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Molecular mechanisms of acquired antifungal drug resistance in principal fungal pathogens and EUCAST guidance for their laboratory detection and clinical implications

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The increasing incidence and changing epidemiology of invasive fungal infections continue to present many challenges to their effective management. The repertoire of antifungal drugs available for treatment is still limited although there are new antifungals on the horizon. Successful treatment of invasive mycoses is dependent on a mix of pathogen-, host- and antifungal drug-related factors. Laboratories need to be adept at detection of fungal pathogens in clinical samples in order to effectively guide treatment by identifying isolates with acquired drug resistance. While there are international guidelines on how to conduct *in vitro* antifungal susceptibility testing, these are not performed as widely as for bacterial pathogens. Furthermore, fungi generally are recovered in cultures more slowly than bacteria, and often cannot be cultured in the laboratory. Therefore, non-culture-based methods, including molecular tests, to detect fungi in clinical specimens are increasingly important in patient management and are becoming more reliable as technology improves. Molecular methods can also be used for detection of target gene mutations or other mechanisms that predict antifungal drug resistance. This review addresses acquired antifungal drug resistance in the principal human fungal pathogens and describes known resistance mechanisms and what in-house and commercial tools are available for their detection. It is emphasized that this approach should be complementary to culture-based susceptibility testing, given the range of mutations, resistance mechanisms and target genes that may be present in clinical isolates, but may not be included in current molecular assays.

Introduction

Amphotericin B was the first antifungal drug for systemic treatment of invasive fungal infections (IFIs), and has been the 'gold standard' of antifungal therapy for nearly 50 years.[1] It has activity against a broad range of human pathogenic fungi, that includes those under review, and extremely few develop resistance following exposure to the drug.

The introduction to the clinic of the triazole antifungals itraconazole and fluconazole in the 1980s offered more therapeutic options for treating IFIs. Additionally, azoles present fewer class-related side effects compared with amphotericin B.[2]

Fluconazole resistance in *Candida albicans* was first reported in the 1990s in AIDS patients who were receiving prolonged low-dose treatment courses for recurrent mucosal candidiasis.[3] Investigations revealed that multiple molecular mechanisms could be involved in acquired resistance in *C. albicans*.[4] *Candida krusei* species (new taxonomic name: *Pichia kudriavzevii*) are intrinsically resistant to fluconazole, while *Candida glabrata* has reduced susceptibility. There are increasing reports of acquired

fluconazole resistance emerging in *Candida tropicalis* and *Candida parapsilosis*. The newly identified *Candida auris*, which has spread widely across the globe, is characterized by frequent acquired resistance to fluconazole, amphotericin B and often multi-antifungal drug resistance.[5]

Although most clinical isolates of *Cryptococcus neoformans* are susceptible to fluconazole, the emergence of drug-resistant strains has been reported, usually linked to prior drug exposure.[6]

Subsequent to its introduction for clinical use, acquired resistance to itraconazole emerged in *Aspergillus fumigatus*.[7,8] The third-generation triazoles, voriconazole, posaconazole and isavuconazole have greater activity against mould fungi than itraconazole. Voriconazole was licensed for the treatment of invasive aspergillosis (IA) in the early 2000s and soon became the drug of choice for that indication. However, within a few years, reports of multi-triazole resistance impacting these triazoles emerged from the Netherlands and the *A. fumigatus* isolates involved were found to have novel molecular changes in the gene encoding the target of the azoles, *cyp51A*.[9] Additionally, some triazole-resistant *A. fumigatus* isolates have *cyp51*-independent resistance mechanisms.

The echinocandins caspofungin, micafungin and anidulafungin are members of the most recent major class of antifungals to be licensed for clinical use. They are established first-line therapy in patients with candidaemia and other forms of invasive candidiasis.[10] Since their introduction, reports have appeared of acquired echinocandin resistance in *Candida* spp., most notably in *C. glabrata*.[11] By contrast, resistance to echinocandins in *Aspergillus* spp. appears to be uncommon[12] but their clinical use as monotherapy in aspergillosis is limited because their *in vivo* activity is lower, a potential consequence of their *in vitro* antifungal effect on *Aspergillus* spp. being only fungistatic.[13]

Flucytosine has its main antifungal activity against *Cryptococcus* and *Candida* spp., but because of the rapid development of fungal resistance during flucytosine monotherapy, its use is mainly limited to combination therapy with amphotericin B. Acquired resistance is associated with mutations affecting cellular drug uptake and the target nucleic acid synthetic pathway. Flucytosine is not recommended for the treatment of aspergillosis because of apparent intrinsic drug resistance, but its efficacy is pH dependent and so it could show *in vitro* antifungal activity at anatomical sites of *Aspergillus* infection where there is an acidic environment.[14,15]

Terbinafine has fungicidal activity and main indication in dermatophyte infections.[16] It is also occasionally used in combination with another antifungal agent for the treatment of mould infections. This is because of its potential to act synergistically in drug combination where there is shared inhibitory action on ergosterol biosynthesis. There are recent reports of terbinafine resistance in the dermatophytes *Trichophyton mentagrophytes*/*Trichophyton interdigitale* complex and *Trichophyton rubrum* particularly from India,[17] but this is increasingly being reported in other countries as well.[18–21] Recently, the taxonomy for the *T. mentagrophytes*/*T. interdigitale* complex was revised and a new species, *Trichophyton indotineae*, proposed for the highly terbinafine-resistant Indian isolates.[22,23]

Trimethoprim/sulfamethoxazole, although principally used as an antibacterial agent, is established as first choice to treat *Pneumocystis jirovecii* pneumonia (PcP). While mutations in target fungal genes are well characterized, their clinical relevance for predicting drug resistance is less clearly established.

The EUCAST sub-committee on Antifungal Susceptibility Testing (as well as the CLSI) has developed susceptibility testing methods for human pathogenic yeasts and moulds, including dermatophytes, with freely available evidence-based breakpoints for categorizing susceptible versus resistant isolates (www.eucast.org/astoffungi) that correlate with clinical outcomes. For less frequent organisms where MICs are available but MIC-outcome data are not, epidemiological cut-off values (ECOFFs) allow detection of isolates with acquired resistance mechanisms. However, susceptibility testing is dependent on culture of the isolate, which is not always possible or available within a reasonable time frame. When cultured samples yield one of the principal fungal pathogens discussed here, this is usually achieved within 48–72 h, whereas dermatophytes require week(s) of incubation, and culture of *P. jirovecii* is not possible in the diagnostic laboratory.

Recognizing the above limitations, molecular tests have been developed and evaluated for rapid fungal detection in body fluids and tissues, and for fungal identification where growth is detected in clinical samples such as blood cultures; these have been extensively and critically reviewed elsewhere.[24–26] Non-culture-based molecular tests to detect antifungal drug resistance in fungal pathogens, whether in-house or commercial, are more limited in number and are reviewed here and summarized in Table 1 for *Aspergillus* spp.,[27–38] *Candida* spp.,[39–41] *Trichophyton* spp.[42,43] and *P. jirovecii*.[44,45] This is accompanied by guidance points on their use in clinical practice (Table 2). Of note, in this review, the term 'mutation' is used for non-synonymous changes in resistance genes that are confirmed or suspected to be related to resistance because they occur in phenotypically resistant strains. The consequent amino acid changes are referred to as alterations.

Aspergillus spp.

Background

Acquired antifungal drug resistance has been described in different *Aspergillus* spp. and for various antifungals, but research has focused mainly on triazole resistance in *A. fumigatus*. Triazoles are not mutagenic, but genetic variation (including triazole resistance mutations) may arise through spontaneous mutations, mitotic recombination or meiotic recombination.[46] When a population of *Aspergillus* conidia is exposed to triazole selection pressure, isolates harbouring a resistance mutation will thrive in comparison with WT isolates and become dominant in the population. This process of resistance selection can take place in a patient who receives prolonged treatment with triazoles (in-host selection) or in the environment where residues of azole fungicides with activity against *A. fumigatus* may provide selection pressure.

Acquired triazole resistance in *A. fumigatus*

Cyp51-mediated triazole resistance

In *A. fumigatus*, triazole resistance mechanisms are mainly associated with alterations in the *cyp51A* gene that encodes the

Table 1. In-house and commercial non-culture methods that detect molecular resistance mechanisms in reviewed fungal pathogens

Fungal pathogen (Specimen types)	Antifungal drug	In-house detection of drug resistance mechanism(s)	Commercially available assays for detection of drug resistance mechanism(s)
Aspergillus spp. (Sputum, bronchoalveolar lavage, serum, or plasma)	Triazoles	Real-time PCR studies[24–30] Pyrosequencing[31]	Aspergenius® multiplex real-time PCR detects *Aspergillus* TR_{34}/L98H; TR_{46}/T289A; TR_{46}/Y121F gene mutations in *cyp51A*[32,33,36–38] Aspergenius® multiplex real-time PCR detects G54 and M220 RUO in *cyp51A* of *A. fumigatus*[34] MycoGENIE® detects *A. fumigatus* TR_{34}/L98H gene mutations[35–38] FungiplexR® Aspergillus Azole-R IVD real-time PCR detects *A. fumigatus* TR_{34} and TR_{46}[24,38]
Candida spp.	Triazoles	No assays for azole resistance due to multiple mechanisms playing in concert	
	Echinocandins	PCR assay to detect Glucan synthase (*FKS*) gene(s) for subsequent sequencing to identify mutations in the hotspots of *fks1* and *fks2* (*C. glabrata* only)[39,40]	
Cryptococcus spp.	Fluconazole	No assays due to variable mechanisms of resistance	
Dermatophytes	Terbinafine	PCR assay to detect squalene epoxidase (*SQLE*) gene for subsequent sequencing to identify mutations in *Trichophyton mentagrophytes/interdigitale* and *T. rubrum*[17,42]	DermaGenius® Resistance Multiplex real-time PCR kit.[43] Detects: *T. rubrum/Trichophyton soudanense*, *T. interdigitale/mentagrophytes*, *T. mentagrophytes* (*ITS type IV*), *T. tonsurans*, *T. violaceum*, *Trichophyton quinckeanum/ Trichophyton schoenleinii* and SQLE alterations: L393F, F397L, L393S, F397I, F397V.
P. jirovecii	Trimethoprim/ sulfamethoxazole	Dihydropteroate synthase gene mutations: Detected using RFLP, PCR sequencing, SSCP, MLST, PCR pyrosequencing[44] Atovaquone mutations: Sequencing of Cytochrome b substitutions in the Qo region T121I, L123F, T100I, I120V, S125A, P239L and L248F (see Table S1)	PneumoGenius® real-time PCR detects mutations at codons 55 and 57 in dihydropteroate synthase (DPHS) gene-encoding sulphonamide resistance[45]

14α-demethylase, an enzyme responsible for the final step of the ergosterol biosynthesis pathway. Ergosterol is the major sterol component of fungal membranes and is critical for membrane permeability and fluidity, thereby being essential for fungal growth and survival.[47] *A. fumigatus* carries one Cyp51A and one Cyp51B protein, where Cyp51B is constitutively expressed and Cyp51A expression is inducible.[48] Lanosterol 14α-demethylase point amino acid mutations mainly appear in Cyp51A and can lead to amino acid changes that result in modifications to ligand access channels through which azoles gain access to the enzyme active site and bind to the haem molecule.[48] Hotspots for amino acid substitutions include G54, G138, M220 and G448, which correspond with specific azole resistance phenotypes (Table 3).[49–82] These single resistance mutations are commonly found in patients with prior exposure to triazole therapy. Non-synonymous substitutions of *A. fumigatus* Cyp51A protein, such as L98H, Y121F and T289A, are commonly accompanied by tandem repeats (TRs) in the gene promoter,[30] which up-regulate the expression of the *cyp51A* gene.[83] Resistance mutations that involve TRs, such as TR_{34}/L98H and TR_{46}/Y121F/T289A (Table 3), are commonly associated with environmental resistance selection through exposure to azole fungicides.[84] Molecule similarity between triazole fungicides and medical triazoles is believed to be responsible for cross-resistance.[85] Single resistance mutations are frequently found in patients treated with triazoles, but they have also been recovered from the environment.[86] While TR-mediated resistance mutations are associated with environmental resistance selection, in-host selection of a TR_{120} resistance mutation,[78] and also an in-host selection of a TR variation TR^{3}_{34}/L98H, has been reported.[87] Ultimately, characteristics of

Table 2. Key points to guide the use of molecular tests for detection of antifungal drug resistance (AFDR)

General:	*The clinical need for molecular testing for AFDR may be considered:*
	In patients who fail a course of appropriate antifungal therapy, who have a subsequent relapse of their infection after an appropriate course of therapy, or who develop a new fungal infection after an earlier prolonged course of antifungal therapy.
	Where local epidemiology suggests that there are high rates of resistance in a particular fungal species, examples include *C. glabrata* resistance to echinocandins, and *A. fumigatus* resistance to voriconazole.
	Where there is limited access to local EUCAST/CLSI compliant phenotypic antifungal susceptibility testing.
	Where phenotypic testing gives borderline susceptible/resistant result and determining if there is a resistance mechanism present can be helpful to guide clinical decision making.
Pathogen-specific:	*Aspergillus:*
	Molecular tests have mostly been applied to detect triazole resistance mechanisms in *A. fumigatus*, less data are available for other *Aspergillus* spp.
	The analytical sensitivity of PCR tests for triazole resistance in *A. fumigatus* due to *cyp51* gene mutation(s) (single copy gene) is lower than sensitivity of PCR to detect presence of the fungus (targets multi-copy gene) in clinical samples.
	A positive PCR for a resistance mechanism to triazoles in *A. fumigatus* suggests avoidance of triazole therapy. However, a negative PCR result for resistance does not confirm triazole susceptibility.
	Acquired resistance to amphotericin B or echinocandins in *A. fumigatus* is rare.
	Candida:
	For triazoles, and in particular fluconazole, the existence of multiple resistance mechanisms in individual strains of *Candida* spp. (e.g. combined *erg11* target gene mutation + efflux mechanism) means that molecular tests to detect AFDR have had limited clinical application; furthermore non-detection of a particular resistance mechanism does not infer triazole susceptibility.
	For echinocandins PCR protocols have been developed that target the hotspot regions of the *FKS* genes, but these are technically demanding and species-specific.
	Acquired echinocandin resistance is most frequent in *C. glabrata*. It is unlikely to occur in patients not previously exposed to echinocandin therapy unless when part of a nosocomial outbreak.
	Acquired resistance to amphotericin B is considered rare.
	There are PCR protocols to detect flucytosine resistance, but as this drug is uncommonly used in candidiasis treatment, phenotypic susceptibility testing is preferred.
	Cryptococcus:
	Because fluconazole and flucytosine resistance mechanisms in *Cryptococcus* spp. are complex, phenotypic testing is preferred over molecular methods.
	Dermatophytes:
	Because of slow growth in laboratory cultures, and limited experience with phenotypic sensitivity testing, the use of molecular tests to detect resistance mechanisms is attractive.
	Both in-house and, recently, commercial PCR tests are available to detect the most common *sqle* gene mutations reported to be associated with terbinafine-resistant *Trichophyton* spp.
	Molecular tests to detect resistance mechanisms for triazoles, in particular itraconazole, are less well developed and not recommended for routine diagnostic use.
	P. jirovecii:
	Because of inability to culture *P. jirovecii* in the diagnostic laboratory there are no correlates between phenotypic sensitivity and putative resistance mutations in therapeutic drug target genes.
	The detection of mutations in drug target genes is not always associated with treatment failure, as this is likely also associated with initial disease severity, but mutations may be related to diminished efficacy of trimethoprim/sulfamethoxazole for PcP prophylaxis.
	Molecular tests may be warranted for clinical decision making in cases of PcP considered refractory to treatment (no improvement post >7 days treatment).

