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Does the use of topical azoles have an impact on antifungal resistance?

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Introduction

Pathogenic fungi have an increasing impact on human health but their study, control or prevention is often a low priority in many health systems. It has been estimated that nearly 1 billion people have skin, nail and hair fungal infections, with mucosal candidiasis affecting tens of millions.[1] Life-threatening fungal diseases affect more than 150 million people, with immunocompromised and immunosuppressed populations being more at risk,[2,3] notably those with AIDS or patients suffering from respiratory conditions such as tuberculosis, chronic obstructive pulmonary disease and asthma.[2,4] There have been comprehensive reviews investigating the incidence of serious fungal diseases per countries, socio-economic background and co-morbidity.[5,6] Perhaps not surprisingly, aspergillosis is predominant as a serious fungal infection with an estimated 6.5 million people suffering from invasive fungal aspergillosis and chronic pulmonary aspergillosis annually.[6] The burden of fungal diseases, incidence and impact has been reviewed by Bongomin *et al*.[5] Although, candidiasis and aspergillosis are prominent infections,[5,7] dermatomycoses, despite their incidence (1 billions infected)[5] are less studied, with Tinea infections being the most reported.[8,9] Among the emerging concerns, dermatomycoses caused by *Trichophyton indotineae* are particularly noteworthy. Initially identified in India,[10] this pathogen has now spread globally.[11] Infections caused by *T. indotineae* tend to be more severe and inflammatory compared to those caused by *Trichophyton rubrum*.[12] A significant challenge in managing *T. indotineae* infections is the widespread clinical resistance to terbinafine, whereas resistance to azoles has been reported less frequently.[13] Terbinafine resistance is primarily attributed to mutations in the squalene epoxidase gene, although additional mutations are linked to a decreased in susceptibility to azoles.[14–16]

Recognizing the importance of fungal infections, the World Health Organization now list 10 antifungals (Amphotericin B, clotrimazole, fluconazole, flucytosine, griseofulvin, itraconazole, nystatin, voriconazole, micafungin and miconazole) in its *List of Essential Medicines*.[17] The choice of antifungals depends on the causative agent and the severity of the infection. For non-severe fungal infections of skin and mucous membranes, amorolfine, allylamines, azoles, ciclopiroxolamine and tolnaftate, amphotericin B and nystatin, and miconazole are used. The use of fluconazole, itraconazole and terbinafine is associated with severe fungal infections of skin and mucous membranes.[18] Other actives are also use, for the treatment of superficial tinea infections, although evidence of their efficacy is lacking.[19] Sahoo and Mahajan[9] reviewed available treatments for cutaneous dermatomycoses. Reported efficacy of an antifungal treatment depends on what is being measured and end points; e.g. MIC, MIC_{90}, MIC_{50} or mycological cure. As such efficacy for a given antifungal can vary between studies. In addition, some dermatomycoses (for examples, *Tinea capitis*, *Tinea* affecting the nails and *Tinea* involving more than one body region simultaneously) are empirically treated with systemic antifungals rather than topical ones.[9] Yet, for many conditions initial antifungal treatment is often acquired over-the-counter (OTC) and this may be without previous consultation with a physician. OTC application of antifungals is normally indicated for non-invasive, local, benign or self-limiting conditions and result in application for limited periods of time, usually 2–6 weeks, although for some infections such as athlete's foot and onychomycosis, treatment can be longer (2–6 months).

Azoles are a major group of antifungals used both for topical and systemic severe fungal infections.[20] Their low cost compared to other antifungals and their effectiveness make azoles the antifungal drugs of choice worldwide.[21] A 2010 survey among Spanish dentists (840 respondents) reported the use of miconazole oral gel (59.3%), followed by nystatin (57.7%) for the topical treatment of oral candidiasis. For most dentists, the first-line treatment was topical antifungals. Experienced dentists, particularly stomatologists, and male rather than female dentists, preferred systemic antifungals (percentage of prescriptions: itraconazole: 14.4%; ketoconazole: 14.3%; fluconazole: 13.1%). Of note, 44.5% of dentists administered chlorhexidine for candidal infections of the oral mucosa.[22] A recent national point prevalence survey in residents of Australian aged-care facilities showed that tinea was one of the most common infections (38.3%) for which the topical antifungals clotrimazole (85.3%) and miconazole (9.1%) were prescribed.[23]

The mechanism of action of azoles has been well described in the literature. Azole antifungal action is driven by the inhibition of C_{14}-demethylation of lanosterol, a precursor of ergosterol, resulting in the accumulation of toxic methylated sterol intermediates,

which subsequently inhibit fungal growth.[24,25] The target site of azoles is the $C_{14}\alpha$-demethylase enzyme, encoded by the ERG11 gene.[22] Additional effects of azoles on fungal cells have been observed. Thevissen *et al.*[26] reported that miconazole induces changes in the actin cytoskeleton, followed by the production of reactive oxygen species (ROS) in yeasts. The accumulation of ROS contributes to miconazole's fungicidal effect,[27–29] but this effect has not been observed with other azoles.[26] Fluconazole has been reported to decrease the adherence of *Candida albicans* to buccal epithelial cells by interfering with candidal receptors.[30]

One of the main concerns about antifungals is the development of fungal resistance.[31] resistance has been widely reported in yeasts, predominantly *Candida* spp.,[32] and in *Aspergillus* spp.,[33] but also in superficial mycoses such as *Trichophyton* spp.[13,32] However, the impact of inappropriate antifungal test interpretation, the use of non-standardized susceptibility methods, and the lack of consensus on resistance definitions have questioned the significance of resistance in pathogenic yeasts and its clinical implications.[34]

This review aims to investigate the impact of antifungals, mainly miconazole and ketoconazole, on emerging resistance in fungi associated with topical infections. Miconazole was approved by the US Food and Drug Administration for topical treatment in 1974.[35] Ketoconazole was approved for systemic use in 1981.[36] In 2022, the estimated number of prescriptions for ketoconazole in the USA was 2 955 996 (https://clincalc.com/DrugStats/Drugs/Ketoconazole). Given the global burden of skin, hair, nail fungal infections and oral candidiasis, investigating evidence of the impact of the widespread use of topical antifungals on emerging fungal resistance, if any, seems justified.

Mechanisms of resistance to azoles

The mechanisms of fungal resistance to antifungals have been well documented, with some mechanisms being specific to the type of compounds.[37] Overall, antimicrobials exert selective pressure on their microbial targets, resulting in the selection of the least susceptible microorganisms and/or the expression of mechanisms that enable survival at the antimicrobial concentration they are exposed to. The use of antifungals in clinical, industrial and agricultural settings has likely contributed to the evolution of resistance in pathogenic fungi.[38] Among azoles, fungal resistance to fluconazole has been widely reported,[37,38] although resistance has been described for all antifungals currently available for the treatment of human infections.[37–39]

Mechanisms of fungal resistance to azoles

There are several mechanisms of fungal resistance to azoles (Figure 1). A number of these involve modifications to the ergosterol synthetic pathway.[38] Ergosterols play an essential role in maintaining membrane integrity and fluidity, and in facilitating the activity of membrane-bound enzymes.[38]

In *Candida* spp., mutations in the ERG11 gene, which encodes for lanosterol $C_{14}\alpha$-demethylase, prevent azole binding and result in decreased efficacy.[40] Mutation of the ERG3 gene prevents the formation of toxic 14α-methyl-3,6-diol, the by-product of the inhibition of the C_{14}-demethylation of lanosterol, thus negating the impact of azole treatment and inhibiting fungal growth.[40] Up-regulation of the ERG11 gene also results in an increased intracellular concentration of lanosterol $C_{14}\alpha$-demethylase, reducing the efficacy of a set concentration of azole.[40] Additionally,

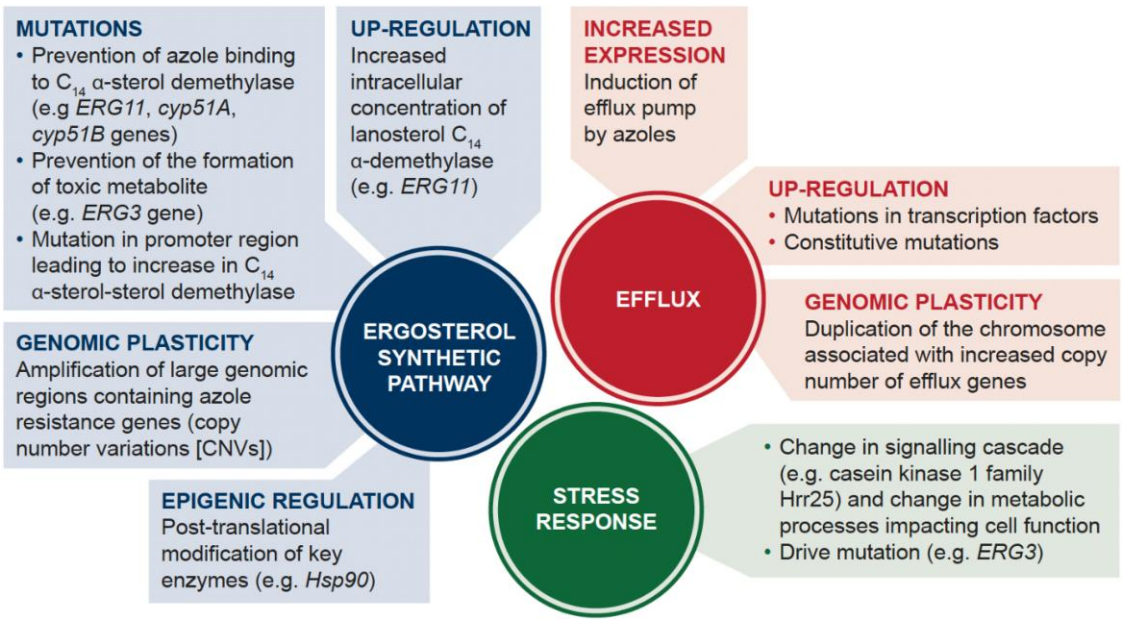

Figure 1. Mechanisms of fungal resistance to azoles. Several of these mechanisms can be expressed simultaneously, reducing the effective concentration of azoles, increasing *in vitro* MIC, and leading to clinical resistance. The main mechanisms are related to ergosterol biosynthesis and/or efflux, although stress responses can involve a broader metabolic response. Many studies have observed that efflux expression can result in a greater reduction in azole susceptibility compared to modifications in ergosterol synthesis (see text).

