The Journal of Infectious Diseases
SUPPLEMENT ARTICLE

Multidrug-Resistant *Candida*: Epidemiology, Molecular Mechanisms, and Treatment

Maiken Cavling Arendrup[1,2,3] and Thomas F. Patterson[4]

[1]Unit of Mycology, Statens Serum Institut, [2]Department of Clinical Microbiology, Rigshospitalet, and [3]Department of Clinical Medicine, University of Copenhagen, Denmark; and [4]Division of Infectious Diseases, San Antonio Center for Medical Mycology, UT Health San Antonio, and the South Texas Veterans Health Care System

Invasive *Candida* infections remain an important cause of morbidity and mortality, especially in hospitalized and immunocompromised or critically ill patients. A limited number of antifungal agents from only a few drug classes are available to treat patients with these serious infections. Resistance can be either intrinsic or acquired. Resistance mechanisms are not exchanged between *Candida*; thus, acquired resistance either emerges in response to an antifungal selection pressure in the individual patient or, more rarely, occur due to horizontal transmission of resistant strains between patients. Although multidrug resistance is uncommon, increasing reports of multidrug resistance to the azoles, echinocandins, and polyenes have occurred in several *Candida* species, most notably *Candida glabrata* and more recently *Candida auris*. Drivers are overall antifungal use, subtherapeutic drug levels at sites of infection/colonization, drug sequestration in the biofilm matrix, and, in the setting of outbreaks, suboptimal infection control. Moreover, recent research suggests that DNA mismatch repair gene mutations may facilitate acquisition of resistance mutations in *C. glabrata* specifically. Diagnosis of antifungal-resistant *Candida* infections is critical to the successful management of patients with these infections. Reduction of unnecessary use of antifungals via antifungal stewardship is critical to limit multidrug resistance emergence.

Keywords. multidrug resistance; echinocandin; fluconazole; azole; amphotericin B; *Candida*; *Candida glabrate*; *Candida C. auris*.

Infections due to *Candida* species are major causes of morbidity and mortality and are associated with a wide variety of clinical manifestations ranging from superficial and mucosal infections to widely disseminated and bloodstream infections [1]. Global estimates suggest that invasive candidiasis occurs in more than a quarter of a million patients every year with incidence rates for candidemia of 2–14 per 100 000 inhabitants in population-based studies [1–4]. *Candida albicans* is still a leading cause of candidemia, but other species (non-*albicans*) of *Candida* now comprise >50% of bloodstream infections in many parts of the world [1]. Antifungal resistance is less common in *C. albicans* but has been reported with long-term antifungal use and with recurrent infections, such as those with chronic mucocutaneous candidiasis or recurrent oropharyngeal candidiasis in patients with uncontrolled human immunodeficiency virus infection. Several of the non-*albicans Candida* species, such as *Candida krusei*, are intrinsically resistant or less susceptible to several classes of antifungals, whereas others, including *Candida glabrata*, develop acquired resistance following exposure to antifungal agents. Resistance to >1 drug class (multidrug resistance) remains uncommon but has been increasingly reported, such as in *Candida auris*. Genetic and molecular mechanisms of resistance have been described for many strains, so that knowledge of these mechanisms may help guide selection of therapy. In these patients at risk for serious *Candida* infection, diagnosis remains difficult but is critical in allowing detection of resistance and determination of optimal treatment regimens.

In this review, we discuss the epidemiology of multidrug resistance, molecular mechanisms for resistance, and strategies for treatment and prevention.

DEFINITION AND LIMITATIONS

Standard definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacteria were recently established through a joint initiative by the European Centre for Disease Prevention and Control and the US Centers for Disease Control and Prevention (CDC) [5]. MDR was defined as acquired nonsusceptibility to at least 1 agent in 3 or more antimicrobial categories; XDR was defined as nonsusceptibility to at least 1 agent in all but 2 or fewer antimicrobial categories (ie, bacterial isolates remain susceptible to only 1 or 2 categories); and PDR was defined as nonsusceptibility to all agents in all antimicrobial categories. These definitions cannot be directly adopted for resistance in *Candida*. The main reason is that only 4 drug classes are available for systemic treatment of *Candida* infections including the azoles (fluconazole, itraconazole, isavuconazole, posaconazole, and voriconazole), polyenes (conventional amphotericin B and its lipid formulations), echinocandins (anidulafungin, caspofungin, and

Correspondence: M. C. Arendrup, MD, PhD, DrMedSci, Unit of Mycology, Statens Serum Institut, building 43/317, Artillerivej 5, DK-2300 Copenhagen, Denmark (maca@ssi.dk).

The Journal of Infectious Diseases® 2017;216(S3):S445–51

DOI: 10.1093/infdis/jix131

micafungin), and, finally, the pyrimidine analogue flucytosine. Among these drug classes, only members of the first 3 are licensed for monotherapy against *Candida* infections and only fluconazole and echinocandins are recommended as first-line agents for invasive candidiasis. On this background and in absence of a standard definition for MDR *Candida*, we defined MDR as an isolate nonsusceptible to ≥1 agent in ≥2 drug classes and XDR as an isolate nonsusceptible to ≥1 agent in ≥3 drug classes.

