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Review article

Epidemiology and molecular mechanisms of antifungal resistance in *Candida* and *Aspergillus*

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Summary

The significant increase in the use of antifungal agents, both for the treatment of candidiasis and invasive aspergillosis and as azole fungicides in agricultural crop protection has resulted in the emergence of resistant clinical isolates, particularly to triazoles and echinocandins. Notably, among isolates that were primarily sensitive to fluconazole such as *Candida parapsilosis* and *Candida tropicalis* have witnessed an emerging resistance development. Also for echinocandins, the occurrence of *Candida* isolates with lower susceptibility to these drugs has been reported, which is possibly due to its broad clinical use. Triazole resistance among *Aspergillus fumigatus* and other *Aspergillus* species is commonly found in European and Asian countries. Specific mutations are associated with azole resistance in *A. fumigatus* and these mutations are now reported globally from six continents. Therefore, we highlight the need to conduct antifungal resistance surveillance studies using clinical isolates of *Candida* and *Aspergillus* in different geographical regions and monitoring of the infection rates in distinct population groups for early detection of resistance to these drugs and implementation of efficient policies for infection control and treatment.

Key words: Antifungal resistance, *Candida* spp., *Aspergillus* spp., azoles, echinocandins, amphotericin B.

Introduction

In recent decades, there has been a substantial increase in the occurrence of invasive fungal infections (IFIs) due to *Candida* and *Aspergillus* in tertiary hospitals throughout the world.[1–3] Despite the large geographical variability in their incidence rates, IFIs have undoubtedly become very important worldwide, particularly among critically ill patients, those with degenerative or neoplastic diseases and in patients with organ transplantation.[3–5] Given the difficulties in diagnosing IFIs due to *Candida* and *Aspergillus* and their high mortality rates, prevention strategies and empirical antifungal therapies have been increasingly used in different risk groups.[2,6–9]

In addition the use of antifungal drugs has drastically increased in medical centres throughout the world although this increase is not always compatible with good clinical practice. In fact, some authors have reported a large number of inappropriate prescriptions of antifungal drugs even in teaching hospitals, a factor that increases not only cost and the risk of toxicity but also the possibility of resistance development to antifungal agents.[10–13] Concurrent with the global increased consumption of antifungal drugs, there has been an increase in the number of reported cases of resistance to different therapeutic antifungal classes among *Candida* and *Aspergillus* species. The present

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review will discuss various antifungal resistance patterns that have emerged in these two most important fungal pathogens and will also update about the risk factors and epidemiology of associated fungal infections.

Epidemiology of triazole resistance in *Candida* spp. and *Aspergillus* spp.

In IFI involving *Candida*, the gradual use of fluconazole and other triazoles in therapeutic regimens for prophylaxis, empirical therapy and diagnostic-driven therapy has led to the development of selective pressure with the emergence of less sensitive species and secondary resistance among isolates primarily sensitive to these drugs.[14] At first, different medical centres worldwide reported the increase in infections due to *Candida glabrata* and *C. krusei* among patients previously exposed to triazoles. At present, *C. glabrata* accounts for 18.1%–40.7% of the cases of candidaemia in the US and 8.5%–31.0% in Europe.[15–27] Until 2005, epidemiological studies conducted in Brazil reported a low frequency (<5%) of candidaemia due to *C. glabrata*. Recently, consistent with the epidemiology in the northern hemisphere, a substantial increase in cases of fungaemia due to *C. glabrata* has also been observed in Brazil. In fact, *C. glabrata* has accounted for 11.2%–13.1% of the reported cases of candidaemia in different regions of Brazil.[28–30] The same phenomenon was recently reported in Saudi Arabia by Omrani *et al.* [31]. According to these authors, the proportion of *C. glabrata* has significantly increased between January 2003 and December 2012.

Candidaemia surveillance in six countries in Asia namely China, Hong Kong, India, Singapore, Taiwan and Thailand reported *C. albicans* (41.3%) as the most common species.[32] Among non-*albicans Candida* species *C. tropicalis* (25.4%) was followed by *C. glabrata* (13.9%) and *C. parapsilosis* (12.1%).[32–34] Although the relative distributions of *Candida* species varied among the countries, the proportion of *C. tropicalis* among blood isolates was higher in tropical areas (India, Thailand and Singapore) than other geographical regions. *Candida parapsilosis* accounted for 33% of candidaemia cases in one Indian hospital, 26% in a Chinese hospital and 14% in a Taiwanese hospital.[32] However *C. glabrata* and *C. krusei* contributed to 26% and 12.2% candidaemia cases in tropical countries (China, India and Singapore).[32] Although, most of the Indian studies demonstrate *C. albicans* as a predominant yeast causing invasive candidiasis, nosocomial *C. tropicalis* candidaemia ranged from 67% to 90% in some Indian hospitals.[34,35] In Qatar, a retrospective analysis covering the period from January 2004 to December 2010 identified 201 episodes of candidaemia, of which 66.2% was due to non-*albicans Candida* species.[36] Similar data were obtained in the United Arab Emirates. In this study, non-*albicans Candida* species occurred more frequently than *C. albicans* in adults (67%), haematological malignancy patients (58%) and in cases with break through candidaemia (83%).[37]

First described in Japan in 2009, *C. auris* is increasingly encountered from cases of candidaemia especially from the Indian subcontinent.[38–41] These findings represent the beginning of an epidemiological shift which is different from Western countries where the increase in uncommon *Candida* species, as *C. kefyr* and *C. lusitaniae*, is likely due to broad use of echinocandins.[42]

A second epidemiological change, first described in developed countries, was the increasing occurrence of fluconazole resistance among isolates of the *C. parapsilosis* complex and *C. tropicalis*. Recent studies that performed susceptibility tests using reference methods reported variations in resistance rates among *C. parapsilosis* isolates of 3.4%–7.5% in the US and 0%–6% in Europe.[17,18,22,28,43] For *C. tropicalis*, these rates were 2.4%–9% in the US and 1.7%–22.0% in Europe.[17,18,20–22,26,28,43] Although infrequent, this phenomenon has been documented in some South American countries, as shown in Table 1. In Brazil, the rates of fluconazole dose-dependent susceptibility and/or resistance in *C. tropicalis* and *C. parapsilosis* are 0%–26.8% and 0%–26.9% respectively. Recently, Pinhati *et al.* [44] reported a candidaemia outbreak due to fluconazole-resistant *C. parapsilosis* in patients admitted to intensive care units (ICUs) and Souza *et al.* [45] reported mutations associated with this resistance.

Conceptually, the azole resistance phenotype in *Candida* spp. results from the combination of more than one resistance mechanism.[14] Table 2 summarises the major mechanisms of resistance to this class of antifungal drugs.

In IFI involving *Aspergillus*, two phenomena associated with resistance to antifungal agents were described: (i) increase in the number of infections due to non-*Aspergillus fumigatus*, including emerging species primarily resistant to triazoles and eventually to amphotericin B; and (ii) occurrence of secondary azole resistance in *A. fumigatus* isolates. Table 3 summarises the major species of non-*A. fumigatus* and those belonging to the section *Fumigati* associated with the phenotype of resistance to the main antifungal agents

Table 1 Distribution of *Candida* species and epidemiology of fluconazole resistance in Latin America.

Countries	Author [Reference]	Number of centres	Number of isolates	Species distribution[1] (%)					DDS/Resistance to FCZ[2] (%)				
				Ca	Ct	Cp	Cgla	Ck	Ca	Ct	Cp	Cgla	Ck
Latin America	Nucci *et al.* [290]	21	672	37.6	17.6	26.5	6.3	2.7	0.4	0	1.1	100	100
Argentina	Córdoba *et al.* [291]	41	457	38.4	15.4	26	4.33	0.5	0	4.2	2.5	20	ND
Chile	Silva *et al.* [292]	13	130	47	14	21	3	1	0	7	4	33	100
Venezuela	Dolande *et al.* [293]	6	154	46.7	19	6	9.2	2.7	7.4	12.9	10	43.4	96
Colombia	Maldonado *et al.* [294]	15	300	48.3	22.3	15	6.7	2.7	7.6	10.3	24.4	100	100
Mexico	Corzo-Leon *et al.* [295]	2	74	46	26	5	13.5	5	0	0	0	11	ND
Brazil	Aquino *et al.* [296]	1	131	45	15.3	24.4	6.9	4.6	0	0	0	45	100
Brazil	Colombo *et al.* [297]	11	712	40.9	20.9	20.5	4.9	1.1	0.3	0	0	6	37.5
Brazil	Bruder-Nascimento *et al.* [298]	1	212	33	17.9	31.1	11.8	0	7.1	18.4	1.5	68	ND
Brazil	Colombo *et al.* [29]	9	300	34	24	26	7	3	0	3	0	64	100
Brazil	Santos *et al.* [299]	1	422	35.7	9.71	46.6	3.5	0.9	9.9	26.8	7.1	100	100
Brazil	da Costa *et al.* [300]	1	93	28.7	30.5	24.1	8.3	1.8	3.7	3.2	26.9	0	ND

[1]Ca, *C. albicans*; Cd, *C. dubliniensis*; Ct, *C. tropicalis*; Cgla, *C. glabrata*; Cp, *C. parapsilosis*; Ck, *C. krusei*; Cgui, *C. guilliermondii*; FCZ, fluconazole; DDS, dose-dependent susceptibility; ND, not determined.

[2]Rates of DDS/Resistance to FCZ reflects the breakpoints available in that particularly period.

Table 2 Mechanisms of azole resistance in different *Candida* species.

Mechanism	Due to:	Result	Occurrence in *Candida* species[1]					
			Ca	Cd	Ct	Cgla	Cp	Ck
Decrease in the intracellular concentration of the target enzyme	- Expression of ABC transporters	Drug efflux	++	+	+	++	++	++
	- Expression of MF transporters		++	++	+	+	++	ND
Changes in the drug target	Mutations in the *ERG11* gene	Decreased affinity of the target enzyme for the azole	++	++	++	+	++	++
Increased production of lanosterol 14α-demethylase	Multiple factors[2]	Increased synthesis of ergosterol	++	++	++	++	++	++

[1]Ca, *C. albicans*; Cd, *C. dubliniensis*; Ct, *C. tropicalis*; Cgla, *C. glabrata*; Cp, *C. parapsilosis*; Ck, *C. krusei*; +, mechanism not yet observed in clinical isolates; ++, mechanism observed in clinical isolates; ND, mechanism not yet described.

[2]gene duplication, mutations in the promoter gene or mutations in the gene encoding the target enzyme.

commonly used in clinical practice.[46–49] The rate of infection due to emerging species of *Aspergillus* is not fully known because only a few case series have performed accurate molecular identification, such as DNA sequencing of *Aspergillus* at the species level. However, there are a substantial number of case series on *A. flavus*, *A. lentulus* and *A. terreus.*[50–52] Primary resistance to amphotericin B (AMB) is well known in *A. terreus,* however, in a recent study by Kathuria *et al.* [52] low AMB minimum inhibitory concentration (MIC) (range 0.5–1 mg/L) in 8% of Indian *A. terreus* isolates were observed. Furthermore, in some isolates of *A. flavus* and *A. ustus* also amphotericin B resistance was reported.[53–55] More recently, this phenomenon has been demonstrated in several species of the section *Fumigati*, including *A. fumigatiaffinis* and *A. lentulus.*[44,46,48,49,56]

Secondary azole resistance in *Aspergillus* has been well documented among isolates of *A. fumigatus.*[12,57] Acquired azole resistance has been reported also in *A. flavus* and *A. terreus*, although there is no evidence of developing acquired resistance to other antimycotic drugs in other species of this genus.[46,48,56,58,59]

Table 4 summarises the key studies that determined the rates of azole resistance in clinical isolates of *A. fumigatus*. The resistance rate varied between 0.6%

and 29.6%, and the lowest rate was observed in isolates from the US, where resistance was <1% and mutations in the *CYP51A* are rare.[12,60,61] Although triazole resistance is still considered unusual, with rates of <5% in many countries, azole resistance in *A. fumigatus* seems to be increasing in several European countries, and high rates have been observed primarily in medical centres in Denmark (4.5%), France (0.8%–8%) and UK (6%–27%).[47,62–67] In these countries, particularly in the UK (specifically in Manchester), high resistance rates (6%–27%) have been reported among patients with chronic cavitary aspergillosis who used triazoles for long periods.[68,69] To find the proper empirical treatment of invasive aspergillosis in areas with a high rate of azole resistance is challenging especially in patients with chronic pulmonary aspergillosis.[12,70]

A second epidemiological model of the development of triazole resistance in *A. fumigatus* has been described in the Netherlands, where the rates of resistance to these drugs vary between 1.7% and 30% in patients not previously exposed to antifungal therapy, thus suggesting a possible environmental route for the development of triazole resistance.[67,71–75] An epidemiological study conducted in Japan involving 196 clinical isolates reported that 11.2% of the isolates were resistant to triazoles.[76]

In Brazil, only two studies reported the prevalence and rates of triazole resistance among emerging species of non-*A. fumigatus*. These studies reported higher MIC values to these drugs among isolates of *A. calidoustus*, *A. clavatus*, *A. tamarii*, *A. nomius*, *A. ochraceus* and *A. terreus*.[54,77]

The rate of azole resistance in clinical *A. fumigatus* isolates in India was found to be 1.7%–1.9%.[57,78–80] However, in China the ARTEMIS global sentinel surveillance programme demonstrated a pan-azole-resistant phenotype in 27.5% of *A. fumigatus* isolates.[81] In contrast, Japan reported 5.2% azole-resistant *A. fumigatus* isolates.[76,82] A similar range of resistance prevalence of 3.2% and 12.5% *A. fumigatus* isolates was observed in clinical samples from chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA) patients from Iran and Kuwait respectively.[83,84]

Molecular mechanisms of azole resistance in *Candida* spp. and *Aspergillus* spp.

Azoles inhibit enzymes involved in the final stages of ergosterol biosynthesis, particularly lanosterol 14α-demethylase. Therefore, sterol precursors – including methylated sterols – accumulate in the cell, resulting in cell membrane instability and consequent fungal growth impairment.[85–87]

In *Candida* and *Aspergillus*, azole resistance appears to be associated with three major molecular mechanisms, which will be discussed in this review.

Table 3 Primary resistance among *Aspergillus* species.

Species	Invasive disease	Resistance profile	Section	References
A. lentulus	Relatively common	Primary resistance to AMB, azoles and varied resistance to CFG	*Fumigati*	[48, 301–304]
A. udagawae	Rare	Primary resistance to AMB and VRC	*Fumigati*	[66, 301, 302, 305, 306]
N. pseudofisheri	Rare	Varied susceptibility to AMB and lower susceptibility to azoles	*Fumigati*	[48, 302, 307]
A. fumigatiaffinis	Not reported	Primary resistance to AMB and lower resistance to azoles	*Fumigati*	[49]
A. viridinutans	Rare	Lower susceptibility to AMB and azoles	*Fumigati*	[48, 308]
A. flavus	Relatively common	Primary resistance to AMB and varied susceptibility to CFG	*Flavi*	[54, 66, 302, 309–313]
A. allilaceus	Relatively common	Lower susceptibility to AMB and CFG	*Flavi*	[302, 314–317]
A. terreus	Relatively common	Primary resistance to AMB	*Terrei*	[66,276,280,318–321]
A. alabamensis	Not reported	Primary resistance to AMB	*Terrei*	[322]
A. niger	Rare	Varied and lower susceptibility to azoles	*Nigri*	[60,310,323–325]
A. calidoustus	Rare	Primary resistance to AMB and azoles and varied susceptibility to CFG	*Ustis*	[77,302,326,327]
A. versicolor	Rare	Varied susceptibility to AMB and lower susceptibility to azoles	*Nidulantes*	[302,325,328]
A. sydowii	Rare	Varied susceptibility to AMB and lower susceptibility to azoles	*Nidulantes*	[60,302,328]

AMB, amphotericin B; CFG, caspofungin; IA, invasive aspergillosis; VRC, voriconazole.