preventing the binding of azoles to lanosterol-4,4-dimethylcholesta-8,14,24-trienol through steric hindrance or by decreasing hydrogen bond interactions may result in decreased azole efficacy.[41]

In *Aspergillus* spp., alteration of the $C_{14}\alpha$-demethylase enzyme encoded by the *cyp51A* and *cyp51B* genes through mutations in these genes can lead to decreased susceptibility to azoles or cross-resistance between azoles such as itraconazole and posaconazole. Mutation in the promoter region of *cyp51A* can also lead to an increase in the $C_{14}\alpha$-demethylase enzyme.[39,40] Mutational changes might depend on the antifungal concentration to which the fungus is exposed, which impacts the degree of stress imparted on the cell.[42]

In yeasts, one well-described mechanism of resistance is the expression of efflux pumps. In *Candida* spp., the expression of efflux pumps is regulated by the CDR1, CDR2 and MDR1 genes, which encode ABC transporters.[38,39] Overexpression of ABC transporters is associated with decreased azole efficacy[40] and may be more effective than ERG11 mutations. In *Candida glabrata*, the up-regulation of CgCDR1 and CgCDR2 (encoding efflux pumps) has been shown to reduce fluconazole efficacy, even in the presence of modifications to ERG11.[43] Mutations in transcription factors that positively regulate CDR1 expression can also lead to its overexpression, which correlates with fluconazole resistance in *Candida auris*.[44] Mutations in regulatory genes for efflux pumps, which confer decreased azole susceptibility, have also been described in other *Candida* species.[38,39] Overall, increased expression of efflux genes, due to altered regulation, leads to reduced susceptibility to azoles.[39]

In moulds, multidrug-resistant efflux pumps have been described in *Aspergillus* species[45–47] and *Penicillium* spp.[48] Overexpression of the *cyp51A* gene, encoding $C_{14}\alpha$-demethylase in *A. fumigatus*, has been observed in azole-resistant isolates.[39] Conversely, the loss of an ABC transporter has resulted in increased susceptibility to itraconazole, posaconazole and voriconazole in clinical *A. fumigatus* susceptible isolates, but also a 4-fold reduction in itraconazole MIC in a clinical resistant isolate.[45] In *T. indotineae*, up-regulation of ABC transporters or mutation in *ERG11/CYP51* gene was associated with azole resistance.[49,50]

Genomic plasticity is another source of resistance to azoles.[39] In diploid *C. albicans*, which are azole-resistant, duplication of chromosomes has been associated with increased expression of genes encoding Erg11and Tac1, a transcriptional regulator of efflux pumps.[39] Similarly, azole resistance has been associated with extensive copy number variations, which result in the amplification of large genomic regions containing azole resistance genes.[39] This same mechanism of azole resistance has been observed in *C. glabrata* and *Cryptococcus neoformans*.[39] Epigenetic regulation, particularly post-translational modification of key enzymes such as Hsp90 in *C. albicans*, impacts susceptibility to fluconazole, itraconazole and voriconazole.[51] Other examples of epigenetic regulation contributing to decreased yeast susceptibility to azoles have been reported with post-modification of histones, such as RPH1 and H3K4 in *C. glabrata*.[52,53]

Stress responses are also involved in resistance to azoles. In fungi, the molecular chaperone Hsp90 is associated with tolerance and resistance.[39] Fu *et al.*[54] observed that depletion of Hsp90 in *C. neoformans* increased susceptibility to fluconazole. Resistance in *C. albicans* to azoles can be mediated by mutations in ERG3, which block the formation of toxic metabolites following azole exposure.[39] ERG3 mutations and the subsequent decreased susceptibility to fluconazole are impacted by stress responses.[55–57] Similarly, the complete signalling cascade following stress response in *C. albicans* has highlighted the potential role of Hrr25 (a casein kinase 1 family protein) in fluconazole tolerance.[58] Finally, tolerance to miconazole can be impacted by several metabolic processes, including tryptophan biosynthesis, membrane trafficking (including endocytosis), regulation of the actin cytoskeleton, and regulation of gene expression.[26] The alteration of these processes is likely part of general stress tolerance mechanisms, which are induced following miconazole exposure.[26] Stress responses are common microbial mechanisms activated following exposure to detrimental factors, whether physical (e.g. heat) or chemical (e.g. antimicrobials), and are not unique to miconazole.

Parameters impacting fungal resistance

Several factors can contribute to the development of clinical resistance to antifungals, including: (i) clinical factors, (ii) host immune system, (iii) socio-economic conditions, (iv) prior exposure to antifungals and stress factors, (v) biofilm formation, (vi) interpretation and type of antifungal tests and (vii) non-clinical use of antifungals (Figure 2).

Clinical Factors that contribute to antifungal resistance include: (i) incorrect or complex diagnosis leading to treatment failure of pre-emptive strategies or prevalence of the immune reconstitution inflammatory syndrome which can overshadow the treatment effectiveness to control fungal growth; (ii) immunosuppression; (iii) high fungal burden impinging the efficacy of antifungals at initiation of treatment; (iv) high virulence characteristics of fungal pathogen; (v) toxicity of treatment, drug-drug interactions and serum drug level; (vi) site of infection; (vii) sub-optimal length of treatment and patient compliance; (viii) underlying disease; (ix) fungal biofilm formation.[33]

Patients' immune status is a significant factor in the development of antifungal resistance. Oral *Candida* infections, and subsequent antifungal use, which can promote antifungal resistance, are more common in immunocompromised individuals.[59,60] In particular, fluconazole resistance is more prominent among IV drug users compared to heterosexuals, homosexuals or blood transfusion recipients.[61] It has also been observed that non-*C. albicans* isolates are more frequently encountered in patients who have recently received antifungal treatment.[61] Additionally, non-*C. albicans* strains are more commonly isolated in immunocompromised patients.[62,63]

The use of antifungals (including fungicides) is essential in agricultural and some industrial processes. Antifungals are also heavily used in veterinary medicine and can be found in consumer products.[64] The selective pressure exerted by antifungals is global and is not exclusively associated with their use to treat human infections. Azole-resistant fungi have been isolated from the environment.[64] Hence, the One Health approach needs to be considered.[38] With this in mind, it may be difficult to attribute emerging fungal resistance to antifungals solely to their use in human medicine. Indeed, mutations and overexpression of C14α-demethylase in *Aspergillus* spp. are also found in the environment where azoles are used in agriculture and have been

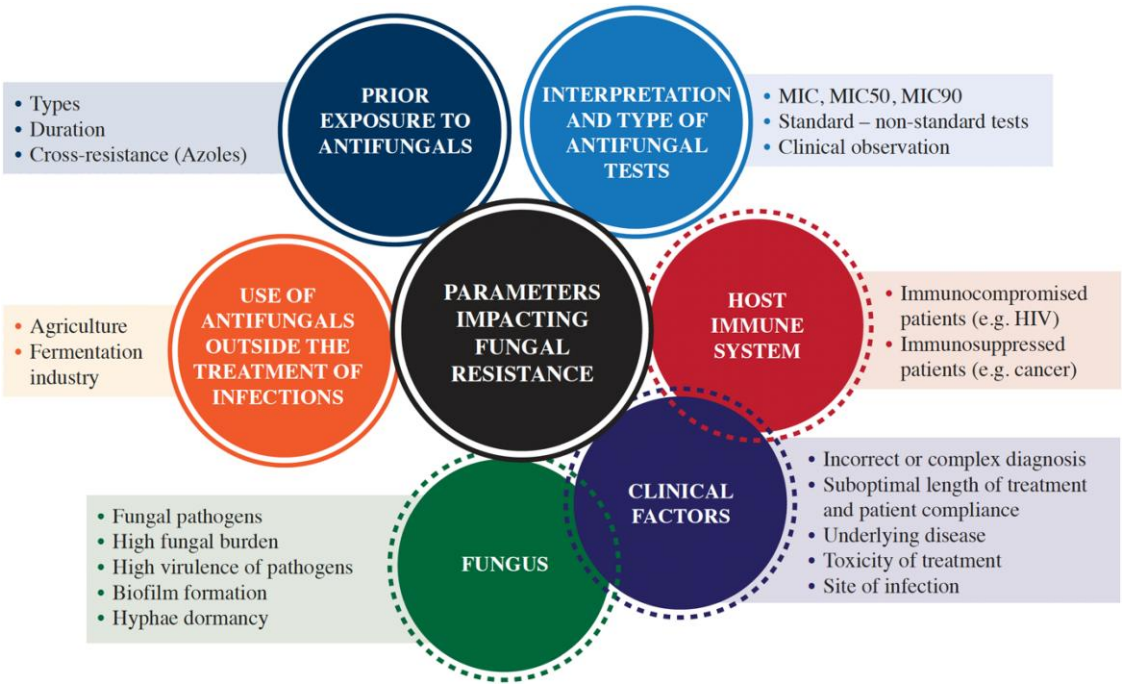

Figure 2. Factors impacting fungal resistance. The use of antifungals to treat infections is not the sole factor contributing to the emergence of resistance. Clinical factors, characteristics of the pathogenic fungus, and the host immune response are all closely intertwined. The type of *in vitro* antifungal test used, along with its interpretation, as well as its relevance to clinical observations, plays a significant role in how 'resistance' is defined and perceived. Additionally, antifungals are widely used for purposes beyond infection treatment, which also contributes to the development of resistance.

reported in patients with no prior exposure to antifungals. As such, environmental isolates of *Aspergillus* spp. may become a reservoir for antifungal resistance.[39]

Resistance refers to a fungal pathogen with a MIC to a given antifungal that exceeds the susceptibility breakpoint for that organism. Intrinsic resistance denotes the innate ability of a fungus to survive antifungal exposure, whereas acquired resistance refers to a previously susceptible strain becoming resistant to the antifungal, usually following mutations that alter gene expression. Clinical resistance refers to the failure of an antifungal agent to eradicate the pathogen in a patient. The CLSI has published protocols for measuring the MIC of yeasts[65,66] and filamentous fungi[67] to antifungals. Other guidelines on antifungal testing have been published by EUCAST.[68,69] Unfortunately, MIC values and breakpoints do not always inform the response to antifungal therapy.[70] MIC determination for moulds does not necessarily reflect the effect of an antifungal on hyphal structures. Nevertheless, the clinical challenge posed by emerging fungal resistance should be guided by *in vitro* susceptibility testing,[38,71] although predicting fungal resistance from laboratory testing is not straightforward.[34]