the fungus and its (azole) environment will determine the supply of mutations and subsequent selection rather than whether resistance was selected in a host or in the environment.[86,88]

Other triazole resistance mechanisms in A. fumigatus

In 10%–50% of triazole-resistant *A. fumigatus* isolates, a WT *cyp51A* gene sequence is found, indicating that other pathways or resistance mutations are likely to be present.[54] The *hap* gene complex member *hapE* has been shown to be associated with an azole-resistant phenotype.[89,90] Another target involves the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase-encoding gene (*hmg1*), which represents a rate-limiting enzyme in the ergosterol biosynthetic pathway.[91] *Hmg1* mutations have been proposed as an underlying mechanism in azole-resistant isolates lacking *cyp51A* mutations.[91–94] Other mechanisms include the overexpression of *cyp51B*,[95] the overexpression of efflux pumps (e.g. Cdr113 and AtrF),[96,97] and mutations in regulators and the transcriptional network (e.g. SrbA).[98] The negative cofactor two A and B (NCT) complex was recently identified as a key regulator of triazole resistance by modulating the expression levels of the transcription factors associated with ergosterol biosynthesis and triazole resistance.[99]

Trends in triazole resistance phenotypes and genotypes in A. fumigatus

SNPs are commonly found in the *cyp51A* gene of *A. fumigatus* isolates of which the significance for the resistance phenotype is unknown. In a recent resistance survey involving 640 clinical *A. fumigatus* isolates, 445 isolates harboured TR_{34}/L98H of which 24 (5%) exhibited one or more additional mutations, including F495I (9 isolates), Q259H (5), S297T (4), D262N (1), N326H (1), P337L (1), Y341H (1), I364V (1), G328A (1) and L399V (1).[49] These SNPs might or might not impact on the triazole resistance phenotype. F495I was recently shown to be associated with resistance to imidazole fungicides, such as imazalil and prochloraz, which was confirmed by recombination experiments.[58] However, TR_{34}/L98H with F495I showed lower voriconazole MICs compared with TR_{34}/L98H without F495I, indicating an effect of this SNP on the activity of voriconazole.[58] *Hmg1* gene mutations have been found in isolates harbouring TR_{34}/L98H and TR_{46}/Y121F/T289A and have been suggested to alter the triazole-resistant phenotype.[91] However, although *hmg1* mutations were found in 24% of *A. fumigatus* isolates with a triazole-resistant phenotype, 8% of isolates with a WT phenotype also harboured SNPs in this gene.[100] The location and type of SNP may determine their significance for the triazole-resistant phenotype, but this requires further studies.

In addition to SNPs, an increased number of TRs have been observed in triazole-resistant *A. fumigatus* isolates, including three or four copies of TR_{46}.[76] However, no specific phenotype change could be demonstrated in these isolates.

Finally, a significant trend towards decreased voriconazole resistance was noted in TR_{34}/L98H isolates in the above-mentioned resistance survey.[49] In 2013 96% (44 of 46) of TR_{34}/L98H isolates were classified as voriconazole resistant, while in 2018 only 55% (59 of 108) of TR_{34}/L98H isolates exhibited a voriconazole-resistant phenotype ($P=0.0001$).[49] However, no underlying mechanism was found that could explain the voriconazole phenotype shift. Through known and unknown resistance mechanisms, an increasing diversity in azole-resistant phenotypes and genotypes is emerging, which is likely to result from the dynamic environments *A. fumigatus* is exposed to and its ability to adapt to these changes.

Resistance testing of A. fumigatus and clinical implications

Although susceptibility testing remains the cornerstone for guidance of antifungal therapy choices, there are increasing challenges with respect to *A. fumigatus* resistance. Firstly, many cases of IA are diagnosed in the absence of a positive *Aspergillus* culture. Mixed triazole-susceptible and triazole-resistant isolates causing infection in patients with IA have been reported and testing of multiple *A. fumigatus* colonies is therefore recommended.[101] As susceptibility testing of multiple colonies is very laborious, an agar-based screening strategy has proven useful to detect resistant colonies.[102] Clinical breakpoints are available for triazoles and *A. fumigatus*, although it remains unclear if susceptible isolates can be safely treated with a triazole if the isolate is resistant for one or more other triazoles.[103] In culture-negative patients, commercial resistance PCR assays are available to detect TR_{34}/L98H and/or TR_{46}/Y121F/T289A alterations directly in clinical specimens (Table 1).[38,104] As these assays rely on the amplification of specific targets, which are confirmed as the cause of azole resistance, additional/alternative resistance mutations and single resistance mutations are not detected. With increasing variation in resistance phenotypes, and thus of the proportion of resistance due to the mechanisms not targeted by the PCR, the correlation between resistance PCR and resistance phenotype will become less well defined. Furthermore, the analytical sensitivity of resistance PCRs in bronchoalveolar lavage (BAL) (which target the single copy *cyp51A* gene) is lower than the *Aspergillus* PCR (where a multi-copy gene is amplified), resulting in the inability to obtain resistance target amplification in 30% of patients despite resistant infection.[105] Thus, although resistance PCR positivity documents resistance, and triazole therapy thereby can be avoided if a resistance mutation is detected, PCR negativity does not confirm azole susceptibility, which in some circumstances, such as CNS aspergillosis, is critical. New approaches are warranted to enable detection of a broad range of resistance mutations, such as sequence-based strategies.[31]

Acquired triazole resistance in other Aspergillus species

Much less is known about the prevalence and underlying mechanisms of acquired triazole resistance in other *Aspergillus* spp. This is probably in part because they are less often causes of invasive disease, but also because acquired resistance is less frequently researched. *Cyp51A* alterations have been reported in azole-resistant *Aspergillus terreus* including M217I and G51A,[84] D344N and M217I,[106] and M217T and M217V.[107] The codons G51 and M217 in *A. terreus* correspond, respectively, to codons G54 and M220 in *A. fumigatus* which, as described above, are also linked to triazole resistance. Molecular resistance mechanisms have also been investigated in *Aspergillus flavus*. P214L has been found in Cyp51A, and S240A and H349R in Cyp51C in triazole-resistant isolates, but not in susceptible counterparts.[108,109] Moreover, MDR2, atrF and *mfs1* up-regulation

Table 3. *Cyp51* gene-related mutations and corresponding phenotype (EUCAST methodology) in triazole-resistant clinical *A. fumigatus* isolates

	Phenotype (MIC mg/L)[a]				
Resistance mutation	Itraconazole	Voriconazole	Posaconazole	Comment	References
TR_{34}/L98H	2–>16	0.5–>16	0.25–2	A significant trend towards lower VRC MICs was observed between 2013 and 2018 in a national surveillance program[49]	49–68
TR_{34}/R65K/L98H	>16	8	4		69
TR_{34}/L98H/S297T	>16	2	0.5	S297T not considered relevant for azole resistance[29]	51
TR_{34}/L98H/S297T/F495I	>16	1–8	0.5–>8	S297T not considered relevant for azole resistance;[29] F495I associated with imidazole resistance[58]	51, 54, 58, 60, 67, 71
TR^{3}_{34}/L98H	>16	4 (–8)	1		87
TR_{46}/Y121F/T289A	0.5–>16	>16	0.125–2		56, 58–61, 64–68, 72–74
TR_{46}/Y121F/M172I/T289A	1	>16	0.5	M172I is also found in WT isolates[29]	75
TR^{3}_{46}/Y121F/M172I/T289A/G448S	>16	>16	1	M172I is also found in WT isolates[29]	74, 76
TR_{53}	>16	16	0.25		77
TR_{120}/F46Y/M172V/E427K	16–>16	4	0.5	In-host selection of TR_{120} in an isolate harbouring F46Y/M172V/E427K with a triazole WT phenotype	78
F46Y/M172V/E427K	4	0.5–8	1–4	F46Y, M172V and E427K have also been found in WT isolates[29]	50, 57, 79
F46Y/M172V/N248T/D255E/E427K	>8	2	0.5	F46Y, M172V, N248T, D255E and E427K have also been found in WT isolates[29]	53, 57, 71
G54W,E,R,V	>16	0.06–2	0.5–>8		50, 52, 53, 55, 63
G138C	>8	8–>8	1–>8		53, 80, 81
P216L	>16	0.5–2	0.25–0.5	POS MIC >16 described in one study,[61] possibly suggesting accumulation of additional resistance mutations	52, 53, 61, 63, 67
F219L	>16	0.25	0.25		52
M220I,K,L,R,T,V	>16	0.5–4	0.5–>8		50, 51, 53, 59, 63
Y431C	>8	2–4	1–2		81
G448S	>8	2–8	0.25–1		53

VRC, voriconazole; POS, posaconazole.

[a]MIC ranges may represent a single isolate or the accumulation of MICs of multiple isolates from different studies. Broad MIC ranges could reflect technical variation in MIC determination, factors related to the resistance mechanism detected or the presence of (undetected) additional resistance mutations. Isavuconazole resistance classification is highly similar to that of voriconazole.[82]

resulting in efflux were reported in azole-resistant *A. flavus* from South Korea.[110] Thus, as for *A. fumigatus*, target gene sequencing can help detect acquired resistance, but it is not enough to rule out additional mechanisms that cause triazole resistance in these species.

Acquired echinocandin and amphotericin B resistance in A. fumigatus

Although echinocandin resistance was successfully promoted in laboratory strains *of A. fumigatus* with *fks* gene mutations as early as in 2005, and this mechanism is common in *Candida*, echinocandin resistance has rarely been reported in *A. fumigatus*. The first example of echinocandin resistance in clinical *A. fumigatus* isolates was reported in 2008 in a clinical isolate displaying overexpression of the *fks1* gene[111] whereas the second involved a point mutation in *fks1* hotspot 1.[12] Recently, it was shown that caspofungin may induce cellular stress, promoting formation of mitochondrion-derived reactive oxygen species and triggering an alteration in the composition of plasma membrane lipids surrounding glucan synthase, rendering it non-susceptible to echinocandins.[112] None of these mechanisms is easily detectable in clinical microbiology laboratories. Finally, we are unaware of any documented clinically relevant acquired amphotericin B resistance mechanisms in *A. fumigatus*.

Candida species

Background

Acquired azole drug resistance rates in *C. albicans* are relatively low worldwide (≤1%).[113,114] In a 20 year global surveillance study, using CLSI criteria, azole resistance in *C. glabrata* isolates ranged from 5.6% to 10.1%; however, these rates were much higher in North America (10.6%) than in Asia-Pacific (6.8%), Europe (4.9%), or Latin America (2.6%).[115] Resistance rates in *C. parapsilosis* and *C. tropicalis* were as high as 5.4% and 4.9%,

respectively, with remarkable differences between continents.[115] *C. krusei* is intrinsically resistant to fluconazole with MIC values usually >32 mg/L.[113–115] Other species with intrinsically elevated fluconazole MIC values include *C. glabrata*, *Candida inconspicua*, *Candida lipolytica*, *Candida norvegensis*, *Candida rugosa*, *Candida pelliculosa* and *Candida guilliermondii*.[114]

Acquired azole resistance in Candida spp.

The gene encoding the target enzyme for azoles in *Candida* is the *erg11* (equivalent to the *cyp51A* gene in *Aspergillus*). Acquired azole resistance in *Candida* spp. is quite uncommon and when found is often preceded by months of therapy.[116] It can be caused by a variety of resistance mechanisms[117–119] that often work concurrently in clinical isolates and consequently the underlying mechanisms in isolates with azole resistance are rarely dissected. Elevated non-WT fluconazole MICs are most commonly observed in *C. glabrata* and *C. auris*.

Alterations in erg11

Amino acid substitutions in Erg11 can affect the optimal binding of the azoles to their target. Erg11 amino acid substitutions have been reported in *C. albicans* by Morio *et al.*[120]

A study looking at 63 fluconazole-resistant *C. albicans* clinical isolates observed that 55 carried at least one mutation in *erg11*.[121] When these mutations are introduced into an azole-susceptible *C. albicans* strain, an increase in fluconazole MIC values is observed and is most prominent if the mutations are homozygous.[121,122]

C. tropicalis isolates displaying azole resistance alone, or combined with amphotericin B, carried *erg11* mutations, which lead to alterations at G464D and Y132F among isolates displaying fluconazole MIC values >64 mg/L and voriconazole MIC values of >8 mg/L.[123] Beyond the Erg11 alterations, these isolates displayed Erg3 substitutions S258F and S113G. In a study evaluating azole resistance among 431 *C. parapsilosis* and 227 *C. tropicalis* isolates collected worldwide, 38 of 46 *C. parapsilosis* and 3 of 6 *C. tropicalis* isolates had the Erg11 alteration Y132F.[114]

In *C. auris*, a small number of *erg11* gene mutations cause azole resistance and these are usually clade specific.[124,125] Y132F causes high resistance rates to fluconazole and voriconazole with CLSI MIC values of 1 or 2 mg/L, while K143R and F126T increase fluconazole MIC values, but voriconazole values remain below 0.5 mg/L.[124]

Up-regulation of erg11

Up-regulation of *erg11* is not commonly noted in clinical isolates and seems to only have a modest effect on azole MIC values.[113,114]

Alterations in erg3

Missense or nonsense mutations in *erg3* have been reported to enable fungal cells to develop resistance to polyenes and azoles[126] but these are uncommon in clinical isolates.