EPIDEMIOLOGY AND DRIVERS OF RESISTANCE

Reports on resistance to antifungal agents are relatively rare (when compared to antibacterial agents) but became much more common with the introduction of additional classes of antifungal agents, particularly the azoles (especially fluconazole), which have been widely used against *Candida* infections. Thus, the development of resistance to the current clinically used azole antifungal agents has become an increasing problem. This is particularly true in patients requiring long-term treatment and in those receiving antifungal prophylaxis, highlighting the importance of antifungal stewardship [6, 7]. Widespread *acquired* azole resistance was frequently described in patients with AIDS and oropharyngeal or mucosal candidiasis (particularly in the era prior to active antiretroviral therapy) and less frequently in invasive infections. In these patients resistance can be stable or transient, in response to azole treatment [8]. Acquired echinocandin resistance has emerged over the past decade and particularly so in *C. glabrata*. Most cases occur after 3–4 weeks of therapy, but resistant mutants have been reported after short-term therapy and even in echinocandin-naive patients in high-incidence settings, suggesting potential transfer among hospitalized patients [9, 10]. In addition, there is a growing awareness of the changing epidemiology of fungal infections, with a shift toward species that are *intrinsically* resistant to the most commonly used antifungal agents (namely, fluconazole) [11, 12] (Table 1).

Intrinsic Resistance

Intrinsic or primary resistance is inherent (not acquired) resistance, which is a characteristic of all or almost all representatives of the species, and it is predictive of clinical failure. Examples of intrinsic resistance are the resistance of *C. krusei* to fluconazole and of many of the newly described *C. auris* strains associated with the global outbreaks of infection in healthcare settings with elevated minimum inhibitory concentrations (MICs) to several classes of antifungal drugs, including azoles, echinocandins, and polyenes (Table 1). The widespread use of azole antifungals has been associated with the emergence of resistant (*C. krusei*) or less susceptible species (particularly *C. glabrata*) in many regions of the world and in specific patient populations such as in transplantation, where azole use is widespread and continued for long-term for prophylaxis and therapy of infection [19, 20].

Other *Candida* species with intrinsic resistance to fluconazole also have decreased susceptibility to the echinocandins so that they can be classified as MDR including *Candida guilliermondii* and the closely related species *Candida fermentati*, which are uncommon causes of infection but are occasionally associated with serious disease, including bloodstream infections [21].

More widespread has been the global emergence of *C. auris*, which has emerged as an important cause of healthcare-associated infections worldwide and can exhibit intrinsic MDR [22]. Initially *C. auris* was described from an external ear canal drainage in Japan in 2009 and later from bloodstream infection in Korea in 2011 [23]. Most subsequent cases occured in India where a high degree of clonality among the isolates has been documented [24, 25]. Recent reports have documented infection throughout the world, including the United States, most typically associated with healthcare-associated infections including transmission in healthcare facilities [16, 24, 26–29]. Patients with *C. auris* have similar risk factors to other *Candida* species infections, including abdominal surgery, broad-spectrum antibiotics, central venous catheters, and comorbid conditions [23]. The diagnosis is often difficult due to misidentification with commercial identification test methods, although matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) can accurately identify the organism if *C. auris* is included in the library [30]. Overall mortality is high, ranging from 30% to 60%, and infections occur several weeks into the patient's hospital admission. Infection control has been difficult as colonization and environmental contamination has been extensive [22, 23]. Although breakpoints are not available for *C. auris*, most strains are resistant to fluconazole (MICs ≥64 mg/L) and about one-third of the isolates have elevated MICs (≥2) against voriconazole and amphotericin B. A few strains (7%) have elevated MICs to the echinocandins as well [23]. Overall, 41% were resistant to 2 drug classes (MDR) and 4% were resistant to 3 (azoles, echinocandins, and polyenes), which make them XDR [16]. Phylogenetically, *C. auris* is related to *Candida haemulonii,* for which it can be confused, which is known for its intrinsic resistance to fluconazole and amphotericin B.

Acquired Resistance

MDR *Candida* most commonly involves acquired resistance in species with intrinsic resistance, but occasionally MDR occurs in normally susceptible species. Most *Candida* species have a low rate (<3%) of echinocandin resistance [31]. An exception to that finding is for *C. glabrata*, which is noted to have increased rates of resistance, particularly in the setting of extensive echinocandin use [32, 33]. These isolates are often associated with decreased susceptibility to other antifungals, particularly fluconazole and other azoles. A report from one US medical center demonstrated that over a 10 year-period, echinocandin resistance rose from 2%–3% to >13% in 2009–2010 [34]. Similar