Prior exposure to antifungals, including azoles, has been associated with decreased efficacy of antifungal treatments.[72–74] Some studies have highlighted that the use of certain azoles, such as itraconazole and fluconazole, has been a major driver of azole resistance.[75,76] The impact of fluconazole use on decreasing fluconazole susceptibility has been well documented over the years,[61,74] with a strong correlation between the use of fluconazole and increased fluconazole resistance in *C. albicans*.[61,77] Other studies, however, have reported little difference in the susceptibility of fungal isolates that had been previously exposed to antifungals compared to those that had not,[72] or minimal changes in the susceptibility of clinical isolates of *Candida* spp. over the years, with the exception of *C. glabrata*,[78] or no increase in fluconazole resistance in *Candida* spp. isolates despite increased clinical use of the azole.[79] Fluconazole therapy has also been associated with the selection of non-*C. albicans* isolates,[61] though this may not be universal.[80]

This review further investigates the available evidence on the impact of miconazole and ketoconazole on emerging fungal resistance. As mentioned earlier, these imidazoles were approved for use in 1972 and 1981, respectively, and have been in use ever since. Ketoconazole has remained heavily used in the USA, according to recent prescription estimates (https://clincalc.com/DrugStats/Drugs/Ketoconazole), and miconazole is on the WHO list of essential medicines.[17]

Impact of miconazole or ketoconazole on fungal resistance

To document the potential impact of these antifungals on fungal resistance, the peer-reviewed literature database searched was Web of Science, which was cross-checked with Scopus, PubMed, and Google Scholar. Initially, a total of 470 scientific articles were analysed (159 concerning miconazole and 311 concerning ketoconazole). Additional articles were added following cross-referencing and analysis of the initially selected literature. After duplicate outputs were removed and the exclusion/

inclusion criteria applied, only 67 articles were retained for an in-depth analysis (29 concerning miconazole and 38 concerning ketoconazole). The literature was analysed up to 30 September 2024.

This section concerns fungi of interest associated with skin or oral infections in humans, including both yeasts and moulds. Based on an initial scoping, papers reporting fungal systemic infections and their treatments were not considered. Papers in which miconazole or ketoconazole were not mentioned were also excluded. Fungal infections in animals were not included. Only peer-reviewed scientific articles written in English were considered. Peer-reviewed review articles, conference abstracts, and editorials were excluded.

Evidence of the impact of miconazole on emerging antifungal resistance

Overall, 29 papers on miconazole were analysed in more depth. Nineteen papers focused on *in vitro* antifungal MIC determination of various clinical fungal isolates. Only one paper described the efficacy of a miconazole product *in vivo*. Nine papers investigated emerging fungal resistance *in vitro*, including four papers on fungal biofilms.

Eighteen studies investigated the *in vitro* efficacy of miconazole against a range of clinical *Candida* spp. isolates, with *C. albicans* being the principal microorganism.[59,61,71–74,78,81–90] Three studies investigated other yeasts, including *Malassezia* spp.;[90] *Pichia kudriavzevii*,[81] *Rhodotorula rubra*,[59] and only two studies concerned moulds: *T. rubrum*[91] and *Neoscytalidium dimidiatum*.[92] Most of the studies (16/18) focused on oral *Candida* isolates. Only two studies concerned skin isolates (Table 1).[82,87]

When miconazole susceptibility was determined, the MIC for *C. albicans* oral isolates varied from 0.016 to 16 mg/L. Only two studies provided MIC_{50} or MIC_{50}/MIC_{90} (Table 1).[72,73] When considering the evolution of miconazole resistance,[78] reported no increase in MIC between *Candida* spp. isolated from patients with oral candidiasis between 2006–2007 and 2012–2013, with the exception of *C. glabrata* (increased in MIC90 from 4 to 16 mg/L, although not statistically significant). In this study, resistance to miconazole was arbitrarily defined as ≥4 mg/L (Table 1). One study reported that *C. albicans* isolated from patients living with HIV and presenting clinical symptoms had a higher MIC than isolates from healthy patients5[9] It has also been observed that there is a shift in *Candida* species in immunocompromised patients, with non-*albicans* species being more frequently isolated.[62,63] However, MIC determination of non-*albicans* species

Table 1. *Candida* spp investigated for their susceptibility to miconazole

Fungal isolates	Number of isolates or (%)	Origin	MIC (mg/L)	MIC_{50}	MIC_{90}	Reference
C. albicans	37	Dental plaques	0.016–2	—	—	Aslani *et al.*[81]
	664	Skin isolates	—	—	—	Bilal *et al.*[82]
	88	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	75	Carries and chronic periodontitis	0.03–4	—	—	De-la-Torre *et al.*[84]
	466	Oral (HIV)	0.007–2.00	—	—	Blignaut *et al.*[83]
	250	Oral candidiasis	0.016–4	—	—	Hamza *et al.*[74]
	116	Oral candidiasis	≥4 (3)[a](6)[b]	< 0.03	0.06	Kamikawa *et al.*[78]
	521	Oral	—	0.03	0.5	Kuriyama *et al.*[72]
	177	Oral candidiasis (diabetic patients)	—	—	—	Manfredi *et al.*[86]
	100	Oral candidiasis (HIV)	0.06–4[c]/0.125–16[d]	—	—	Melo *et al.*[59]
	43	Clinical specimens	<0.031–0.5	—	—	Taghipour *et al.*[87]
	70	Oral periodontitis	0.03–16	—	—	Waltimo *et al.*[89]
	500	Oral candidiasis	≤0.015–>16	0.12	—	Yu *et al.*[73]
	29	Skin (psoriasis)	0.03–1[e]	—	—	de Aguiar Cordeiro *et al.*[90]
C. glabrata	18	Skin isolates	—	—	—	Bilal *et al.*[82]
	9	Oral (HIV)	0.06–0.25	—	—	Blignaut *et al.*[83]
	6	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	5	Carries and chronic periodontitis	0.03	—	—	De-la-Torre *et al.*[84]
	20	Oral candidiasis	0.063–0.5	—	—	Hamza *et al.*[74]
	36	Oral candidiasis	≥4 (2)[a] (11)[b]	0.125[a,b]	>16 [a]/1[b]	Kamikawa *et al.*[78]
	59	Oral	—	0.06	0.25	Kuriyama *et al.*[72]
	5	Oral candidiasis (HIV)	0.5–4[d]	—	—	Melo *et al.*[59]
	21	Clinical specimens	<0.031–0.5	—	—	Taghipour *et al.*[87]
	29	Oral candidiasis	0.03–2	—	—	Yu *et al.*[73]
C. tropicalis	3	Skin isolates	—	—	—	Bilal *et al.*[82]
	9	Oral (HIV)	0.015–0.5	—	—	Blignaut *et al.*[83]

Continued

Table 1. *Continued*

Fungal isolates	Number of isolates or (%)	Origin	MIC (mg/L)	MIC_{50}	MIC_{90}	Reference
	5	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	4	Carries and chronic periodontitis	0.03–1	—	—	De-la-Torre *et al.*[84]
	8	Oral candidiasis	0.125–4	—	—	Hamza *et al.*[74]
	3	Oral candidiasis	≥4 (1)[b]	0.06[a]/<0.03[b]	0.06[a,b]	Kamikawa *et al.*[78]
	8	Oral candidiasis (HIV)	0.5[c]/2–8[d]	—	—	Melo *et al.*[59]
	12	Clinical specimens	<0.031–0.5	—	—	Taghipour *et al.*[87]
	16	Oral candidiasis	≤0.015–16	—	—	Yu *et al.*[73]
C. parapsilosis	1	Skin isolates	—	—	—	Blignaut *et al.*[83]
	9	Oral (HIV)	0.12–0.4	—	—	Blignaut *et al.*[83]
	1	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	18	Carries and chronic periodontitis	0.03–0.35	—	—	Lomeli-Martinez *et al.*[85]
	2	Oral candidiasis	<0.03	<0.03	<0.03	Kamikawa *et al.*[78]
	12	Oral	—	0.25	0.5	Kuriyama *et al.*[72]
	8	Oral candidiasis (HIV)	0.5–8[d]	—	—	Melo *et al.*[59]
	6	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
	4	Oral candidiasis	1–2	—	—	Yu *et al.*[73]
C. krusei	8	Skin isolates	—	—	—	Bilal *et al.*[82]
	38	Oral (HIV)	0.03–8	—	—	Blignaut *et al.*[83]
	3	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	2	Carries and chronic periodontitis	0.5	—	—	De-la-Torre *et al.*[84]
	10	Oral candidiasis	1–2	—	—	Hamza *et al.*[74]
	1	Oral candidiasis	≥4 (1)[b]	0.5[b]	0.5[b]	Kamikawa *et al.*[78]
	7	Oral	—	0.5	8	Kuriyama *et al.*[72]
	13	Oral candidiasis (HIV)	2[c]/4–16[d]	—	—	Melo *et al.*[59]
	3	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. rugosa	2	Oral (HIV)	0.06	—	—	Blignaut *et al.*[83]
	2	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. famata	2	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. kefyr	1	Oral (HIV)	0.015	—	—	Blignaut *et al.*[83]
	2	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	3	Oral candidiasis	0.016	—	—	Hamza *et al.*[74]
	3	Oral candidiasis (HIV)	0.06–4[d]	—	—	Melo *et al.*[59]
	1	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. lusitaniae	8	Oral (HIV)	0.03–0.12	—	—	Blignaut *et al.*[83]
	9	Oral candidiasis (HIV)	0.25–4[d]	—	—	Melo *et al.*[59]
	1	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. guilliermondii	2	Oral (HIV)	1–16	—	—	Blignaut *et al.*[83]
	6	Carries and chronic periodontitis	0.03–1	—	—	De-la-Torre *et al.*[84]
	3	Oral candidiasis (HIV)	1[c]/0.25–8[d]	—	—	Melo *et al.*[59]
	1	Clinical specimens	<0.031	—	—	Taghipour *et al.*[87]
C. dubliniensis	10	Oral (HIV)	0.015–0.06	—	—	Blignaut *et al.*[83]
	11	Carries and chronic periodontitis	0.03	—	—	De-la-Torre *et al.*[84]
C. lipolytica	5	Carries and chronic periodontitis	0.03	—	—	De-la-Torre *et al.*[84]
C. intermedia	2	Oral candidiasis (HIV)	1[c]/3[d]	—	—	Melo *et al.*[59]
C. norvegensis	1	Oral candidiasis (HIV)	1[c]/4[d]	—	—	Melo *et al.*[59]

—: No data provided.
[a]Number of isolates from 2006 to 2007 with a high miconazole MIC.
[b]Number of isolates from 2021 to 2013 with a high miconazole MIC.
[c]Combined data from healthy patients.
[d]Combine data from patients with HIV.
[e]In healthy patients, miconazole MIC range: 0.03–0.5 mg/L.