Table 4 Rates of triazole resistance in clinical isolates of *Aspergillus fumigatus.*

Countries	Study period	Number of samples	Resistance rate (%)	Methodology	References
UK (single centre)	1997–2007	519	6.6	EUCAST	[68]
UK (single centre)	2008–2009	230	27.8	EUCAST	[69]
The Netherlands (multicentre)	1945–1998	170	1.7	CLSI M38-P	[71]
The Netherlands (single centre)	1994–2007	2061	3.1	CLSI M38-A	[67]
The Netherlands (multicentre)	2007–2009	1792	4.6	ITZ agar	[72]
The Netherlands (multicentre, 8 centres)	2009–2011	921	6.8	4D plates[1]	[73]
The Netherlands (multicentre)	2010–2013	952	24	EUCAST	[74]
Spain (multicentre, 29 centres)	2010–2011	156	0.6	EUCAST	[65]
Spain (single centre)	1999–2011	353	4.2	CLSI (M38-A2)	[328]
France (single centre)	2006–2009	118	0.8	Etest®	[329]
France (single centre)	2010–2011	85	8.0	EUCAST	[183]
Germany (single centre)	2011–2012	527	3.2	EUCAST	[141]
Germany (multicentre)	2010–2013	526	1.1	EUCAST	[64]
Germany (multicentre)	2012–2013	27	29.6	Etest®	[157]
Denmark (single centre)	2007 and 2009	133	4.5	EUCAST	[177]
Japan (single centre)	1994–2010	196	11.2	CLSI (M38-A2)	[76]
India (single centre)	2005–2010	103	1.9	CLSI (M38-A2)	[330]
Iran (single centre)	2003–2009	124	3.2	CLSI M38-A	[82]
US (multicentre, 23 centres)	2001–2006	181	0.6	CLSI (M38-A2)	[66]

[1]Triazoles diluted in agar; CLSI, Clinical Laboratory Standard Institute; ITZ, itraconazole; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

Decrease in the intracellular drug concentration

The activation of efflux pumps located in the cell membrane has already been described as an important mechanism of resistance to antifungal agents in *Candida* spp. Two different drug efflux systems have been associated with azole resistance: (i) the ATP-binding cassette (ABC) protein superfamily, and (ii) the major facilitator superfamily (MFS) of transporters.

Proteins belonging to the ABC superfamily are primary transporters that hydrolyse ATP molecules and are essential for substrate transport. Many ABC transporters with different topologies have been described in fungal species. In *C. albicans*, it is believed that there are 28 types of transporters, and although their role in azole resistance is not fully understood, two transporters – *Candida* drug resistance 1 (CDR1) and *Candida* drug resistance 2 (CDR2) – are well characterised.[15,88,89]

Sanglard *et al.* [90] reported that the deletion of both alleles in the *CDR1* gene in *C. albicans* resulted in increased intracellular levels of fluconazole. Moreover, this mutant is hypersensitive to azoles, terbinafine, amorolfine and several other metabolic inhibitors, including cycloheximide, brefeldin A and fluphenazine. Azole-resistant *C. albicans* isolates showed high *CDR2* gene expression levels, even higher than the *CDR1* expression levels. Furthermore, the concomitant deletion of *CDR1* and *CDR2* resulted in increased susceptibility to azoles.[88] Other studies in agreement with these findings have shown that *CDR1* and *CDR2* overexpression plays an important role in the azole resistance phenotype observed in clinical isolates of *C. albicans.*[91–94]

In other *Candida* species, functional homologues of *CDR1* and *CDR2* and other genes encoding ABC transporters have been described and were associated with drug resistance, including *CgCDR1*, *CgCDR2* (also known as *PDH1*) and *CgSNQ2* in *C. glabrata*; *CdCDR1* and *CdCDR2* in *C. dubliniensis* and *ABC1* in *C. krusei.*[15,95–102] In *C. tropicalis*, isolates with *in vitro*-induced resistance showed overexpression of *CtCDR1.*[103] However, this profile was not observed in clinical isolates of *C. tropicalis.*[104,105] In *C. parapsilosis*, Silva *et al.* [106] reported the increased expression of *CDR1* in isolates with induced resistance to fluconazole due to the expression of the transcription factor Ndt80. In *C. albicans*, Ndt80 is involved in *CDR1* regulation. In addition, this transcription factor binds to a wide range of genes with diverse biological functions, including other transporter factor, as well as genes that encode ergosterol biosynthesis enzymes.[107,108]

The second efflux system associated with azole resistance involves MFS transporters, which transport various substrates using the proton gradient generated in the plasma membrane.[89,109] On the basis of *in silico* analysis, 95 MFS transporters are believed to occur in *C. albicans*. Of these, only the product of the multidrug

resistance 1 (*MDR1*) gene has been associated with azole resistance in clinical isolates.[15] Importantly, unlike *CDR1* and *CDR2* overexpression, which culminates in resistance to various azoles, *MDR1* overexpression leads to resistance to fluconazole alone and does not appear to be associated with cross-resistance among the azoles.[15,89] Different mechanisms are involved in *MDR1* regulation, including the transcription factor Mrr1 (multidrug-resistance regulator 1).[14] Inactivation of *MRR1* in clinical *C. albicans* strains blocked *MDR1* expression.[110] Gain-of-function mutations in *MRR1* causing constitutive up-regulation of *MDR1* have been reported in different *Candida* species.[111–113]

Wirsching *et al.* [114] using gene deletion techniques, found that *C. albicans* isolates without the *MDR1* gene were extremely susceptible to fluconazole. In fact, the correlation between *MDR1* overexpression and the fluconazole resistance phenotype has been previously demonstrated.[93,94,115,116]

MDR1 homologues genes have been found in different species of *Candida*. Although the *CgFLR1* gene confers resistance to fluconazole when expressed in *Saccharomyces cerevisiae*, the actual role of this gene in the resistance phenotype in *C. glabrata* isolates is not known.[89,97] In *C. dubliniensis*, the *MDR1* gene appears to play an important role in azole resistance.[95,115,117] In *C. tropicalis*, no association was found between drug resistance of clinical isolates and *MDR1* overexpression.[104,105] In *C. parapsilosis*, *MDR1* expression has been associated with a fluconazole resistance phenotype.[106,113]

Much less is known about the role of efflux pumps in resistance development in *Aspergillus* species compared with the number of studies on resistance in *Candida*. Most studies used *A. fumigatus* as a reference, and genes coding for efflux transporters are abundant in this species. On the basis of *in silico* analysis, at least 50 genes encoding ABC transporters and 300 genes encoding MFS transporters were predicted in the genome of this fungal species.[118,119] Despite the large number of genes encoding these transporters, little is known about the association between gene overexpression and triazole resistance in *A. fumigatus*. In this species, the overexpression of the transporter genes *AfuMDR1*, *AfuMDR2*, *AfuMDR3*, *AfuMDR4* and *ATRF* is associated with triazole resistance.[119,120] The *ATRF* gene product (AtrF) is 1547 amino acids sequence and has characteristic MDR motifs.[121] The *AfuMDR4* gene is also involved in drug resistance of biofilms produced by *A. fumigatus.*[118] Da Silva Ferreira *et al.* [121] reported the expression of five ABC transporters (abcA–E) and three MFS transporters (mfsA–C) in response to the *in vitro* induction of voriconazole resistance in a clinical, azole susceptible, isolate. However, to date, the association between these findings and clinical resistance to azoles has not been fully elucidated.

Among the transporters characterised in drug-resistant clinical isolates of *A. fumigatus*, the levels of *CDR1B* have been most consistent and prominently overexpressed. In fact, the mRNA expression levels for *CDR1B* were 5- to 30-fold higher in these isolates. *CDR1B* deletion also resulted in a 4-fold increase in the susceptibility to itraconazole in isolates both resistant and susceptible to this drug.[122] Of note, the data available on the effect of efflux pumps on the decrease in the susceptibility of *A. fumigatus* to azoles were obtained from studies on resistance induced *in vitro* and not from clinical isolates for which resistance was acquired during infection of human hosts.[122]

Changes in the target site of the drug (*ERG11/CYP51*)

The inhibitory activity of triazoles via changes in the target site has been described as a frequent cause of resistance. In this setting, non-synonymous mutations in *ERG11* (*CYP51* for *Aspergillus*) can change the three-dimensional structure of lanosterol 14α-demethylase, resulting in decreased affinity of this molecule to azoles and, consequently, decreased inhibition of ergosterol biosynthesis.[15,123,124]

According to Morio *et al.* [125], more than 140 types of substitutions in the amino acid sequence of *ERG11* have been described in *C. albicans.* However, few of these substitutions have been associated with azole resistance and, when present, may not contribute to the same extent to the development of the phenotype of resistance to this drug class. To address this hypothesis, some of these substitutions, including K143R, S405F, G464S, I471T and R467K, were described only in azole-resistant isolates, whereas others, including E266D and V488I, were observed in isolates both resistant and susceptible to azoles and, therefore, do not appear to contribute to the resistance phenotype.[125–130]

Mutations in *ERG11* have also been described in other species of *Candida*, including *C. dubliniensis*, *C. krusei*, *C. tropicalis* and, more recently, *C. kefyr* and *C. parapsilosis.*[104,105,113,131–134] Although the primary mechanism involved in azole resistance appears to be the expression of efflux pumps, Hull *et al.* [135] reported the presence of a non-synonymous mutation in *ERG11* in a clinical *C. glabrata* isolate with cross-resistance between azoles and amphotericin B.

Unlike yeasts, *Aspergillus* has two distinct genes – *CYP51A* and *CYP51B* – that encode Cyp51. Most studies indicate that point mutations in the *CYP51A* gene in *A. fumigatus* confer resistance to triazoles and innate resistance to fluconazole.[136] In contrast, *CYP51B* is associated with the growth rate and maintenance of the fungal cell, but its role in the susceptibility to azoles is still unclear.[49] Clinical data suggest that *CYP51A* has an important role in regulating the activity of 14-α-demethylase, whereas *CYP51B* is a redundant gene whose expression becomes important only in the absence of *CYP51A.*[136,137]

Specific mutations in *CYP51A* may confer resistance to one, two or all triazoles, and various mutations in this gene have been described in clinical isolates and laboratory-generated mutants.[63,138] Nonsynonymous point mutations are reported more frequently in this gene, particularly in codons 54, 98, 138, 220, 431, 434 and 448.[72,139] Among these mutants, isolates with changes in codons 98, 138, 431, 434 and 448 generally have the pan-azole resistance phenotype, i.e. resistance to all azoles.[68] Denning *et al.* [140] evaluated 25 sputum samples positive for *Aspergillus* from patients with chronic pulmonary aspergillosis and allergic bronchopulmonary aspergillosis and detected mutations in codon M220 in four (16%) samples. Three of the four patients with this marker experienced treatment failure when itraconazole and/or posaconazole were used. To date, more than 30 different point mutations in *CYP51A* conferring azole resistance in *A. fumigatus* have been identified.[141–143]

Another type of azole resistance mechanism that occurs very frequently is a tandem repeat in a 34-bp sequence in the promoter region (TR_{34}) of the *CYP51A* gene. This mechanism, combined with a leucine to histidine substitution at codon 98 (L98H), resulted in increased *CYP51A* expression.[63,67,144,145] This mutation is probably associated with use of azole fungicides in agriculture and was first reported in Europe.[67] The same resistance mechanism was subsequently reported in clinical and environmental azole-resistant *A. fumigatus* from all over the world including Africa, Americas, Asia, Australia and the Middle East.[12,57,80,146,147] In contrast, more recently few studies from Japan, described azole resistance in clinical *A. fumigatus* isolates but interestingly none of them exhibited TR_{34}/L98H resistance mechanism, instead several SNPs and novel mutations, F332K and P216L were reported.[148,149] A single centre study from Japan reported 5.2% azole-resistant isolates harbouring only G54E/R/W and I266N mutation.[76,82]

Recently, a novel mechanism of resistance involving the tandem repeat of a 46-bp sequence (TR_{46}), together with the substitution of a few amino acids (Y121F/T289A), was associated with treatment failure in patients using voriconazole in the Netherlands.[73] In addition to Belgium and the Netherlands, clinical and environmental isolates carrying the TR_{46}/Y121F/T289A mutation have been identified in China, Colombia, Denmark, France, Germany, India, Spain, Tanzania and US.[12,64,150–158] The TR_{34}/L98H mutation induces pan-azole resistance, whereas the TR_{46}/Y121F/T289A mutation induces high resistance to voriconazole.[73,74,151–156]

Hodiamont *et al.* [159] evaluated a patient with chronic granulomatous disease and osteomyelitis due to *A. fumigatus* and found a mutation associated with the tandem repeat of a 53-bp sequence in the promoter region of *CYP51A* in this species. However, this mutation did not involve any amino acid substitution. It is of note that the patient received prophylaxis with itraconazole and that the isolate showed resistance to itraconazole and voriconazole and lower susceptibility to posaconazole. This same TR_{53} has recently been found in resistant environmental *A. fumigatus* isolates from Colombia.[158]

In fact, not all isolates with increased MIC values have a mutation in the *CYP51A* gene, and not every mutation implies fungal resistance to antifungal agents.[67]

Increased production and inhibition of the target enzyme by the drug

The increased production of ergosterol due to the increase in the expression of the *ERG11* gene (*CYP51*) constitutes a common azole-resistance mechanism in *C. albicans* and *A. fumigatus.*[92–94,123,160] In *C. albicans*, two mechanisms are associated with *ERG11* overexpression: (i) an increase in the expression of the transcription factor Upc2, which is responsible for regulating the expression of most genes involved in ergosterol biosynthesis; and (ii) formation of an isochromosome with two copies of the left arm of chromosome 5, where the *ERG11* gene is located, or full duplication of the chromosome.[14]

The association between increased expression of *ERG11* and azole resistance has also been reported in *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis.*[45,105,113,131,161–163]

In *A. fumigatus*, the increased production of ergosterol may be associated with the transcriptional regulation of the *CYP51A* gene, and this regulation

may be mediated by both transcription factors and tandem repeats in the promoter regions. SrbA, a transcriptional regulator belonging to the sterol regulatory element binding protein (SREBP) family, has important implications in triazole resistance in *Aspergillus.*[164] The deletion of the *SRBA* gene in *A. fumigatus* induces hyper susceptibility to azoles and increases fungal susceptibility to fluconazole. This phenomenon may be associated with decreased expression of the *CYP51A* gene in the absence of SrbA because isolates with repressed *CYP51A* also exhibited increased susceptibility to this drug.[136,165,166]

Recently, the complete genome sequences of two inbred isolates of *A. fumigatus* (one susceptible and one resistant) isolated from immunocompromised patients with chronic pulmonary aspergillosis were used to identify mutations that confer azole resistance. This study identified several non-synonymous mutations. However, only the mutation in the transcription factor associated with the CCAAT-sequence in a subunit of the HapE complex conferred resistance of this progeny to azoles by increasing the *CYP51A* gene expression levels.[167] CCAAT-sequences are present in the promoters of a large number of eukaryotic genes. In general, they are formed by approximately 50- to 200-bp sequences upstream of the transcription initiation site and may occur in any orientation of the gene. These sequences are attached to different transcription factors, which can explain the diversity of functions associated with the modulation of the transcription levels in eukaryotic cells.[168] Coincidentally, the CCAAT-sequence is present in the promoter of the *CYP51A* gene. Therefore, the *hapE* mutant can induce the increase in the expression of the *CYP51A* mRNA, demonstrating that transcription factors can regulate the expression of these genes.[167] However, to date, these changes have not yet been described among resistant clinical isolates.