Table 1. Intrinsic Susceptibility Patterns for *Candida* Species

Species	AMB	Echinocandins	Fluconazole	Comments
Common *Candida* species				
C. albicans	S	S	S	
C. dubliniensis	S	S	S	Closely related to *C. albicans;* fluconazole resistance easily acquired [13]
C. glabrata	S	S	I	Efflux pumps often induced during azole therapy [14]
C. krusei	S	S	R	
C. parapsilosis	S	S/I	S	Harbors an *FKS1* hot spot alteration responsible for elevated echinocandin MICs. Wild-type population is categorized as susceptible by CLSI and as intermediate by EUCAST [15]
C. tropicalis	S	S	S	
Uncommon *Candida* species				
C. auris	(X)	(X)	X	93% resistant to fluconazole, 35% to amphotericin B, and 7% to echinocandins; 41% resistant to 2 antifungal classes and 4% resistant to 3 classes [16]
C. bracharensis			X	Closely related to *C. glabrata*
C. lusitaniae	X			
C. fermentati		X		
C. guilliermondii		S/X	X	Harbors an *FKS1* hot spot alteration responsible for elevated echinocandin MICs. Wild-type population is categorized as susceptible by CLSI but not by EUCAST due to insufficient evidence to indicate whether the wild-type population of this pathogen can be considered susceptible to echinocandins [17, 18]
C. metapsilosis		X		Closely related to *C. parapsilosis*
C. nivariensis			X	Closely related to *C. glabrata*
C. orthopsilosis		X		Closely related to *C. parapsilosis*
C. ciferrii			X	
C. inconspicua			X	
C. humicula			X	
C. lambica			X	
C. lipolytica			X	
C. norvegensis			X	
C. palmioleophila			X	
C. rugosa			X	
C. valida			X	
S. cerevisiae[a]			X	Closely related to *C. glabrata*

CLSI and EUCAST breakpoints have been established for the common *Candida* species allowing classification of wild-type isolates into S (susceptible), I (intermediate), and R (resistant) categories. For the uncommon *Candida* species, breakpoints have not been established. For these species, an "X" denotes that the MICs for the antifungal compound are elevated compared to those for *C. albicans*.

Abbreviations: AMB, amphotericin B; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

[a]Anamorphic state is *C. robusta*.

findings were reported at other US hospitals, and a CDC survey showed rates of 3.1%–3.6% from 4 US cities in varying geographic regions [32]. Importantly, echinocandin resistance was associated with cross-resistance to azole antifungals in 36% of the echinocandin-resistant strains, so that concerns regarding MDR *C. glabrata* significantly increased. Also importantly, "hot spot" *FKS* mutations associated with resistance were common in those isolates with elevated MICs and more likely to be associated with clinical failure [33].

Other *Candida* species have also been reported to acquire MDR after antifungal exposure, including *C. albicans* and others, such as *Candida kefyr* and *Candida lusitaniae* [35–38]. *Candida albicans* resistance mutations have not been particularly common, although they can occur in the setting of long-term echinocandin use or in patients with lack of source control of the infection. These echinocandin and potentially MDR *C. albicans* isolates are associated with poorer responses in animal models of infection as well as in clinical infections. Less significant mutations may be associated with higher MICs, but can in some cases be overcome in animal models with higher doses of therapy [36, 39].

MOLECULAR MECHANISMS OF RESISTANCE

Amphotericin B exerts fungicidal activity via binding to ergosterol in the fungal cell membrane (Table 2). Upon binding, 6 ergosterol molecules form a pore through the membrane, leading to loss of intracellular compounds and cell death. Ergosterol is formed from a precursor, lanosterol, via several intermediate sterols. This biosynthesis involves a number of enzymatic steps encoded by *ERG6, ERG11, ERG24, ERG25, ERG26, ERG27,*

Table 2. Summary of Molecular Resistance Mechanisms Described in *Candida*

	Drug Class			
	Amphotericin B	Echinocandins	Azoles	Flucytosine
Drug target	Ergosterol	Glucan synthase	P450 demethylase	DNA and RNA synthesis
Resistance mechanism				
Target gene mutation	*ERG2, 3, 5, 6* and *11* → less ergosterol	*FKS1* and *FKS2* → less binding	*ERG11* → less binding	
Target up-regulation			*UPC2*, Duplication of chromosome 5 Isochromosomes	
Efflux pumps			*CDR*[a], *MFS*[a] *CgSNQ2, PDH1* (*C. glabrata* specifically)	
Reduced drug uptake				Loss of permease
Reduced intracellular activation				*FCA1*[b] (*C. albicans*), *FCY1*[b] (*C. glabrata*) *FUR1*[c]

[a]ATP-binding cassette (ABC) transporters including *CDR1* and *CDR2* are regulated by a zinc cluster finger transcription regulator and major facilitator superfamily transporters by transcription factors *MMR1* in *C. albicans*. In *C. glabrata*, other transcription regulators are described including *PDR1* that regulates *CgCDR1*, *CgCDR2*, and *CgSNQ2* [40–42].

[b]*FCA1* and *FCY1* encodes cytosine deaminase, and mutations in these genes therefore inhibits the conversion of flucytosine into 5-F-fluorouridine [43].