(although not necessarily from immunocompromised or immunosuppressed patients) did not reveal a change in miconazole susceptibility (Table 1). Non-*Candida* yeasts have also been studied for their susceptibility to miconazole.[90] de Aguiar Cordeiro *et al.*[90] reported that miconazole susceptibility for *Malassezia* spp. isolated from patients with psoriasis (MIC range 0.5->16 mg/L) was not different from those isolated from healthy patients (MIC range 0.25->16 mg/L).

The interest in providing MIC and notably MIC_{50}/MIC_{90} data is to understand changes in antifungal susceptibility of clinical isolates. Several studies highlighted that miconazole susceptibility/resistance status could not be provided in the absence of appropriate breakpoints for *Candida* spp. or other fungi.[74,81,83,91,92] Other studies provided miconazole resistance information in clinical isolates, although the determination of how resistance was calculated is not explicit,[61,82,86,89] or actual MIC data is not provided.[61,82,85,86] Many studies used arbitrary breakpoint values to determine miconazole resistance against *Candida* spp. These breakpoints were based on the published literature,[59,61,71–73,78,87] the use of other antifungal breakpoints,[84] or on the interpretation of the zone of inhibition (following the CLSI M44-A2 disk diffusion test).[71,88] Whilst many studies claim the isolation of *Candida* spp. resistant to miconazole, Kuriyama *et al.*[72] reported that all 618 *C. albicans* dental isolates were susceptible to miconazole. Likewise, Taghipour *et al.*[87] reported that all clinical *Candida* isolates tested were susceptible to miconazole, as their MIC ≤ 5 mg/L (arbitrary breakpoint). Tantivitayakul *et al.*[88] reported that all *Candida* isolates from healthy patients (100) and patients suffering from oral candidiasis (100) were susceptible to miconazole.

Studies of mould susceptibility to miconazole are scarce. An increase in azole MIC above the MIC_{90} (miconazole MIC50: 0.25 mg/L; MIC90: 0.5 mg/L) was observed for *T. rubrum* from patients suffering from onychomycosis who had received prior antifungal treatment, although the type of prior treatment was not specified[92] reported a miconazole MIC ranging from 0.031 to 1 mg/L for *N. dimidiatum*.

Only one manuscript reported results from an *in vivo* study. Takahashi *et al.*[93] described that a miconazole–nitrate soap used in elderly patients wearing diapers was effective in inhibiting the conversion of pseudohyphae/blastoconidia. There are no reports of the use of mono topical miconazole therapy against *T. indotineae*. Therapy combining topical and systemic treatments have achieved complete resolution of the clinical condition, although the benefit of such combined therapy is still questioned.[94] Topical miconazole and ciclopirox olamine has been used successfully against a terbinafine resistant isolate in a young patient, although lesions reappear when the treatment was stopped.[95] Studies reporting on the impact of miconazole on the selection of azole-resistant *T. indotineae* could not be found.

The remaining miconazole studies concerned emerging miconazole resistance *in vitro* (Table 2). Four studies investigated the impact of fungal biofilms on the efficacy of miconazole.[96–99] Gebremedhin *et al.*[96] and Kean *et al.*[97] used sedimentation biofilms to explore the miconazole concentration needed to decrease biofilm metabolic activity by 50%; miconazole concentration ranged from 50 to >96 mg/L depending on the *Candida* species, with *C. parapsilosis* being the least susceptible.[96] In a mixed *C. albicans*/*Staphylococcus aureus* biofilm, 40 mg/L of miconazole was necessary to achieve a 50% reduction in metabolic activity.[97] Lamfon *et al.*[98,99] used a constant-depth fermenter to study the impact of biofilm on miconazole efficacy. In the first study, *C. albicans* biofilms needed 256 mg/L to be inhibited, a > 1000-fold increase in MIC from planktonic *C. albicans*.[99] The second study investigated complex multispecies biofilms and recorded that *C. albicans* in a complex biofilm grew in the presence of 256 mg/L miconazole. Interestingly, to reflect product usage, when a miconazole gel solution (48 mg/L) was pulsed into the biofilm for 168 h, a 2 $\log_{10}$ reduction in *Candida* spp. was recorded initially, but after 120 h, growth resumed.[98] These studies indicate that *Candida* spp. in biofilms are more resilient than their planktonic counterparts to miconazole and raise questions about the efficacy of current oral miconazole treatments when complex biofilms are present.

Other studies looked at the impact of miconazole on emerging azole resistance. Rautemaa *et al.*[100] investigated factors behind fluconazole resistance in *Candida* spp. They studied the susceptibility of 162 clinical *Candida* spp. isolates to fluconazole (mean MIC = 19.5 mg/L) and antifungal usage over 30 years. Notably, two patients from whom fluconazole-resistant *C. albicans* were isolated had only ketoconazole and miconazole as prior treatments. However, the main conclusion regarding emerging fluconazole resistance was that it correlated more strongly with the amount of fluconazole used rather than the duration of its use. In a follow-up study, 43 *C. albicans* isolates (18 fluconazole dose-dependent isolates: 16 ≤ MIC ≤ 32 and 25 fluconazole-susceptible: MIC ≤ 8) were investigated, and antifungal usage prior to isolating the strains was obtained from patients' records.[101] Significantly longer azole usage was associated with the isolation of fluconazole-resistant strains, particularly for ketoconazole ($P = 0.0241$) and miconazole ($P = 0.0012$). Conversely, Blanco and van Rossem[102] observed no increase in miconazole resistance (arbitrary breakpoint MIC of 2 mg/L) in *Candida* spp. following single or repeated treatment courses of 0.25% miconazole–nitrate ointment. In this study, 158/200 patients completed the initial treatment phase that investigated miconazole susceptibility in isolates from patients together with clinical outcomes. Hamza *et al.*[74] reported a decreased susceptibility to miconazole in *C. albicans* isolates from patients who had previously received azole treatment (likely fluconazole, based on the narrative). Additionally, the authors observed that prior azole treatment correlated with recurrent oropharyngeal candidiasis. Isham and Ghannoum[103] reported that miconazole MIC increased between *Candida* isolates that were susceptible (0.12 mg/L) and resistant (0.5 mg/L) to fluconazole. The effect of repeated exposure to miconazole in *Candida* spp. was investigated *in vitro* by Ghannoum *et al.*[104] After 15 passages in different concentrations of miconazole ranging from 0.5 to 4 times the MIC, 2/6 strains showed a 4- to 5-fold increase in MIC, although the adjusted MIC remained low at 0.5 mg/L.

Overall, these studies, with the exception of Rautemaa *et al.*[101], showed that *Candida* spp. exposure to miconazole, *in vitro* or *in situ*, was not associated with a decrease in *Candida* spp. susceptibility to miconazole. Rautemaa *et al.*[101] raised the possibility of miconazole treatment being associated with fluconazole resistance *in situ*, based on the correlation between fluconazole-resistant isolates and prior antifungal treatment in 23 patients (43 isolates) suffering from autoimmune

Table 2. *Candida* spp. biofilm resistance to miconazole

Biofilms					
Isolates	MIC (mg/L)	Method	Assay	Concentration (mg/L) resulting in 50% reduction of biofilm metabolic activity	References
C. albicans	MYA-2732[a]: 1	48 h sedimentation biofilm on acrylic disks	CLSI microdilution method (M27-A3) and biofilm metabolic assay	MYA-2732[a]: 96	Gebremedhin *et al.*[96]
	Isolate[b]: 0.5			Isolate[b]: 50	Gebremedhin *et al.*[96]
C. glabrata	MYA-275[a]: 0.25			MYA-275[a]: 50	Gebremedhin *et al.*[96]
	Isolate[b]: 0.5			Isolate[b]: 50	Gebremedhin *et al.*[96]
C. tropicalis	Isolate[b]: 0.016			Isolate[b]: 50	Gebremedhin *et al.*[96]
C. parapsilosis	Isolate[b]: 2			Isolate[b]: >96	
Dual species *Candida* and *Staphylococcus aureus*	—	24 h sedimentation biofilm	24 h treatment with miconazole range 320 to 0.63 mg/L	40	Kean *et al.*[97]
Isolates	**MIC (mg/L)**	**Method**	**Biofilm susceptibility**		**References**
C. albicans	0.25	Constant depth film fermenter biofilms on acrylic disks	MIC >256 mg/L (>1024 increase in MIC)	24 h treatment with 256 mg/L miconazole of 2 and 6 h biofilm: 99.2% and 99.9% reduction in viability	Gebremedhin *et al.*[96]
Complex biofilms from patient isolates include bacteria and *C. albicans, C. kefyr, C. tropicalis* and *C. famata*	—	Constant depth film fermenter biofilms on acrylic disks in mucin-containing artificial saliva	*Candida* spp grew in 256 mg/L miconazole	Microcosm denture plaque treated by pulsing in miconazole gel solution (48 mg/L) for 168 h: 2 $\log_{10}$ reduction in *Candida* spp. until after 120 h then growth resumed	Lamfon *et al.*[98]

[a]Standard strain.
[b]Clinical isolate.

polyendocrinopathy candidiasis ectodermal dystrophy syndrome. However, their previous study[100] is not mentioned, despite the conclusion being pertinent: the use of low concentrations of fluconazole in multiple courses annually was identified as the major risk factor for persistent colonization with fluconazole-resistant *C. albicans*.