Efflux up-regulation

Up-regulation of efflux systems from the ATP-binding cassette (ABC) transporters and the major facilitator superfamily (MFS) can cause lower intracellular accumulation of the azoles, leading to resistance.[119] The ABC transporters involved in azole resistance in *C. albicans* are Cdr1 and Cdr2. Functional homologues of these are noted in other *Candida* spp. The most important MFS transporter in *Candida* spp. is the Mdr1 pump, previously named BenR. In contrast to Cdr1 and Cdr2, which have all azoles as substrates, Mdr1 only extrudes fluconazole. Their substrate specificity in *C. albicans* and *C. glabrata* was confirmed in a study by Sanglard and Coste[127] who evaluated the Cdr1, Cdr2 and CgCdr1 from *C. glabrata*, as well as Mdr1 and Erg11 alterations. They demonstrated that fluconazole MIC values were affected by all resistance mechanisms, whereas the MIC values of itraconazole, isavuconazole, posaconazole and voriconazole were unchanged when only Mdr1 was overexpressed.

Alterations in various zinc cluster transcription factors (ZCFs) have been identified as being responsible for the up-regulation of the efflux systems in *Candida* spp. In *C. albicans*, the promoter Tac1 is known to up-regulate Cdr1 and Cdr2.[128,129] Mutations in multidrug-resistant regulator (*mrr*) *2* increasing the expression of Cdr1 have also been described.[130] In *C. glabrata*, resistance to azoles is mediated by the overexpression of the ABC multidrug transporter regulated by the ZCF CgPdr1.[131] This regulator is important for fungal–host interactions. Mutations in this transcription factor have been demonstrated to increase azole resistance *in vitro* and *in vivo*.[131]

Overexpression of Mdr1 in *C. albicans* has been associated with a gain-of-function mutation in the promoter *mrr1*.[132] *C. albicans* isolates that became homozygous to *mrr1* with a single nucleotide substitution, resulting in P683S and G997V, demonstrated elevated fluconazole MIC values. In a recent global surveillance study, overexpression of Mdr1 was detected in 38 of 46 azole-resistant *C. parapsilosis* isolates exhibiting azole non-susceptible or non-WT phenotypes.[114]

Combinations of resistance mechanisms

In most *Candida* spp. azole resistance is the result of a combination of mechanisms. In *C. albicans* laboratory isolates, resistance usually occurs through the gain-of-function alterations in the transcription factors Mrr1, Tac1 and Upc2, which results in the up-regulation of efflux pumps and ergosterol biosynthesis genes concomitantly. The combination of these mechanisms generated a 500-fold increase in fluconazole MIC values.[133] In addition to up-regulating genes encoding efflux transporters and *erg11*, clinical isolates had Erg11 amino acid substitutions known to cause resistance.[116]

Molecular detection of azole resistance in Candida

Due to this plethora of resistance mechanisms that can co-exist, susceptibility testing is a better tool for clinical laboratories to detect resistance to azole agents than genetic methods. Molecular detection of *erg11* gene mutations known to cause azole resistance could be used to detect or confirm resistance.[134] However, resistance could not be ruled out in the absence of these alterations.

Echinocandin resistance

The 1,3-β-D-glucan synthase (GS) complex mainly comprises two subunits Fks and Rho, of which Fks1p is the main target of the

	Anidulafungin EUCAST ECOFF (mg/L)	Fks1 Hotspot 1 1st AA no.	Fks1 Hotspot 1 AA sequence	Fks1 Hotspot 2 1st AA no.	Fks1 Hotspot 2 AA sequence	Fks2 Hotspot 1 1st AA no.	Fks2 Hotspot 1 AA sequence	Fks2 Hotspot 2 1st AA no.	Fks2 Hotspot 2 AA sequence
C. albicans	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. auris	NA	635	FLTLSLRDP	1350	DWIRRYTL				
C. dubliniensis	0.03	641	FLTLSLRDP	1357	DWIRRYTL				
C. glabrata[a]	0.06	625	FLILSLRDP	1340	DWVRRYTL	659	FLILSLRDP	1374	DWIRRYTL
C. kefyr	(0.03)	651	FLTLSLRDP	1366	DWVRRYTL				
C. krusei	0.06#	655	FLILSIRDP	1364	DWIRRYTL				
C. lusitaniae	(0.06)	634	FLTLSLRDP	1348	DWIRRYTL				
C. tropicalis	0.06	650	FLTLSLRDP	1366	DWIRRYTL				
C. parapsilosis	4	652	FLTLSLRDA	1369	DWIRRYTL				
C. metapsilosis	(4)	*104**	FLTLSLRDA	*821**	DWIRRYTL				
C. orthopsilosis	(4)	645	FLTLSLRDA	1362	DWVRRYTL				
C. guilliermondii	(4)	632	FMALSLRDP	1347	DWIRRYTL				
C. lipolytica	NA	662	FLILSLRDP	1387	DWIRRCVL				
S. cerevisiae	(1)	639	FLVLSLRDP	1353	DWVRRYTL	658	FLILSLRDP	1372	DWVRRYTL

Figure 1. Amino acid (AA) sequences of Fks1 and Fks2 in 10 WT *Candida* species. Amino acid codons associated with increased MIC are underlined and in bold font. In the online version a colour indication is applied to inform origin (naturally occurring or acquired) and impact on the MIC (strong, weak or silent). **Red**: 'strong R' mutation, subscript at codons involving a mutation or deletion; superscript at codon involving a mutation or stop codon. **Yellow**: 'weak R' mutation. **Blue**: inherent AA difference with proven or possible relation to intrinsic lower susceptibility. **Grey**: inherent AA difference of unknown importance. Green: inherent AA difference, probably with no effect. [a]Of note: combination of the following alterations outside the defined hotspots has also been confirmed as cause of echinocandin resistance: Fks1 W508stop combined with Fks2 E655K. ECOFFs indicated in () are estimated WT upper limits (peak MIC + 2 dilutions) based upon the MICs of Danish blood isolates. **Inaccurate annotation, sequencing of entire gene-sequence required.* #The micafungin (but not anidulafungin) ECOFF for *C. krusei* is noticeably higher (0.25 mg/L) than for *C. albicans* (0.015 mg/L) and *C. glabrata* (0.03 mg/L). NA, not available. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

echinocandins.[135,136] Fks1, Fks2 and Fks3 are encoded by the genes *fks1*, *fks2* and *fks3*, respectively. Resistance in *Candida* spp. is caused by mutations in *fks1* for most *Candida* spp. and also in *fks2* in *C. glabrata*.[137] These occur within two specific regions, known as hotspots (HS) 1 and 2 (Figure 1).[136] These regions are highly conserved within each species, but have different amino acid sequences. Intrinsically elevated echinocandin MIC values have been associated with inherent genetic polymorphisms within the *fks* sequence among certain species, notably including *C. parapsilosis*.[138]

Echinocandin resistance rates are low for most *Candida* spp., with the exception of *C. glabrata*. Results for 20 years of the SENTRY Antifungal Surveillance Program demonstrated that echinocandin resistance did not show dramatic changes for the five most common *Candida* spp.[115] This is different to the findings of Alexander *et al.*[139] who showed an increase in echinocandin resistance in *C. glabrata* of 7.3%. However, a large survey performed by the CDC, Atlanta, USA, found that the rate of echinocandin non-susceptible *C. glabrata* isolates increased from 4.2% to 7.8% between 2008 and 2014.[140] Recent data from a global surveillance study demonstrate a decrease in caspofungin resistance rates among *C. glabrata*. From 2017 to 2020, only 1.9% of the 1448 *C. glabrata* collected in 29 countries exhibited caspofungin resistance and 2.7% displayed resistance to any echinocandin tested (M. Castanheira, unpublished). In a nationwide Danish survey of *Candida* spp. isolates from cases of candidaemia (2004–18), the proportion of *Candida* spp. susceptible to fluconazole decreased while there was a slight increase in echinocandin resistance and this was associated with a proportional decrease in *C. albicans* isolates and an increase in *C. glabrata* isolates.[141] In one single-centre survey of *Candida* spp. isolates, also from candidaemia cases collected between 2002–19, there was an increase in fluconazole resistance (3.5%–6.8%) while echinocandin resistance remained stable at around 3%.[142]

C. albicans is the second most common *Candida* sp. to display echinocandin resistance, most likely due to its high prevalence in clinical settings. Heterozygous and homozygous mutations in *C. albicans* may generate different phenotypes.[143]

In a study of *C. glabrata* isolates over a 10 year period, 119 isolates displaying non-WT MIC values for echinocandins were screened for *fks* gene mutations.[144] A total of 28 had alterations in *fks* hotspots. The most common alterations were *fks2* HS1 S663P or F659S/V/Y, followed by *fks1* HS1 S629P. This is also true for the emerging species *C. auris*, which is a major problem in certain countries.[145–147]

Candida spp. isolates from recent studies have mostly demonstrated mutations of the corresponding codons in both *C. glabrata* and *C. albicans*, and other species, confirming that these are the dominant amino acid alterations (Figure 1).[113,114,136]

Isolates with double mutations have higher MIC values. Lackner *et al.*[143] demonstrated that *C. albicans* laboratory mutants with homozygous double mutations significantly enhance resistance in an *in vivo* model when compared with heterozygous single mutations.

Although alterations in Fks2p and Fks3p are deemed unimportant for most *Candida* spp., homozygous deletions of *fks2* and *fks3* in laboratory-engineered strains of *C. albicans* decrease their susceptibility to echinocandins since they result in a compensatory overexpression of Fks1 and increase in cell wall glucan.[148]

Interestingly, the MIC values for isolates with the same *fks* gene mutations are not always the same. This finding suggests the presence of compensatory mutations that could lead to changes that would improve the binding of the echinocandins —although not to the same level as a WT enzyme.[149] Unlike with azoles, drug efflux and biosynthesis pathway modulation of the fungal cell wall does not seem to affect *Candida* spp. susceptibility to echinocandin agents.

Clinical aspects of echinocandin resistance

Candida spp. isolates can develop resistance to echinocandins after short therapy courses or long-term treatment (median ~1 month),[150] and resistance has not been described in drug-naive patients except where it has been acquired nosocomially.[149] This means that echinocandin use is the main driver of mutations and resistance. Thus, isolates from breakthrough infection have elevated MIC values and amino acid substitutions in the Fks hotspots.[151]

In a study evaluating the risk factors for patients having *C. glabrata* candidaemia with an isolate harbouring an *fks* mutation, patients who received 3 or more days of echinocandin therapy were more likely to have isolates that developed echinocandin resistance and to carry *fks* mutations.[152]

Molecular detection of echinocandin resistance in Candida spp.

Molecular methods for detection of echinocandin resistance have been developed.[153–155] The most commonly used was PCR targeting the hotspot regions for the *fks* sequences, followed by conventional sequencing methods. However, *fks* genes are over 3 kb in length and sequencing the entire gene can be cumbersome. Furthermore, primers for *fks* gene amplification are species specific, and this increases both the need for expertise and added laboratory workload. A pyrosequencing method was described for detecting *fks* mutations[156] and, more recently, next-generation sequencing has been used to evaluate several different traits in fungal isolates, including echinocandin resistance in *Candida* spp.[113,114,157]

Amphotericin B and flucytosine resistance

Acquisition of amphotericin B resistance in *Candida* spp. is apparently rare,[158] but current MIC-based methods may fail to detect resistant isolates. Overall, low rates of breakthrough candidemia in patients treated with amphotericin B have been described.[159]

Resistance to flucytosine in *Candida* spp. is primarily related to impaired drug uptake by the cytosine permease encoded by *fcy2*, and alterations in enzymes involved in the conversion of flucytosine to the active compound 5-fluorouracil (Fcy1 and Fur1).[160]

Molecular detection of amphotericin B and flucytosine resistance in Candida

Amphotericin B resistance in *Candida* spp. is mainly due to changes in the ergosterol biosynthesis pathway.[161] In contrast to azole resistance, where *erg11* mutations have been directly associated with a resistance phenotype, there is no clear marker for detection of amphotericin B resistance.[162] Mutations in *fcy2* or *fur1* that are associated with flucytosine resistance can be detected by PCR.[163,164] However, as flucytosine is rarely used for *Candida* infections knowledge on resistance mutations is still limited and phenotypic testing remains the more attractive method to detect resistance.

Cryptococcus spp.

Background

Cryptococcal meningitis occurs mainly in people living with HIV and represents an important fungal cause of mortality, particularly in sub-Saharan Africa.[165] Recommended treatment for cryptococcal meningitis is an induction regimen comprising amphotericin B combined with flucytosine, followed by a consolidation phase with fluconazole.[166,167] Regimens may vary depending on drug availability.[167] For maintenance therapy, fluconazole is the treatment of choice. Indeed, amphotericin B, flucytosine and the azoles show good *in vitro* activity against *Cryptococcus* spp.[168] In contrast, *Cryptococcus* spp. are intrinsically resistant to echinocandins.[169,170]

Acquired resistance to amphotericin B in *Cryptococcus* spp. seems very uncommon.[168] Resistance rates to fluconazole and flucytosine remained low, at least in Europe and the USA, while higher rates of fluconazole resistance have been reported from other parts of the world such as Cambodia, South Africa and Taiwan.[171–173] In a comprehensive review of 29 studies published between 1988 and 2017, the mean fluconazole resistance rate was 10.6% for incident isolates, while it rose to 24.1% for relapse isolates.[173] Of note, in Africa specifically, 70% of isolates from cases of relapse were resistant.[172] Exposure to fluconazole during therapy is probably the main driving force for emergence of resistance.[168] However, exposure to fungicides in the environment may also be responsible for fluconazole resistance.[174–176] Indeed, it has been shown that *in vitro* exposure to both azole or even non-azole fungicides used in agriculture can select fluconazole cross-resistant isolates of *C. neoformans* and *Cryptococcus gattii*.[174,175] Although primary flucytosine resistance is rare in *C. neoformans*,[168] acquired resistance is common when the drug is used as monotherapy.[177,178]

In vitro antifungal susceptibility testing

Although there are reference methods for antifungal susceptibility testing, clinical breakpoints are currently not available for *Cryptococcus* with the exception of 1 mg/L for amphotericin B for EUCAST (www.eucast.org).[173] Nevertheless, the values of >32 and >16 mg/L for fluconazole and flucytosine, respectively, and mainly obtained by the CLSI methodology, are often used as

Table 4. Summary of mechanisms of resistance in *Cryptococcus* spp. to the main antifungal drug classes[a]

Drug class	Mode of resistance[b]	Molecular mechanisms	References
Azoles[c]	Decreased affinity of 14α-demethylase, mutations in ERG11	G484S, G470R, Y145F, (G344S)	182–188
	ERG11 overexpression		182, 189
	Decreased intracellular concentration of azoles and efflux pumps	Up-regulation of ABC transporter CnAFR1	179, 182, 183, 190–192
	Heteroresistance, chromosome duplication	Chromosome 1	193–200
Amphotericin B	Ergosterol depletion	Defect in delta 8-7 isomerase	183, 205
	Unknown		190, 204
Flucytosine	Decreased uptake or metabolism	Alterations of cytosine permease (FCY2), cytosine deaminase (FCY1), uracil phosphoribosyltransferase (FUR1)	177, 209
	Unknown	Unknown	208

[a]*Cryptococcus* spp. are inherently resistant to echinocandin drug class.
[b]Several mechanisms of resistance may be present in a single isolate.
[c]Applies mainly to fluconazole.

breakpoints in the literature.[168] For fluconazole specifically, heteroresistance, where clinical resistance is not related to the MIC (see below), further complicates MIC interpretation. Despite these technical issues, there are many supportive data that correlated the high *in vitro* MICs to fluconazole, flucytosine, or amphotericin B, with clinical failure both in experimental animal models[179–181] and in patients.[168]

Acquired azole resistance

Mechanisms of fluconazole resistance in *Cryptococcus* are varied and include mutations in *erg11*,[182–188] overexpression of *erg11*,[182,189] efflux pumps[179,182,183,190–192] and heteroresistance.[193–200] (Table 4).