Risk factors for the development of candidaemia by fluconazole-resistant isolates

The first epidemiological studies that assessed the risk for developing bloodstream infection (BI) due to *C. glabrata* and *C. krusei,* which are intrinsic resistant to fluconazole, provided clinicians with information necessary for the early detection of patients at risk of developing IFIs due to fluconazole-resistant *Candida* isolates. In these cases, the independent variables associated with BI due to *C. glabrata* included the previous use of fluconazole or caspofungin, recent exposure to metronidazole, surgical procedures, the presence of central venous catheter (CVC) position, cancer, mechanical ventilation and senescence.[169–171] For infections due to *C. krusei*, the independent risk factors included previous exposure to fluconazole or caspofungin, neutropenia, solid tumours, organ transplantation, splenectomy and use of antibiotics with antianaerobic coverage.[170–172]

Over the past few years, an increase in the number of cases of candidaemia due to fluconazole-resistant isolates belonging to species primarily sensitive to this drug, including *C. parapsilosis* and *C. tropicalis* were noted.[17,18,20–22,26,28,38,44] Several studies have explored the predisposing factors for the development of candidaemia due to fluconazole-resistant *Candida* species.

In this scenario, Garnacho-Monteiro *et al.* [173] observed that neutropenia, chronic kidney disease and previous exposure to fluconazole were independent risk factors for the isolation of fluconazole-resistant *Candida* isolates. Recently, Cuervo *et al.* [174], on the basis of clinical data easily obtained at the bedside, developed and validated a score that allows for the estimation of the risk of candidaemia due to fluconazole-non-susceptible (Flu-NS) isolates. The multivariate analysis indicated that the following variables were classified as risk factors for fluconazole resistance: previous use of azoles for 3 days, hospitalisation in units with a prevalence of candidaemia due to Flu-NS isolates >15%, and previous history of transplantation.

Risk factors for resistance development in *A. fumigatus* worldwide: epidemiological model in the Netherlands and the UK

High rates of triazole resistance in *A. fumigatus* were reported in the Netherlands and in the UK.[68] The first clinical case of resistance to itraconazole was reported in 1997.[175] In 2000, Moore *et al.* [70,176] tested the susceptibility of more than 900 isolates to amphotericin B and itraconazole. The percentage of patients harbouring *A. fumigatus* isolates resistant to this drug class increased to 15% in 2007 and to 20% in 2009. In the Netherlands, azole resistance increased dramatically from 2.5% in 2000, to 4.9% in 2002, to 6.6% in 2004, to 10% in 2009.[46] By 2013 in certain centres resistance rates of up to 15% are found in high risk haematology patients.[75] This trend represents a yearly increase of 6%, and in this case, the patients were infected by resistant isolates but without prior exposure to antifungal therapy.[62] Therefore, although higher rates of resistance in *A. fumigatus* were described in the Netherlands and the UK (Manchester),

it is essential to recognise that the mode of resistance development had distinct determinants in these countries.

In Manchester, where the National Aspergillosis Centre is located, the hypothesis of resistance is attributed to the use of long-term antifungal therapy in patients with chronic forms of pulmonary aspergillosis, more specifically, aspergilloma and chronic cavitary aspergillosis. In the latter case, some researchers suggested the occurrence of microevolution of the fungus in the lung tissue because both sensitive and resistant isolates with identical or closely related genotypes were reported to infect the same patient.[177,178]

In the Netherlands, the occurrence of triazole resistance in *Aspergillus* is closely associated with so called environmental mutations. It is believed that the use of azoles as fungal pesticides has led to the emergence of mutations in the *CYP51A* gene.[46,57] From approximately 30 azole fungicides commonly used in agriculture, seven showed cross-resistance with azoles in clinical use.[79,179] Two resistance mechanisms associated with point mutations in *CYP51A* (TR_{34}/L98H and TR_{46}/Y121F/T289A) have been reported in clinical and environmental isolates, and the latter were recovered from patients not exposed to antifungal agents.[151–153]

An increasing number of cases involving azole-resistant environmental isolates of *A. fumigatus* have been documented in Asia and Europe.[78,79,83,180] It is suggested that most of the clinical cases of resistance reported between 2009 and 2011 from Dutch patients were due to the dissemination of resistant environmental isolates.[181] Between 2012 and 2015, other countries, including Austria, Belgium, Denmark, France, Germany, Ireland, Poland, Spain, Sweden and the UK, also reported the identification of clinical isolates of *A. fumigatus* harbouring the TR_{34}/L98H mutation.[12,65,141,152,181–183]

The wide geographical distribution of the TR_{34}/L98H mutation has raised questions about the origin of azole-resistant *Aspergillus* isolates, including (i) the geographical migration of resistant conidia carrying this mutation, (ii) the independent local development of this mutation and subsequent selection of the TR_{34}/L98H mutation in unrelated isolates and (iii) the previous two hypotheses occurring simultaneously.[144] To clarify the origin and dissemination of this genotype, Camps *et al.* [181], using molecular markers, evaluated the genetic relationship of 142 European isolates recovered between 1998 and 2007. The authors noted that the isolates with the TR_{34}/L98H resistance mechanism showed less genetic variation compared with wild-type susceptible isolates or with those with other resistance mechanisms. In addition, the crossing of these isolates indicated that the TR_{34}/L98H isolates could cross with azole susceptible isolates of different genetic origins, suggesting that TR_{34}/L98H isolates could complete their natural sexual cycles. This finding suggests the occurrence of a common ancestry for the TR_{34}/L98H mechanism.[181]

Chowdhary *et al.* [79] evaluated nine loci in azole-resistant and susceptible isolates from India and observed the occurrence of a wide genetic diversity among environmental and clinical isolates susceptible to azoles, and these isolates were highly polymorphic. The azole-resistant isolates harbouring the TR_{34}/L98H mutation shared the same genotype, indicating clonal propagation. However, this genotype was not detected in other isolates from other countries, such as China, France, Germany and the Netherlands. Therefore, the authors suggested that the Indian genotype was an adaptive recombinant progeny derived from the crossing between an azole-resistant isolate (that migrated into India) and an azole-sensitive isolate (that was native to India); this crossing underwent mutation and quickly dispersed to various parts of India.[78]

Of note, 61% of the global agricultural fungicide market is concentrated in Western Europe and Asia, suggesting that the prevalence of the TR_{34}/L98H mutation in the *CYP51A* gene is directly associated with geographical parameters and the indiscriminate use of these antifungal fungicides.[184]

With regard to the emerging TR_{46}/Y121F/T289A mutation, to date, clinical isolates carrying this mutation have been detected in China, Belgium, Denmark, France, Germany, the Netherlands, Spain and the US, whereas environmental isolates with this mechanism have been reported in Belgium, Colombia, France, Germany, India, the Netherlands and Tanzania.[12,64,150–152,157,158,184]

At this point, an increased concern about the rapid spread of isolates harbouring these two mutations should be underscored because these cases have been reported in hospitals and homes throughout Europe.[12,73] Furthermore, the exposure of *A. fumigatus* to azole fungicides can induce the emergence of novel resistance mechanisms over time, thereby compromising the clinical use of azoles in the treatment of diseases associated with *Aspergillus*.

Rates of echinocandin resistance in *Candida* spp. and *Aspergillus* spp. worldwide

Echinocandins represent the most recent antifungal class, consisting of three agents – micafungin,

anidulafungin and caspofungin – that act by inhibiting cell wall synthesis. Caspofungin was the first echinocandin approved by the Food and Drug Administration (FDA) in 2001 in the US, followed by micafungin and anidulafungin, which were approved in 2005 and 2006, respectively, and the three drugs are available only in parenteral preparations.[185–187] Since their introduction, echinocandins have been widely used for the treatment and empirical therapy of fungal infections due to *Candida* and *Aspergillus.*[188–190] Although echinocandin resistance is still considered unusual, cases of resistance by using this therapeutic class have become increasingly frequent.[42,191]

Recently, Cleveland *et al.* [192] conducted a surveillance study in two US cities between 2008 and 2013 involving invasive candidiasis in a prospective cohort. In this series, increases in cases of echinocandin resistance were observed in Atlanta (from 1.2% to 2.9%, a 147% increase) and Baltimore (2% to 3%, a 77% increase). In addition the authors reported that 44% of the multidrug-resistant isolates that exhibited an echinocandin- and fluconazole resistance phenotype were recovered from patients without prior exposure to echinocandins, suggesting the occurrence of horizontal transmission of this resistant phenotype.

Although this resistance mechanism remains uncommon in isolates of *C. albicans*, *C. krusei* and *C. tropicalis*, echinocandin resistance has become increasingly common among isolates of *C. glabrata* and other species such as *C. lusitaniae* and *C. kefyr.*[42,193] Recently, Alexander *et al.* [194] reported that in the US, the resistance rate of *C. glabrata* isolates increased from 4.9% to 12.3% between 2001 and 2010. In Europe, the same trend is observed, although on a smaller scale. Accordingly, a study reported that the resistance rate of *C. glabrata* isolates increased from 0% to 3.1% between 2004 and 2013 in Denmark.[195] It is important to mention that the factors associated with the increased development of resistance in *C. glabrata* isolates remain unknown.[195]

In Latin America, a single case report has demonstrated clinical and microbiological echinocandin resistance in *Candida*. Bizerra *et al.* [196], using sequencing methodologies for the study of *FKS* genes and quantification of glucan synthesis, reported the occurrence of a mutation associated with this resistance phenotype in *C. glabrata* isolated from a single patient.

To date, little is known about echinocandin resistance in *Aspergillus*, particularly because susceptibility tests are not performed routinely and present technical difficulties and problems associated with reproducibility and the clinical-*in vitro* testing correlation.[197,198]

Because the therapeutic outcome of treatment of aspergillosis with echinocandins is poorer than with polyenes and triazoles, the use of echinocandins in these patients is limited and restricted to cases involving empirical or combination therapies.[189,198,199] Consequently, few cases of secondary resistance of *Aspergillus* to this therapeutic class have been reported. However, non-*Aspergillus fumigatus* species may exhibit primary resistance, as is the case in *A. lentulus.*[200,201]

Molecular mechanisms of echinocandin resistance in *Candida* spp. and *Aspergillus* spp.

Echinocandins present predominantly fungicidal activity against *Candida* spp. and fungistatic activity against *Aspergillus* spp.[202–204]

Experimental data have shown that the primary mechanism of action of echinocandins is associated with inhibition of the synthesis of the fungal cell wall via inhibition of β-1,3-glucan synthase (Fks1/AfFks1 for *Aspergillus fumigatus*), which is responsible for the synthesis of β-1,3-glucans.[205] In fungi belonging to the phylum *Ascomycota*, the paralogous genes *FKS1*, *FKS2* and *FKS3* are responsible for the synthesis of β-1,3-glucan synthase. In most species, the *FKS1* gene is more active, whereas *FKS2* is expressed in adverse conditions and only during the sexual cycle and sporulation.[205] The *FKS3* gene is involved in the formation of the cell walls of spores.[206] In some species, including *C. glabrata*, *FKS1* and *FKS2* are functionally redundant.[207] In *Aspergillus* spp., only *FKS1* has been described.[208] The echinocandin resistance mechanisms reported in *Candida* spp. and *Aspergillus* spp. will be discussed below.

Mutations in the FKS genes

The main resistance mechanism described for echinocandins involves the occurrence of mutations in the *FKS1* gene, resulting in conformational changes in the enzyme encoded by this gene (Fks1), decreased affinity between echinocandins and Fks1, and the consequent resistance to these compounds.[209–212] These mutations occur in two specific, highly conserved regions of the *FKS1* gene known as hot spot 1 (HS1), corresponding to positions 641-649 in the amino acid sequence of the Fks1 protein, and hot spot 2 (HS2), spanning positions 1345-1365 of the amino acid sequence.[211,213]

In *C. albicans*, among the mutations already described, the replacement of serine with proline,

phenylalanine or tyrosine at position 645 has been described as the most frequent event.[14,211,213–215] In fact, Balashov *et al.* [209] found that, among 85 caspofungin-resistant isolates of *C. albicans*, 93% had a mutation in the serine residue at position 645.

Mutations in *FKS1* have been described not only in *C. albicans* but also in *C. krusei, C. parapsilosis* and *C. tropicalis* and, more recently, in *C. kefyr*.[201,210,213,214,216,217] In *C. glabrata*, in addition to mutations in *FKS1*, mutations in the *FKS2* gene have been associated with echinocandin resistance.[196,218–221]

In contrast to the mutations described for the *Candida* species that develop secondary resistance to echinocandins, isolates of the complexes *C. guilliermondii* and *C. parapsilosis* develop natural mutations, not due to exposure to the drug, which explain their higher MIC values to all echinocandins.[222–224] In fact, the Fks1 coding regions of all members of the *C. parapsilosis* complex contain a mutation at position 660 of the amino acid sequence involving the substitution of proline with alanine.[216] Similarly, it has been reported that the Fks1 coding regions of all isolates of *C. guilliermondii* contain a substitution at position 642 of the amino acid sequence.[211] Despite the higher MIC values, various experimental and clinical studies have demonstrated that these drugs exhibit inhibitory activity against *C. parapsilosis* infections. However, their antifungal activity is only fungistatic and not fungicidal, which may explain the higher incidence of persistent candidaemia with *C. parapsilosis* in patients treated with echinocandins.[225–229]

Despite the advances made in the characterisation of echinocandin resistance mechanisms in *Candida*, to date, little is known about the molecular basis of *Aspergillus* resistance to these drugs.[56] Moreover, few clinical isolates associated with treatment failure have been investigated. Arendrup *et al.* [230] evaluated the echinocandin resistance mechanism of an isolate of *A. fumigatus* isolated from a patient with invasive pulmonary aspergillosis who experienced treatment failure with caspofungin; this isolate did not harbour mutations in the *AfFKS1* gene; therefore, the β-1,3-glucan synthase was completely sensitive *in vitro*. However, the *AfFKS1* gene was overexpressed, indicating that treatment with caspofungin did not decrease the expression of this gene. Similarly, Arendrup *et al.* [231] studied the amplified DNA of *A. fumigatus* from liver and lung tissue samples from a patient with aspergillosis subjected to long-term therapy with caspofungin, but did not find any mutations in the sequence of the hot spot region of *AfFKS1*.

Because the reports of echinocandin resistance in clinical isolates are rare, several researchers have sought *in vitro* models for the induction of resistance in the laboratory with the aim of better characterising the resistance mechanisms associated with these drugs. Gardiner *et al.* [232] inserted a site-directed mutation in *AfKS1* consisting of a substitution of serine with tyrosine at codon 678 (S678Y). This mutation decreased the susceptibility to caspofungin 16-fold, with a minimum effective concentration (MEC) of 4 μg/mL. This same study used mini-array analysis of 220 target genes to evaluate the expression of relevant genes that could contribute to the decrease in the susceptibility to caspofungin in mutant isolates. Their results allowed the identification of (i) increased expression levels of 28 genes encoding structural components of the cell wall (*AGS2*, *CWP1*, *MCI36*), (ii) enzymes responsible for cell wall biosynthesis (Kre1, Mci35) and (iii) signal transducers (MAPK, MCI106). The expression levels of genes encoding proteins involved in transportation, such as *MDR1* and *MDR4*, were also increased in this mutant.[232]

Rocha *et al.* [233] generated a mutant by the substitution of serine with proline at codon 678 (S678P) in *AfKS1*, and this change was sufficient to confer resistance to three echinocandins, demonstrating that the modification in Fks1 is a conserved mechanism in echinocandin resistance among pathogenic fungi.