Evidence of the impact of ketoconazole on emerging antifungal resistance

Thirty-eight papers on ketoconazole were retained. The majority of studies[61,76,82,83,86,105–117] investigated *Candida* spp., with *C. albicans* being the most isolated fungus (Table 3). Oral isolates (124) from Chinese patients suffering from neck and throat cancer presented a large yeast diversity, with species such as *Kodamaea ohmeri*, *Hanseniaspora opuntiae*, *Pichia terricola*, *Trichosporon asahii* and *Pichia norvegensis*, in addition to *Candida* spp. These non-*Candida* spp. were found not to be resistant to ketoconazole, although ketoconazole breakpoints were arbitrary in this study.[116] A much larger study investigated 589 yeasts from oral swabs from patients living with HIV and healthy patients; 554 *Candida* spp. and 35 non-*Candida* yeasts, including *Saccharomyces cerevisiae* (Ketoconazole MIC range: 0.03–0.25 mg/L; MIC50: 0.12 mg/L; MIC90: 0.25 mg/L), *Rhodotorula* spp. (Ketoconazole MIC range: 0.25–1 mg/L; MIC50: 0.25 mg/L), *Geotrichum* spp. (Ketoconazole MIC range: 0.5–1 mg/L; MIC50: 1 mg/L), *Hansenula anomala* (Ketoconazole MIC range: 0.06–0.25 mg/L; MIC50: 0.12 mg/L), *Cryptococcus luteolus* (Ketoconazole 4 mg/L), and *Cryptococcus albidus* (Ketoconazole MIC: 0.06 mg/L).[82] The ketoconazole MIC range of *C. albicans* isolates from the oral cavity of patients with HIV from different years and reported in various studies is remarkably conserved (0.007–0.5 mg/L). Higher ketoconazole MICs were obtained in isolates from patients with cancer or diabetes, or from predominantly blood cultures (Table 3).[76,105,107] There has been concern that in immunocompromised and immunosuppressed patients, antifungal treatments—particularly fluconazole—shift the species

Table 3. *Candida* spp investigated for their susceptibility to ketoconazole

Fungal isolates	Number of isolates	Origin	MIC (mg/L)	MIC_{50}	MIC_{90}	Reference
C. albicans	664	Skin isolates	—	—	—	Bilal *et al.*[82]
	88	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	100	Oral (diabetic patients)	<0.02–>16[a]	0.06–>16[a]	0.5–>16[a]	Bremenkamp *et al.*[105]
	49	Oral (Cancer patients)	0.015–≥16	—	—	Davies *et al.*[107]
	466	Oral (HIV)	0.007–0.5	0.07	0.03	Blignaut *et al.*[83]
	177	Oral candidiasis (diabetic patients)	—	—	—	Manfredi *et al.*[86]
	137	Oral (HIV)	—	—	—	Magaldi *et al.*[110]
	85	Oral (HIV)	0.03–0.25	0.03	0.06	Milan *et al.*[111]
	29	Skin (psoriasis)	0.03–0.25[b]	—	—	de Aguiar Cordeiro *et al.*[90]
	49	Oral (HIV)	—	—	—	Khan *et al.*[113]
	90	Oral (HIV)	0.03–0.5	—	—	Chunchanur *et al.*[114]
	170	Various specimens	—	—	—	Wu *et al.*[115]
	66	Oral (cancer)	—	—	—	Wu *et al.*[116]
	32	Various specimens	<0.008–>16	—	≤ 0.008	Santhanam *et al.*[76]
C. glabrata	18	Skin isolates	—	—	—	Bilal *et al.*[82]
	9	Oral (HIV)	0.25–0.5	0.25	—	Blignaut *et al.*[83]
	6	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	5	Oral (diabetic patients)	< 0.02–2[a]	< 0.02–0.5[a]	0.25–2[a]	Bremenkamp *et al.*[105]
	20	Oral (HIV)	—	—	—	Magaldi *et al.*[110]
	10	Oral (HIV)	0.125–1	0.25	0.5	Chunchanur *et al.*[114]
	68	Various specimens	—	—	—	Wu *et al.*[115]
	17	Various specimens	<0.008–4	—	1	Santhanam *et al.*[76]
C. tropicalis	3	Skin isolates	—	—	—	Bilal *et al.*[82]
	9	Oral (HIV)	0.007–0.03	0.03	—	Blignaut *et al.*[83]
	5	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	5	Oral (diabetic patients)	0.02–2[a]	0.02–2[a]	0.5–2[a]	Bremenkamp *et al.*[105]
	26	Oral (HIV)	—	—	—	Magaldi *et al.*[110]
	4	Oral (HIV)	—	—	—	Khan *et al.*[113]
	39	Various specimens	—	—	—	Wu *et al.*[115]
	28	Oral (cancer)	—	—	—	Wu *et al.*[116]
	15	Various specimens	<0.008–8	—	1	Santhanam *et al.*[76]
C. parapsilosis	1	Skin isolates	—	—	—	Bilal *et al.*[82]
	9	Oral (HIV)	0.015–0.25	0.03	—	Blignaut *et al.*[83]
	1	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	3	Oral (diabetic patients)	< 0.02–4[a]	< 0.02–4[a]	< 0.02–4[a]	Bremenkamp *et al.*[105]
	1	Oral (Cancer patients)	0.13	—	—	Davies *et al.*[107]
	10	Oral (HIV)	—	—	—	Magaldi *et al.*[110]
	6	Oral (HIV)	—	—	—	Khan *et al.*[113]
	3	Various specimens	—	—	—	Wu *et al.*[115]
	6	Oral (cancer)	—	—	—	Wu *et al.*[116]
	13	Various specimens	0.015–0.5	—	0.25	Santhanam *et al.*[76]
C. krusei	8	Skin isolates	—	—	—	Bilal *et al.*[82]
	38	Oral (HIV)	0.12–2	1	1	Blignaut *et al.*[83]
	3	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	2	Oral (diabetic patients)	0.25–0.5	0.25	0.5	Bremenkamp *et al.*[105]
	2	Oral (Cancer patients)	0.25	—	—	Davies *et al.*[107]
	2	Oral (HIV)	—	—	—	Magaldi *et al.*[110]
	7	Oral (HIV)	0.03–2	1	1	Milan *et al.*[111]
	4	Oral (HIV)	—	—	—	Khan *et al.*[113]
	10	Various specimens	—	—	—	Wu *et al.*[115]
	8	Oral (cancer)	—	—	—	Wu *et al.*[116]
C. rugosa	2	Oral (HIV)	0.03	—	—	Blignaut *et al.*[83]

Continued

Table 3. *Continued*

Fungal isolates	Number of isolates	Origin	MIC (mg/L)	MIC_{50}	MIC_{90}	Reference
C. kefyr	1	Oral (HIV)	0.03	—	—	Blignaut *et al.*[83]
	2	Oral candidiasis	—	—	—	Chavanet *et al.*[61]
	1	Oral (diabetic patients)	0.02	0.02	0.02	Bremenkamp *et al.*[105]
C. lusitaniae	8	Oral (HIV)	0.03–0.06	0.03	—	Blignaut *et al.*[83]
C. guilliermondii	2	Oral (HIV)	0.06–0.25	0.06	—	Blignaut *et al.*[83]
	13	Oral (HIV)	—	—	—	Khan *et al.*[113]
C. dubliniensis	10	Oral (HIV)	0.015–0.06	0.015	0.06	Blignaut *et al.*[83]
	36	Oral (HIV and non-HIV, vaginal)	≤ 0.03–>16	≤ 0.03	4	Quindós *et al.*[112]
	22	Oral (HIV)	0.06–16			Chunchanur *et al.*[114]
	4	Oral (HIV)	—	—	—	Khan *et al.*[113]
	22	Oral (HIV)	0.03–0.5	—	—	Chunchanur *et al.*[114]
	4	Various specimens	—	—	—	Wu *et al.*[115]
	2	Oral (diabetic patients)	< 0.02–0.25[a]	< 0.02–0.25[a]	< 0.02–0.2[a]	Bremenkamp *et al.*[105]
C. intermedia	2	Various specimens	—	—	—	Wu *et al.*[115]
C. stellatoidea	20	Oral (diabetic patients)	< 0.02–0.16[a]	< 0.02–0.25[a]	0.25–16[a]	Bremenkamp *et al.*[105]
C. metapsilosis	1	Various specimens	—	—	—	Wu *et al.*[115]
	2	Oral (cancer)	—	—	—	Wu *et al.*[116]

—: No data provided.
[a]All *Candida* grouped together from diabetic group 1, diabetic group 2, control 1 and control 2.
[b]MIC range in healthy patients: 0.03–0.06 mg/L.

distribution to non-*albicans* isolates, which are less susceptible to azoles.

In this review, when efficacy data were collated, non-*C. albicans* isolates did not generally display increased MICs to ketoconazole compared to *C. albicans*, based on MIC range data obtained from several studies (Table 3).[76,83,105,107,111,112,114] However, oral isolates of *C. dubliniensis* from patients with HIV and from patients with cancer presented the highest ketoconazole MIC (>16 mg/L).[112,114] This highlights the limitation of MIC measurements in relation to clinical findings. For example, Milan *et al.*[111] reported that 18/21 isolates identified as azole-susceptible dose-dependent (SDD)/resistant belonged to non-*albicans* species.

While some studies have based ketoconazole breakpoints on CLSI (NCCLS) standards or antifungal protocols (ATB Fungus system, bioMérieux),[61,82,105,108,110] others have relied on published literature (Table 4).[76,111,114,116] Yet, latest 2017 edition of the CLSI M27, CLSI M38 standards or EUCAST do not provide breakpoints for ketoconazole.[118–120] For yeast, the CLSI M27 refers to the CLSI document M59 titled 'Epidemiological Cut-off values for Antifungal Susceptibility Testing'. That document does not provide data on epidemiological cut-off values for ketoconazole and *Candida* spp. or moulds.[121] Furthermore, the CLSI M38 standard states 'data are not available to confirm a correlation between MIC and treatment outcome with ketoconazole'.[67]

Two studies did not provide either MIC or breakpoint information,[86,113] although Manfredi *et al.*[86] used a qualitative test rather than an established CLSI (NCCLS) standard (Table 4). Resistance breakpoints, when provided, varied greatly between studies. Perhaps not surprisingly, when the resistance breakpoint was lower, the percentage of ketoconazole-resistant isolates was higher (Table 4), with the exception of Bremenkamp *et al.*[105], who reported very high (47.1%) ketoconazole resistance in isolates from diabetic type 1 patients (but not in diabetic type 2 patients; only 6.2% resistance).