[c]*FUR1* encodes uracil phosphoribosyltransferase, and mutations in this gene therefore inhibits the conversion of 5-F-fluorouridine into 5-fluorodeoxyuridylic acid monophosphate [44].

ERG2, ERG3, ERG5, and *ERG4* in sequential order. Combined mutations in *ERG11* and in *ERG3* or *ERG5* and single mutations in *ERG6* or in *ERG2* have been associated with depletion of ergosterol and amphotericin B resistance in *Candida*; however, acquired amphotericin B resistance is a rare event [45–49].

Azoles are fungistatic against *Candida* and act by binding to and inhibiting the intracellular target enzyme ERG11p involved in the biosynthesis of ergosterol. More than 140 alterations have been described in the *ERG11* target gene, some of which have been found exclusively in azole-resistant isolates, whereas others have also been found in susceptible isolates [50]. The impact of the alteration on azole resistance depends on location and specific substitution and has been challenging to dissect as several target gene mutations often occur simultaneously and often also play in concert with other resistance mechanisms [42, 50–52]. Gain of function (GOF) mutations in the transcription gene *UPC2* that regulates *ERG11* expression lead to supernumerary ERG11p concentration and consequently insufficient azole activity [52]. Finally, efflux pumps contribute to azole resistance in *Candida* [42]. The pleiotropic class of the ATP binding cassette (ABC) transporters (PDR) include the major azole drug transporters *CDR1, CDR2*, and specifically for *C. glabrata* also the *CgSNQ2*, and confer panazole resistance [40–42, 53]. The *MDR1* transporter belonging to the major facilitator superfamily (MFS) is also involved in azole resistance in *Candida* but apparently does not confer resistance to posaconazole, itraconazole, or isavuconazole [53]. Expression levels of these drug transporters are determined by their specific regulators *TAC1* for *CDR1* and *CDR2*, and *MRR1* for regulation of *MDR1* expression. Hence, GOF mutations in these regulators have been related to overexpression of the efflux pumps and hence azole resistance [54, 55].

Echinocandin resistance has only been convincingly linked to one molecular mechanism, namely mutations in 2 hot spot regions of the target gene *FKS1* (wild-type AA sequences for *C. albicans* hot spot 1: FLTLSLRDP and hot spot 2: DWIRRYTL) or, in the case of *C. glabrata*, also in *FKS2* (wild-type AA sequences hot spot 1: FLILSLRDP and hot spot 2: DWIRRYTL) [56]. In the vast majority of cases only a single mutation is responsible, although rare cases several alterations are found [57]. The level of resistance depends on the specific codon involved, the specific alteration, and in which species it is occurring. In example, a D to Y alteration at the eighth codon in hot spot 1 of FKS1 affects the MIC much more in *C. krusei* than in *C. albicans*, with a ≥3 to ≥5 two-fold dilution steps MIC elevation in *C. krusei* but only 1–2 steps in *C. albicans* [58]. The most significant MIC elevation is found for alterations involving the first and fifth amino acids (F [phenylalanine] and S [serine], respectively) in the hot spot 1 region of the *FKS1* or *FKS2* target genes [56]. Differential in vivo activity has been observed when comparing the 3 echinocandins against *C. glabrata*. In example, micafungin, but not anidulafungin and caspofungin, retained its activity against *C. glabrata* harboring the Fks2p-S663F alteration in an animal model, and anidulafungin remained clinically efficacious though caspofungin therapy failed in a clinical case involving a *C. albicans* isolate with heterologous Fks1p-R647R/G and P649P/L double mutations [56, 57]. However, these are the exceptions from the general rule that *FKS1* and *FKS2* hot spot alterations affect all 3 echinocandins. An overview of all the specific alterations and their impact can be found in [56].

Flucytosine is actively transported into the fungal cell by permease (encoded by *FCY2*). It is subsequently converted to 5-fluorouracil or to 5-fluorouridine monophosphate by the enzymes cytosine deaminase or uracil phosphoribosyltransferase

encoded by the *FCY1* and *FUR1* genes, respectively, and act by inhibiting transcription, DNA replication, and protein synthesis. Resistance emerge rapidly if used as monotherapy and has been ascribed to mutations in the *FCY2*, *FCY1*, and *FUR1* genes [43, 44]. Moreover, 3 new biological processes that affect flucytosine resistance in *C. glabrata* was recently proposed including arginine homeostasis, cell wall remodeling, and the aquaglyceroporins of the Fps family [59].