Of note are the differences in the *in vitro* susceptibility profile to echinocandins between different species of *Aspergillus*. For example *A. niger* exhibited higher MECs to echinocandins compared with other species, suggesting the occurrence of differences in the cell wall composition in these fungal species.[234] In contrast, *A. lentulus* was less susceptible to caspofungin, although it was apparently susceptible to micafungin and anidulafungin. The analysis of the *FKS1* region of this fungal species indicated no polymorphism in the hot spot region of this gene.[56,197] One hypothesis to explain this phenomenon is the excessive production of glucan by this species, which could limit the effective concentrations of antifungal agents.[208]

Increase in chitin synthesis

The increase in chitin synthesis has been described as an important resistance mechanism against echinocandins. Walker *et al.* [235] have shown that the exposure of *C. albicans* to low levels of echinocandins induces the expression of genes encoding chitin synthases, increases the concentration of chitin in the cell wall and consequently decreases the activity of

echinocandin. Corroborating this hypothesis, Plaine *et al.* [236] demonstrated that *C. albicans* mutants with higher levels of chitin in the cell wall were resistant to caspofungin, whereas those with decreased levels of chitin were very susceptible to this drug.

Increased chitin concentrations after exposure to echinocandins have also been reported in *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis.*[237,238]

Risk factors for the development of candidaemia with echinocandin-resistant isolates

Although still rare, cases of echinocandin resistance in clinical isolates of *Candida* have been increasingly reported, in particular among *C. glabrata* isolates.[193]

Using case–control studies, several authors have highlighted that prior exposure to echinocandins results in a higher risk of developing candidaemia due to the lower susceptibility of these isolates to this class of antifungal agents.[215,239,240] For infections due to *C. glabrata*, in addition to the previous exposure to antifungal agents, exposure to total parenteral nutrition (TPN) has also been reported to be a predisposing factor.[241–243] Recent studies have indicated the development of echinocandin resistance after short periods of exposure to these antifungal agents. In fact, Ruggero and Topal identified a *C. albicans* isolate with a *FKS1* mutation after 14 days of prophylactic therapy with micafungin.[244]

Resistance to amphotericin B

Amphotericin B is an antifungal agent belonging to the class of polyenes which are natural antifungal compounds produced by species of the genus *Streptomyces*. Since the 1950s until the discovery of azoles, standard therapy for IFIs involved the use of amphotericin B. Because of its broad spectrum of activity against yeasts, filamentous and dimorphic fungi and its fungicidal activity, amphotericin B based drugs are still considered the gold standard for the treatment of most fungal infections, particularly in cases of severe IFI, when a quick response is required.[89,200,245–247]

The classical mechanism of action of amphotericin B involves the irreversible binding to ergosterol present in the plasma membranes of fungal cells, resulting in the formation of pores in the cell membrane, with loss of K^+ and Na^+ ions, which leads to impairment of the cellular osmotic balance and consequent cell death.[89,248,249] Furthermore, recent findings have shown that exposure to amphotericin B induces the production and accumulation of reactive oxygen species, which eventually causes cell death.[250,251]

Although amphotericin B has a broad spectrum of activity, this antifungal drug is highly toxic to the host. Nephrotoxicity is the major side-effect, which has limited its clinical use.[188,252–256] To decrease drug toxicity, lipid formulations have been developed which are currently first choice of treatment of IFIs.[257–261]

Secondary resistance to amphotericin B is rare among isolates of *Candida* spp., but some cases of resistance have been reported.[135,262–267] Several studies have shown resistance of *C. lusitaniae* to this drug. Although these studies reported that *C. lusitaniae* isolates have low rates of resistance *in vitro*, this species seems to develop secondary resistance during treatment with amphotericin B, and several cases of therapeutic failure are on record.[267–274]

Similar to *Candida*, secondary resistance to amphotericin B has not been observed in *Aspergillus*, although some patients experienced treatment failure when using this drug.[262,263,275] However, primary resistance to amphotericin B has been reported among isolates of *A. flavus*, *A. lentulus*, *A. terreus* and *A. ustus.*[48,53,276–280]

Previous studies have also shown that isolates of *A. flavus* exhibit higher MIC values to amphotericin B compared with isolates belonging to the section *Fumigati.*[65,199,276,277,279,281]

Hadrich *et al.* [277] evaluated the *in vitro* susceptibility to amphotericin B of 37 *A. flavus* isolates recovered from 14 patients with haematological malignancies, of which 13 were treated with amphotericin B deoxycholate. Of the total isolates evaluated, 84% ($N = 31$) exhibited resistance to this drug *in vitro*. Among the patients infected with resistant isolates, nine were treated with amphotericin B, and 88% ($N = 7$) died.[277] Similar results were observed by Lass-Flörl *et al.* [281], who compared the *in vitro* susceptibility to amphotericin B in strains of *A. flavus* with clinical outcome of patients undergoing bone narrow transplantation. In this study, 12 *A. flavus sensu lato* isolates were evaluated. Only patients infected with susceptible isolates (MIC to AMB <2 μg/mL, $N = 4$) survived, whereas those infected with resistant isolates (CIM to AMB ≥2 μg/mL, $N = 8$) died.[281]

Alastruey-Izquierdo *et al.* [65] evaluated 280 clinical isolates of *Aspergillus* from 29 Spanish hospitals and reported a rate of resistance to amphotericin B of 10.8%. In this series, four of 27 (14.8%) *A. flavus* isolates and seven of 26 (27%) *A. terreus* isolates were resistant to this drug. In Brazil, Gonçalves *et al.* [54]

observed a high rate of resistance (49%) to amphotericin B in a series consisting of 77 clinical isolates belonging to the section *Flavi*. Likewise studies from India show high amphotericin B MICs in 31 out of 37 isolates (84%) [277]. However, the genetic mechanisms responsible for acquired resistance to amphotericin B have not been elucidated. Some studies have indicated that mutations in the genes involved in the biosynthetic pathway of ergosterol (*ERG*), including *ERG2*, *ERG3*, *ERG5*, *ERG6* and *ERG11*, may lead to decreased concentration of ergosterol and the formation of intermediate sterols in the fungal cell membrane.[104,282–286] These quantitative and qualitative changes in plasma membrane sterols decrease the affinity of amphotericin B for its target sites and constitute the major mechanisms of resistance of *Candida* isolates to this drug.

Although most of the data related to resistance mechanisms for amphotericin B have been observed in trials with yeast, Chamilos and Kontoyiannis thoroughly reviewed these mechanisms in *Aspergillus*. These authors reported that these mechanisms can be attributed to two phenomena: (i) a decreased concentration of membrane ergosterol via mutations in the biosynthetic pathway of ergosterol and/or prior exposure to triazoles, and (ii) increased production of reducing enzymes (e.g. catalase), which confer resistance to oxidative stress and can also act as oxidising agents.[287]

Although *A. terreus* is the best studied species, its intrinsic mechanism of resistance to amphotericin B is not fully elucidated but has been attributed to the high expression levels of catalase, an enzyme that causes damage to the fungal cell membrane via generation of reactive oxygen species.[288] A recent study showed that application of pro-oxidants significantly affects amphotericin B efficacy by rendering resistant isolates more susceptible to this drug which was also confirmed *in vivo* in a *Galleria* model.[289]

Conclusions

Over the past few years, the significant increase in the use of antifungal agents for the treatment of candidiasis and invasive aspergillosis has resulted in the emergence of resistant clinical isolates, particularly against triazoles and echinocandins. In addition, a recent emergence of fluconazole resistance among isolates that were primarily sensitive to this drug, including *C. parapsilosis* and *C. tropicalis* has been noted. For echinocandins, despite the low resistance rates, the occurrence of isolates with lower susceptibility to this drug has been increasingly reported, particularly among *C. glabrata* isolates.

Therefore, we highlight the need to conduct antifungal resistance surveillance studies using clinical isolates of *Candida* and *Aspergillus* in different geographical regions and monitoring of the infection rates in distinct population groups with the aims of early detection of resistance to these drugs and implementation of efficient policies for infection control and treatment.

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Potential conflicts of interest

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References

1 Magill SS, Edwards JR, Bamberg W *et al.* Multistate point-prevalence survey of health care-associated infections. *N Engl J Med* 2014; **370**: 1198–208.
2 Garey KW, Aitken SL, Dima-Ala A *et al.* Echinocandin use in hospitalized patients: a multi-institutional study. *Am J Med Sci* 2015; **349**: 316–20.
3 Meersseman W, Lagrou K, Maertens J, Van Wijngaerden E. Invasive aspergillosis in the intensive care unit. *Clin Infect Dis* 2007; **45**: 205–16.
4 Arendrup MC, Dzajic E, Jensen RH *et al.* Epidemiological changes with potential implication for antifungal prescription recommendations for fungaemia: data from a nationwide fungaemia surveillance programme. *Clin Microbiol Infect* 2013; **19**: E343–53.
5 Nucci M, Queiroz-Telles F, Tobon AM, Restrepo A, Colombo AL. Epidemiology of opportunistic fungal infections in Latin America. *Clin Infect Dis* 2010; **51**: 561–70.
6 Fraczek MG, Kirwan MB, Moore CB, Morris J, Denning DW, Richardson MD. Volume dependency for culture of fungi from

respiratory secretions and increased sensitivity of *Aspergillus* quantitative PCR. *Mycoses* 2014; **57**: 69–78.
7 Allou N, Allyn J, Montravers P. When and how to cover for fungal infections in patients with severe sepsis and septic shock. *Curr Infect Dis Rep* 2011; **13**: 426–32.
8 Bow EJ, Vanness DJ, Slavin M *et al.* Systematic review and mixed treatment comparison meta-analysis of randomized clinical trials of primary oral antifungal prophylaxis in allogeneic hematopoietic cell transplant recipients. *BMC Infect Dis* 2015; **15**: 128.
9 Candoni A, Caira M, Cesaro S *et al.* Multicentre surveillance study on feasibility, safety and efficacy of antifungal combination therapy for proven or probable invasive fungal diseases in haematological patients: the SEIFEM real-life combo study. *Mycoses* 2014; **57**: 342–50.
10 Apisarnthanarak A, Yatrasert A, Mundy LM. Impact of education and an antifungal stewardship program for candidiasis at a Thai tertiary care center. *Infect Control Hosp Epidemiol* 2010; **31**: 722–7.
11 Valerio M, Munoz P, Rodriguez-Gonzalez C *et al.* Training should be the first step toward an antifungal stewardship program. *Enferm Infecc Microbiol Clin* 2015; **33**: 221–7.
12 Verweij PE, Chowdhary A, Melchers WJ, Meis JF. Azole resistance in *Aspergillus fumigatus*: Can we retain the clinical use of mold-active antifungal azoles? *Clin Infect Dis* 2015; **2016**(62): 362–8. pii: civ885. [Epub ahead of print].
13 Sanguinetti M, Posteraro B, Lass-Florl C. Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. *Mycoses* 2015; **58**(Suppl 2): 2–13.
14 Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of antifungal drug resistance. *Cold Spring Harb Perspect Med* 2015; **5**: a019752.
15 Das I, Nightingale P, Patel M, Jumaa P. Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK. *Int J Infect Dis* 2011; **15**: e759–63.
16 Arendrup MC, Bruun B, Christensen JJ *et al.* National surveillance of fungemia in Denmark (2004 to 2009). *J Clin Microbiol* 2011; **49**: 325–34.
17 Peman J, Canton E, Minana JJ *et al.* [Changes in the epidemiology of fungaemia and fluconazole susceptibility of blood isolates during the last 10 years in Spain: results from the FUNGEMYCA study]. *Rev Iberoam Micol* 2011; **28**: 91–9.
18 Tragiannidis A, Fegeler W, Rellensmann G *et al.* Candidaemia in a European Paediatric University Hospital: a 10-year observational study. *Clin Microbiol Infect* 2012; **18**: E27–30.
19 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.
20 Lockhart SR, Iqbal N, Cleveland AA *et al.* Species identification and antifungal susceptibility testing of *Candida* bloodstream isolates from population-based surveillance studies in two U.S. cities from 2008 to 2011. *J Clin Microbiol* 2012; **50**: 3435–42.
21 Asmundsdottir LR, Erlendsdottir H, Gottfredsson M. Nationwide study of candidemia, antifungal use, and antifungal drug resistance in Iceland, 2000 to 2011. *J Clin Microbiol* 2013; **51**: 841–8.
22 Ericsson J, Chryssanthou E, Klingspor L *et al.* Candidaemia in Sweden: a nationwide prospective observational survey. *Clin Microbiol Infect* 2013; **19**: E218–21.
23 Klingspor L, Tortorano AM, Peman J *et al.* Invasive *Candida* infections in surgical patients in intensive care units: a prospective, multicentre survey initiated by the European Confederation of Medical Mycology (ECMM) (2006-2008). *Clin Microbiol Infect* 2015; **21**: e1–10.
24 Puig-Asensio M, Padilla B, Garnacho-Montero J *et al.* Epidemiology and predictive factors for early and late mortality in *Candida* bloodstream infections: a population-based surveillance in Spain. *Clin Microbiol Infect* 2014; **20**: O245–54.
25 Pfaller MA, Jones RN, Castanheira M. Regional data analysis of *Candida* non-*albicans* strains collected in United States medical sites over a 6-year period, 2006-2011. *Mycoses* 2014; **57**: 602–11.
26 Pfaller MA, Andes DR, Diekema DJ *et al.* Epidemiology and outcomes of invasive candidiasis due to non-albicans species of *Candida* in 2,496 patients: data from the Prospective Antifungal Therapy (PATH) registry 2004-2008. *PLoS ONE* 2014; **9**: e101510.
27 Guinea J, Zaragoza O, Escribano P *et al.* Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. *Antimicrob Agents Chemother* 2014; **58**: 1529–37.
28 Moretti ML, Trabasso P, Lyra L *et al.* Is the incidence of candidemia caused by Candida glabrata increasing in Brazil? Five-year surveillance of *Candida* bloodstream infection in a university reference hospital in southeast Brazil. *Med Mycol* 2013; **51**: 225–30.
29 Colombo AL, Garnica M, Aranha Camargo LF *et al. Candida glabrata*: an emerging pathogen in Brazilian tertiary care hospitals. *Med Mycol* 2013; **51**: 38–44.
30 Colombo AL, Guimaraes T, Sukienik T *et al.* Prognostic factors and historical trends in the epidemiology of candidemia in critically ill patients: an analysis of five multicenter studies sequentially conducted over a 9-year period. *Intensive Care Med* 2014; **40**: 1489–98.
31 Omrani AS, Makkawy EA, Baig K *et al.* Ten-year review of invasive *Candida* infections ina tertiary care center in Saudi Arabia. *Saudi Med J* 2014; **35**: 821–6.
32 Tan BH, Chakrabarti A, Li RY *et al.* Incidence and species distribution of candidaemia in Asia: a laboratory-based surveillance study. *Clin Microbiol Infect* 2015; **21**: 946–53.
33 Cheng YR, Lin LC, Young TG, Liu CE, Chen CH, Tsay RW. Risk factors for candidemia-related mortality at a medical center in central Taiwan. *J Microbiol Immunol Infect* 2006; **39**: 155–61.
34 Chowdhary A, Becker K, Fegeler W *et al.* An outbreak of candidemia due to *Candida tropicalis* in a neonatal intensive care unit. *Mycoses* 2003; **46**: 287–92.
35 Kothavade RJ, Kura MM, Valand AG, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol* 2010; **59**: 873–80.
36 Taj-Aldeen SJ, Kolecka A, Boesten R *et al.* Epidemiology of candidemia in Qatar, the Middle East: performance of MALDI-TOF MS for the identification of *Candida* species, species distribution, outcome, and susceptibility pattern. *Infection* 2014; **42**: 393–404.
37 Ellis M, Hedstrom U, Jumaa P, Bener A. Epidemiology, presentation, management and outcome of candidemia in a tertiary care teaching hospital in the United Arab Emirates, 1995-2001. *Med Mycol* 2003; **41**: 521–8.
38 Chowdhary A, Sharma C, Duggal S *et al.* New clonal strain of *Candida auris*, Delhi. *India. Emerg Infect Dis* 2013; **19**: 1670–3.
39 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.
40 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.
41 Sharma C, Kumar N, Meis JF, Pandey R, Chowdhary A. Draft genome sequence of a fluconazole-resistant *Candida auris* strain from a candidemia patient in India. *Genome Announc* 2015; pii: e00722-15. doi: 10.1128/genomeA.00722-15.
42 Jung DS, Farmakiotis D, Jiang Y, Tarrand JJ, Kontoyiannis DP. Uncommon *Candida* species fungemia among cancer patients, Houston, Texas. *USA. Emerg Infect Dis* 2015; **21**: 1942–50.
43 Minea B, Nastasa V, Moraru RF *et al.* Species distribution and susceptibility profile to fluconazole, voriconazole and MXP-4509 of 551 clinical yeast isolates from a Romanian multi-centre study. *Eur J Clin Microbiol Infect Dis* 2015; **34**: 367–83.
44 Pinhati HM, Bizerra FC, Siqueira RA, Castro FF, Colombo AL. Outbreak of candidemia due fluconazole resistant Candida parapsilosis strains in a tertiary care hospital in Brazil. Fifty-third Interscience Conference