One study reported that prior fluconazole treatment resulted in a higher number of *Candida* spp. isolates resistant to ketoconazole (4.9% versus 11.9% resistance), although the number of isolates was small and the standard used to calculate breakpoints was not defined.[110] Clinical resistance in two *C. albicans* isolates from patients who received extensive azole therapy correlated with high MICs to fluconazole (>100 mg/L) and ketoconazole (>50 mg/L).[117] Of note, co-infection may also impact the relevance of *in vitro* MIC data.[106] This is particularly relevant, as the isolation of several yeast species from a patient is common (Table 3). A cross-resistance correlation between azoles was provided in one study, which analysed 102 *Candida* spp. MICs to azoles.[111] The authors reported great agreement between itraconazole and ketoconazole MICs (kappa coefficient: 0.85), a moderate agreement between fluconazole and itraconazole MICs (kappa coefficient: 0.49), and between fluconazole and ketoconazole MICs (kappa coefficient: 0.50), indicating cross-resistance potential when these azoles are used sequentially.[111]

Metzger and Hofmann[122] reported a high number of fluconazole-resistant *C. albicans* isolates that were also resistant to itraconazole and ketoconazole. Wu *et al.*[115] also reported on azole cross-resistance in non-*C. albicans* species, with 14.3% of isolates resistant to two or more antifungals, notably *C. glabrata* and *C. krusei*, which displayed the highest frequency of antifungal resistance. In their study, there was no indication of prior azole usage in patients at least 3 months before sampling, and they

Table 4. Pathogenic yeasts reported resistance to ketoconazole

Yeasts	Breakpoint used MIC (mg/L)	Literature reference for breakpoint calculation	Resistance to ketoconazole (% of isolates)	Reference
Candida spp.	S: ≤8; R: ≥16[a]	CLSI M27-A2 2002	47.2% (diabetic type 1patient group 1; 63 isolates) versus 2.8% (control; 76 isolates) 6.2% (diabetic patient type 2 group 1; 76 isolates) versus 3% (control; 66 isolates)	Bremenkamp *et al.*[105]
	Not provided	CLSI M60 or epidemiological cut-off values guidelines	87.75% susceptible, 9.9% intermediate, 2.45% resistant (490 isolates over 10 years)	Bilal *et al.*[82]
	S: ≤1; I: 2–8; R≥16	PHLS' tentative interpretative guidelines	7% resistant; 19% intermediate (82 isolates)	Davies *et al.*[107]
	Not provided	ATB Fungus system	100% susceptible (106 isolates)	Chavanet *et al.*[61]
	S: ≤0.125; R: ≥1; SDD: 0.25–0.5	Literature	13% (106 isolates)	Milan *et al.*[111]
	Not provided	Not provided	13.5% isolates from patients with HIV versus 7.5% isolated from control	Khan *et al.*[113]
C. albicans	Not provided	CLSI M44-A2	28% (50 isolates)	Tamai *et al.*[108]
	Not provided	Not provided	15% (229 isolates)	Manfredi *et al.*[86]
	S: ≤1; I: 1–4; R≥4	Literature	10%[b]	Chunchanur *et al.*[114]
	Not provided	NCCLS[c]	4.9% (2 isolates with no prior treatment).; 11.9% (8 isolates with prior fluconazole treatment) and 10.5% SDD	Magaldi *et al.*[110]
Non-*albicans*	Not provided	Not provided	23% (229 isolates)	Manfredi *et al.*[86]
C. glabrata	Not provided	NCCLS[c]	70% (7 isolates); 10% SDD	Magaldi *et al.*[110]
C. dublieniensis	R: ≥1	Not provided	24.1%	Quindós *et al.*[112]
	S: ≤1; I: 1–4; R≥4	Literature	18.2% (22 isolates)	Chunchanur *et al.*[114]
Candida spp. and other yeasts	Not provided	Literature	9.7% (124 isolates)	Wu *et al.*[116]
	R>0.125	Literature	20.9% (82 isolates)	Santhanam *et al.*[76]

PHLS, Public Health England; SDD, susceptibility dose dependent (see text).
[a]S: susceptible; I: intermediate; R: resistant.
[b]Data not provided.
[c]Exact stand not mentioned.

theorised that resistance could have originated from the use of antifungals in agriculture, the increased use of antifungals in hospitals, or antifungal-resistant yeasts from other parts of China.[115] In patients with HIV, several factors affected the presence of azole-resistant *Candida* spp., including prior use of azoles (fluconazole and itraconazole), co-infections, and a low number of circulating CD4+ T-cells.[109]

Non-*Candida* spp. have also been investigated. Two publications reported on *Malassezia* spp., which are commonly associated with skin infections.[90,123] Skin isolates from patients with *Pityriasis versicolor* and healthy patients included *M. furfur*, *M. sympodialis* and *M. globosa*, all of which were susceptible to ketoconazole, although there were antifungal susceptibility (MIC) differences between species.[123] In another study, higher MIC values for ketoconazole were observed in *Malassezia* strains obtained from psoriasis patients (MIC range: 0.06–2 mg/L) compared to healthy patients (MIC range: 0.03–1 mg/L).[90]

Seven studies concerned moulds, with *Trichophyton* spp. being the most studied (Table 5).[91,92,124–128] Gupta and Kohli[124] reported that the ketoconazole MIC of *T. rubrum* did not correlate with clinical resistance or susceptibility. Additionally, ketoconazole MICs were not necessarily affected by prior treatments with itraconazole or terbinafine. This, however, was not the finding of de Assis Santos *et al.*[91], who reported an increase in *T. rubrum* MIC beyond the MIC_{90} (from 0.5 to >0.91 mg/L) in isolates from patients who had received prior treatment (not disclosed in the paper), indicative of potential treatment failure. A concentration of 0.91 mg/L has been deemed achievable in the cutaneous layer after taking 200 mg orally of ketoconazole.[126] Shaw *et al.*[127] and Kong *et al.*[128] reported good susceptibility of *T. mentagrophytes* complex isolates to ketoconazole (Table 5), but elevated MIC for terbinafine in isolates associated with mutations in the squalene epoxidase gene.[127,128] Maurya *et al.*[125] indicated that the MIC_{90} for 75 dermatophyte isolates was generally higher against fluconazole and

Table 5. Moulds investigated for their susceptibility to ketoconazole

Fungal isolates	Number of isolates	Susceptibility/resistance to ketoconazole	MIC (mg/L)	MIC_{50}	MIC_{90}	References
Trichophyton spp.						
T. rubrum	18	MIC not reflective of clinical outcome	0.06–32	0.06	—	Gupta and Kohli[124]
	40	MIC >0.91 mg/L associated with prior treatment	—	0.25	0.5	de Assis Santos *et al.*[91]
	32	Concentration in the range to eliminate *T. rubrum*	05–2	—	—	Korting *et al.*[126]
	13	All isolates susceptible (Breakpoint value used R >1 mg/L)[a]	0.03–0.125	0.06	0.06	Maurya *et al.*[125]
T. mentagrophytes	16	Concentration in the range to eliminate *T. mentagrophytes*	05–2	—	—	Korting *et al.*[126]
	31	All isolates susceptible (Breakpoint value used R >1 mg/L)	0.03–0.25	0.06	0.125	Maurya *et al.*[125]
	498	Concentration in the range to eliminate *T. mentagrophytes*	0.0078–2	0.125	0.5	Shaw *et al.*[127]
	35		0.016–2	0.25	1	Kong *et al.*[128]
T. interdigitale	36	Concentration in the range to eliminate *T. interdigitale*	0.125–2	0.25	1	Kong *et al.*[128]
T. indotineae	64	Concentration in the range to eliminate *T. indotineae*	0.063–4	0.5	1	Kong *et al.*[128]
T. tonsurans	12	All isolates susceptible (Breakpoint value used *R*	0.03–0.06	0.03	0.06	Maurya *et al.*[125]
T. verrucosum	9	>1 mg/L)a	0.06–0.5	0.125	0.5	Maurya *et al.*[125]
T. violaceum	1		0.25	0.25	0.25	Maurya *et al.*[125]
Others						
Neoscytalidium dimidiatum	29	Ketoconazole inactive	16	—	—	James *et al.*[92]
Microsporum gypseum	5	All isolates susceptible (Breakpoint value used *R*	0.03–0.125	0.06	0.125	Maurya *et al.*[125]
Epidermophyton floccosum	4	>1 mg/L)a	0.03–0.25	0.06	0.125	Maurya *et al.*[125]

—: No data provided.
[a]Use of arbitrary breakpoints to define susceptibility/resistance.

terbinafine than ketoconazole (Table 5). Despite the high MIC_{90} reported in some publications, clinical treatment with ketoconazole (200 to 400 mg daily) was effective against griseofulvin-clinically resistant *T. rubrum*, resulting in complete mycologic cure in 6 patients, although onychomycosis persisted in some patients, and in 4/6 patients, *T. rubrum* infection recurred despite achieving early clearing of skin and nails.[129]

Itraconazole appears to be the most commonly prescribed azole for treating *T. indotineae* infections.[94] Systemic ketoconazole (200 mg twice daily for 6 weeks), used without a topical agent, led to some improvement in a patient but did not result in complete healing.[94] Several successful combination therapies have however been reported. For example, a patient infected with a terbinafine-resistant *T. indotineae* strain was successfully treated with oral itraconazole (200 mg/day for 1 week) combined with topical ketoconazole for 2 weeks.[130] In another case, the combination of topical ketoconazole with oral terbinafine (250 mg/day) for 4 months resulted in complete healing.[131] However, topical ketoconazole alone, applied for 3 months, failed to control the infection in a different patient.[131] Similarly, oral ketoconazole (1200 mg/day in divided doses every 12 h) was ineffective against a terbinafine-resistant *T. indotineae* isolate.[132] These two studies[131,132] did not assess the phenotypic susceptibility of the *T. indotineae* isolates to ketoconazole following the treatment period.