Alteration of the target enzyme, 14α-demethylase has been reported in several studies (Table 4). A reduced affinity of the enzyme for azoles, associated with other mechanisms of resistance, was demonstrated in fluconazole-resistant clinical isolates of *C. neoformans*.[182,183] More recently, several mutations in *erg11* associated with azole resistance have been reported, including G484S (corresponding to G464S in *C. albicans*),[184,186] G470R[187] and Y145F (equivalent to Y132F in *C. albicans*).[185] A G344S mutation in *erg11* has also been observed in a multi-azole-resistant laboratory mutant.[188]

Overexpression of *erg11* is also a common mechanism of azole resistance in yeasts. In an early study, it was shown that there was an increased level of 14α-demethylase within fluconazole-resistant *C. neoformans* isolates from AIDS patients.[182] Similarly, overexpression of *erg11* in the presence of fluconazole, associated with overexpression of an efflux pump, was observed in an animal strain of *C. neoformans*.[187]

Several studies demonstrated a decreased intracellular content of fluconazole in fluconazole-resistant isolates of *C. neoformans* supporting the presence of active multi-drug efflux mechanisms.[182,183,190] Subsequently, genes encoding multidrug transporters have been demonstrated in *C. neoformans*,[201] and up-regulation of the ABC transporter CnAFR1 was shown to be involved in fluconazole resistance in laboratory mutants, confirmed *in vivo* in a murine model of cryptococcosis and observed (in association with overexpression of *erg11*) in an isolate of *C. neoformans* from a case of feline cryptococcosis.[179,189,192] Efflux pumps have been demonstrated in *C. gattii* and shown to confer fluconazole resistance when expressed in *Saccharomyces cerevisiae* although their contributions to azole resistance in clinical strains remain to be confirmed.[191]

Heteroresistance is another mechanism that could be associated with treatment failure in *Cryptococcus* spp. Heteroresistance is defined by the presence, in a single isolate, of different populations with differing susceptibility to a drug.[196,200–203] Heteroresistance, first described in 1999 in *C. neoformans*,[193] is a dynamic and heterogenous trait, not related to initial MIC[194] but temperature dependent.[193,195] It is an intrinsic phenomenon, as it was demonstrated in strains isolated before the discovery and use of azole drugs.[196,200] It has been reported both in *C. neoformans* and *C. gattii* and seems to be more frequent in the latter species.[200] Heteroresistance has been linked to chromosome duplication, in particular a disomy of chromosome 1, which carries the genes coding for Afr1 and Erg11.[197,199] It is strain dependent and more frequent after fluconazole treatment both in an animal model[198] and in patients.[199]

Amphotericin B resistance

Laboratory-generated amphotericin B-resistant mutants have been characterized and showed no alteration in ergosterol content.[190,204] In contrast, in amphotericin B-resistant clinical isolates, a low content of ergosterol was observed and could be attributed to a defect in the delta 8-7 isomerase, which plays a role in ergosterol synthesis.[183,205]

Flucytosine resistance

Mechanisms of resistance to flucytosine in *C. neoformans* may be due to mutation in cytosine permease (*fcy2*), cytosine deaminase (*fcy1*), or uracil phosphoribosyltransferase (*fur1*).[206,207] Flucytosine resistance has also been reported in clinical isolates of *C. gattii*, but with an as yet unknown mechanism(s).[208]

Nevertheless, *in vitro* experiments showed that defects in DNA mismatch repair (MSH2) promote mutations responsible for flucytosine resistance in *Cryptococcus deuterogattii*.[209] It should be pointed out that combination therapy with amphotericin B and flucytosine may still be effective even in a case of flucytosine resistance, depending on the mechanism of resistance involved.[210]

Molecular detection of drug resistance in Cryptococcus

Although antifungal drug resistance has been clearly demonstrated in *Cryptococcus* spp., the mechanisms involved are diverse and not fully understood. There are currently no commercialized nor easy diagnostic methods for the detection of the molecular mechanisms of antifungal resistance in *Cryptococcus* spp. in clinical microbiology laboratories.

Dermatophytes

Background

The majority of dermatophyte infections are caused by three genera: *Epidermophyton*, *Microsporum* and *Trichophyton*. Mild infections are treated topically with terbinafine, azoles (ketoconazole, miconazole, clotrimazole, luliconazole, sertaconazole, eberconazole), amorolfine or ciclopirox. Serious infections, as well as scalp and nail infections are treated systemically with terbinafine, triazoles (itraconazole or fluconazole) or griseofulvin and often in combination with topical treatment. Newer systemic triazoles including voriconazole and posaconazole are increasingly used off label in failing case settings.[211,212] Resistance in dermatophytes was first reported shortly after the turn of the millennium and as of today has been found in *Trichophyton* and *Microsporum* spp. but not in *Epidermophyton*.[213] Although drug resistance in dermatophytes is not routinely investigated, resistance in *Trichophyton* is increasingly reported worldwide.[42,214–217] The highest rates are observed in India, i.e. 36% for terbinafine (MIC ≥4 mg/L) and 68% for fluconazole (MICs ≥16 mg/L) and apparently involve the spread of an early diverging unique clade referred to as *T. indotineae* and previously reported as *T. mentagrophytes*/*T. interdigitale* complex and *T. mentagrophytes* genotype VIII.[23] Recently, a study carried out under the auspices of the European Academy of Dermatology and Venereology task force of Mycology reported that among 20 European countries, only one country reported no known resistance although susceptibility testing is not a routine test in clinical laboratories.[21] A total of 126 cases were reported as having either clinical and/or microbiological-confirmed antifungal resistance with infections located in the scalp, body (some very widespread), groin, palm, soles (and co-infection of nails) and genital areas. In Denmark, an increasing number of resistant cases are reported.[218] Finally, terbinafine resistance was also found in two isolates from wild hedgehogs in Poland, illustrating that resistant isolates may be shed in the environment.[219]

In vitro antifungal susceptibility testing

A number of studies have reported terbinafine MICs for *Trichophyton* by the CLSI M38-A2 method.[213,220–226] In these studies, MIC ranges vary from <0.007–0.031 to 0.125–≥32 mg/L and 0.004–0.06 to 0.06–0.06 mg/L for *T. interdigitale* and *T. rubrum*, respectively, suggesting some interlaboratory variation.[220–223] Moreover, differential criteria have been adopted for identification of resistant isolates, e.g. >0.25 and >2 mg/L, suggesting a need for standardization.[221,226] No formal CLSI clinical breakpoints are established but it is stated that most *Trichophyton* MICs are ≤0.25 mg/L but some *T. rubrum* have MICs of >0.5 mg/L.[226] A EUCAST method (E.Def 11.0) has been developed and validated in a multicentre study.[227] This method adopted an objective spectrophotometric endpoint reading to improve reproducibility and facilitates a broader implementation of antifungal susceptibility testing for dermatophytes. Tentative ECOFFs have been established but no breakpoints so far. These, as well as tentative MIC targets and ranges for two Quality Control strains, can be found at the EUCAST website (www.eucast.org).

Terbinafine resistance

Terbinafine resistance has been linked to hotspot mutations in the squalene epoxidase (SQLE) target gene of *Trichophyton* spp.[20,42,214,221,228] The corresponding amino acid alterations detected so far are summarized in Table 5. High-level resistance (≥5 2-fold dilution elevation of the modal MIC) is associated with alterations involving L393 or F397 and these two codons are the most common ones involved in terbinafine resistance in both *T. mentagrophytes*, *T. interdigitale*, *T. indotineae*, *T. rubrum* and *Trichophyton tonsurans*. Q408L is a novel alteration recently described in highly resistant *T. mentagrophytes* isolates causing refractory infection in a married couple and L437P has recently been found in a Danish *T. rubrum* isolate with high-level resistance.[218,229] Less prominent MIC elevation (2–3 2-fold dilution elevation of the modal MIC) has been associated with Q408L, F415S, H440Y, S443P and combined I121M and V237I alterations.[42,214,218] I121M combined with V237I is a novel alteration, possibly associated with resistance given both codons are in close proximity to the terbinafine binding site and are also situated in a region that is conserved across several fungal species.[42] Importantly, however, although most terbinafine-resistant *Trichophyton* isolates harbour SQLE mutations, resistant isolates without such mutations have also been described, suggesting additional mechanisms may play a role.[230] Indeed, ABC transporters and MDR2, in particular, appear to be involved in resistance to terbinafine in *Trichophyton* and also to other antifungal compounds.[231,232]

Terbinafine resistance in *Microsporum* is to our knowledge only described in a single isolate from a feline patient treated unsuccessfully with topical terbinafine for 3 months for severe skin infection.[217] In this isolate, the underlying mechanism involved overexpression of the *pdr1*, *mdr1*, *mdr2* and *mdr4* genes encoding ABC transporter proteins.[233]

Azole resistance

Within the *T. rubrum* genome, four ATP-binding cassette (ABC) transporters (TruMdr1, TruMdr2, TruMdr3 and TruMdr5) and TruMfs2 transporter belonging to the MFS have been shown to be able to operate as azole efflux pumps.[234] TruMdr3 and TruMfs1 can act with all azole compounds, while TruMdr1 and TruMfs2 only export fluconazole and voriconazole, and TruMdr2 and TruMdr5 are specific for itraconazole.[216] As mentioned above, efflux pumps can also transport terbinafine and can account for azole–terbinafine cross resistance.[234] Similarly,

Table 5. Overview of mutations in the squalene epoxidase (SQLE) target gene of *T. rubrum* and *T. interdigitale* and their implication for terbinafine susceptibility (for references, see text)

	High-level resistance	Low-level resistance	Unknown impact (Direct sequencing, unable to grow in EUCAST AFST)	Unrelated to resistance
T. rubrum	L437P, L393F, L393S, F397L, F397I	I121M/V237I, F415S, H440Y/F484Y, S443P	Y414C/L438C, F415V	F484Y, I479V
T. indotineae (T. mentagrophytes/ T. interdigitale)	L393F, L393S, S395P, F397L, Q408L, H440T	S443P		L335F, A448T

itraconazole and fluconazole resistance in *Microsporum canis* has been linked to efflux pumps.[235]

Molecular detection of drug resistance in dermatophytes

Molecular detection of recognized resistance mutations provides a possibility for rapid detection of resistance and particularly so when compared with susceptibility testing methods for the slow-growing dermatophytes. It also provides an option to detect resistance when the isolate is not growing sufficiently well in the susceptibility testing medium, and potentially also for direct detection of resistance in clinical material. SQLE target gene amplification and sequencing allow detection of mutations conferring high- and low-level resistance (Table 5 and described above). Moreover, a PCR test for the detection of the two most common alterations T1189C and C1191A conferring alterations in L393F and F397L, respectively, was recently published,[236] and a commercial PCR kit detecting alterations L393F, F397L, L393S, F397I and F397V (Table 1).[43]

P. jirovecii

Background

Potentially drug-resistant PcP was first documented in the 1990s, but it continues to be difficult to determine its current scale.[237,238] Polymorphisms in the genes encoding targets for anti-PcP therapies are recognized as a potential reason for resistant disease. Trimethoprim/sulfamethoxazole is recommended as agent of first choice for treatment and prophylaxis of PcP. Certain aspects of the disease itself might represent a problem for molecular diagnostic testing. Low fungal burdens in HIV-negative patients can limit the applicability of PCR-based detection. Second, many cases of PcP are caused by multiple co-infecting strains, which include both WT and mutant genotypes.[239,240] Finally, unlike most fungal diseases, laboratory culture plays no role in the diagnosis and management of PcP.

In vitro antifungal susceptibility testing

The difficulty in culturing *Pneumocystis* has hindered both PcP diagnosis and research and precludes the possibility of *in vitro* susceptibility testing.[241]

Trimethoprim/sulfamethoxazole and atovaquone resistance

A wide range of mutations have been identified in dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) genes, although even the presence of the most common polymorphisms (DHPS codons T55A and S57P) associated with sulfamethoxazole-based resistance are not necessarily related to treatment failure[242] (Table S1, available as Supplementary data at *JAC* Online). Mutations in cytochrome B gene have been associated with potential atovaquone resistance, but the impact on patient survival is unclear.[243,244]

It is very difficult to determine the prognostic impact of PcP caused by a potentially resistant strain, irrespective of the type of anti-PcP therapy, but in patients infected with strains possessing *dhps* gene mutants an increased duration of hospital stay and the need for mechanical ventilation have been noted.[245] The impact of drug-resistant disease in the non-HIV cohort remains difficult to determine, due to the fulminant nature of the infection in this broad population. Some studies describe prior drug exposure as the factor behind resistance, yet others describe resistant disease in sulfamethoxazole-naive patients.[246,247] This could reflect the nature of the infection, and the need for infection control measures, which are currently not widely applied.[246,247]

The geographical distribution of mutations potentially associated with PcP resistance is broad, with the studies originating from all continents with the exception of Antarctica[248–270] (Table S1). The number of documented cases is likely limited by the number of studies investigating this issue, and evidence would suggest that *Pneumocystis* strains with potential resistance to therapy will be widespread. Of the available studies, mutation rates associated with sulfamethoxazole treatment failure vary between 0% and 100% (Table S1), with the higher mutation rates documented in the USA compared with Europe and the lowest rates documented in resource-limited countries.[44]

Various routes of acquisition of resistant PcP have been described, including human-to-human transmission of a resistant strain, *de novo* development of resistance due to use of (possibly sub-optimal) therapy, infection with resistant strains from an environmental source and reactivation of latent disease.[44]

Molecular detection of drug resistance

Of the novel diagnostic approaches, only molecular testing has the potential to detect drug resistance. A variety of methods (RFLP, DNA sequencing, SSCP, MLST and real-time PCR) have been used, but DNA sequencing of target genes is usually applied.[44] A commercial real-time PcP PCR assay capable of detecting mutations at codons 55 and 57 in the *dhps* gene potentially associated with sulphonamide treatment failure

is available,[45] but clinical validation is limited. One of the major drawbacks of molecular testing for PcP is the lack of knowledge regarding which target gene alterations affect drug susceptibility. Indeed, it may be that other molecular mechanisms are involved in combination with the polymorphisms already identified and persistent PcP PCR positivity where Ct values indicate a consistent or increasing burden despite therapy could be associated with treatment failure. Therefore, molecular detection of drug resistance in PcP is yet not ready for clinical use.[44]

Conclusions

Molecular detection of antifungal drug resistance is, from a clinical perspective, still in its infancy, but technically feasible and potentially helpful, at least for the rapid detection of azole resistance in *A. fumigatus*. Other important applications include detection of echinocandin resistance in *C. glabrata* and terbinafine resistance in *Trichophyton* spp. New technologies, including next-generation and metagenomic sequencing, have the potential to enable screening of pathogens and clinical specimens for the presence of known and yet to be identified resistance mechanisms.[24,271] However, it is important to be aware that molecular testing alone cannot determine an organism's antifungal drug susceptibility.