on Antimicrobial Agents and Chemotherapy. American Society for Microbiology: Denver, CO, 2013.
45 Souza AC, Fuchs BB, Pinhati HM *et al. Candida parapsilosis* resistance to fluconazole: Molecular mechanisms and in vivo impact in infected Galleria mellonella larvae. *Antimicrob Agents Chemother* 2015; **59**: 6581–7.
46 Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009; **9**: 789–95.
47 van der Linden JW, Snelders E, Arends JP, Daenen SM, Melchers WJ, Verweij PE. Rapid diagnosis of azole-resistant aspergillosis by direct PCR using tissue specimens. *J Clin Microbiol* 2010; **48**: 1478–80.
48 Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of A. fumigatus. *Eukaryot Cell* 2005; **4**: 625–32.
49 Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, Cuenca-Estrella M, Rodriguez-Tudela JL. *Aspergillus* section *Fumigati*: antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother* 2008; **52**: 1244–51.
50 Bastos VR, Santos DW, Padovan AC *et al.* Early invasive pulmonary aspergillosis in a kidney transplant recipient caused by *Aspergillus lentulus*: first Brazilian report. *Mycopathologia* 2015; **179**: 299–305.
51 Barchiesi F, Mazzocato S, Mazzanti S *et al.* Invasive aspergillosis in liver transplant recipients: epidemiology, clinical characteristics, treatment, and outcomes in 116 cases. *Liver Transpl* 2015; **21**: 204–12.
52 Kathuria S, Sharma C, Singh PK *et al.* Molecular epidemiology and in-vitro antifungal susceptibility of *Aspergillus terreus* species complex isolates in Delhi, India: evidence of genetic diversity by amplified fragment length polymorphism and microsatellite typing. *PLoS ONE* 2015; **10**: e0118997.
53 Azzola A, Passweg JR, Habicht JM *et al.* Use of lung resection and voriconazole for successful treatment of invasive pulmonary *Aspergillus ustus* infection. *J Clin Microbiol* 2004; **42**: 4805–8.
54 Goncalves SS, Stchigel AM, Cano J, Guarro J, Colombo AL. In vitro antifungal susceptibility of clinically relevant species belonging to *Aspergillus* section *Flavi*. *Antimicrob Agents Chemother* 2013; **57**: 1944–7.
55 Shivaprakash MR, Geertsen E, Chakrabarti A, Mouton JW, Meis JF. In vitro susceptibility of 188 clinical and environmental isolates of *Aspergillus flavus* for the new triazole isavuconazole and seven other antifungal drugs. *Mycoses* 2011; **54**: e583–9.
56 Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Med Mycol* 2011; **49**(Suppl 1): S90–5.
57 Chowdhary A, Kathuria S, Xu J, Meis JF. Emergence of azole-resistant *Aspergillus fumigatus* strains due to agricultural azole use creates an increasing threat to human health. *PLoS Pathog* 2013; **9**: e1003633.
58 Paul RA, Rudramurthy SM, Meis JF, Mouton JW, Chakrabarti A. A novel Y319H substitution in *Cyp51C* associated with azole resistance in *Aspergillus flavus*. *Antimicrob Agents Chemother* 2015; **59**: 6615–9.
59 Arendrup MC, Jensen RH, Grif K *et al.* In vivo emergence of *Aspergillus terreus* with reduced azole susceptibility and a *Cyp51A* M217I alteration. *J Infect Dis* 2012; **206**: 981–5.
60 Pfaller MA, Messer SA, Boyken L *et al.* In vitro survey of triazole cross-resistance among more than 700 clinical isolates of *Aspergillus* species. *J Clin Microbiol* 2008; **46**: 2568–72.
61 Pham CD, Reiss E, Hagen F, Meis JF, Lockhart SR. Passive surveillance for azole-resistant *Aspergillus fumigatus*, United States, 2011-2013. *Emerg Infect Dis* 2014; **20**: 1498–503.
62 Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. *Bull World Health Organ* 2011; **89**: 864–72.
63 Mellado E, Garcia-Effron G, Alcazar-Fuoli L *et al.* A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of *cyp51A* alterations. *Antimicrob Agents Chemother* 2007; **51**: 1897–904.
64 Fischer J, van Koningsbruggen-Rietschel S, Rietschel E *et al.* Prevalence and molecular characterization of azole resistance in *Aspergillus* spp. isolates from German cystic fibrosis patients. *J Antimicrob Chemother* 2014; **69**: 1533–6.
65 Alastruey-Izquierdo A, Mellado E, Pelaez T *et al.* Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). *Antimicrob Agents Chemother* 2013; **57**: 3380–7.
66 Baddley JW, Marr KA, Andes DR *et al.* Patterns of susceptibility of Aspergillus isolates recovered from patients enrolled in the Transplant-Associated Infection Surveillance Network. *J Clin Microbiol* 2009; **47**: 3271–5.
67 Snelders E, van der Lee HA, Kuijpers J *et al.* Emergence of azole resistance in Aspergillus fumigatus and spread of a single resistance mechanism. *PLoS Med* 2008; **5**: e219.
68 Howard SJ, Cerar D, Anderson MJ *et al.* Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009; **15**: 1068–76.
69 Bueid A, Howard SJ, Moore CB *et al.* Azole antifungal resistance in *Aspergillus fumigatus*: 2008 and 2009. *J Antimicrob Chemother* 2010; **65**: 2116–8.
70 Schweer KE, Bangard C, Hekmat K, Cornely OA. Chronic pulmonary aspergillosis. *Mycoses* 2014; **57**: 257–70.
71 Verweij PE, Te Dorsthorst DT, Rijs AJ, De Vries-Hospers HG, Meis JF. Nationwide survey of in vitro activities of itraconazole and voriconazole against clinical *Aspergillus fumigatus* isolates cultured between 1945 and 1998. *J Clin Microbiol* 2002; **40**: 2648–50.
72 van der Linden JW, Snelders E, Kampinga GA *et al.* Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007-2009. *Emerg Infect Dis* 2011; **17**: 1846–54.
73 van der Linden JW, Camps SM, Kampinga GA *et al.* Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. *Clin Infect Dis* 2013; **57**: 513–20.
74 van Ingen J, van der Lee HA, Rijs TA *et al.* Azole, polyene and echinocandin MIC distributions for wild-type, TR34/L98H and TR46/Y121F/T289A *Aspergillus fumigatus* isolates in the Netherlands. *J Antimicrob Chemother* 2015; **70**: 178–81.
75 Fuhren J, Voskuil WS, Boel CH *et al.* High prevalence of azole resistance in *Aspergillus fumigatus* isolates from high-risk patients. *J Antimicrob Chemother* 2015; **70**: 2894–8.
76 Tashiro M, Izumikawa K, Hirano K *et al.* Correlation between triazole treatment history and susceptibility in clinically isolated Aspergillus fumigatus. *Antimicrob Agents Chemother* 2012; **56**: 4870–5.
77 Negri CE, Goncalves SS, Xafranski H *et al.* Cryptic and rare *Aspergillus* species in Brazil: prevalence in clinical samples and in vitro susceptibility to triazoles. *J Clin Microbiol* 2014; **52**: 3633–40.
78 Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH, Meis JF. Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the cyp51A gene in India. *J Antimicrob Chemother* 2012; **67**: 362–6.
79 Chowdhary A, Kathuria S, Xu J *et al.* Clonal expansion and emergence of environmental multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR_{34}/L98H mutations in the *cyp51A* gene in India. *PLoS ONE* 2012; **7**: e52871.
80 Chowdhary A, Sharma C, Kathuria S, Hagen F, Meis JF. Prevalence and mechanism of triazole resistance in *Aspergillus fumigatus* in a referral chest hospital in Delhi, India and an update of the situation in Asia. *Front Microbiol* 2015; **6**: 428.
81 Lockhart SR, Frade JP, Etienne KA, Pfaller MA, Diekema DJ, Balajee SA. Azole resistance in *Aspergillus fumigatus* isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the cyp51A gene. *Antimicrob Agents Chemother* 2011; **55**: 4465–8.
82 Tashiro M, Izumikawa K, Minematsu A *et al.* Antifungal susceptibilities of *Aspergillus fumigatus* clinical isolates obtained in Nagasaki, Japan. *Antimicrob Agents Chemother* 2012; **56**: 584–7.

83 Seyedmousavi S, Hashemi SJ, Zibafar E *et al.* Azole-resistant *Aspergillus fumigatus*. *Iran. Emerg Infect Dis* 2013; **19**: 832–4.
84 Ahmad S, Joseph L, Hagen F, Meis JF, Khan Z. Concomitant occurrence of itraconazole-resistant and -susceptible strains of *Aspergillus fumigatus* in routine cultures. *J Antimicrob Chemother* 2015; **70**: 412–5.
85 Vanden Bossche H. Biochemical targets for antifungal azole derivatives: hypothesis on the mode of action. *Curr Top Med Mycol* 1985; **1**: 313–51.
86 Chen SC, Sorrell TC, Chang CC, Paige EK, Bryant PA, Slavin MA. Consensus guidelines for the treatment of yeast infections in the haematology, oncology and intensive care setting, 2014. *Intern Med J* 2014; **44**: 1315–32.
87 Parker JE, Warrilow AG, Price CL, Mullins JG, Kelly DE, Kelly SL. Resistance to antifungals that target CYP51. *J Chem Biol* 2014; **7**: 143–61.
88 Sanglard D, Ischer F, Monod M, Bille J. Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. *Microbiology* 1997; **143**: 405–16.
89 Sanglard D, Coste A, Ferrari S. Antifungal drug resistance mechanisms in fungal pathogens from the perspective of transcriptional gene regulation. *FEMS Yeast Res* 2009; **9**: 1029–50.
90 Sanglard D, Ischer F, Monod M, Bille J. Susceptibilities of *Candida albicans* multidrug transporter mutants to various antifungal agents and other metabolic inhibitors. *Antimicrob Agents Chemother* 1996; **40**: 2300–5.
91 Sanglard D, Kuchler K, Ischer F, Pagani JL, Monod M, Bille J. Mechanisms of resistance to azole antifungal agents in *Candida albicans* isolates from AIDS patients involve specific multidrug transporters. *Antimicrob Agents Chemother* 1995; **39**: 2378–86.
92 White TC. Increased mRNA levels of ERG16, CDR, and MDR1 correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 1997; **41**: 1482–7.
93 Goldman GH, da Silva Ferreira ME, dos Reis Marques E *et al.* Evaluation of fluconazole resistance mechanisms in *Candida albicans* clinical isolates from HIV-infected patients in Brazil. *Diagn Microbiol Infect Dis* 2004; **50**: 25–32.
94 Hu L, Du X, Li T *et al.* Genetic and phenotypic characterization of *Candida albicans* strains isolated from infectious disease patients in Shanghai. *J Med Microbiol* 2015; **64**: 74–83.
95 Moran GP, Sanglard D, Donnelly SM, Shanley DB, Sullivan DJ, Coleman DC. Identification and expression of multidrug transporters responsible for fluconazole resistance in *Candida dubliniensis*. *Antimicrob Agents Chemother* 1998; **42**: 1819–30.
96 Sanglard D, Ischer F, Bille J. Role of ATP-binding-cassette transporter genes in high-frequency acquisition of resistance to azole antifungals in *Candida glabrata*. *Antimicrob Agents Chemother* 2001; **45**: 1174–83.
97 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**: 2753–65.
98 Katiyar SK, Edlind TD. Identification and expression of multidrug resistance-related ABC transporter genes in *Candida krusei*. *Med Mycol* 2001; **39**: 109–16.
99 Pinjon E, Moran GP, Jackson CJ *et al.* Molecular mechanisms of itraconazole resistance in *Candida dubliniensis*. *Antimicrob Agents Chemother* 2003; **47**: 2424–37.
100 Torelli R, Posteraro B, Ferrari S *et al.* The ATP-binding cassette transporter-encoding gene CgSNQ2 is contributing to the CgPDR1-dependent azole resistance of *Candida glabrata*. *Mol Microbiol* 2008; **68**: 186–201.
101 Caudle KE, Barker KS, Wiederhold NP, Xu L, Homayouni R, Rogers PD. Genomewide expression profile analysis of the *Candida glabrata* Pdr1 regulon. *Eukaryot Cell* 2011; **10**: 373–83.
102 Miyazaki H, Miyazaki Y, Geber A *et al.* Fluconazole resistance associated with drug efflux and increased transcription of a drug transporter gene, PDH1, in *Candida glabrata*. *Antimicrob Agents Chemother* 1998; **42**: 1695–701.
103 Barchiesi F, Calabrese D, Sanglard D *et al.* Experimental induction of fluconazole resistance in *Candida tropicalis* ATCC 750. *Antimicrob Agents Chemother* 2000; **44**: 1578–84.
104 Vandeputte P, Larcher G, Berges T, Renier G, Chabasse D, Bouchara JP. Mechanisms of azole resistance in a clinical isolate of *Candida tropicalis*. *Antimicrob Agents Chemother* 2005; **49**: 4608–15.
105 Jiang C, Dong D, Yu B *et al.* Mechanisms of azole resistance in 52 clinical isolates of *Candida tropicalis* in China. *J Antimicrob Chemother* 2013; **68**: 778–85.
106 Silva AP, Miranda IM, Guida A *et al.* Transcriptional profiling of azole-resistant *Candida parapsilosis* strains. *Antimicrob Agents Chemother* 2011; **55**: 3546–56.
107 Sellam A, Tebbji F, Nantel A. Role of Ndt80p in sterol metabolism regulation and azole resistance in *Candida albicans*. *Eukaryot Cell* 2009; **8**: 1174–83.
108 Chen CG, Yang YL, Shih HI, Su CL, Lo HJ. CaNdt80 is involved in drug resistance in *Candida albicans* by regulating CDR1. *Antimicrob Agents Chemother* 2004; **48**: 4505–12.
109 Pao SS, Paulsen IT, Saier MH Jr. Major facilitator superfamily. *Microbiol Mol Biol Rev* 1998; **62**: 1–34.
110 Morschhauser J, Barker KS, Liu TT, Bla BWJ, Homayouni R, Rogers PD. The transcription factor Mrr1p controls expression of the MDR1 efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathog* 2007; **3**: e164.
111 Dunkel N, Blass J, Rogers PD, Morschhauser 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.
112 Schubert S, Rogers PD, Morschhauser J. Gain-of-function mutations in the transcription factor MRR1 are responsible for overexpression of the MDR1 efflux pump in fluconazole-resistant *Candida dubliniensis* strains. *Antimicrob Agents Chemother* 2008; **52**: 4274–80.
113 Grossman NT, Pham CD, Cleveland AA, Lockhart SR. Molecular mechanisms of fluconazole resistance in *Candida parapsilosis* isolates from a U.S. surveillance system. *Antimicrob Agents Chemother* 2015; **59**: 1030–7.
114 Wirsching S, Michel S, Morschhauser J. Targeted gene disruption in *Candida albicans* wild-type strains: the role of the MDR1 gene in fluconazole resistance of clinical *Candida albicans* isolates. *Mol Microbiol* 2000; **36**: 856–65.
115 Wirsching S, Moran GP, Sullivan DJ, Coleman DC, Morschhauser J. MDR1-mediated drug resistance in *Candida dubliniensis*. *Antimicrob Agents Chemother* 2001; **45**: 3416–21.
116 Fuentes M, Hermosilla G, Alburquenque C, Falconer MA, Amaro J, Tapia C. [Characterization of azole resistance mechanisms in Chilean clinical isolates of Candida albicans]. *Rev Chilena Infectol* 2014; **31**: 511–7.
117 Moran G, Sullivan D, Morschhauser J, Coleman D. The *Candida dubliniensis* CdCDR1 gene is not essential for fluconazole resistance. *Antimicrob Agents Chemother* 2002; **46**: 2829–41.
118 Rajendran R, Mowat E, McCulloch E *et al.* Azole resistance of *Aspergillus fumigatus* biofilms is partly associated with efflux pump activity. *Antimicrob Agents Chemother* 2011; **55**: 2092–7.
119 Ferreira ME, Colombo AL, Paulsen I *et al.* The ergosterol biosynthesis pathway, transporter genes, and azole resistance in *Aspergillus fumigatus*. *Med Mycol* 2005; **43**(Suppl 1): S313–9.
120 Slaven JW, Anderson MJ, Sanglard D *et al.* Increased expression of a novel *Aspergillus fumigatus* ABC transporter gene, atrF, in the presence of itraconazole in an itraconazole resistant clinical isolate. *Fungal Genet Biol* 2002; **36**: 199–206.
121 da Silva Ferreira ME, Malavazi I, Savoldi M *et al.* Transcriptome analysis of *Aspergillus fumigatus* exposed to voriconazole. *Curr Genet* 2006; **50**: 32–44.
122 Fraczek MG, Bromley M, Buied A *et al.* The cdr1B efflux transporter is associated with non-cyp51a-mediated itraconazole resistance in *Aspergillus fumigatus*. *J Antimicrob Chemother* 2013; **68**: 1486–96.