The remaining 10 papers retained provide information on mechanisms of fungal resistance to ketoconazole.[133–142] Efflux is a primary mechanism of resistance to antifungals (Figure 1) and has been implicated in ketoconazole resistance and cross-resistance between fluconazole and ketoconazole.[135,140–142] Importantly, ketoconazole has been reported to increase efflux pump expression levels in *Candida* spp.,[135,138] and also in moulds (*T. rubrum*).[133] Ketoconazole acts as a stressor, resulting in a positive transcriptional response that leads to an up-regulation of efflux.[139] Increased copy numbers of efflux genes (tandem repeats) (e.g. ERG11) have also been reported in clinically isolated ketoconazole-resistant strains.[136,137]

Impact of miconazole and ketoconazole in cross-resistance to antifungals

One of the key questions this review aimed to answer was the impact of miconazole or ketoconazole on fungal resistance and cross-resistance to other azoles. There is no doubt that fungi

Table 6. Reported multiple resistance to azoles based on ketoconazole and miconazole literature

Isolates	Multiple resistance[a]	Prior antifungal use	Susceptibility of isolates	Reference
C. glabrata, C krusei	Fluconazole, ketoconazole, itraconazole	Extensive period of azole (not defined) treatment	—	Cartledge *et al.*[106]
Candida spp.	Fluconazole, 5-fluorocytosine	Fluconazole	Isolates susceptible to miconazole or ketoconazole	Chavanet *et al.*[61]
Malassezia spp.	Fluconazole, itraconazole, amphotericin B	—	Isolates susceptible to ketoconazole and posaconazole	Chebil *et al.*[123]
Candida spp.	Fluconazole and itraconazole or ketoconazole	—	—	Davies *et al.*[107]
Candida spp.	Fluconazole, itraconazole	Fluconazole, clotrimazole, nystatin, miconazole and ketoconazole	—	Hamza *et al.*[74]
Candida spp.	Fluconazole, itraconazole, ketoconazole	Not significant—same results with patients with prior exposure and patients with no prior exposure	—	Kuriyama *et al.*[72]
C. dubliniensis	Fluconazole, econazole, voriconazole, fluorocytosine	—	All non-*albicans* susceptible to miconazole	Lomeli-Martinez *et al.*[85]
Candida spp.	Fluconazole, itraconazole	Fluconazole	—	Magaldi *et al.*[110]
C. albicans	Fluconazole and itraconazole or ketoconazole or miconazole	No prior treatment 6 months before sampling	—	Manfredi *et al.*[86]
Trichophyton spp.	Fluconazole, itraconazole	Fluconazole, terbinafine (not explicit in the text)	Isolates susceptible to ketoconazole	Maurya *et al.*[125]
C. albicans	Fluconazole, itraconazole, ketoconazole	Fluconazole	—	Metzger and Hofmann[122]
C. glabrata and *C. parapsilosis.*	Fluconazole, itraconazole	—	One isolate intermediate to miconazole and 2 susceptible	Espinel-Ingroff *et al.*[69]
C. parapsilosis.	Miconazole and itraconazole	—	1 isolate	Miranda-Cadena *et al.*[71]
C. dubliniensis	Fluconazole and itraconazole, or ketoconazole	—	—	Quindós *et al.*[112]
Candida spp.	Fluconazole, ketoconazole, posaconazole, itraconazole	Heavy use of itraconazole	—	Santhanam *et al.*[76]
C. dubliniensis	Fluconazole, ketoconazole	—	—	Chunchanur *et al.*[114]
Candida spp.	Fluconazole, itraconazole,	Prior treatment with fluconazole	—	Tumbarello *et al.*[109]
C. albicans	Fluconazole, miconazole, clortimazole	—	—	Waltimo *et al.*[89]
Candida spp.	Fluconazole, itraconazole, ketoconazole, fluorocytosine	No prior treatment 3 months before sampling	—	Wu *et al.*[116]
Candida spp.	Fluconazole, itraconazole, ketoconazole, fluorocytosine	No prior treatment 3 months before sampling	—	Wu *et al.*[115]

[a]Classified as resistant or susceptible dose dependent.

have the ability to adapt to the stress imparted by antifungal treatment. In addition, some mechanisms of fungal resistance can lead to cross-resistance (Figure 1). Prior azole treatments, especially fluconazole, have been associated with emerging azole resistance, particularly in *Candida* spp.[61,75,117,143–147] In the literature on miconazole and ketoconazole retained for this review, several studies observed cross-resistance between azoles (Table 6).

Whilst some publications mentioned that no azole agent had been used during the 3–6 month period prior to sample collection,[86,116] several studies noted that fluconazole had been used (Table 6).[61,74,109,110,122,125] As reported above, one study reported increased MIC to miconazole in *C. albicans* isolated from patients who had received prior azole treatment (fluconazole is inferred but not explicitly mentioned).[74] High MIC correlations between itraconazole and ketoconazole or fluconazole and

ketoconazole,[111] or miconazole and fluconazole,[103] have also been reported.

Conclusion

The literature on the development of fungal resistance to miconazole and ketoconazole is limited, with most studies focusing on the MIC determination of patient isolates alongside other azoles (Tables 1 and 3). Notably, miconazole and ketoconazole were not the primary azoles investigated, with a greater emphasis placed on fluconazole and itraconazole, as well as other antifungals such as amphotericin B, nystatin, and fluorocytosine. Despite their long history of clinical use, very few studies have investigated the mechanisms of fungal resistance to these two imidazoles. Moreover, none of the studies on ketoconazole examined the impact of biofilms on antifungal efficacy, and none of the miconazole studies explored resistance mechanisms.

This review set out to explore miconazole and ketoconazole when used clinically as topical or oral antifungals. However, only a few papers specifically mentioned the type of antifungal product used, with most publications focusing on *in vitro* antifungal testing (Tables 1 and 3).

As noted in previous reviews, the correlation between *in vitro* MIC values and clinical resistance remains problematic.[34,70] Clinical resistance refers to the failure of an antifungal agent to eradicate the pathogen in a patient, and laboratory MIC values and breakpoints do not always reflect the response to treatment in clinical practice. The measure of MIC itself is not always a reliable indicator of treatment efficacy, and the use of MIC_{90} is preferred by some authors, particularly for *Candida* spp. and yeast infections. For moulds, MIC determination may not correlate with antifungal efficacy, even if initial clearing is observed. The lack of reliable breakpoints for miconazole and ketoconazole in some fungi has led to the use of arbitrary values, potentially explaining the variability in the reported percentage of ketoconazole-resistant isolates across studies (Table 4).

The impact of miconazole and fluconazole on emerging fungal resistance remains unclear based on the current literature. One key factor influencing resistance development is the clinical use of sub-optimal azole concentrations.[146,148] Interestingly, repeated exposure of *Candida* spp. to miconazole (0.5–4×MIC) *in vitro* resulted in an increased MIC in some strains, though the MIC remained at 0.5 mg/L in others,[104] indicating a disconnect between *in vitro* findings and clinical outcomes.

The use of fluconazole has been linked to the selection of non-*C. albicans* species.[61,106,149] Recurring fungal infections after multiple antifungal treatments have been observed,[74,109] sometimes associated with a shift to non-*C. albicans* species,[106,149] though this is not always the case.[74] In this review, when compiling information on miconazole and ketoconazole, the MIC ranges were similar between *C. albicans* and non-*C. albicans* species (Tables 2 and 3).

Miconazole[17] and ketoconazole remain important clinical agents for the treatment of fungal infections (https://clincalc.com/DrugStats/Drugs/Ketoconazole). Based on the limited available literature, there is no evidence to suggest that these imidazoles, which have been in clinical use for many years, have led to the emergence of clinically resistant fungi. However, more benefit/risk analyses are needed, as factors beyond the clinical use of antifungals (Figure 2) may have a greater impact on the development of resistance.

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Transparency declarations

Jean-Yves Maillard is the Director of Biocide Consult Ltd. Kenvue Brands LLC has not been involved in any decision making, execution, analysis or reporting of the review. AI has not been used for the writing of this manuscript.

Authors contribution

Conducted the search for, read and analysed, all articles, wrote the review, design the tables and figures, and edited the manuscript: J.-Y.M.

Data availability

All data used in this review are provided in the tables.

References

1 Brown GD, Denning DW, Gow NAR *et al*. Hidden killers: human fungal infections. *Sci Transl Med* 2012; **4**: 165rv13. https://doi.org/10.1126/scitranslmed.3004404

2 Denning DW. Minimizing fungal disease deaths will allow the UNAIDS target of reducing annual AIDS deaths below 500 000 by 2020 to be realized. *Philos Trans R Soc Lond B Biol Sci* 2016; **371**: 20150468. https://doi.org/10.1098/rstb.2015.0468

3 Marr KA, Carter RA, Boeckh M *et al*. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* 2002; **100**: 4358–66. https://doi.org/10.1182/blood-2002-05-1496

4 Guinea J, Torres-Narbona M, Gijón P *et al*. Pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: incidence, risk factors, and outcome. *Clin Microbiol Infect* 2010; **16**: 870–7. https://doi.org/10.1111/j.1469-0691.2009.03015.x

5 Bongomin F, Gago S, Oladele R *et al*. Global and multi-national prevalence of fungal diseases-estimate precision. *J Fungi (Basel)* 2017; **3**: 57. https://doi.org/10.3390/jof3040057

6 Denning DW. Global incidence and mortality of severe fungal disease. *Lancet Infect Dis* 2024; **24**: e428–38. https://doi.org/10.1016/S1473-3099(23)00692-8

7 Deepa AG, Nair BJ, Sivakumar TT *et al*. Uncommon opportunistic fungal infections of oral cavity: a review. *J Oral Maxillofac Pathol* 2014; **18**: 235–43. https://doi.org/10.4103/0973-029X.140765

8 Leung AK, Lam JM, Leong KF *et al*. Tinea corporis: an updated review. *Drugs Context* 2020; **9**: 2020–5-6. https://doi.org/10.7573/dic.2020-5-6

9 Sahoo AK, Mahajan R. Management of tinea corporis, tinea cruris, and tinea pedis: a comprehensive review. *Indian Dermatol Online J* 2016; **7**: 77–86. https://doi.org/10.4103/2229-5178.178099

10 Kano R, Kimura U, Kakurai M *et al*. *Trichophyton indotineae* sp.: a new highly terbinafine-resistant anthropophilic dermatophyte species. *Mycopathologia* 2020; **185**: 947–58. https://doi.org/10.1007/s11046-020-00455-8

11 Chowdhary A, Singh A, Kaur A *et al*. The emergence and worldwide spread of the species *Trichophyton indotineae* causing difficult-to-treat dermatophytosis: a new challenge in the management of dermatophytosis. *PLoS Pathog* 2022; **18**: e1010795. https://doi.org/10.1371/journal.ppat.1010795

12 Gupta AK, Venkataraman M, Hall DC *et al*. The emergence of Trichophyton indotineae: implications for clinical practice. *Int J Dermatol* 2023; **62**: 857–61. https://doi.org/10.1111/ijd.16362

13 Jabet A, Normand A-C, Brun S *et al*. *Trichophyton indotineae*, from epidemiology to therapeutic. *Med Mycol* 2023; **33**: 101383. https://doi.org/10.1016/j.mycmed.2023.101383

14 Burmester A, Hipler U-C, Uhrlaß S *et al*. Indian *Trichophyton mentagrophytes* squalene epoxidase *erg1* double mutants show high proportion of combined fluconazole and terbinafine resistance. *Mycoses* 2020; **63**: 1175–80. https://doi.org/10.1111/myc.13150

15 Yamada T, Maeda M, Nagai H *et al*. Two different types of tandem sequences mediate the overexpression of TinCYP51B in azole-resistant *Trichophyton indotineae*. *Antimicrob Agents Chemother* 2023; **67**: e0093323. https://doi.org/10.1128/aac.00933-23

16 Bhattacharyya A, Sadhasivam S, Sinha M *et al*. Treatment recalcitrant cases of tinea corporis/cruris caused by *T. mentagrophytes*—interdigitale complex with mutations in ERG11 ERG 3, ERG4, MDR1 MFS genes & SQLE and their potential implications. *Int J Dermatol* 2023; **62**: 637–48. https://doi.org/10.1111/ijd.16622

17 World Health Organisation (WHO). List of Essential Medicines 2023. (https://iris.who.int/bitstream/handle/10665/371090/WHO-MHP-HPS-EML-2023.02-eng.pdf?sequence=1.