MDR Resistance Mechanisms

Most MDR *Candida* infections involve isolates belonging to species with intrinsic resistance, for example, echinocandin resistance in *C. glabrata* and *C. krusei* [34, 58, 60, 61] or infections with *C. guilliermondii* or *C. auris*, which is intrinsically multidrug resistant and currently emerging in India and other continents as described above [16]. Multidrug resistance in species that possess no intrinsic resistance is rare, as in general it requires acquisition of several resistance mechanisms and these often come at a fitness cost [62, 63]. However, *ERG3* and *ERG2* alterations have individually been associated with azole and amphotericin B cross-resistance in *C. albicans* and *Candida dubliniensis* [45, 62, 64, 65]. Moreover, azole and polyene resistance have been found in *Candida* isolates harboring *ERG11* mutations in combination with either *ERG3* or *ERG5* alterations [45, 46]. Finally, XDR has been described in a very few patients undergoing long-term and alternating antifungal therapy. A stepwise development of azole, echinocandin, and amphotericin B resistance was observed in *C. albicans* from a patient with mucosal infection over a 5-year period [49], and azole, flucytosine, and echinocandin resistance was acquired in *C. glabrata* due to acquisition of mutations in *FUR1* (*CgFUR1*) and *CgFKS2* and overexpression of *CgCDR1* and *CgCDR2* during 20 weeks of antifungal therapy in a hematopoietic stem cell transplant recipient [66].

Multidrug-Resistant *C. glabrata*

Over the past years, a rise in MDR *C. glabrata* has been reported. *Candida glabrata* has been significantly associated with prior fluconazole exposure and, when causing invasive infection, prompt echinocandin therapy without de-escalation [1, 12, 67–69]. In this context of prolonged and more broad-spectrum antifungal exposure, it was less surprising that this species was particularly prone to become MDR. However, it has recently become evident that other mechanisms may contribute to the overrepresentation of *C. glabrata* among MDR *Candida*. First, it was shown that *MSH2* DNA mismatch repair gene mutations in *C. glabrata* facilitate rapid acquisition of fluconazole, echinocandin, and amphotericin B resistance [70]. The underlying resistance mechanisms were identical to those seen in clinical isolates with resistance; moreover, *MSH2* mutations were found among clinical isolates [71]. These findings may also help explain why echinocandin resistance in *C. glabrata* in some studies has been associated to prior fluconazole exposure, although the drug targets and resistance mechanisms are completely different [10]. The implications of this finding are concerning, as it raises the question if fluconazole prescription even in the primary healthcare sector is also a potential driver of MDR *C. glabrata*. Second, Jensen et al reported a high rate of echinocandin-resistant *C. glabrata* in the oral microflora after candidemia treatment with ≥7 days of echinocandins, and Shields et al reported a high prevalence among *C. glabrata* isolates from abdominal candidiasis [72, 73]. These findings suggest that (1) subtherapeutic drug concentrations (potentially linked to the high protein binding of echinocandin drugs) at mucosal surfaces and in focal infections may facilitate resistance development and/or (2) that *Candida* biofilm of the oral cavity or on intraabdominal devices may be involved in resistance selection. Drug sequestration in the biofilm matrix reduces drug efficacy but also provides an environment of lower exposure that may facilitate selection of acquired resistance [74].

Treatment and Prevention of MDR *Candida* Infection

Treatment options for MDR *Candida* infections remain limited. Expert guidelines have few evidence-based data to guide their recommendations [12, 69]. Anecdotal experience has shown that in patients with *C. glabrata* infections that have elevated MICs to the echinocandins and fluconazole resistance as well (MDR strains), clinical failure rate is increased [9, 75]. In those patients, both expert panels and clinical experience suggest that liposomal amphotericin B combined with an extensive search for undrained or unremoved foci of infection (such as central catheters, abdominal abscesses, other hardware, or thrombophlebitis) are critical to a successful outcome [34]. In patients with MDR *C. albicans* infection, experimental animal data suggest that some less critical *FKS* mutations may be associated with higher response rates with higher doses of therapy, but clinical correlation of those data are lacking [36, 39].

In patients with MDR *C. auris*, limited experience is available to guide optimal approaches to therapy, but susceptibility data suggest that echinocandin resistance is less frequent than for the azoles such that, for clinically available antifungal agents, an echinocandin or amphotericin B remains the likely drug of choice in those infections [16, 22, 23]. In those settings, antifungal susceptibility testing is strongly recommended and complete mycological evaluation for underlying resistance mechanisms for those strains with elevated MICs may be reasonable. Data regarding combinations of antifungal agents from different classes are limited, so conclusive recommendations cannot be made. Newer therapies from additional drug classes are needed to improve outcomes in those patients.

Prevention strategies to limit development of MDR and XDR infections are critical. As previously discussed, effective source control for abdominal and device-related infections will reduce the burden of infection and eliminate persister cells, which may

become drug tolerant over time [36]. Additionally, devices and hardware should be removed to eliminate biofilms, which can be a drug-protected nidus of infection. Antifungal drugs should not be given in subtherapeutic doses, which can encourage acquisition of resistance, and therapy should not be continued for durations longer than indicated. Prophylaxis of infection is needed to prevent infection in established high-risk patients, but a careful attempt should be made to establish a mycological diagnosis of infection so that susceptibility testing can assist in antifungal management.

CONCLUSIONS

Antifungal drug resistance including MDR *Candida* species has become increasingly important in the management of invasive fungal infections. These infections are associated with high morbidity and mortality and can be associated with healthcare-associated transmission. The emergence of MDR *C. glabrata* has become common in many medical centers and presents significant management challenges. Similarly, *C. auris* has emerged as an important clonally spread species that is associated with MDR and XDR characteristics and is associated with long-term colonization and extensive environmental contamination. Outcomes are poor with these resistant infections; thus, an accurate mycological diagnosis and therapy guided by susceptibility testing should be used to optimize management. New therapies are needed to improve the outcome of patients with these infections.