123 Monk BC, Tomasiak TM, Keniya MV *et al.* Architecture of a single membrane spanning cytochrome P450 suggests constraints that orient the catalytic domain relative to a bilayer. *Proc Natl Acad Sci USA* 2014; **111**: 3865–70.
124 Snelders E, Karawajczyk A, Verhoeven RJ *et al.* The structure-function relationship of the *Aspergillus fumigatus* cyp51A L98H conversion by site-directed mutagenesis: the mechanism of L98H azole resistance. *Fungal Genet Biol* 2011; **48**: 1062–70.
125 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.
126 Chau AS, Mendrick CA, Sabatelli FJ, Loebenberg D, McNicholas PM. Application of real-time quantitative PCR to molecular analysis of *Candida albicans* strains exhibiting reduced susceptibility to azoles. *Antimicrob Agents Chemother* 2004; **48**: 2124–31.
127 Kakeya H, Miyazaki Y, Miyazaki H, Nyswaner K, Grimberg B, Bennett JE. Genetic analysis of azole resistance in the Darlington strain of *Candida albicans*. *Antimicrob Agents Chemother* 2000; **44**: 2985–90.
128 Lamb DC, Kelly DE, White TC, Kelly SL. The R467K amino acid substitution in *Candida albicans* sterol 14alpha-demethylase causes drug resistance through reduced affinity. *Antimicrob Agents Chemother* 2000; **44**: 63–7.
129 Loffler J, Kelly SL, Hebart H, Schumacher U, Lass-Florl C, Einsele H. Molecular analysis of cyp51 from fluconazole-resistant *Candida albicans* strains. *FEMS Microbiol Lett* 1997; **151**: 263–8.
130 Sanglard D, Ischer F, Koymans L, Bille J. Amino acid substitutions in the cytochrome P-450 lanosterol 14alpha-demethylase (CYP51A1) from azole-resistant *Candida albicans* clinical isolates contribute to resistance to azole antifungal agents. *Antimicrob Agents Chemother* 1998; **42**: 241–53.
131 Perea S, Lopez-Ribot JL, Wickes BL *et al.* Molecular mechanisms of fluconazole resistance in *Candida dubliniensis* isolates from human immunodeficiency virus-infected patients with oropharyngeal candidiasis. *Antimicrob Agents Chemother* 2002; **46**: 1695–703.
132 Forastiero A, Mesa-Arango AC, Alastruey-Izquierdo A *et al. Candida tropicalis* antifungal cross-resistance is related to different azole target (Erg11p) modifications. *Antimicrob Agents Chemother* 2013; **57**: 4769–81.
133 Ricardo E, Miranda IM, Faria-Ramos I, Silva RM, Rodrigues AG, Pina-Vaz C. In vivo and in vitro acquisition of resistance to voriconazole by *Candida krusei*. *Antimicrob Agents Chemother* 2014; **58**: 4604–11.
134 Couzigou C, Gabriel F, Biteau N, Fitton-Ouhabi V, Noel T, Accoceberry I. Two missense mutations, E123Q and K151E, identified in the ERG11 allele of an azole-resistant isolate of *Candida kefyr* recovered from a stem cell transplant patient for acute myeloid leukemia. *Med Mycol Case Rep* 2014; **5**: 12–5.
135 Hull CM, Parker JE, Bader O *et al.* Facultative sterol uptake in an ergosterol-deficient clinical isolate of *Candida glabrata* harboring a missense mutation in ERG11 and exhibiting cross-resistance to azoles and amphotericin B. *Antimicrob Agents Chemother* 2012; **56**: 4223–32.
136 Mellado E, Garcia-Effron G, Buitrago MJ, Alcazar-Fuoli L, Cuenca-Estrella M, Rodriguez-Tudela JL. Targeted gene disruption of the 14-alpha sterol demethylase (cyp51A) in *Aspergillus fumigatus* and its role in azole drug susceptibility. *Antimicrob Agents Chemother* 2005; **49**: 2536–8.
137 Hu W, Sillaots S, Lemieux S *et al.* Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. *PLoS Pathog* 2007; **3**: e24.
138 Rodriguez-Tudela JL, Alcazar-Fuoli L, Cuesta I *et al.* Clinical relevance of resistance to antifungals. *Int J Antimicrob Agents* 2008; **32** (Suppl 2): S111–3.
139 Mellado E, Garcia-Effron G, Alcazar-Fuoli L, Cuenca-Estrella M, Rodriguez-Tudela JL. Substitutions at methionine 220 in the 14alpha-sterol demethylase (Cyp51A) of *Aspergillus fumigatus* are responsible for resistance in vitro to azole antifungal drugs. *Antimicrob Agents Chemother* 2004; **48**: 2747–50.
140 Denning DW, Park S, Lass-Florl C *et al.* High-frequency triazole resistance found In nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis* 2011; **52**: 1123–9.
141 Bader O, Weig M, Reichard U *et al.* cyp51A-Based mechanisms of *Aspergillus fumigatus* azole drug resistance present in clinical samples from Germany. *Antimicrob Agents Chemother* 2013; **57**: 3513–7.
142 Becher R, Wirsel SG. Fungal cytochrome P450 sterol 14alpha-demethylase (CYP51) and azole resistance in plant and human pathogens. *Appl Microbiol Biotechnol* 2012; **95**: 825–40.
143 Alcazar-Fuoli L, Mellado E. Current status of antifungal resistance and its impact on clinical practice. *Br J Haematol* 2014; **166**: 471–84.
144 Vermeulen E, Lagrou K, Verweij PE. Azole resistance in *Aspergillus fumigatus*: a growing public health concern. *Curr Opin Infect Dis* 2013; **26**: 493–500.
145 Paul S, Moye-Rowley WS. Multidrug resistance in fungi: regulation of transporter-encoding gene expression. *Front Physiol* 2014; **5**: 143.
146 Kidd SE, Goeman E, Meis JF, Slavin MA, Verweij PE. Multi-triazole-resistant *Aspergillus fumigatus* infections in Australia. *Mycoses* 2015; **58**: 350–5.
147 Wu CJ, Wang HC, Lee JC *et al.* Azole-resistant *Aspergillus fumigatus* isolates carrying TR_{34}/L98H mutations in Taiwan. *Mycoses* 2015; **58**: 544–9.
148 Asano M, Kano R, Makimura K, Hasegawa A, Kamata H. Molecular typing and in-vitro activity of azoles against clinical isolates of *Aspergillus fumigatus* and *A. niger* in Japan. *J Infect Chemother* 2011; **17**: 483–6.
149 Hagiwara D, Takahashi H, Watanabe A *et al.* Whole-genome comparison of *Aspergillus fumigatus* strains serially isolated from patients with aspergillosis. *J Clin Microbiol* 2014; **52**: 4202–9.
150 Abdolrasouli A, Rhodes J, Beale MA *et al.* Genomic context of azole resistance mutations in Aspergillus fumigatus determined using whole-genome sequencing. *MBio* 2015; **6**: e00536. doi:10.1128/mBio. 00536-15.
151 Astvad KM, Jensen RH, Hassan TM *et al.* First detection of TR46/Y121F/T289A and TR34/L98H alterations in Aspergillus fumigatus isolates from azole-naive patients in Denmark despite negative findings in the environment. *Antimicrob Agents Chemother* 2014; **58**: 5096–101.
152 Chowdhary A, Sharma C, Kathuria S, Hagen F, Meis JF. Azole-resistant *Aspergillus fumigatus* with the environmental TR46/Y121F/T289A mutation in India. *J Antimicrob Chemother* 2014; **69**: 555–7.
153 Chowdhary A, Sharma C, Hagen F, Meis JF. Exploring azole antifungal drug resistance in *Aspergillus fumigatus* with special reference to resistance mechanisms. *Future Microbiol* 2014; **9**: 697–711.
154 Chen Y, Wang H, Lu Z *et al.* Emergence of TR46/Y121F/T289A in an *Aspergillus fumigatus* isolate from a Chinese patient. *Antimicrob Agents Chemother* 2015; **59**: 7148–50.
155 Pelaez T, Monteiro MC, Garcia-Rubio R, Bouza E, Gomez-Lopez A, Mellado E. First detection of *Aspergillus fumigatus* azole-resistant strain due to Cyp51A TR46/Y121F/T289A in an azole-naive patient in Spain. *New Microbes New Infect* 2015; **6**: 33–4.
156 Lavergne RA, Morio F, Favennec L *et al.* First description of azole-resistant Aspergillus fumigatus due to TR46/Y121F/T289A mutation in France. *Antimicrob Agents Chemother* 2015; **59**: 4331–5.
157 Steinmann J, Hamprecht A, Vehreschild MJ *et al.* Emergence of azole-resistant invasive aspergillosis in HSCT recipients in Germany. *J Antimicrob Chemother* 2015; **70**: 1522–6.
158 Le Pape P, Lavergne RA, Morio F, Alvarez C. Multiple fungicide-driven alterations in azole-resistant *Aspergillus fumigatus*, Colombia, 2015. *Emerg Infect Dis* 2016; **22**: 156–7. in press. http://dx.doi.org/10.3201/eid2201.1510978.

159 Hodiamont CJ, Dolman KM, Ten Berge IJ, Melchers WJ, Verweij PE, Pajkrt D. Multiple-azole-resistant *Aspergillus fumigatus* osteomyelitis in a patient with chronic granulomatous disease successfully treated with long-term oral posaconazole and surgery. *Med Mycol* 2009; **47**: 217–20.

160 Morschhauser J. The genetic basis of fluconazole resistance development in *Candida albicans*. *Biochim Biophys Acta* 2002; **1587**: 240–8.

161 Redding SW, Kirkpatrick WR, Coco BJ *et al. Candida glabrata* oropharyngeal candidiasis in patients receiving radiation treatment for head and neck cancer. *J Clin Microbiol* 2002; **40**: 1879–81.

162 Rogers PD, Vermitsky JP, Edlind TD, Hilliard GM. Proteomic analysis of experimentally induced azole resistance in *Candida glabrata*. *J Antimicrob Chemother* 2006; **58**: 434–8.

163 Tavakoli M, Zaini F, Kordbacheh M, Safara M, Raoofian R, Heidari M. Upregulation of the ERG11 gene in *Candida krusei* by azoles. *Daru* 2010; **18**: 276–80.

164 Blatzer M, Barker BM, Willger SD *et al.* SREBP coordinates iron and ergosterol homeostasis to mediate triazole drug and hypoxia responses in the human fungal pathogen *Aspergillus fumigatus*. *PLoS Genet* 2011; **7**: e1002374.

165 Moye-Rowley WS. Multiple mechanisms contribute to the development of clinically significant azole resistance in *Aspergillus fumigatus*. *Front Microbiol* 2015; **6**: 70.

166 Willger SD, Puttikamonkul S, Kim KH *et al.* A sterol-regulatory element binding protein is required for cell polarity, hypoxia adaptation, azole drug resistance, and virulence in *Aspergillus fumigatus*. *PLoS Pathog* 2008; **4**: e1000200.

167 Camps SM, van der Linden JW, Li Y *et al.* Rapid induction of multiple resistance mechanisms in *Aspergillus fumigatus* during azole therapy: a case study and review of the literature. *Antimicrob Agents Chemother* 2012; **56**: 10–6.

168 Tuncher A, Sprote P, Gehrke A, Brakhage AA. The CCAAT-binding complex of eukaryotes: evolution of a second NLS in the HapB subunit of the filamentous fungus *Aspergillus nidulans* despite functional conservation at the molecular level between yeast, *A.nidulans* and human. *J Mol Biol* 2005; **352**: 517–33.

169 Ben-Ami R, Olshtain-Pops K, Krieger M *et al.* Antibiotic exposure as a risk factor for fluconazole-resistant *Candida* bloodstream infection. *Antimicrob Agents Chemother* 2012; **56**: 2518–23.

170 Mikulska M, Del Bono V, Ratto S, Viscoli C. Occurrence, presentation and treatment of candidemia. *Expert Rev Clin Immunol* 2012; **8**: 755–65.

