutoff values for eight *Candida* species and the CLSI M27-A3 broth microdilution method. *Antimicrob Agents Chemother* **2014**; 58:916–22.
46. Pfaller MA, Messer SA, Woosley LN, Jones RN, Castanheira M. Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance. *J Clin Microbiol* **2013**; 51:2571–81.
47. Pfaller MA, Diekema DJ, Ostrosky-Zeichner L, et al. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. *J Clin Microbiol* **2008**; 46:2620–9.
48. Andes D, Diekema DJ, Pfaller MA, Bohrmuller J, Marchillo K, Lepak A. In vivo comparison of the pharmacodynamic targets for echinocandin drugs against *Candida* species. *Antimicrob Agents Chemother* **2010**; 54:2497–506.
49. Pfaller MA, Diekema DJ, Andes D, et al. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat* **2011**; 14:164–76.
50. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW. Breakpoints for antifungal agents: an update from EUCAST focussing on echinocandins against *Candida* spp. and triazoles against *Aspergillus* spp. *Drug Resist Updat* **2013**; 16:81–95.
51. Arendrup MC, Rodriguez-Tudela JL, Lass-Flörl C, et al. EUCAST technical note on anidulafungin. *Clin Microbiol Infect* **2011**; 17:E18–20.
52. Espinel-Ingroff A, Arendrup MC, Pfaller MA, et al. Interlaboratory variability of Caspofungin MICs for *Candida* spp. Using CLSI and EUCAST methods: should the clinical laboratory be testing this agent? *Antimicrob Agents Chemother* **2013**; 57:5836–42.
53. Ben-Ami R, Hilerowicz Y, Novikov A, Giladi M. The impact of new epidemiological cutoff values on *Candida glabrata* resistance rates and concordance between testing methods. *Diagn Microbiol Infect Dis* **2014**; 79:209–13.
54. Pfaller MA, Messer SA, Diekema DJ, Jones RN, Castanheira M. Use of micafungin as a surrogate marker to predict susceptibility and resistance to caspofungin among 3,764 clinical isolates of *Candida* by use of CLSI methods and interpretive criteria. *J Clin Microbiol* **2014**; 52:108–14.
55. Pfaller MA, Diekema DJ, Jones RN, Castanheira M. Use of anidulafungin as a surrogate marker to predict susceptibility and resistance to caspofungin among 4,290 clinical isolates of *Candida* by using CLSI methods and interpretive criteria. *J Clin Microbiol* **2014**; 52:3223–9.
56. Arendrup MC, Perlin DS. Echinocandin resistance: an emerging clinical problem? *Curr Opin Infect Dis* **2014**; 27:48492.
57. Espinel-Ingroff A, Pfaller MA, Bustamante B, et al. Multilaboratory study of epidemiological cutoff values for detection of resistance in eight *Candida* species to fluconazole, posaconazole, and voriconazole. *Antimicrob Agents Chemother* **2014**; 58:2006–12.
58. Shields RK, Nguyen MH, Press EG, et al. The presence of an FKS mutation rather than MIC is an independent risk factor for failure of echinocandin therapy among patients with invasive candidiasis due to *Candida glabrata*. *Antimicrob Agents Chemother* **2012**; 56:4862–9.
59. Perlin DS. Echinocandin resistance, susceptibility testing and prophylaxis: implications for patient management. *Drugs* **2014**; 74:1573–85.
60. Thompson GR 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS 2nd, Patterson TF. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother* **2008**; 52:3783–5.
61. Fekkar A, Dannaoui E, Meyer I, et al. Emergence of echinocandin-resistant *Candida* spp. in a hospital setting: a consequence of 10 years of increasing use of antifungal therapy? *Eur J Clin Microbiol Infect Dis* **2014**; 33:1489–96.
62. Fekkar A, Meyer I, Brossas JY, et al. Rapid emergence of echinocandin resistance during *Candida kefyr* fungemia treatment with caspofungin. *Antimicrob Agents Chemother* **2013**; 57:2380–2.
63. Lewis JS 2nd, Wiederhold NP, Wickes BL, Patterson TF, Jorgensen JH. Rapid emergence of echinocandin resistance in *Candida glabrata* resulting in clinical and microbiologic failure. *Antimicrob Agents Chemother* **2013**; 57:4559–61.
64. Koh AY, Kohler JR, Cogshall KT, Van Rooijen N, Pier GB. Mucosal damage and neutropenia are required for *Candida albicans* dissemination. *PLoS Pathog* **2008**; 4:e35.

65. Magill SS, Swoboda SM, Shields CE, et al. The epidemiology of *Candida* colonization and invasive candidiasis in a surgical intensive care unit where fluconazole prophylaxis is utilized: follow-up to a randomized clinical trial. *Ann Surg* **2009**; 249:657–65.
66. Miranda LN, van der Heijden IM, Costa SF, et al. *Candida* colonisation as a source for candidaemia. *J Hosp Infect* **2009**; 72:9–16.
67. Voss A, Hollis RJ, Pfaller MA, Wenzel RP, Doebbeling BN. Investigation of the sequence of colonization and candidemia in nonneutropenic patients. *J Clin Microbiol* **1994**; 32:975–80.
68. Richet HM, Andremon A, Tancrede C, Pico JL, Jarvis WR. Risk factors for candidemia in patients with acute lymphocytic leukemia. *Rev Infect Dis* **1991**; 13:211–5.
69. Harriott MM, Noverr MC. Importance of *Candida*-bacterial polymicrobial biofilms in disease. *Trends Microbiol* **2011**; 19:557–63.
70. Mitchell KF, Taff HT, Cuevas MA, Reinicke EL, Sanchez H, Andes DR. Role of matrix beta-1,3 glucan in antifungal resistance of non-*albicans* *Candida* biofilms. *Antimicrob Agents Chemother* **2013**; 57:1918–20.
71. Cheng S, Clancy C, Hartman D, Hao B, Nguyen M. *Candida glabrata* intra-abdominal candidiasis is characterized by persistence within the peritoneal cavity and abscesses. *Infect Immun* **2014**; 82:3015–22.
72. Scott LJ. Micafungin: a review of its use in the prophylaxis and treatment of invasive *Candida* infections. *Drugs* **2012**; 72:2141–65.
73. de la Torre P, Reboli AC. Micafungin: an evidence-based review of its place in therapy. *Core Evid* **2014**; 9:27–39.
74. Chou LS, Lewis RE, Ippoliti C, Champlin RE, Kontoyiannis DP. Caspofungin as primary antifungal prophylaxis in stem cell transplant recipients. *Pharmacotherapy* **2007**; 27:1644–50.
75. Mattiuzzi GN, Alvarado G, Giles FJ, et al. Open-label, randomized comparison of itraconazole versus caspofungin for prophylaxis in patients with hematologic malignancies. *Antimicrob Agents Chemother* **2006**; 50:143–7.
76. Ziakas PD, Kourbeti IS, Mylonakis E. Systemic antifungal prophylaxis after hematopoietic stem cell transplantation: a meta-analysis. *Clin Ther* **2014**; 36:292–306.e1.
77. Ruggero MA, Topal JE. Development of echinocandin-resistant *Candida albicans* candidemia following brief prophylactic exposure to micafungin therapy. *Transpl Infect Dis* **2014**; 16:469–72.