Notes

Supplement sponsorship. This work is part of a supplement sponsored by grants from Astellas Pharma Global Development, Inc. and Merck & Co., Inc.

Potential conflicts of interest. M. C. A. has received grants from Amplyx, Basilea, Cidara, F2G, and Gilead to her institution and speaker's fees from Astellas, Basilea, Gilead, MSD, Pfizer, and T2 Biosystems. T. F. P. has been a consultant for or served on advisory boards to Amplyx, Astellas, Cidara Therapeutics, F2G, Gilead, Merck, Pfizer, Scynexis, Toyama, Viamet, and Vical. Both authors have 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. Kullberg BJ, Arendrup MC. Invasive candidiasis. N Engl J Med **2015**; 373:1445–56.
2. Bitar D, Lortholary O, Le Strat Y, et al. Population-based analysis of invasive fungal infections, France, 2001–2010. Emerg Infect Dis **2014**; 20:1149–55.
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. Magill SS, Edwards JR, Bamberg W, et al.; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. N Engl J Med **2014**; 370:1198–208.
5. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **2012**; 18:268–81.
6. Revankar SG, Kirkpatrick WR, McAtee RK, et al. Detection and significance of fluconazole resistance in oropharyngeal candidiasis in human immunodeficiency virus-infected patients. J Infect Dis **1996**; 174:821–7.
7. Redding S, Smith J, Farinacci G, et al. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by in vitro susceptibility testing and DNA subtype analysis. Clin Infect Dis **1994**; 18:240–2.
8. Marr KA, Lyons CN, Rustad TR, Bowden RA, White TC, Rustad T. Rapid, transient fluconazole resistance in *Candida albicans* is associated with increased mRNA levels of CDR. Antimicrob Agents Chemother **1998**; 42:2584–9.
9. 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.
10. Vallabhaneni S, Cleveland AA, Farley MM, et al. Epidemiology and risk factors for echinocandin nonsusceptible *Candida glabrata* bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008–2014. Open Forum Infect Dis **2015**; 2:ofv163.
11. Redding SW, Marr KA, Kirkpatrick WR, Coco BJ, Patterson TF. *Candida glabrata* sepsis secondary to oral colonization in bone marrow transplantation. Med Mycol **2004**; 42:479–81.
12. Pappas PG, Kauffman CA, Andes DR, et al. Executive summary: clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis **2016**; 62:409–17.
13. Moran GP, Sullivan DJ, Henman MC, et al. Antifungal drug susceptibilities of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. Antimicrob Agents Chemother **1997**; 41:617–23.
14. Tumbarello M, Sanguinetti M, Trecarichi EM, et al. Fungaemia caused by *Candida glabrata* with reduced susceptibility to fluconazole due to altered gene expression: risk factors, antifungal treatment and outcome. J Antimicrob Chemother **2008**; 62:1379–85.
15. 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.
16. Lockhart SR, Etienne KA, Vallabhaneni S, et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis **2017**; 64:134–40.
17. Arendrup MC, Cuenca-Estrella M, Lass-Flörl C, Hope WW; European Committee on Antimicrobial Susceptibility Testing–Subcommittee on Antifungal Susceptibility Testing (EUCAST-AFST). EUCAST technical note on *Candida* and micafungin, anidulafungin and fluconazole. Mycoses **2014**; 57:377–9.
18. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts; 4th informational supplement. Document M27-S4. Wayne, PA: CLSI, **2012**.
19. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N Engl J Med **1991**; 325:1274–7.
20. Westbrook SD, Kirkpatrick WR, Freytes CO, et al. *Candida krusei* sepsis secondary to oral colonization in a hemopoietic stem cell transplant recipient. Med Mycol **2007**; 45:187–90.
21. Cheng JW, Yu SY, Xiao M, et al. Identification and antifungal susceptibility profile of *Candida guilliermondii* and *Candida fermentati* from a multicenter study in China. J Clin Microbiol **2016**; 54:2187–9.
22. Vallabhaneni S, Kallen A, Tsay S, et al.; MSD. Investigation of the first seven reported cases of *Candida auris*, a globally emerging invasive, multidrug-resistant fungus—United States, May 2013–August 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:1234–7.
23. Chowdhary A, Voss A, Meis JF. Multidrug-resistant *Candida auris*: 'new kid on the block' in hospital-associated infections? J Hosp Infect **2016**; 94:209–12.
24. Chowdhary A, Anil Kumar V, Sharma C, et al. Multidrug-resistant endemic clonal strain of *Candida auris* in India. Eur J Clin Microbiol Infect Dis **2014**; 33:919–26.
25. Prakash A, Sharma C, Singh A, et al. Evidence of genotypic diversity among *Candida auris* isolates by multilocus sequence typing, matrix-assisted laser desorption ionization time-of-flight mass spectrometry and amplified fragment length polymorphism. Clin Microbiol Infect **2016**; 22:277 e1–9.
26. Morales-López SE, Parra-Giraldo CM, Ceballos-Garzón A, et al. Invasive infections with multidrug-resistant yeast *Candida auris*, Colombia. Emerg Infect Dis **2017**; 23:162–4.
27. Ruiz Gaitán AC, Moret A, López Hontangas JL, et al. Nosocomial fungemia by *Candida auris*: first four reported cases in continental Europe. Rev Iberoam Micol **2017**; 34:23–7.
28. Schelenz S, Hagen F, Rhodes JL, et al. First hospital outbreak of the globally emerging *Candida auris* in a European hospital. Antimicrob Resist Infect Control **2016**; 5:35.
29. Sharma C, Kumar N, Pandey R, Meis JF, Chowdhary A. Whole genome sequencing of emerging multidrug resistant *Candida auris* isolates in India demonstrates low genetic variation. New Microbes New Infect **2016**; 13:77–82.
30. Kathuria S, Singh PK, Sharma C, et al. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: characterization by matrix-assisted laser desorption

ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. J Clin Microbiol **2015**; 53:1823–30.
31. Castanheira M, Messer SA, Rhomberg PR, Pfaller MA. Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). Diagn Microbiol Infect Dis **2016**; 85:200–4.
32. 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.
33. Beyda ND, John J, Kilic A, Alam MJ, Lasco TM, Garey KW. FKS mutant *Candida glabrata*: risk factors and outcomes in patients with candidemia. Clin Infect Dis **2014**; 59:819–25.
34. 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.
35. 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.
36. Perlin DS. Echinocandin resistance in *Candida*. Clin Infect Dis **2015**; 61:S612–7.
37. Perlin DS. Mechanisms of echinocandin antifungal drug resistance. Ann N Y Acad Sci **2015**; 1354:1–11.
38. Asner SA, Giulieri S, Diezi M, Marchetti O, Sanglard D. Acquired multidrug antifungal resistance in *Candida lusitaniae* during therapy. Antimicrob Agents Chemother **2015**; 59:7715–22.
39. 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.
40. 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.
41. Vermitsky JP, Earhart KD, Smith WL, Homayouni R, Edlind TD, Rogers PD. Pdr1 regulates multidrug resistance in *Candida glabrata*: gene disruption and genome-wide expression studies. Mol Microbiol **2006**; 61:704–22.
42. Sanglard D. Emerging threats in antifungal-resistant fungal pathogens. Front Med (Lausanne) **2016**; 3:11.
43. Erbs P, Exinger F, Jund R. Characterization of the *Saccharomyces cerevisiae* FCY1 gene encoding cytosine deaminase and its homologue FCA1 of *Candida albicans*. Curr Genet **1997**; 31:1–6.
44. Charlier C, El Sissy C, Bachelier-Bassi S, et al. Acquired flucytosine resistance during combination therapy with caspofungin and flucytosine for *Candida glabrata* cystitis. Antimicrob Agents Chemother **2015**; 60:662–5.
45. Martel CM, Parker JE, Bader O, et al. Identification and characterization of four azole-resistant erg3 mutants of *Candida albicans*. Antimicrob Agents Chemother **2010**; 54:4527–33.
46. Martel CM, Parker JE, Bader O, et al. A clinical isolate of *Candida albicans* with mutations in ERG11 (encoding sterol 14alpha-demethylase) and ERG5 (encoding C22 desaturase) is cross resistant to azoles and amphotericin B. Antimicrob Agents Chemother **2010**; 54:3578–83.
47. 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**; 52:3701–9.
48. Hull CM, Bader O, Parker JE, et al. Two clinical isolates of *Candida glabrata* exhibiting reduced sensitivity to amphotericin B both harbor mutations in ERG2. Antimicrob Agents Chemother **2012**; 56:6417–21.
49. Jensen RH, Astvad KM, Silva LV, et al. Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. J Antimicrob Chemother **2015**; 70:2551–5.
50. Morio F, Loge C, Besse B, Hennequin C, Le Pape P. Screening for amino acid substitutions in the *Candida albicans* Erg11 protein of azole-susceptible and azole-resistant clinical isolates: new substitutions and a review of the literature. Diagn Microbiol Infect Dis **2010**; 66:373–84.
51. Li X, Brown N, Chau AS, et al. Changes in susceptibility to posaconazole in clinical isolates of *Candida albicans*. J Antimicrob Chemother **2004**; 53:74–80.
52. Dunkel N, Liu TT, Barker KS, Homayouni R, Morschhäuser J, Rogers PD. A gain-of-function mutation in the transcription factor Upc2p causes upregulation of ergosterol biosynthesis genes and increased fluconazole resistance in a clinical *Candida albicans* isolate. Eukaryot Cell **2008**; 7:1180–90.
53. Sanglard D, Coste AT. Activity of isavuconazole and other azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. Antimicrob Agents Chemother **2015**; 60:229–38.
54. Dunkel N, Blass J, Rogers PD, Morschhäuser J. Mutations in the multi-drug resistance regulator MRR1, followed by loss of heterozygosity, are the main cause of MDR1 overexpression in fluconazole-resistant *Candida albicans* strains. Mol Microbiol **2008**; 69:827–40.