171 Lortholary O, Renaudat C, Sitbon K *et al.* Worrisome trends in incidence and mortality of candidemia in intensive care units (Paris area, 2002-2010). *Intensive Care Med* 2014; **40**: 1303–12.

172 Schuster MG, Meibohm A, Lloyd L, Strom B. Risk factors and outcomes of *Candida krusei* bloodstream infection: a matched, case-control study. *J Infect* 2013; **66**: 278–84.

173 Garnacho-Montero J, Diaz-Martin A, Garcia-Cabrera E *et al.* Risk factors for fluconazole-resistant candidemia. *Antimicrob Agents Chemother* 2010; **54**: 3149–54.

174 Cuervo G, Puig-Asensio M, Garcia-Vidal C *et al.* A simple prediction score for estimating the risk of candidaemia caused by fluconazole non-susceptible strains. *Clin Microbiol Infect* 2015; **21**: e1–9.

175 Denning DW, Radford SA, Oakley KL, Hall L, Johnson EM, Warnock DW. Correlation between in-vitro susceptibility testing to itraconazole and in-vivo outcome of *Aspergillus fumigatus* infection. *J Antimicrob Chemother* 1997; **40**: 401–14.

176 Moore CB, Sayers N, Mosquera J, Slaven J, Denning DW. Antifungal drug resistance in *Aspergillus*. *J Infect* 2000; **41**: 203–20.

177 Mortensen KL, Jensen RH, Johansen HK *et al. Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. *J Clin Microbiol* 2011; **49**: 2243–51.

178 Howard SJ, Pasqualotto AC, Anderson MJ *et al.* Major variations in *Aspergillus fumigatus* arising within aspergillomas in chronic pulmonary aspergillosis. *Mycoses* 2013; **56**: 434–41.

179 Snelders E, Melchers WJ, Verweij PE. Azole resistance in *Aspergillus fumigatus*: a new challenge in the management of invasive aspergillosis? *Future Microbiol* 2011; **6**: 335–47.

180 Badali H, Vaezi A, Haghani I *et al.* Environmental study of azole-resistant *Aspergillus fumigatus* with TR34/L98H mutations in the cyp51A gene in Iran. *Mycoses* 2013; **56**: 659–63.

181 Camps SM, Rijs AJ, Klaassen CH *et al.* Molecular epidemiology of *Aspergillus fumigatus* isolates harboring the TR34/L98H azole resistance mechanism. *J Clin Microbiol* 2012; **50**: 2674–80.

182 Hamprecht A, Buchheidt D, Vehreschild JJ *et al.* Azole-resistant invasive aspergillosis in a patient with acute myeloid leukaemia in Germany. *Euro Surveill* 2012; **17**: 20262.

183 Burgel PR, Baixench MT, Amsellem M *et al.* High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. *Antimicrob Agents Chemother* 2012; **56**: 869–74.

184 Stensvold RC, Jørgensen LN, Arendrup MC. Azole-resistant invasive aspergillosis: relationship to agriculture. *Curr Fung Infect Rep* 2012; **6**: 178–91.

185 Deresinski SC, Stevens DA. Caspofungin. *Clin Infect Dis* 2003; **36**: 1445–57.

186 Morris MI, Villmann M. Echinocandins in the management of invasive fungal infections, Part 2. *Am J Health Syst Pharm* 2006; **63**: 1813–20.

187 Chandrasekar PH, Sobel JD. Micafungin: a new echinocandin. *Clin Infect Dis* 2006; **42**: 1171–8.

188 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.

189 Viscoli C, Herbrecht R, Akan H *et al.* An EORTC Phase II study of caspofungin as first-line therapy of invasive aspergillosis in haematological patients. *J Antimicrob Chemother* 2009; **64**: 1274–81.

190 Colombo AL, Guimaraes T, Camargo LF *et al.* Brazilian guidelines for the management of candidiasis - a joint meeting report of three medical societies: Sociedade Brasileira de Infectologia, Sociedade Paulista de Infectologia and Sociedade Brasileira de Medicina Tropical. *Braz J Infect Dis* 2013; **17**: 283–312.

191 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.

192 Cleveland AA, Harrison LH, Farley MM *et al.* Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008-2013: results from population-based surveillance. *PLoS ONE* 2015; **10**: e0120452.

193 Maubon D, Garnaud C, Calandra T, Sanglard D, Cornet M. Resistance of *Candida* spp. to antifungal drugs in the ICU: where are we now? *Intensive Care Med* 2014; **40**: 1241–55.

194 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.

195 Arendrup MC, Perlin DS. Echinocandin resistance: an emerging clinical problem? *Curr Opin Infect Dis* 2014; **27**: 484–92.

196 Bizerra FC, Jimenez-Ortigosa C, Souza AC *et al.* Breakthrough candidemia due to multidrug-resistant *Candida glabrata* during prophylaxis with a low dose of micafungin. *Antimicrob Agents Chemother* 2014; **58**: 2438–40.

197 Arendrup MC. Invasive fungal infections: past achievements and challenges ahead. *Clin Microbiol Infect* 2009; **15**: 599–601.

198 Marr KA, Schlamm HT, Herbrecht R *et al.* Combination antifungal therapy for invasive aspergillosis: a randomized trial. *Ann Intern Med* 2015; **162**: 81–9.

199 Walsh TJ, Anaissie EJ, Denning DW *et al.* Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **46**: 327–60.

200 Staab JF, Kahn JN, Marr KA. Differential *Aspergillus lentulus* echinocandin susceptibilities are Fksp independent. *Antimicrob Agents Chemother* 2010; **54**: 4992–8.

201 Eschertzhuber S, Velik-Salchner C, Hoermann C, Hoefer D, Lass-Florl C. Caspofungin-resistant *Aspergillus flavus* after heart transplantation and mechanical circulatory support: a case report. *Transpl Infect Dis* 2008; **10**: 190–2.

202 Barchiesi F, Spreghini E, Tomassetti S *et al.* Effects of caspofungin against Candida guilliermondii and *Candida parapsilosis*. *Antimicrob Agents Chemother* 2006; **50**: 2719–27.

203 Kahn JN, Hsu MJ, Racine F, Giacobbe R, Motyl M. Caspofungin susceptibility in *Aspergillus* and non-*Aspergillus* molds: inhibition of glucan synthase and reduction of beta-D-1,3 glucan levels in culture. *Antimicrob Agents Chemother* 2006; **50**: 2214–6.

204 Enache-Angoulvant A, Girard A, Poirot JL, Hennequin C. In vitro activity of caspofungin and voriconazole against uncommon *Candida* spp. *Int J Antimicrob Agents* 2009; **33**: 595–6.

205 Mazur P, Morin N, Baginsky W *et al.* Differential expression and function of two homologous subunits of yeast 1,3-beta-D-glucan synthase. *Mol Cell Biol* 1995; **15**: 5671–81.

206 Ishihara S, Hirata A, Nogami S, Beauvais A, Latge JP, Ohya Y. Homologous subunits of 1,3-beta-glucan synthase are important for spore wall assembly in *Saccharomyces cerevisiae*. *Eukaryot Cell* 2007; **6**: 143–56.

207 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.

208 Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol* 2011; **6**: 441–57.

209 Balashov SV, Park S, Perlin DS. Assessing resistance to the echinocandin antifungal drug caspofungin in *Candida albicans* by profiling mutations in FKS1. *Antimicrob Agents Chemother* 2006; **50**: 2058–63.

210 Garcia-Effron G, Chua DJ, Tomada JR *et al.* Novel FKS mutations associated with echinocandin resistance in *Candida* species. *Antimicrob Agents Chemother* 2010; **54**: 2225–7.

211 Perlin DS. Resistance to echinocandin-class antifungal drugs. *Drug Resist Updat* 2007; **10**: 121–30.

212 Walker LA, Gow NA, Munro CA. Fungal echinocandin resistance. *Fungal Genet Biol* 2010; **47**: 117–26.

213 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.

214 Desnos-Ollivier M, Bretagne S, Raoux D *et al.* Mutations in the fks1 gene in *Candida albicans*, *C. tropicalis*, and *C. krusei* correlate with elevated caspofungin MICs uncovered in AM3 medium using the method of the European Committee on Antibiotic Susceptibility Testing. *Antimicrob Agents Chemother* 2008; **52**: 3092–8.

215 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.

216 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.

217 Jensen RH, Johansen HK, Arendrup MC. Stepwise development of a homozygous S80P substitution in Fks1p, conferring echinocandin resistance in *Candida tropicalis*. *Antimicrob Agents Chemother* 2013; **57**: 614–7.

218 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.

219 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**: 2263–5.

220 Thompson GR 3rd, Wiederhold NP, Vallor AC, Villareal NC, Lewis JS, Patterson TF. Development of caspofungin resistance following prolonged therapy for invasive candidiasis secondary to *Candida glabrata* infection. *Antimicrob Agents Chemother* 2008; **52**: 3783–5.

221 Katiyar S, Pfaller M, Edlind T. *Candida albicans* and *Candida glabrata* clinical isolates exhibiting reduced echinocandin susceptibility. *Antimicrob Agents Chemother* 2006; **50**: 2892–4.

222 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.

223 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.

224 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.

225 Colombo AL, Ngai AL, Bourque M *et al.* Caspofungin use in patients with invasive candidiasis caused by common non-*albicans Candida* species: review of the caspofungin database. *Antimicrob Agents Chemother* 2010; **54**: 1864–71.

226 Foldi R, Kovacs R, Gesztelyi R *et al.* Comparison of in vitro and vivo efficacy of caspofungin against *Candida parapsilosis*, *C. orthopsilosis*, *C. metapsilosis* and *C. albicans*. *Mycopathologia* 2012; **174**: 311–8.

227 Spreghini E, Orlando F, Tavanti A *et al.* In vitro and in vivo effects of echinocandins against *Candida parapsilosis* sensu stricto, *Candida orthopsilosis* and *Candida metapsilosis*. *J Antimicrob Chemother* 2012; **67**: 2195–202.

228 Szilagyi J, Foldi R, Gesztelyi R *et al.* Comparison of the kidney fungal burden in experimental disseminated candidiasis by species of the *Candida parapsilosis* complex treated with fluconazole, amphotericin B and caspofungin in a temporarily neutropenic murine model. *Chemotherapy* 2012; **58**: 159–64.

229 Fernandez-Ruiz M, Aguado JM, Almirante B *et al.* Initial use of echinocandins does not negatively influence outcome in *Candida parapsilosis* bloodstream infection: a propensity score analysis. *Clin Infect Dis* 2014; **58**: 1413–21.

230 Arendrup MC, Perkhofer S, Howard SJ *et al.* Establishing in vitro-in vivo correlations for *Aspergillus fumigatus*: the challenge of azoles versus echinocandins. *Antimicrob Agents Chemother* 2008; **52**: 3504–11.

231 Arendrup MC, Garcia-Effron G, Buzina W *et al.* Breakthrough *Aspergillus fumigatus* and *Candida albicans* double infection during caspofungin treatment: laboratory characteristics and implication for susceptibility testing. *Antimicrob Agents Chemother* 2009; **53**: 1185–93.

232 Gardiner RE, Souteropoulos P, Park S, Perlin DS. Characterization of Aspergillus fumigatus mutants with reduced susceptibility to caspofungin. *Med Mycol* 2005; **43**(Suppl 1): S299–305.

233 Rocha EM, Garcia-Effron G, Park S, Perlin DS. A Ser678Pro substitution in Fks1p confers resistance to echinocandin drugs in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2007; **51**: 4174–6.

234 Imhof A, Balajee SA, Marr KA. New methods to assess susceptibilities of *Aspergillus* isolates to caspofungin. *J Clin Microbiol* 2003; **41**: 5683–8.

235 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.

236 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.

237 Walker LA, Gow NA, Munro CA. Elevated chitin content reduces the susceptibility of *Candida* species to caspofungin. *Antimicrob Agents Chemother* 2013; **57**: 146–54.

238 Cota JM, Grabinski JL, Talbert RL *et al.* Increases in SLT2 expression and chitin content are associated with incomplete killing of *Candida glabrata* by caspofungin. *Antimicrob Agents Chemother* 2008; **52**: 1144–6.

239 Blanchard E, Lortholary O, Boukris-Sitbon K *et al.* Prior caspofungin exposure in patients with hematological malignancies is a risk factor for subsequent fungemia due to decreased susceptibility in