Not all resistance mechanisms are suitable for molecular detection, as diagnostic accuracy may be unknown, and new mechanisms of resistance continue to arise. Moreover, interpretation of the clinical impact of a given mutation requires expertise about the level and spectrum of resistance dependent on the codon involved as well as the specific amino acid substitution.

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Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

References

1 Anderson TM, Clay MC, Cioffi AG *et al*. Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nat Chem Biol* 2014; **10**: 400–6.

2 Shafiei M, Peyton L, Hashemzadeh M *et al*. History of the development of antifungal azoles: A review on structures, SAR, and mechanism of action. *Bioorg Chem* 2020; **104**: 104240.

3 Law D, Moore CB, Wardle HM *et al*. High prevalence of antifungal resistance in *Candida* spp. from patients with AIDS. *J Antimicrob Chemother* 1994; **34**: 659–68.

4 White TC, Holleman S, Dy F *et al*. Resistance mechanisms in clinical isolates of *Candida albicans*. *Antimicrob Agents Chemother* 2002; **46**: 1704–13.

5 Jeffery-Smith A, Taori SK, Schelenz S *et al*. *Candida auris*: A review of the literature. *Clin Microbiol Rev* 2017; **31**: e00029-17.

6 Cheong JWS, McCormack J. Fluconazole resistance in cryptococcal disease: Emerging or intrinsic? *Med Mycol* 2013; **51**: 261–9.

7 Denning DW, Venkateswarlu K, Oakley KL *et al*. Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 1997; **41**: 1364–8.

8 Dannaoui E, Borel E, Monier MF *et al*. Acquired itraconazole resistance in *Aspergillus fumigatus*. *J Antimicrob Chemother* 2001; **47**: 333–40.

9 Verweij PE, Mellado E, Melchers WJG. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 2007; **356**: 1481–3.

10 Pappas PG, Kauffman CA, Andes DR *et al*. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2016; **62**: e1–50.

11 Singh-Babak SD, Babak T, Diezmann S *et al*. Global analysis of the evolution and mechanism of echinocandin resistance in *Candida glabrata*. *PLoS Path* 2012; **8**: e1002718.

12 Jiménez-Ortigosa C, Moore C, Denning DW *et al*. Emergence of echinocandin resistance due to a point mutation in the *fks* gene of *Aspergillus fumigatus* in a patient with chronic pulmonary aspergillosis. *Antimicrob Agents Chemother* 2017; **61**: e01277-17.

13 Aruanno M, Glampedakis E, Lamoth F. Echinocandins for the treatment of invasive aspergillosis: From laboratory to bedside. *Antimicrob Agents Chemother* 2019; **63**: e00399-19.

14 Verweij PE, Te Dorsthorst DTA, Janssen WHP *et al*. *In vitro* activities at pH 5.0 and pH 7.0 and *in vivo* efficacy of flucytosine against *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2008; **52**: 4483–5.

15 Gsaller F, Furukawa T, Carr PD *et al*. Mechanistic basis of pH-dependent 5-flucytosine resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2018; **62**: e02593-17.

16 McClellan KJ, Wiseman LR, Markham A. Terbinafine: an update of its use in superficial mycoses. *Drugs* 1999; **58**: 179–202.

17 Ebert A, Monod M, Salamin K *et al*. Alarming India-wide phenomenon of antifungal resistance in dermatophytes: A multicentre study. *Mycoses* 2020; **63**: 717–28.

18 Nenoff P, Verma SB, Ebert A *et al*. Spread of terbinafine-resistant *Trichophyton mentagrophytes* type VIII (India) in Germany: 'the tip of the iceberg?' *J Fungi (Basel)* 2020; **6**: 207.

19 Sacheli R, Harag S, Dehavay F *et al*. Belgian national survey on Tinea capitis: Epidemiological considerations and highlight of terbinafine-resistant *T. mentagrophytes* with a mutation on SQLE Gene. *J Fungi (Basel)* 2020; **6**: 195.

20 Siopi M, Efstathiou I, Theodoropoulos K *et al*. Molecular epidemiology and antifungal susceptibility of *Trichophyton isolates* in Greece: emergence of terbinafine-resistant *Trichophyton mentagrophytes* type VIII locally and globally. *J Fungi (Basel)* 2021; **7**: 419.

21 Saunte DML, Pereiro-Ferreirós M, Rodríguez-Cerdeira C *et al*. Emerging antifungal treatment failure of dermatophytosis in Europe: take care or it may become endemic. *J Eur Acad Dermatol Venereol* 2021; **35**: 1582–6.

22 Kano R, Kimura U, Kakurai M *et al*. *Trichophyton indotineae* sp. nov.: A new highly terbinafine-resistant anthropophilic dermatophyte species. *Mycopathologia* 2020; **185**: 947–58.

23 Tang C, Kong X, Ahmed SA *et al*. Taxonomy of the *Trichophyton mentagrophytes*/*T. interdigitale* species complex harboring the highly virulent, multiresistant genotype *T. indotineae*. *Mycopathologia* 2021; **186**: 315–26.

24 Kidd SE, Chen SC-A, Meyer W *et al*. A new age in molecular diagnostics for invasive fungal disease: are we ready? *Front Microbiol* 2020; **10**: 2903.

25 Consortium Opathy, Gabaldon T. Recent trends in molecular diagnostics of yeast infections: from PCR to NGS. *FEMS Microbiol Rev* 2019; **43**: 517–47.

26 White PL. Recent advances and novel approaches in laboratory-based diagnostic mycology. *Med Mycol* 2019; **57**: S259–66.

27 Camp I, Manhart G, Schabereiter-Gurtner C *et al*. Clinical evaluation of an in-house panfungal real-time PCR assay for the detection of fungal pathogens. *Infection* 2020; **48**: 345–55.

28 Rath P-M, Steinmann J. Overview of commercially available PCR assays for the detection of *Aspergillus* spp. DNA in patient samples. *Front Microbiol* 2018; **9**: 740.

29 Dudakova A, Speiss B, Tangwattanachuleeporn M *et al*. Molecular tools for the detection and deduction of azole antifungal drug resistance phenotypes in *Aspergillus* species. *Clin Microbiol Rev* 2017; **30**: 1065–91.

30 Buil JB, Zoll J, Verweij PE *et al*. Molecular detection of azole-resistant *Aspergillus fumigatus* in clinical samples. *Front Microbiol* 2018; **9**: 515.

31 van der Torre MH, Novak-Frazer L, Rautemaa-Richardson R. Detecting azole antifungal resistance in *Aspergillus fumigatus* by pyrosequencing. *J Fungi (Basel)* 2020; **6**: 12.

32 Chong G-LM, van de Sande WWJ, Dingemans GJH *et al*. Validation of a new *Aspergillus* real-time PCR assay for direct detection of *Aspergillus* and azole resistance of *Aspergillus fumigatus* on bronchoalveolar lavage fluid. *J Clin Microbiol* 2015; **53**: 868–74.

33 Montesinos I, Argudin MA, Hites M *et al*. Culture-based methods and molecular tools for azole-resistant *Aspergillus fumigatus* detection in a Belgian University Hospital. *J Clin Microbiol* 2017; **55**: 2391–9.

34 Singh A, Sharma B, Mahto KK *et al*. High frequency direct detection of triazole resistance in *Aspergillus fumigatus* from patients with chronic pulmonary fungal diseases in India. *J Fungi (Basel)* 2020; **6**: 67.

35 Mikulska M, Furfaro E, De Carolis E *et al*. Use of *Aspergillus fumigatus* real-time PCR in broncho-alveolar lavage (BAL) samples for diagnosis of invasive aspergillosis, including azole-resistant cases, in high risk haematology patients: the need for a combined use with galactomannan. *Med Mycol* 2019; **57**: 987–96.

36 Guegan H, Chevrier S, Belleguic C *et al*. Performance of molecular approaches for Aspergillus detection and azole resistance surveillance in cystic fibrosis. *Front Microbiol* 2018; **9**: 531.

37 Dannaoui E, Gabriel F, Gaboyard M *et al*. Molecular diagnosis of invasive aspergillosis and detection of azole resistance by a newly commercialized PCR kit. *J Clin Microbiol* 2017; **55**: 3210–8.

38 Scharmann U, Kirchhoff L, Hain A *et al*. Evaluation of three commercial PCR assays for the detection of azole-resistant *Aspergillus fumigatus* from respiratory samples of immunocompromised patients. *J Fungi (Basel)* 2021; **7**: 132.

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

40 Kordalewska M, Lee A, Park S *et al*. Understanding echinocandin resistance in the emerging pathogen *Candida auris*. *Antimicrob Agents Chemother* 2018; **62**: e00238-18.

41 Leach L, Russell A, Zhu Y *et al*. A rapid and automated sample-to-result *Candida auris* real-time PCR assay for high-throughput testing of surveillance samples with the BD Max Open System. *J Clin Microbiol* 2019; **57**: e00630-19.

42 Saunte DML, Hare RK, Jørgensen KM *et al*. Emerging terbinafine resistance in *Trichophyton*: clinical characteristics, squalene epoxidase gene mutations and a reliable EUCAST method for detection. *Antimicrob Agents Chemother* 2019; **63**: e01126-19.

43 Singh A, Singh P, Dingemans P *et al*. Evaluation of DermaGenius® resistance real time polymerase reaction for detection of terbinafine-resistant Trichophyton species. *Mycoses* 2021; **64**: 721–6.

44 De la Horra C, Friaza V, Morilla R *et al*. Update on dihydropteroate-synthase (dhps) mutations in *Pneumocystis jirovecii*. *J Fungi (Basel)* 2021; **7**: 856.

45 Montesinos I, Delforge M-L, Ajjaham F *et al*. Evaluation of a new commercial real-time PCR assay for diagnosis of *Pneumocystis jirovecii* pneumonia and identification of dihydropteroate synthase (DHPS) mutations. *Diagn Microbiol Infect Dis* 2017; **87**: 32–6.

46 Verweij PE, Zhang J, Debets AJM *et al*. In-host adaptation and acquired triazole resistance in *Aspergillus fumigatus*: a dilemma for clinical management. *Lancet Infect Dis* 2016; **16**: 251–60.

47 Alcazar-Fuoli L, Mellado E. Ergosterol biosynthesis in *Aspergillus fumigatus*: its relevance as an antifungal target and role in antifungal drug resistance. *Front Microbiol* 2013; **3**: 439.

48 Zhang J, Li L, Lv Q *et al*. The fungal CYP51s: their functions, structures, related drug resistance, and inhibitors. *Front Microbiol* 2019; **10**: 691.

49 Lestrade PPA, Buil JB, van der Beek MT *et al*. Paradoxical trends in azole-resistant *Aspergillus fumigatus* in a national multicenter surveillance program, 2013–2018. *Emerg Infect Dis* 2020; **26**: 1447–55.

50 Rodriguez-Tudela JL, Alcazar-Fuoli L, Mellado E *et al*. Epidemiological cutoffs and cross resistance to azole drugs in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2008; **52**: 2468–72.

51 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.

52 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.

53 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.

54 Liu M, Zeng R, Zhang L *et al*. Multiple cyp51A-based mechanisms identified in azole-resistant isolates of *Aspergillus fumigatus* from China. *Antimicrob Agents Chemother* 2015; **59**: 4321–5.

55 Lazzarini C, Esposto MC, Prigitano A *et al*. Azole resistance in *Aspergillus fumigatus* clinical isolates from an Italian culture collection. *Antimicrob Agents Chemother* 2015; **60**: 682–5.

56 van Paassen J, Russcher A, In't Veld-van Wingerden AW *et al*. Emerging aspergillosis by azole-resistant Aspergillus fumigatus at an intensive care unit in the Netherlands, 2010 to 2013. *Euro Surveill* 2016; **21**: pii=30300.

57 Dauchy C, Bautin N, Nseir S *et al*. Emergence of *Aspergillus fumigatus* azole resistance in azole-naïve patients with chronic obstructive pulmonary disease and their homes. *Indoor Air* 2018; **28**: 298–306.

58 Chen Y, Li Z, Han X *et al*. Elevated MIC values of imidazole drugs against *Aspergillus fumigatus* isolates with TR(34)/L98H/S297T/F495I mutation. *Antimicrob Agents Chemother* 2018; **62**: e01549-17.

59 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.

60 Astvad KM, Jensen RH, Hassan TM *et al*. First detection of TR_{46}/Y121F/T289A and TR_{34}/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.

61 van Ingen J, van der Lee HA, Rijs TA *et al*. Azole, polyene and echinocandin MIC distributions for wild-type, TR_{34}/L98H and TR_{46}/Y121F/T289A *Aspergillus fumigatus* isolates in the Netherlands. *J Antimicrob Chemother* 2015; **70**: 178–81.

62 Choukri F, Botterel F, Sitterlé E *et al*. Prospective evaluation of azole resistance in *Aspergillus fumigatus* clinical isolates in France. *Med Mycol* 2015; **53**: 593–6.

63 Jensen RH, Hagen F, Astvad KM *et al*. Azole-resistant *Aspergillus fumigatus* in Denmark: a laboratory-based study on resistance mechanisms and genotypes. *Clin Microbiol Infect* 2016; **22**: 1–9.

64 Resendiz-Sharpe A, Mercier T, Lestrade PPA *et al*. Prevalence of voriconazole-resistant invasive aspergillosis and its impact on mortality in haematology patients. *J Antimicrob Chemother* 2019; **74**: 2759–66.

65 Buil JB, Snelders E, Denardi LB *et al*. Trends in azole resistance in *Aspergillus fumigatus*, the Netherlands, 1994-2016. *Emerg Infect Dis* 2019; **25**: 176–8.

66 Buil JB, Rijs AJMM, Meis JF *et al*. *In vitro* activity of the novel antifungal compound F901318 against difficult-to-treat *Aspergillus* isolates. *J Antimicrob Chemother* 2017; **72**: 2548–52.