55. Coste AT, Karababa M, Ischer F, Bille J, Sanglard D. TAC1, transcriptional activator of CDR genes, is a new transcription factor involved in the regulation of *Candida albicans* ABC transporters CDR1 and CDR2. Eukaryot Cell **2004**; 3:1639–52.
56. Arendrup MC, Perlin DS, Jensen RH, Howard SJ, Goodwin J, Hope W. Differential in vivo activities of anidulafungin, caspofungin, and micafungin against *Candida glabrata* isolates with and without FKS resistance mutations. Antimicrob Agents Chemother **2012**; 56:2435–42.
57. Lackner M, Tscherner M, Schaller M, et al. Positions and numbers of FKS mutations in *Candida albicans* selectively influence in vitro and in vivo susceptibilities to echinocandin treatment. Antimicrob Agents Chemother **2014**; 58:3626–35.
58. Jensen RH, Justesen US, Rewes A, Perlin DS, Arendrup MC. Echinocandin failure case due to a previously unreported FKS1 mutation in *Candida krusei*. Antimicrob Agents Chemother **2014**; 58:3550–2.
59. Costa C, Ponte A, Pais P, et al. New mechanisms of flucytosine resistance in *C. glabrata* unveiled by a chemogenomics analysis in *S. cerevisiae*. PLoS One **2015**; 10:e0135110.
60. Bordallo-Cardona MA, Escribano P, de la Pedrosa EG, et al. In vitro exposure to increasing micafungin concentrations easily promotes echinocandin resistance in *Candida glabrata* isolates. Antimicrob Agents Chemother **2017**; 61. pii:e01542-16.
61. Forastiero A, Garcia-Gil V, Rivero-Menendez O, et al. Rapid development of *Candida krusei* echinocandin resistance during caspofungin therapy. Antimicrob Agents Chemother **2015**; 59:6975–82.
62. Vincent BM, Lancaster AK, Scherz-Shouval R, Whitesell L, Lindquist S. Fitness trade-offs restrict the evolution of resistance to amphotericin B. PLoS Biol **2013**; 11:e1001692.
63. Ben-Ami R, Kontoyiannis DP. Resistance to echinocandins comes at a cost: the impact of FKS1 hotspot mutations on *Candida albicans* fitness and virulence. Virulence **2012**; 3:95–7.
64. Morio F, Pagniez F, Lacroix C, Miegeville M, Le Pape P. Amino acid substitutions in the *Candida albicans* sterol Δ5,6-desaturase (Erg3p) confer azole resistance: characterization of two novel mutants with impaired virulence. J Antimicrob Chemother **2012**; 67:2131–8.
65. Pinjon E, Moran GP, Jackson CJ, et al. Molecular mechanisms of itraconazole resistance in *Candida dubliniensis*. Antimicrob Agents Chemother **2003**; 47:2424–37.
66. 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**; 54:1360–2.
67. Lortholary O, Desnos-Ollivier M, Sitbon K, Fontanet A, Bretagne S, Dromer F; French Mycosis Study Group. Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2441 patients. Antimicrob Agents Chemother **2011**; 55:532–8.
68. Arendrup MC, Sulim S, Holm A, et al. Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. J Clin Microbiol **2011**; 49:3300–8.
69. Cornely OA, Bassetti M, Calandra T, et al.; ESCMID Fungal Infection Study Group. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: non-neutropenic adult patients. Clin Microbiol Infect **2012**; 18:19–37.
70. Healey KR, Zhao Y, Perez WB, et al. Prevalent mutator genotype identified in fungal pathogen *Candida glabrata* promotes multi-drug resistance. Nat Commun **2016**; 7:11128.
71. Dellière S, Healey K, Gits-Muselli M, et al. Fluconazole and echinocandin resistance of *Candida glabrata* correlates better with antifungal drug exposure rather than with MSH2 mutator genotype in a French cohort of patients harboring low rates of resistance. Front Microbiol **2016**; 7:2038.
72. Jensen RH, Johansen HK, Søes LM, et al. Posttreatment antifungal resistance among colonizing *Candida* isolates in candidemia patients: results from a systematic multicenter study. Antimicrob Agents Chemother **2015**; 60:1500–8.
73. Shields RK, Nguyen MH, Press EG, Clancy CJ. Abdominal candidiasis is a hidden reservoir of echinocandin resistance. Antimicrob Agents Chemother **2014**; 58:7601–5.
74. Nett JE, Crawford K, Marchillo K, Andes DR. Role of Fks1p and matrix glucan in *Candida albicans* biofilm resistance to an echinocandin, pyrimidine, and polyene. Antimicrob Agents Chemother **2010**; 54:3505–8.
75. 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.