Candida spp.: a case-control study in Paris, France. *Antimicrob Agents Chemother* 2011; **55**: 5358–61.
240 Lortholary O, Desnos-Ollivier M, Sitbon K *et al.* 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.
241 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.
242 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.
243 Farmakiotis D, Tarrand JJ, Kontoyiannis DP. Drug-resistant *Candida glabrata* infection in cancer patients. *Emerg Infect Dis* 2014; **20**: 1833–40.
244 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.
245 Perfect JR, Dismukes WE, Dromer F *et al.* Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis* 2010; **50**: 291–322.
246 Wheat LJ, Freifeld AG, Kleiman MB *et al.* Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007; **45**: 807–25.
247 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**: 1519–27.
248 Odds FC, Brown AJ, Gow NA. Antifungal agents: mechanisms of action. *Trends Microbiol* 2003; **11**: 272–9.
249 Masia Canuto M, Gutierrez Rodero F. Antifungal drug resistance to azoles and polyenes. *Lancet Infect Dis* 2002; **2**: 550–63.
250 Mesa-Arango AC, Trevijano-Contador N, Roman E *et al.* The production of reactive oxygen species is a universal action mechanism of amphotericin B against pathogenic yeasts and contributes to the fungicidal effect of this drug. *Antimicrob Agents Chemother* 2014; **58**: 6627–38.
251 Peman J, Canton E, Espinel-Ingroff A. Antifungal drug resistance mechanisms. *Expert Rev Anti Infect Ther* 2009; **7**: 453–60.
252 Mohr JF, Hall AC, Ericsson CD, Ostrosky-Zeichner L. Fatal amphotericin B overdose due to administration of nonlipid formulation instead of lipid formulation. *Pharmacotherapy* 2005; **25**: 426–8.
253 Wang YH, Zhang JP, Chang Y, Hu CQ. A newly identified derivative of amphotericin B: isolation, structure determination and primary evaluation of the activity and toxicity. *J Antibiot (Tokyo)* 2010; **63**: 553–7.
254 Bates DW, Su L, Yu DT *et al.* Mortality and costs of acute renal failure associated with amphotericin B therapy. *Clin Infect Dis* 2001; **32**: 686–93.
255 Falci DR, da Rosa FB, Pasqualotto AC. Comparison of nephrotoxicity associated to different lipid formulations of amphotericin B: a real-life study. *Mycoses* 2015; **58**: 104–12.
256 Falci DR, da Rosa FB, Pasqualotto AC. Hematological toxicities associated with amphotericin B formulations. *Leuk Lymphoma* 2015; **56**: 2889–94.
257 Groll AH, Muller FM, Piscitelli SC, Walsh TJ. Lipid formulations of amphotericin B: clinical perspectives for the management of invasive fungal infections in children with cancer. *Klin Padiatr* 1998; **210**: 264–73.
258 Bowden R, Chandrasekar P, White MH *et al.* A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* 2002; **35**: 359–66.
259 Walsh TJ, Hiemenz JW, Seibel NL *et al.* Amphotericin B lipid complex for invasive fungal infections: analysis of safety and efficacy in 556 cases. *Clin Infect Dis* 1998; **26**: 1383–96.
260 Bowden RA, Cays M, Gooley T, Mamelok RD, van Burik JA. Phase I study of amphotericin B colloidal dispersion for the treatment of invasive fungal infections after marrow transplant. *J Infect Dis* 1996; **173**: 1208–15.
261 Wingard JR. Lipid formulations of amphotericins: are you a lumper or a splitter? *Clin Infect Dis* 2002; **35**: 891–5.
262 da Matta DA, de Almeida LP, Machado AM *et al.* Antifungal susceptibility of 1000 *Candida* bloodstream isolates to 5 antifungal drugs: results of a multicenter study conducted in Sao Paulo, Brazil, 1995-2003. *Diagn Microbiol Infect Dis* 2007; **57**: 399–404.
263 Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; **20**: 133–63.
264 Nolte FS, Parkinson T, Falconer DJ *et al.* Isolation and characterization of fluconazole- and amphotericin B-resistant *Candida albicans* from blood of two patients with leukemia. *Antimicrob Agents Chemother* 1997; **41**: 196–9.
265 Clancy CJ, Nguyen MH. Correlation between in vitro susceptibility determined by E test and response to therapy with amphotericin B: results from a multicenter prospective study of candidemia. *Antimicrob Agents Chemother* 1999; **43**: 1289–90.
266 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**: 938–44.
267 Favel A, Michel-Nguyen A, Peyron F *et al.* Colony morphology switching of *Candida lusitaniae* and acquisition of multidrug resistance during treatment of a renal infection in a newborn: case report and review of the literature. *Diagn Microbiol Infect Dis* 2003; **47**: 331–9.
268 Pfaller MA, Boyken L, Messer SA, Tendolkar S, Hollis RJ, Diekema DJ. Evaluation of the etest method using Mueller-Hinton agar with glucose and methylene blue for determining amphotericin B MICs for 4,936 clinical isolates of *Candida* species. *J Clin Microbiol* 2004; **42**: 4977–9.
269 Zaoutis TE, Foraker E, McGowan KL *et al.* Antifungal susceptibility of *Candida* spp. isolated from pediatric patients: a survey of 4 children's hospitals. *Diagn Microbiol Infect Dis* 2005; **52**: 295–8.
270 Chen SC, Marriott D, Playford EG *et al.* Candidaemia with uncommon *Candida* species: predisposing factors, outcome, antifungal susceptibility, and implications for management. *Clin Microbiol Infect* 2009; **15**: 662–9.
271 Pfaller MA, Diekema DJ, Gibbs DL *et al.* Results from the ARTEMIS DISK Global sntifungal surveillance study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol* 2010; **48**: 1366–77.
272 Atkinson BJ, Lewis RE, Kontoyiannis DP. *Candida lusitaniae* fungemia in cancer patients: risk factors for amphotericin B failure and outcome. *Med Mycol* 2008; **46**: 541–6.
273 Merz WG. *Candida lusitaniae*: frequency of recovery, colonization, infection, and amphotericin B resistance. *J Clin Microbiol* 1984; **20**: 1194–5.
274 Minari A, Hachem R, Raad I. *Candida lusitaniae*: a cause of breakthrough fungemia in cancer patients. *Clin Infect Dis* 2001; **32**: 186–90.
275 Moosa MY, Alangaden GJ, Manavathu E, Chandrasekar PH. Resistance to amphotericin B does not emerge during treatment for invasive aspergillosis. *J Antimicrob Chemother* 2002; **49**: 209–13.
276 Steinbach WJ, Benjamin DK, Kontoyiannis DP *et al.* Infections due to *Aspergillus terreus*: a multicenter retrospective analysis of 83 cases. *Clin Infect Dis* 2004; **39**: 192–8.
277 Hadrich I, Makni F, Neji S *et al.* Amphotericin B in vitro resistance is associated with fatal *Aspergillus flavus* infection. *Med Mycol* 2012; **50**: 829–34.

278 Guinea J, Pelaez T, Alcala L, Ruiz-Serrano MJ, Bouza E. Antifungal susceptibility of 596 *Aspergillus fumigatus* strains isolated from outdoor air, hospital air, and clinical samples: analysis by site of isolation. *Antimicrob Agents Chemother* 2005; **49**: 3495–7.
279 Diekema DJ, Messer SA, Hollis RJ, Jones RN, Pfaller MA. Activities of caspofungin, itraconazole, posaconazole, ravuconazole, voriconazole, and amphotericin B against 448 recent clinical isolates of filamentous fungi. *J Clin Microbiol* 2003; **41**: 3623–6.
280 Lass-Florl C, Griff K, Mayr A *et al.* Epidemiology and outcome of infections due to *Aspergillus terreus*: 10-year single centre experience. *Br J Haematol* 2005; **131**: 201–7.
281 Lass-Florl C, Kofler G, Kropshofer G *et al.* In-vitro testing of susceptibility to amphotericin B is a reliable predictor of clinical outcome in invasive aspergillosis. *J Antimicrob Chemother* 1998; **42**: 497–502.
282 Sanglard D, Ischer F, Parkinson T, Falconer D, Bille J. *Candida albicans* mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents. *Antimicrob Agents Chemother* 2003; **47**: 2404–12.
283 Young LY, Hull CM, Heitman J. Disruption of ergosterol biosynthesis confers resistance to amphotericin B in *Candida lusitaniae*. *Antimicrob Agents Chemother* 2003; **47**: 2717–24.
284 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**: 982–90.
285 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.
286 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.
287 Chamilos G, Kontoyiannis DP. Update on antifungal drug resistance mechanisms of *Aspergillus fumigatus*. *Drug Resist Updat* 2005; **8**: 344–58.
288 Blum G, Perkhofer S, Grif K *et al.* A 1-year *Aspergillus terreus* surveillance study at the University Hospital of Innsbruck: molecular typing of environmental and clinical isolates. *Clin Microbiol Infect* 2008; **14**: 1146–51.
289 Blatzer M, Jukic E, Posch W *et al.* Amphotericin B resistance in *Aspergillus terreus* is overpowered by coapplication of pro-oxidants. *Antioxid Redox Signal* 2015; **23**: 1424–38.
290 Nucci M, Queiroz-Telles F, Alvarado-Matute T *et al.* Epidemiology of candidemia in Latin America: a laboratory-based survey. *PLoS ONE* 2013; **8**: e59373.
291 Cordoba S, Vivot W, Bosco-Borgeat ME *et al.* Species distribution and susceptibility profile of yeasts isolated from blood cultures: results of a multicenter active laboratory-based surveillance study in Argentina. *Rev Argent Microbiol* 2011; **43**: 176–85.
292 Silva V, Diaz MC, Febre N. Chilean Invasive Fungal Infections G. Invasive fungal infections in Chile: a multicenter study of fungal prevalence and susceptibility during a 1-year period. *Med Mycol* 2004; **42**: 333–9.
293 Dolande Franco ME, Reviakina V, Panizo MM *et al.* Distribution and antifungal susceptibility of *Candida* clinical isolations coming from six health care centers in the metropolitan area of Caracas, Venezuela (years 2003-2005). *Rev Iberoam Micol* 2008; **25**: 17–21.
294 Cortes JA, Reyes P, Gomez CH *et al.* Clinical and epidemiological characteristics and risk factors for mortality in patients with candidemia in hospitals from Bogota. *Colombia. Braz J Infect Dis* 2014; **18**: 631–7.
295 Corzo-Leon DE, Alvarado-Matute T, Colombo AL *et al.* Surveillance of *Candida* spp bloodstream infections: epidemiological trends and risk factors of death in two Mexican tertiary care hospitals. *PLoS ONE* 2014; **9**: e97325.
296 Aquino VR, Lunardi LW, Goldani LZ, Barth AL. Prevalence, susceptibility profile for fluconazole and risk factors for candidemia in a tertiary care hospital in southern Brazil. *Braz J Infect Dis* 2005; **9**: 411–8.
297 Colombo AL, Nucci M, Park BJ *et al.* Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. *J Clin Microbiol* 2006; **44**: 2816–23.
298 Bruder-Nascimento A, Camargo CH, Sugizaki MF *et al.* Species distribution and susceptibility profile of Candida species in a Brazilian public tertiary hospital. *BMC Res Notes* 2010; **3**: 1.
299 Santos ER, Dal Forno CF, Hernandez MG *et al.* Susceptibility of *Candida* spp. isolated from blood cultures as evaluated using the M27-A3 and new M27-S4 approved breakpoints. *Rev Inst Med Trop Sao Paulo* 2014; **56**: 477–82.
300 da Costa VG, Quesada RM, Abe AT, Furlaneto-Maia L, Furlaneto MC. Nosocomial bloodstream *Candida* infections in a tertiary-care hospital in South Brazil: a 4-year survey. *Mycopathologia* 2014; **178**: 243–50.
301 Hong SB, Shin HD, Hong J *et al.* New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. *Antonie Van Leeuwenhoek* 2008; **93**: 87–98.
302 Balajee SA, Kano R, Baddley JW *et al.* Molecular identification of *Aspergillus* species collected for the Transplant-Associated Infection Surveillance Network. *J Clin Microbiol* 2009; **47**: 3138–41.
303 Montenegro G, Sanchez Puch S, Jewtuchowicz VM *et al.* Phenotypic and genotypic characterization of *Aspergillus lentulus* and *Aspergillus fumigatus* isolates in a patient with probable invasive aspergillosis. *J Med Microbiol* 2009; **58**: 391–5.
304 Alhambra A, Catalan M, Moragues MD *et al.* Isolation of *Aspergillus lentulus* in Spain from a critically ill patient with chronic obstructive pulmonary disease. *Rev Iberoam Micol* 2008; **25**: 246–9.
305 Vinh DC, Shea YR, Sugui JA *et al.* Invasive aspergillosis due to *Neosartorya udagawae*. *Clin Infect Dis* 2009; **49**: 102–11.
306 Sugui JA, Vinh DC, Nardone G *et al. Neosartorya udagawae* (*Aspergillus udagawae*), an emerging agent of aspergillosis: how different is it from *Aspergillus fumigatus? J Clin Microbiol* 2010; **48**: 220–8.
307 Balajee SA, Marr KA. Phenotypic and genotypic identification of human pathogenic aspergilli. *Future Microbiol* 2006; **1**: 435–45.
308 Vinh DC, Shea YR, Jones PA, Freeman AF, Zelazny A, Holland SM. Chronic invasive aspergillosis caused by *Aspergillus viridinutans*. *Emerg Infect Dis* 2009; **15**: 1292–4.
309 Krishnan S, Manavathu EK, Chandrasekar PH. *Aspergillus flavus*: an emerging non-fumigatus Aspergillus species of significance. *Mycoses* 2009; **52**: 206–22.
310 Gomez-Lopez A, Garcia-Effron G, Mellado E, Monzon A, Rodriguez-Tudela JL, Cuenca-Estrella M. In vitro activities of three licensed antifungal agents against spanish clinical isolates of *Aspergillus* spp. *Antimicrob Agents Chemother* 2003; **47**: 3085–8.
311 Hsueh PR, Lau YJ, Chuang YC *et al.* Antifungal susceptibilities of clinical isolates of *Candida* species, *Cryptococcus neoformans*, and *Aspergillus* species from Taiwan: surveillance of multicenter antimicrobial resistance in Taiwan program data from 2003. *Antimicrob Agents Chemother* 2005; **49**: 512–7.
312 Lionakis MS, Lewis RE, Torres HA, Albert ND, Raad II, Kontoyiannis DP. Increased frequency of non-*fumigatus Aspergillus* species in amphotericin B- or triazole-pre-exposed cancer patients with positive cultures for aspergilli. *Diagn Microbiol Infect Dis* 2005; **52**: 15–20.
313 Koss T, Bagheri B, Zeana C, Romagnoli MF, Grossman ME. Amphotericin B-resistant *Aspergillus flavus* infection successfully treated with caspofungin, a novel antifungal agent. *J Am Acad Dermatol* 2002; **46**: 945–7.
314 Johnson EM, Oakley KL, Radford SA *et al.* Lack of correlation of in vitro amphotericin B susceptibility testing with outcome in a murine model of *Aspergillus* infection. *J Antimicrob Chemother* 2000; **45**: 85–93.
315 Segal BH, DeCarlo ES, Kwon-Chung KJ, Malech HL, Gallin JI, Holland SM. *Aspergillus nidulans* infection in chronic granulomatous disease. *Medicine (Baltimore)* 1998; **77**: 345–54.

316 Kontoyiannis DP, Lewis RE, May GS, Osherov N, Rinaldi MG. *Aspergillus nidulans* is frequently resistant to amphotericin B. *Mycoses* 2002; **45**: 406–7.
317 Verweij PE, Varga J, Houbraken J *et al. Emericella quadrilineata* as cause of invasive aspergillosis. *Emerg Infect Dis* 2008; **14**: 566–72.
318 Lass-Florl C, Alastruey-Izquierdo A, Cuenca-Estrella M, Perkhofer S, Rodriguez-Tudela JL. In vitro activities of various antifungal drugs against *Aspergillus terreus*: Global assessment using the methodology of the European committee on antimicrobial susceptibility testing. *Antimicrob Agents Chemother* 2009; **53**: 794–5.
319 Sutton DA, Sanche SE, Revankar SG, Fothergill AW, Rinaldi MG. In vitro amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. *J Clin Microbiol* 1999; **37**: 2343–5.
320 Caston JJ, Linares MJ, Gallego C *et al.* Risk factors for pulmonary *Aspergillus terreus* infection in patients with positive culture for filamentous fungi. *Chest* 2007; **131**: 230–6.
321 Hachem RY, Kontoyiannis DP, Boktour MR *et al. Aspergillus terreus*: an emerging amphotericin B-resistant opportunistic mold in patients with hematologic malignancies. *Cancer* 2004; **101**: 1594–600.
322 Balajee SA, Baddley JW, Peterson SW *et al. Aspergillus alabamensis*, a new clinically relevant species in the section *Terrei*. *Eukaryot Cell* 2009; **8**: 713–22.
323 Kaya AD, Kiraz N. In vitro susceptibilities of *Aspergillus* spp. causing otomycosis to amphotericin B, voriconazole and itraconazole. *Mycoses* 2007; **50**: 447–50.
324 Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, Cuenca-Estrella M, Rodriguez-Tudela JL. Species identification and antifungal susceptibility patterns of species belonging to *Aspergillus* section *Nigri*. *Antimicrob Agents Chemother* 2009; **53**: 4514–7.
325 Morgan J, Wannemuehler KA, Marr KA *et al.* Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Med Mycol* 2005; **43**(Suppl 1): S49–58.
326 Varga J, Houbraken J, Van Der Lee HA, Verweij PE, Samson RA. *Aspergillus calidoustus* sp. nov., causative agent of human infections previously assigned to *Aspergillus ustus*. *Eukaryot Cell* 2008; **7**: 630–8.
327 Houbraken J, Due M, Varga J, Meijer M, Frisvad JC, Samson RA. Polyphasic taxonomy of *Aspergillus* section *Usti*. *Stud Mycol* 2007; **59**: 107–28.
328 Takahata Y, Hiruma M, Sugita T, Muto M. A case of onychomycosis due to *Aspergillus sydowii* diagnosed using DNA sequence analysis. *Mycoses* 2008; **51**: 170–3.
329 Escribano P, Pelaez T, Munoz P, Bouza E, Guinea J. Is azole resistance in *Aspergillus fumigatus* a problem in Spain? *Antimicrob Agents Chemother* 2013; **57**: 2815–20.
330 Alanio A, Sitterle E, Liance M *et al.* Low prevalence of resistance to azoles in *Aspergillus fumigatus* in a French cohort of patients treated for haematological malignancies. *J Antimicrob Chemother* 2011; **66**: 371–4.