67 Jeanvoine A, Rocchi S, Reboux G *et al*. Azole-resistant *Aspergillus fumigatus* in sawmills of Eastern France. *J Appl Microbiol* 2017; **123**: 172–84.

68 Lestrade PP, Bentvelsen RG, Schauwvlieghe AFAD *et al*. Voriconazole resistance and mortality in invasive aspergillosis: A multicenter retrospective cohort study. *Clin Infect Dis* 2019; **68**: 1463–71.

69 Macedo D, Brito Devoto T, Pola S *et al*. A novel combination of CYP51A mutations confers pan-azole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2020; **64**: e2501-19.

70 Chen Y, Lu Z, Zhao J *et al*. Epidemiology and molecular characterizations of azole resistance in clinical and environmental *Aspergillus fumigatus* isolates from China. *Antimicrob Agents Chemother* 2016; **60**: 5878–84.

71 Lavergne RA, Morio F, Danner-Boucher I *et al*. One year prospective survey of azole resistance in *Aspergillus fumigatus* at a French cystic fibrosis reference centre: prevalence and mechanisms of resistance. *J Antimicrob Chemother* 2019; **74**: 1884–9.

72 Moore CB, Novak-Frazer L, Muldoon E *et al*. First isolation of the pan-azole-resistant *Aspergillus fumigatus* cyp51A TR_{46}/Y121F/T289A mutant in a UK patient. *Int J Antimicrob Agents* 2017; **49**: 512–4.

73 Pelaez T, Monteiro MC, Garcia-Rubio R *et al*. First detection of *Aspergillus fumigatus* azole-resistant strain due to Cyp51A TR_{46}/Y121F/T289A in an azole-naive patient in Spain. *New Microbes New Infect* 2015; **6**: 33–4.

74 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.

75 Rößler S, Bader O, Stölzel F *et al*. Progressive dispersion of azole resistance in *Aspergillus fumigatus*: Fatal invasive aspergillosis in a patient with acute myeloid leukemia infected with an *A. fumigatus* strain with a cyp51A TR46 Y121F M172I T289A allele. *Antimicrob Agents Chemother* 2017; **61**: e00270-17.

76 Zhang J, Snelders E, Zwaan BJ *et al*. A novel environmental azole resistance mutation in *Aspergillus fumigatus* and a possible role of sexual reproduction in its emergence. *mBio* 2017; **8**: e00791-17.

77 Hodiamont CJ, Dolman KM, Ten Berge IJ *et al*. 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.

78 Hare RK, Gertsen JB, Astvad KMT *et al*. *In vivo* selection of a unique tandem repeat mediated azole resistance mechanism (TR(120)) in *Aspergillus fumigatus* cyp51A, Denmark. *Emerg Infect Dis* 2019; **25**: 577–80.

79 Pinto E, Monteiro C, Maia M *et al*. *Aspergillus* species and antifungals susceptibility in clinical setting in the north of Portugal: Cryptic species and emerging azoles resistance in *A. fumigatus*. *Front Microbiol* 2018; **9**: 1656.

80 Howard SJ, Webster I, Moore CB *et al*. Multi-azole resistance in *Aspergillus fumigatus*. *Int J Antimicrob Agents* 2006; **28**: 450–3.

81 Albarrag AM, Anderson MJ, Howard SJ *et al*. Interrogation of related clinical pan-azole resistant *Aspergillus fumigatus* strains: G138C, Y431C, and G434C single nucleotide polymorphisms in cyp51A, upregulation of cyp51A, and integration and activation of transposon Atf1 in the cyp51A promoter. *Antimicrob Agents Chemother* 2011; **55**: 5113–21.

82 Gregson L, Goodwin J, Johnson A *et al*. *In vitro* susceptibility of *Aspergillus fumigatus* to isavuconazole: correlation with itraconazole, voriconazole, and posaconazole. *Antimicrob Agents Chemother* 2013; **57**: 5778–80.

83 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.

84 Verweij PE, Snelders E, Kema GH *et al*. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009; **9**: 789–95.

85 Snelders E, Camps SM, Karawajczyk A *et al*. Triazole fungicides can induce cross-resistance to medical triazoles in *Aspergillus fumigatus*. *PLoS One* 2012; **7**: 31801.

86 Buil JB, Hare RK, Zwaan BJ *et al*. The fading boundaries between patient and environmental routes of triazole resistance selection in *Aspergillus fumigatus*. *PLoS Pathog* 2019; **15**: 1007858.

87 Risum M, Hare RK, Gertsen JB *et al*. Azole resistant *Aspergillus fumigatus* among Danish cystic fibrosis patients: increasing prevalence and dominance of TR34/L98H. *Front Microbiol* 2020; **11**: 1850.

88 Barber AE, Riedel J, Sae-Ong T *et al*. Effects of agricultural fungicide use on *Aspergillus fumigatus* abundance, antifungal susceptibility, and population structure. *mBio* 2020; **11**: e02213-20.

89 Camps SM, Dutilh BE, Arendrup MC *et al*. Discovery of a HapE mutation that causes azole resistance in *Aspergillus fumigatus* through whole genome sequencing and sexual crossing. *PLoS One* 2012; **7**: 50034.

90 Hortschansky P, Misslinger M, Mörl J *et al*. Structural basis of HapE(P88L)-linked antifungal triazole resistance in *Aspergillus fumigatus*. *Life Sci Alliance* 2020; **3**: 202000729.

91 Rybak JM, Ge W, Wiederhold NP *et al*. Mutations in hmg1, challenging the paradigm of clinical triazole resistance in *Aspergillus fumigatus*. *mBio* 2019; **10**: 00437–19.

92 Hagiwara D, Arai T, Takahashi H *et al*. Non-cypA azole-resistant *Aspergillus fumigatus* isolates with mutation in HMG-CoA reductase. *Emerg Infect Dis* 2018; **24**: 1889–97.

93 Liang T, Yang X, Li R *et al*. Emergence of W272C substitution in Hmg1 in a triazole-resistant isolate of *Aspergillus fumigatus* from a Chinese patient with chronic cavitary pulmonary aspergillosis. *Antimicrob Agents Chemother* 2021; **65**: e00263-21.

94 Handelman M, Morogovsky A, Liu W *et al*. Triazole-resistant *Aspergillus fumigatus* in an Israeli patient with chronic cavitary pulmonary aspergillosis due to a novel E306K substitution in Hmg1. *Antimicrob Agents Chemother* 2021; **65**: e1089-21.

95 Buied A, Moore CB, Denning DW *et al*. High-level expression of cyp51B in azole-resistant clinical *Aspergillus fumigatus* isolates. *J Antimicrob Chemother* 2013; **68**: 512–4.

96 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.

97 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.

98 Blosser SJ, Cramer RA. SREBP-dependent triazole susceptibility in *Aspergillus fumigatus* is mediated through direct transcriptional regulation of erg11A (cyp51A). *Antimicrob Agents Chemother* 2012; **56**: 248–57.

99 Furukawa T, van Rhijn N, Fraczek M *et al*. The negative cofactor 2 complex is a key regulator of drug resistance in *Aspergillus fumigatus*. *Nat Commun* 2020; **11**: 427.

100 Resendiz-Sharpe A, Hokken MWJ, Mercier T *et al*. *Hmg1* gene mutation prevalence in triazole-resistant *Aspergillus fumigatus* clinical isolates. *J Fungi (Basel)* 2020; **6**: 227.

101 Ullmann AJ, Aguado JM, Arikan-Akdagli S *et al*. Diagnosis and management of *Aspergillus* diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. *Clin Microbiol Infect* 2018; **24**: S1–38.

102 Guinea J, Verweij PE, Meletiadis J *et al*. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). How to: EUCAST recommendations on the screening procedure E.Def 10.1 for the detection of azole resistance in *Aspergillus fumigatus* isolates using four-well azole-containing agar plates. *Clin Microbiol Infect* 2019; **25**: 681–7.

103 Arendrup MC, Friberg N, Mares M *et al*. How to interpret MICs of antifungal compounds according to the revised clinical breakpoints v. 10.0 European Committee on antimicrobial susceptibility testing (EUCAST). *Clin Microbiol Infect* 2020; **26**: 1464–72.

104 White PL, Posso RB, Barnes RA. Analytical and clinical evaluation of the PathoNostics AsperGenius assay for detection of invasive aspergillosis and resistance to azole antifungal drugs directly from plasma samples. *J Clin Microbiol* 2017; **55**: 2356–66.

105 Chong CM, van der Beek MT, von dem Borne PA *et al*. PCR-based detection of *Aspergillus fumigatus* Cyp51A mutations on bronchoalveolar lavage: a multicentre validation of the AsperGenius assay in 201 patients with haematological disease suspected for invasive aspergillosis. *J Antimicrob Chemother* 2016; **71**: 3528–35.

106 Rivero-Menendez O, Soto-Debran JC, Medina N *et al*. Molecular identification, antifungal susceptibility testing, and mechanisms of azole resistance in *Aspergillus* species received within a surveillance program on antifungal resistance in Spain. *Antimicrob Agents Chemother* 2019; **63**: e00865-19.

107 Zoran T, Sartori B, Sappi L *et al*. Azole-resistance in *Aspergillus terreus* and related species: an emerging problem or a rare phenomenon? *Front Microbiol* 2018; **9**: 516.

108 Lucio J, Gonzalez-Jimenez I, Rivero-Menendez O *et al*. Point mutations in the 14-α sterol demethylase cyp51A or cyp51C could contribute to azole resistance in *Aspergillus flavus*. *Genes (Basel)* 2020; **11**: 1217.

109 Liu W, Sun Y, Chen W *et al*. The T788G mutation in the cyp51C gene confers voriconazole resistance in *Aspergillus flavus* causing aspergillosis. *Antimicrob Agents Chemother* 2012; **56**: 2598–603.

110 Choi MJ, Won EJ, Joo MY *et al*. Microsatellite typing and resistance mechanism analysis of voriconazole-resistant *Aspergillus flavus* isolates in South Korean hospitals. *Antimicrob Agents Chemother* 2019; **63**: e01610-18.

111 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.

112 Satish S, Jiménez-Ortigoza C, Zhao Y *et al*. Stress-Induced Changes in the Lipid Microenvironment of β-(1,3)-d-Glucan Synthase Cause

Clinically Important Echinocandin Resistance in *Aspergillus fumigatus*. *mBio* 2019; **10**: e00779-19.

113 Castanheira M, Deshpande LM, Davis AP *et al*. Monitoring antifungal resistance in a global collection of invasive yeasts and moulds: Application of CLSI epidemiological cutoff values and whole genome sequencing analysis for detection of azole resistance in *Candida albicans*. *Antimicrob Agents Chemother* 2017; **61**: e00906-17.

114 Castanheira M, Deshpande LM, Messer SA *et al*. Analysis of global antifungal surveillance results reveals predominance of Erg11 Y132F alteration among azole-resistant *Candida parapsilosis* and *Candida tropicalis* and country-specific isolate dissemination. *Int J Antimicrob Agents* 2020; **55**: 105799.

115 Pfaller MA, Diekema DJ, Turnidge JD *et al*. Twenty years of the SENTRY Antifungal Surveillance Program: Results for Candida species From 1997-2016. *Open Forum Infect Dis* 2019; **6**: S79–94.

116 Jensen RH, Astvad KM, Silva LV *et al*. Stepwise emergence of azole, echinocandin, and amphotericin B multidrug resistance *in vivo* in *Candida albicans* orchestrated by multiple genetic alterations. *J Antimicrob Chemother* 2015; **70**: 2551–5.

117 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.

118 Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2002; **2**: 73–85.

119 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.

120 Morio F, Loge C, Besse B *et al*. 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. *Diag Microbiol Infect Dis* 2010; **66**: 373–84.

121 Flowers SA, Colon B, Whaley SG *et al*. Contribution of clinically derived mutations in ERG11 to azole resistance in *Candida albicans*. *Antimicrob Agents Chemother* 2015; **59**: 450–60.

122 Mesquida A, Vincente T, Reigadas E *et al*. *In vitro* activity of ibrexafungerp and comparators against *Candida albicans* genotypes from vaginal samples and blood cultures. *Clin Microbiol Infect* 2021; **27**: 915.e5–e8.

123 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.

124 Carolus H, Pierson S, Muñoz JF *et al*. Genome-wide analysis of experimentally evolved *Candida auris* reveals multiple novel mechanisms of multidrug resistance. *mBio* 2021; **12**: e03333-20.

125 Lockhart SR, Etienne KA, Vallabhaneni S *et al*. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. *Clin Infect Dis* 2017; **64**: 134–40.

126 Martel CM, Parker JE, Bader O *et al*. Identification and characterization of four azole-resistant *erg*3 mutants of *Candida albicans*. *Antimicrob Agents Chemother* 2010; **54**: 4527–33.

127 Sanglard D, Coste AT. Activity of isavuconazole and other azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. *Antimicrob Agents Chemother* 2016; **60**: 229–38.

128 Coste AT, Crittin J, Bauser C *et al*. Functional analysis of *cis*- and *trans*-acting elements of the *Candida albicans* CDR2 promoter with a novel promoter reporter system. *Eukaryot Cell* 2009; **8**: 1250–67.

129 Liu Z, Myers LC. Mediator tail module is required for Tac1-activated *CDR1* expression and azole resistance in *Candida albicans*. *Antimicrob Agents Chemother* 2017; **61**: e01342-17.

130 Nishimoto AT, Zhang Q, Hazlett B *et al*. Contribution of clinically derived mutations in the gene encoding the zinc cluster transcription factor Mrr2 to fluconazole antifungal resistance and *CDR1* expression in *Candida albicans*. *Antimicrob Agents Chemother* 2019; **63**: e00078-19.

131 Ferrari S, Sanguinetti M, Torelli R *et al*. Contribution of *CgPDR1*-regulated genes in enhanced virulence of azole-resistant *Candida glabrata*. *PLoS One* 2011; **6**: e17589.

132 Morschhauser J, Barker KS, Liu TT *et al*. The transcription factor Mrr1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathog* 2007; **3**: e164.

133 Sasse C, Dunkel N, Schafer T *et al*. The stepwise acquisition of fluconazole resistance mutations causes a gradual loss of fitness in *Candida albicans*. *Mol Microbiol* 2012; **86**: 539–56.

134 Wang H, Kong F, Sorrell TC *et al*. Rapid detection of EG11 gene mutations in clinical *Candida albicans* isolates with reduced susceptibility to fluconazole by rolling circle amplification and DNA sequencing. *BMC Microbiol* 2009; **9**: 167.

135 Aguilar-Zapata D, Petraitiene R, Petraitis V. Echinocandins: The expanding antifungal armamentarium. *Clin Infect Dis* 2015; **61** Suppl 6: S604–11.

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

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

138 Grossman NT, Chiller TM, Lockhart SR. Epidemiology of echinocandin resistance in *Candida*. *Curr Fungal Infect Rep* 2014; **8**: 243–8.

139 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.

140 Zimbeck AJ, Iqbal N, Ahlquist AM *et al*. *FKS* mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother* 2010; **54**: 5042–7.

141 Risum M, Astvad K, Johansen HK *et al*. Update 2016-2018 of the nationwide Danish fungaemia surveillance study: epidemiologic changes in a 15-year perspective. *J Fungi (Basel)* 2021; **7**: 491.

142 Díaz-García J, Mesquida A, Sánchez-Carrillo C *et al*. Monitoring the epidemiology and antifungal resistance of yeasts causing fungemia in a tertiary care hospital in Madrid, Spain: Any relevant changes in the last 13 years? *Antimicrob Agents Chemother* 2021; **65**: e01827–20.

143 Lackner M, Tscherner M, Schaller M *et al*. Positions and numbers of *FKS* mutations in *Candida albicans* selectively influence *in vitro* and *in vivo* susceptibilities to echinocandin treatment. *Antimicrob Agents Chemother* 2014; **58**: 3626–35.

144 Castanheira M, Woosley LN, Messer SA *et al*. Frequency of *fks* mutations among *Candida glabrata* isolates from a 10-year global collection of bloodstream infection isolates. *Antimicrob Agents Chemother* 2014; **58**: 577–80.

145 Helleberg M, Jørgensen KM, Hare RK *et al*. Rezafungin *in vitro* activity against contemporary Nordic clinical *Candida* isolates and *Candida auris* determined by the EUCAST reference method. *Antimicrob Agents Chemother* 2020; **64**: e02438-19.

146 Biagi MJ, Wiederhold NP, Gibas C *et al*. Development of high-level echinocandin resistance in a patient with recurrent *Candida auris* candidemia secondary to chronic candiduria. *Open Forum Infect Dis* 2019.

147 Hou X, Lee A, Jiménez-Ortigosa C *et al*. Rapid detection of *ERG11*-associated azole resistance and *FKS*-associated echinocandin

resistance in *Candida auris*. *Antimicrob Agents Chemother* 2019; **63**: e01811-18.

148 Suwunnakorn S, Wakabayashi H, Kordalewska M *et al*. FKS2 and FKS3 genes of opportunistic human pathogen *Candida albicans* influence echinocandin susceptibility. *Antimicrob Agents Chemother* 2018; **62**: e02299-17.

149 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.

150 Perlin DS. Echinocandin resistance in *Candida*. *Clin Infect Dis* 2015; **61** Suppl 6: S612–7.

151 Perlin DS. Mechanisms of echinocandin antifungal drug resistance. *Ann N Y Acad Sciences* 2015; **1354**: 1–11.

152 Beyda ND, John J, Kilic A *et al*. *FKS* mutant *Candida glabrata*: Risk factors and outcomes in patients with candidemia. *Clin Infect Dis* 2014; **59**: 819–25.

153 Pfaller MA, Diekema D, Castanheira M *et al*. Definitions and epidemiology of *Candida* species not susceptible to echinocandins. *Curr Fungal Infect Rep* 2011; **5**: 120–7.

154 Shields RK, Nguyen MH, Clancy CJ. Clinical perspectives on echinocandin resistance among *Candida* species. *Curr Opin Infect Dis* 2015; **28**: 514–22.

155 Perlin DS. Echinocandin resistance, susceptibility testing and prophylaxis: implications for patient management. *Drugs* 2014; **74**: 1573–85.

156 Wiederhold NP, Grabinski JL, Garcia-Effron G *et al*. Pyrosequencing to detect mutations in *FKS1* that confer reduced echinocandin susceptibility in *Candida albicans*. *Antimicrob Agents Chemother* 2008; **52**: 4145–8.

157 Garnaud C, Botterel F, Sertour N *et al*. Next-generation sequencing offers new insights into the resistance of *Candida* spp. to echinocandins and azoles. *J Antimicrob Chemother* 2015; **70**: 2556–65.

158 Arendrup MC, Patterson TF. Multidrug-resistant *Candida*: epidemiology, molecular mechanisms, and treatment. *J Infect Dis* 2017; **216**: S445–51.

159 Kimura M, Araoka H, Yamamoto H *et al*. Clinical and microbiological characteristics of breakthrough candidemia in allogeneic hematopoietic stem cell transplant recipients in a Japanese hospital. *Antimicrob Agents Chemother* 2017; **61**: e01791-16.

160 Costa C, Ponte A, Pais P *et al*. New mechanisms of flucytosine resistance in *C. glabrata* unveiled by a chemogenomics analysis in *S. cerevisiae*. *PLoS One* 2015; **10**: e0135110.

161 Carolus H, Pierson S, Lagrou K *et al*. Amphotericin B and other polyenes–discovery, clinical use, mode of action and drug resistance. *J Fungi (Basel)* 2020; **6**: 321.

162 Cuenca-Estrella M. Antifungal drug resistance mechanisms in pathogenic fungi: from bench to bedside. *Clin Microbiol Infect* 2014; **20**: S54–9.

163 Gopinathan S, Janagond AB, Agatha D *et al*. Detection of FUR1 gene in 5-flucytosine resistant Candida isolates in vaginal candidiasis patients. *J Clin Diagn Res* 2013; **7**: 2452–5.

164 Dodgson AR, Dodgson KJ, Pujol C *et al*. Clade-specific flucytosine resistance is due to a single nucleotide change in the FUR1 gene of *Candida albicans*. *Antimicrob Agents Chemother* 2004; **48**: 2223–7.

165 Rajasingham R, Smith RM, Park BJ *et al*. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 2017; **17**: 873–81.

166 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.

167 WHO. Guidelines on the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children: supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva: WHO 2018. https://apps.who.int/iris/handle/10665/260399.

168 Pfaller MA, Lodge JK, Ghannoum MA. Drug resistance in *Cryptococcus*: epidemiology and molecular mechanisms. In: Heitman J Kozel T Kwon-Chung K Perfect J and Casadevall A, eds. *Cryptococcus. American Society of Microbiology*, 2011; 203–16.

169 Maligie MA, Selitrennikoff CP. *Cryptococcus neoformans* resistance to echinocandins: (1,3)β-glucan synthase activity is sensitive to echinocandins. *Antimicrob Agents Chemother* 2005; **49**: 2851–6.

170 Perfect JR, Cox GM. Drug resistance in *Cryptococcus neoformans*. *Drug Resist Updat* 1999; **2**: 259–69.

171 Sar B, Monchy D, Vann M *et al*. Increasing *in vitro* resistance to fluconazole in *Cryptococcus neoformans* Cambodian isolates: April 2000 to March 2002. *J Antimicrob Chemother* 2004; **54**: 563–5.

172 Bicanic T, Harrison T, Niepieklo A *et al*. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. *Clin Infect Dis* 2006; **43**: 1069–73.

173 Bongomin F, Oladele RO, Gago S *et al*. A systematic review of fluconazole resistance in clinical isolates of *Cryptococcus* species. *Mycoses* 2018; **61**: 290–7.

174 Bastos RW, Carneiro HCS, Oliveira LVN *et al*. Environmental triazole induces cross-resistance to clinical drugs and affects morphophysiology and virulence of *Cryptococcus gattii* and *C. neoformans*. *Antimicrob Agents Chemother* 2018; **62**: e01179--17.

175 Bastos RW, Freitas GJC, Carneiro HCS *et al*. From the environment to the host: How non-azole agrochemical exposure affects the antifungal susceptibility and virulence of *Cryptococcus gattii*. *Sci Total Environ* 2019; **681**: 516–23.

176 Takahashi JPF, Feliciano LM, Santos DCS *et al*. Could fungicides lead to azole drug resistance in a cross-resistance manner among environmental *Cryptococcus* strains? *Curr Fungal Infect Rep* 2020; **14**: 9–14.

177 Block ER, Jennings AE, Bennett JE. 5-fluorocytosine resistance in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 1973; **3**: 649–56.

178 Hospenthal DR, Bennett JE. Flucytosine monotherapy for cryptococcosis. *Clin Infect Dis* 1998; **27**: 260–4.

179 Sanguinetti M, Posteraro B, La Sorda M *et al*. Role of AFR1, an ABC transporter-encoding gene, in the *in vivo* response to fluconazole and virulence of *Cryptococcus neoformans*. *Infect Immun* 2006; **74**: 1352–9.

180 Schwarz P, Dromer F, Lortholary O *et al*. Efficacy of amphotericin B in combination with flucytosine against flucytosine-susceptible or flucytosine-resistant isolates of *Cryptococcus neoformans* during disseminated murine cryptococcosis. *Antimicrob Agents Chemother* 2006; **50**: 113–20.

181 Velez JD, Allendoerfer R, Luther M *et al*. Correlation of *in vitro* azole susceptibility with *in vivo* response in a murine model of cryptococcal meningitis. *J Infect Dis* 1993; **168**: 508–10.

182 Lamb DC, Corran A, Baldwin BC *et al*. Resistant P45051A1 activity in azole antifungal tolerant *Cryptococcus neoformans* from AIDS patients. *FEBS Lett* 1995; **368**: 326–30.

183 Venkateswarlu K, Taylor M, Manning NJ *et al*. Fluconazole tolerance in clinical isolates of *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 1997; **41**: 748–51.

184 Rodero L, Mellado E, Rodriguez AC *et al*. G484S amino acid substitution in lanosterol 14-alpha demethylase (ERG11) is related to fluconazole resistance in a recurrent *Cryptococcus neoformans* clinical isolate. *Antimicrob Agents Chemother* 2003; **47**: 3653–6.

185 Sionov E, Chang YC, Garraffo HM *et al*. Identification of a *Cryptococcus neoformans* cytochrome P450 lanosterol 14alpha-demethylase (Erg11) residue critical for differential susceptibility between fluconazole/voriconazole and itraconazole/posaconazole. *Antimicrob Agents Chemother* 2012; **56**: 1162–9.

186 Bosco-Borgeat ME, Mazza M, Taverna CG *et al*. Amino acid substitution in *Cryptococcus neoformans* lanosterol 14-alpha-demethylase involved in fluconazole resistance in clinical isolates. *Rev Argent Microbiol* 2016; **48**: 137–42.

187 Gago S, Serrano C, Alastruey-Izquierdo A *et al*. Molecular identification, antifungal resistance and virulence of *Cryptococcus neoformans* and *Cryptococcus deneoformans* isolated in Seville, Spain. *Mycoses* 2017; **60**: 40–50.

188 Kano R, Okubo M, Hasegawa A *et al*. Multi-azole-resistant strains of *Cryptococcus neoformans* var. *grubii* isolated from a FLZ-resistant strain by culturing in medium containing voriconazole. *Med Mycol* 2017; **55**: 877–82.

189 Kano R, Okubo M, Yanai T *et al*. First isolation of azole-resistant *Cryptococcus neoformans* from feline cryptococcosis. *Mycopathologia* 2015; **180**: 427–33.

190 Joseph-Horne T, Hollomon D, Loeffler RS *et al*. Cross-resistance to polyene and azole drugs in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 1995; **39**: 1526–9.

191 Basso LR, Jr, Gast CE, Bruzual I *et al*. Identification and properties of plasma membrane azole efflux pumps from the pathogenic fungi *Cryptococcus gattii* and *Cryptococcus neoformans*. *J Antimicrob Chemother* 2015; **70**: 1396–407.

192 Posteraro B, Sanguinetti M, Sanglard D *et al*. Identification and characterization of a *Cryptococcus neoformans* ATP binding cassette (ABC) transporter-encoding gene, CnAFR1, involved in the resistance to fluconazole. *Mol Microbiol* 2003; **47**: 357–71.

193 Mondon P, Petter R, Amalfitano G *et al*. Heteroresistance to fluconazole and voriconazole in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 1999; **43**: 1856–61.

194 Xu J, Onyewu C, Yoell HJ *et al*. Dynamic and heterogeneous mutations to fluconazole resistance in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 2001; **45**: 420–7.

195 Yamazumi T, Pfaller MA, Messer SA *et al*. Characterization of heteroresistance to fluconazole among clinical isolates of *Cryptococcus neoformans*. *J Clin Microbiol* 2003; **41**: 267–72.

196 Sionov E, Chang YC, Garraffo HM *et al*. Heteroresistance to fluconazole in *Cryptococcus neoformans* is intrinsic and associated with virulence. *Antimicrob Agents Chemother* 2009; **53**: 2804–15.

197 Sionov E, Lee H, Chang YC *et al*. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog* 2010; **6**: e1000848.

198 Sionov E, Chang YC, Kwon-Chung KJ. Azole heteroresistance in *Cryptococcus neoformans:* emergence of resistant clones with chromosomal disomy in the mouse brain during fluconazole treatment. *Antimicrob Agents Chemother* 2013; **57**: 5127–30.

199 Stone NR, Rhodes J, Fisher MC *et al*. Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. *J Clin Invest* 2019; **129**: 999–1014.

200 Varma A, Kwon-Chung KJ. Heteroresistance of *Cryptococcus gattii* to fluconazole. *Antimicrob Agents Chemother* 2010; **54**: 2303–11.

201 Thornewell SJ, Peery RB, Skatrud PL. Cloning and characterization of CneMDR1: a *Cryptococcus neoformans* gene encoding a protein related to multidrug resistance proteins. *Gene* 1997; **201**: 21–9.

202 El-Halfawy OM, Valvano MA. Antimicrobial heteroresistance: an emerging field in need of clarity. *Clin Microbiol Rev* 2015; **28**: 191–207.

203 Ferreira GF, Santos DA. Heteroresistance and fungi. *Mycoses* 2017; **60**: 562–8.

204 Kim SJ, Kwon-Chung J, Milne GW *et al*. Relationship between polyene resistance and sterol compositions in *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 1975; **7**: 99–106.

205 Kelly SL, Lamb DC, Taylor M *et al*. Resistance to amphotericin B associated with defective sterol delta 8–>7 isomerase in a *Cryptococcus neoformans* strain from an AIDS patient. *FEMS Microbiol Lett* 1994; **122**: 39–42.

206 Vermes A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother* 2000; **46**: 171–9.

207 Whelan WL. The genetic basis of resistance to 5-fluorocytosine in *Candida* species and *Cryptococcus neoformans*. *Crit Rev Microbiol* 1987; **15**: 45–56.

208 Vu K, Thompson GR 3rd, Roe CC *et al*. Flucytosine resistance in *Cryptococcus gattii* is indirectly mediated by the FCY2-FCY1-FUR1 pathway. *Med Mycol* 2018; **56**: 857–67.

209 Billmyre RB, Applen Clancey S, Li LX *et al*. 5-fluorocytosine resistance is associated with hypermutation and alterations in capsule biosynthesis in *Cryptococcus*. *Nature Comm* 2020; **11**: 127.

210 Schwarz P, Janbon G, Dromer F *et al*. Combination of amphotericin B with flucytosine is active *in vitro* against flucytosine-resistant isolates of *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 2007; **51**: 383–5.

211 Liu HB, Liu F, Kong QT *et al*. Successful treatment of refractory Majocchi's Granuloma with voriconazole and review of published literature. *Mycopathologia* 2015; **180**: 237–43.

212 Rouzaud C, Chosidow O, Brocard A *et al*. Severe dermatophytosis in solid organ transplant recipients: A French retrospective series and literature review. *Transpl Infect Dis* 2018; **20**: 1–11.

213 Osborne CS, Leitner I, Favre B *et al*. Amino acid substitution in *Trichophyton rubrum* squalene epoxidase associated with resistance to terbinafine. *Antimicrob Agents Chemother* 2005; **49**: 2840–4.

214 Yamada T, Maeda M, Alshahni MM *et al*. Terbinafine resistance of Trichophyton clinical isolates caused by specific point mutations in the squalene epoxidase gene. *Antimicrob Agents Chemother* 2017; **61**: 1–13.

215 Baudraz-Rosselet F, Ruffieux C, Lurati M *et al*. Onychomycosis insensitive to systemic terbinafine and azole treatments reveals non-dermatophyte moulds as infectious agents. *Dermatology* 2010; **220**: 164–8.

216 Monod M. Antifungal resistance in dermatophytes: Emerging problem and challenge for the medical community. *J Med Mycol* 2019; **29**: 283–4.

217 Hsiao Y-H, Chen C, Han HS *et al*. The first report of terbinafine resistance *Microsporum canis* from a cat. *J Vet Med Sci* 2018; **80**: 898–900.

218 Astvad KMT, Hare RK, Jørgensen KM *et al*. Increasing terbinafine resistance in Danish *Trichophyton* isolates 2019-2020. *J Fungi (Basel)* 2022; **8**: 150.

219 Gnat S, Łagowski D, Dylag M *et al*. European hedgehogs (*Erinaceus europaeus* L.) as a reservoir of dermatophytes in Poland. *Microb Ecol* 2021.

220 Barros MEDS, Santos DDA, Hamdan JS. Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). *J Med Microbiol* 2007; **56**: 514–8.

221 Singh A, Masih A, Khurana A *et al*. High terbinafine resistance in *Trichophyton interdigitale* isolates in Delhi, India harbouring mutations in the squalene epoxidase gene. *Mycoses* 2018; **61**: 477–84.

222 Rezaei-Matehkolaei A, Khodavaisy S, Alshahni MM *et al*. *In Vitro* Antifungal Activity of Novel Triazole Efinaconazole and Five

Comparators against Dermatophyte Isolates. *Antimicrob Agents Chemother* 2018; **62**: 1–6.

223 Altinbaş R, Özakkaş F, Bariş A *et al*. *In vitro* susceptibility of seven antifungal agents against dermatophytes isolated in İstanbul. *Turk J Med Sci* 2018; **48**: 615–9.

224 Chowdhary A, Kathuria S, Singh PK *et al*. Molecular characterization and *in vitro* antifungal susceptibility of 80 clinical isolates of mucormycetes in Delhi, India. *Mycoses* 2014; **57**: 97–107.

225 Khurana A, Masih A, Chowdhary A *et al*. Correlation of *in vitro* susceptibility based on MICs and squalene epoxidase mutations with clinical response to terbinafine in patients with tinea corporis/cruris. *Antimicrob Agents Chemother* 2018; **62**: 1–9.

226 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard—Second Edition: M38-A2*. 2008.

227 Arendrup MC, Jørgensen KM, Guinea J *et al*. Multicentre validation of a EUCAST method for the antifungal susceptibility testing of microconidia-forming dermatophytes. *J Antimicrob Chemother* 2020; **75**: 1807–19.

228 Rudramurthy SM, Shankarnarayan SA, Dogra S *et al*. Mutation in the Squalene epoxidase gene of *Trichophyton interdigitale* and *Trichophyton rubrum* associated with allylamine resistance. *Antimicrob Agents Chemother* 2018; **26**: e02522-17.

229 Hsieh A, Quenan S, Riat A *et al*. A new mutation in the SQLE gene of *Trichophyton mentagrophytes* associated to terbinafine resistance in a couple with disseminated tinea corporis. *J Mycol Med* 2019; **29**: 352–9.

230 Hiruma J, Kitagawa H, Noguchi H *et al*. Terbinafine-resistant strain of *Trichophyton interdigitale* strain isolated from a tinea pedis patient. *J Dermatol* 2019; **46**: 351–3.

231 Fachin AL, Ferreira-Nozawa MS, Maccheroni W *et al*. Role of the ABC transporter TruMDR2 in terbinafine, 4-nitroquinoline N-oxide and ethidium bromide susceptibility in *Trichophyton rubrum*. *J Med Microbiol* 2006; **55**: 1093–9.

232 Martins MP, Franceschini ACC, Jacob TR *et al*. Compensatory expression of multidrug-resistance genes encoding ABC transporters in dermatophytes. *J Med Microbiol* 2016; **65**: 605–10.

233 Kano R, Hsiao Y-H, Han HS *et al*. Resistance mechanism in a terbinafine-resistant strain of *Microsporum canis*. *Mycopathologia* 2018; **183**: 623–7.

234 Monod M, Feuermann M, Salamin K *et al*. *Trichophyton rubrum* Azole Resistance Mediated by a New ABC Transporter, TruMDR3. *Antimicrob Agents Chemother* 2019; **63**: e00863–19.

235 Aneke CI, Rhimi W, Otranto D *et al*. Synergistic Effects of Efflux Pump Modulators on the Azole Antifungal Susceptibility of *Microsporum canis*. *Mycopathologia* 2020; **7**: 279–88.

236 Shankarnarayan SA, Shaw D, Sharma A *et al*. Rapid detection of terbinafine resistance in Trichophyton species by Amplified refractory mutation system-polymerase chain reaction. *Sci Rep* 2020; **10**: 1297.

237 van Dooyeweert DA, Schneider MM, Borleffs JC *et al*. The influence of PCP prophylaxis on bacteriuria incidence and resistance development to trimethoprim/sulfamethoxazole in HIV-infected patients. *Neth J Med* 1996; **49**: 225–7.

238 Walker DJ, Meshnick SR. Drug resistance in *Pneumocystis carinii*: an emerging problem. *Drug Resist Updat* 1998; **1**: 201–4.

239 Alanio A, Gits-Muselli M, Guigue N *et al*. Diversity of *Pneumocystis jirovecii* across Europe: A multicentre observational study. *EBioMedicine* 2017; **22**: 155–63.

240 Latouche S, Lacube P, Maury E *et al*. *Pneumocystis jirovecii* dihydropteroate synthase genotypes in French patients with pneumocystosis: a 1998-2001 prospective study. *Med Mycol* 2003; **41**: 533–7.

241 Cruciani M, Marcati P, Malena M *et al*. Meta-analysis of diagnostic procedures for *Pneumocystis carnii* pneumonia in HIV-1-infected patients. *Eur Resp J* 2002; **20**: 982–9.

242 Stein CR, Poole C, Kazanjian P *et al*. Sulfa use, dihydropteroate synthase mutations, and *Pneumocystis jiroveci* pneumonia. *Emerg Infect Dis* 2004; **10**: 1760–5.

243 Walker DJ, Wakefield AE, Dohn MN *et al*. Sequence polymorphisms in the *Pneumocystis carinii* cytochrome b gene and their association with atovaquone prophylaxis failure. *J Infect Dis* 1998; **178**: 1767–75.

244 Kazanjian P, Armstrong W, Hossler PA *et al*. *Pneumocystis carinii* cytochrome b mutations are associated with atovaquone exposure in patients with AIDS. *J Infect Dis* 2001; **183**: 819–22.

245 Ponce CA, Chabé M, George C *et al*. High Prevalence of *Pneumocystis jirovecii* Dihydropteroate Synthase Gene Mutations in Patients with a First Episode of Pneumocystis Pneumonia in Santiago, Chile, and Clinical Response to Trimethoprim-Sulfamethoxazole Therapy. *Antimicrob Agents Chemother* 2017; **61**: e01290-16.

246 Hauser PM, Nahimana A, Taffe P *et al*. Interhuman transmission as a potential key parameter for geographical variation in the prevalence of *Pneumocystis jirovecii* dihydropteroate synthase mutations. *Clin Infect Dis* 2010; **51**: 28–33.

247 Yiannakis EP, Boswell TC. Systematic review of outbreaks of *Pneumocystis jirovecii* pneumonia: evidence that *P. jirovecii* is a transmissible organism and the implications for healthcare infection control. *J Hosp Infect* 2016; **93**: 1–8.

248 Ozkoc S, Erguden C, Bayram Delibas S. Absence of dihydropteroate synthase gene mutations in *Pneumocystis jirovecii* strains isolated from Aegean region of Turkey. *Parasitol Res* 2018; **117**: 3103–8.

249 Suárez I, Roderus L, van Gumpel E *et al*. Low prevalence of DHFR and DHPS mutations in *Pneumocystis jirovecii* strains obtained from a German cohort. *Infection* 2017; **45**: 341–7.

250 Rabodonirina M, Vaillant L, Taffé P *et al*. *Pneumocystis jirovecii* genotype associated with increased death rate of HIV-infected patients with pneumonia. *Emerg Infect Dis* 2013; **19**: 21–8.

251 Dimonte S, Berrilli F, D'Orazi C *et al*. Molecular analysis based on mtLSU-rRNA and DHPS sequences of *Pneumocystis jirovecii* from immunocompromised and immunocompetent patients in Italy. *Infect Genet Evol* 2013; **14**: 68–72.

252 Friaza V, Morilla R, Respaldiza N *et al*. *Pneumocystis jiroveci* dihydropteroate synthase gene mutations among colonized individuals and Pneumocystis pneumonia patients from Spain. *Postgrad Med* 2010; **122**: 24–8.

253 Nahimana A, Rabodonirina M, Helweg-Larsen JP. Sulfa resistance and dihydropteroate synthase mutants in recurrent *Pneumocystis carinii* pneumonia. *Emerg Infect Dis* 2003; **9**: 864–7.

254 Visconti E, Ortona E, Mencarini P *et al*. Mutations in dihydropteroate synthase gene of *Pneumocystis carinii* in HIV patients with *Pneumocystis carinii* pneumonia. *Int J Antimicrob Agents* 2001; **18**: 547–51.

255 Helweg-Larsen J, Benfield TL, Eugen-Olsen J *et al*. Effects of mutations in Pneumocystis carinii dihydropteroate synthase gene on outcome of AIDS-associated *P. carinii* pneumonia. *Lancet* 1999; **354**: 1347–51.

256 Singh Y, Mirdha BR, Guleria R *et al*. Novel dihydropteroate synthase gene mutation in *Pneumocystis jirovecii* among HIV-infected patients in India: Putative association with drug resistance and mortality. *J Glob Antimicrob Resist* 2019; **17**: 236–9.

257 Ahn A, Chang J, Sung H *et al*. Case of pneumonia caused by *Pneumocystis jirovecii* resistant to SXT in the absence of previous drug exposure. *Lab Med Online* 2016; **6**: 250–4.

258 Singh Y, Mirdha BR, Guleria R *et al*. Molecular detection of DHFR gene polymorphisms in *Pneumocystis jirovecii* isolates from Indian patients. *J Infect Dev Ctries* 2015; **9**: 1250–6.

259 Lee SM, Cho YK, Sung YM *et al*. A case of pneumonia caused by *Pneumocystis jirovecii* resistant to trimethoprim-sulfamethoxazole. *Korean J Parasitol* 2015; **53**: 321–7.

260 Long Y, Zhang C, Su L *et al*. *Pneumocystis jirovecii* dihydropteroate synthase gene mutations in a group of HIV-negative immunocompromised patients with *Pneumocystis* pneumonia. *Exp Ther Med* 2014; **8**: 1825–30.

261 Tyagi AK, Mirdha BR, Luthra K *et al*. Dihydropteroate synthase (DHPS) gene mutation study in HIV-Infected Indian patients with *Pneumocystis jirovecii* pneumonia. *J Infect Dev Ctries* 2010; **4**: 761–6.

262 van Hal SJ, Gilgado F, Doyle T *et al*. Clinical significance and phylogenetic relationship of novel Australian *Pneumocystis jirovecii* genotypes. *J Clin Microbiol* 2009; **47**: 1818–23.

263 Tyagi AK, Mirdha BR, Guleria R *et al*. Study of dihydropteroate synthase (DHPS) gene mutations among isolates of *Pneumocystis jiroveci*. *Indian J Med Res* 2008; **128**: 734–9.

264 Takahashi T, Hosoya N, Endo T *et al*. Relationship between mutations in dihydropteroate synthase of *Pneumocystis carinii* f. sp. hominis isolates in Japan and resistance to sulfonamide therapy. *J Clin Microbiol* 2000; **38**: 3161–4.

265 Yoon C, Subramanian A, Chi A *et al*. Dihydropteroate synthase mutations in Pneumocystis pneumonia: impact of applying different definitions of prophylaxis, mortality endpoints and mutant in a single cohort. *Med Mycol* 2013; **51**: 568–75.

266 Navin TR, Beard CB, Huang L *et al*. Effect of mutations in *Pneumocystis carinii* dihydropteroate synthase gene on outcome of *P. carinii* pneumonia in patients with HIV-1: a prospective study. *Lancet* 2001; **358**: 545–9.

267 Huang L, Beard CB, Creasman J *et al*. Sulfa or sulfone prophylaxis and geographic region predict mutations in the *Pneumocystis carinii* dihydropteroate synthase gene. *J Infect Dis* 2000; **182**: 1192–8.

268 Kazanjian P, Locke AB, Hossler PA *et al*. *Pneumocystis carinii* mutations associated with sulfa and sulfone prophylaxis failures in AIDS patients. *AIDS* 1998; **12**: 873–8.

269 Taylor SM, Meshnick SR, Worodria W *et al*. International HIV-associated Opportunistic Pneumonias Study. Low prevalence of Pneumocystis pneumonia (PCP) but high prevalence of pneumocystis dihydropteroate synthase (dhps) gene mutations in HIV-infected persons in Uganda. *PLoS One* 2012; **7**: 499.

270 Dini L, du Plessis M, Frean J *et al*. High prevalence of dihydropteroate synthase mutations in *Pneumocystis jirovecii* isolated from patients with Pneumocystis pneumonia in South Africa. *J Clin Microbiol* 2010; **48**: 2016–21.

271 Trotter AJ, Ayden A, Strinden MJ *et al*. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. *Curr Opin Microbiol* 2019; **51**: 39–45.