18 Nenoff P, Klonowski E, Uhrlaß S *et al*. Topische und systemische antimykotische behandlung von dermatomykosen. *Dermatologie* 2024; **75**: 655–73. https://doi.org/10.1007/s00105-024-05359-y

19 Weinstein A, Berman B. Topical treatment of common superficial tinea infections. *Am Fam Physician* 2002; **65**: 2095–102.

20 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. https://doi.org/10.1016/j.bioorg.2020.104240

21 Zavrel M, Esquivel BD, White TC. The ins and outs of azole antifungal drug resistance: molecular mechanisms of transport. In: *Handbook of Antimicrobial Resistance*. Springer, 2017; 423–52.

22 Martínez-Beneyto Y, Lopez-Jornet P, Velandrino-Nicolas A *et al*. Use of antifungal agents for oral candidiasis: results of a national survey. *Int J Dent Hygiene* 2010; **8**: 47–52. https://doi.org/10.1111/j.1601-5037.2008.00357.x

23 Bennett N, Walker K, Buising K *et al*. Topical antimicrobial prescribing patterns in residents of Australian aged-care facilities: use of a national point prevalence survey to identify opportunities for quality improvement. *Am J Infect Control* 2021; **49**: 1113–7. https://doi.org/10.1016/j.ajic.2021.03.019

24 Xiao L, Madison V, Chau AS *et al*. Three-dimensional models of wild-type and mutated forms of cytochrome P450 14alpha-sterol demethylases from Aspergillus fumigatus and Candida albicans provide insights into posaconazole binding. *Antimicrob Agents Chemother* 2004; **48**: 568–74. https://doi.org/10.1128/AAC.48.2.568-574.2004

25 Carrillo-Muñoz AJ, Giusiano G, Eziyra PA *et al*. Antifungal agents: mode of action in yeast cells. *Rev Esp Quimioterap* 2006; **19**: 130–9.

26 Thevissen K, Ayscough KR, Aerts AM *et al*. Miconazole induces changes in actin cytoskeleton prior to reactive oxygen species induction in yeast. *J Biol Chem* 2007; **282**: 21592–7. https://doi.org/10.1074/jbc.M608505200

27 Kobayashi D, Kondo K, Uehara N *et al*. Endogenous reactive oxygen species is an important mediator of miconazole antifungal effect. *Antimicrob Agents Chemother* 2002; **46**: 3113–7. https://doi.org/10.1128/AAC.46.10.3113-3117.2002

28 Gourlay CW, Ayscough KR. Identification of an upstream regulatory pathway controlling actin-mediated apoptosis in yeast. *J Cell Sci* 2005; **118**: 2119–32. https://doi.org/10.1242/jcs.02337

29 François I, Cammue B, Borgers M *et al*. A zoles: mode of antifungal action and resistance development. Effect of miconazole on endogenous reactive oxygen species production in *Candida albicans*. *Anti-Infect Agents Med Chem* 2006; **5**: 3–13. https://doi.org/10.2174/187152106774755554

30 Darwazeh AMG, Lamev P-J, Lewis MAO *et al*. Systemic fluconazole therapy and *in vitro* adhesion of *Candida albicans* to human buccal epithelial cells. *J Oral Path Med* 1991; **20**: 17–9. https://doi.org/10.1111/j.1600-0714.1991.tb00881.x

31 Fisher MC, Hawkins NJ, Sanglard D *et al*. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. *Science* 2018; **360**: 739–42. https://doi.org/10.1126/science.aap7999

32 Gupta AK, Venkataraman M. Antifungal resistance in superficial mycoses. *J Dermatol Treat* 2022; **33**: 1888–95. https://doi.org/10.1080/09546634.2021.1942421

33 Dladla M, Gyzenhout M, Marias G *et al*. Azole resistance in *Aspergillus fumigatus*- comprehensive review. *Arch Microbiol* 2024; **206**: 305. https://doi.org/10.1007/s00203-024-04026-z

34 Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2002; **2**: 73–85. https://doi.org/10.1016/S1473-3099(02)00181-0

35 Zhang AY, Camp WL, Elewski BE. Advances in topical and systemic antifungals. *Dermatol Clin* 2007; **25**: 165–83. https://doi.org/10.1016/j.det.2007.01.002

36 Gupta AK, Lyons DCA. The rise and fall of oral ketoconazole. *J Cutaneous Med Surg* 2015; **19**: 352–7. https://doi.org/10.1177/1203475415574970

37 Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: prevalence, mechanisms, and management. *Lancet Infect Dis* 2017; **17**: 383–92. https://doi.org/10.1016/S1473-3099(17)30316-X

38 de Paiva Macedo J, Dias VC. Antifungal resistance: why are we losing this battle? *Future Microbiol* 2024; **19**: 1027–40. https://doi.org/10.1080/17460913.2024.2342150

39 Lee Y, Robbins N, Cowen LE. Molecular mechanisms governing antifungal drug resistance. *NPJ Antimicrob Resist* 2023; **1**: 5. https://doi.org/10.1038/s44259-023-00007-2

40 Kanafani ZA, Perfect JR. Resistance to antifungal agents: mechanisms and clinical impact. *Clin Infect Dis* 2008; **46**: 120–8. https://doi.org/10.1086/524071

41 Nishimoto AT, Sharma C, Rogers PD. Molecular and genetic basis of azole antifungal resistance in the opportunistic pathogenic fungus *Candida albicans*. *J Antimicrob Chemother* 2020; **75**: 257–70. https://doi.org/10.1093/jac/dkz400

42 Todd RT, Soisangwan N, Peters S *et al*. Antifungal drug concentration impacts the spectrum of adaptive mutations in *Candida albicans*. *Mol Biol Evol* 2023; **40**: msad009. https://doi.org/10.1093/molbev/msad009

43 Sanguinetti M, Posteraro B, Fiori B *et al*. Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance. *Antimicrob Agents Chemother* 2005; **49**: 668–79. https://doi.org/10.1128/AAC.49.2.668-679.2005

44 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. https://doi.org/10.1128/mBio.03333-20

45 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. https://doi.org/10.1093/jac/dkt075

46 Tobin MB, Peery RB, Skatrud PL. Genes encoding multiple drug resistance-like proteins in Aspergillus fumigatus and *Aspergillus flavus*. *Gene* 1997; **200**: 11–23. https://doi.org/10.1016/S0378-1119(97)00281-3

47 Del Sorbo G, Andrade AC, Van Nistelrooy JGM *et al*. Multidrug resistance in *Aspergillus nidulans* involves novel ATP-binding cassette transporters. *Mol Gen Genet* 1997; **254**: 417–26. https://doi.org/10.1007/s004380050434

48 Nakaune R, Hamamoto H, Imada J *et al*. A novel ABC transporter gene, PMR5, is involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. *Mol Genet Genomics* 2002; **267**: 179–85. https://doi.org/10.1007/s00438-002-0649-6

49 Gupta AK, Polla Ravi S, Wang T *et al*. Antifungal resistance, susceptibility testing and treatment of recalcitrant dermatophytosis caused by *Trichophyton indotineae*: a north American perspective on management. *Am J Clin Dermatol* 2023; **24**: 927–38. https://doi.org/10.1007/s40257-023-00811-6

50 Burmester A, Hipler U-C, Elsner P *et al*. Point mutations in the squalene epoxidase erg1 and sterol 14-α demethylase erg11 gene of *T indotineae* isolates indicate that the resistant mutant strains evolved independently. *Mycoses* 2022; **65**: 97–102. https://doi.org/10.1111/myc.13393

51 Li X, Cai Q, Mei H *et al*. The Rpd3/Hda1 family of histone deacetylases regulates azole resistance in *Candida albicans*. *J Antimicrob Chemother* 2015; **70**: 1993–2003. https://doi.org/10.1093/jac/dkv070

52 Moirangthem R, Kumar K, Kaur R. Two functionally redundant FK506-binding proteins regulate multidrug resistance gene expression and govern azole antifungal resistance. *Antimicrob Agents Chemother* 2021; **65**: e02415–20. https://doi.org/10.1128/AAC.02415-20

53 Baker KM, Hoda S, Saha D *et al*. The set1 histone H3K4 methyltransferase contributes to azole susceptibility in a species-specific manner by differentially altering the expression of drug efflux pumps and the ergosterol gene pathway. *Antimicrob Agents Chemother* 2022; **66**: e0225021. https://doi.org/10.1128/aac.02250-21

54 Fu C, Beattie SR, Jezewski AJ *et al*. Genetic analysis of Hsp90 function in *Cryptococcus neoformans* highlights key roles in stress tolerance and virulence. *Genetics* 2022; **220**: yab164. https://doi.org/10.1093/genetics/iyab164

55 Cowen LE, Lindquist S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science* 2005; **309**: 2185–9. https://doi.org/10.1126/science.1118370

56 LaFayette SL, Collins C, Zaas AK *et al*. PKC signaling regulates drug resistance of the fungal pathogen *Candida albicans* via circuitry comprised of Mkc1, calcineurin, and Hsp90. *PLoS Pathog* 2010; **6**: e1001069. https://doi.org/10.1371/journal.ppat.1001069

57 Robbins N, Collins C, Morhayim J *et al*. Metabolic control of antifungal drug resistance. *Fungal Genet Biol* 2010; **47**: 81–93. https://doi.org/10.1016/j.fgb.2009.07.004

58 Lee Y, Liston SD, Lee D *et al*. Functional analysis of the *Candida albicans* kinome reveals Hrr25 as a regulator of antifungal susceptibility. *iScience* 2022; **25**: 104432. https://doi.org/10.1016/j.isci.2022.104432

59 Melo NR, Taguchi H, Jorge J *et al*. Oral Candida flora from Brazilian human immunodeficiency virus-infected patients in the highly active antiretroviral therapy era. *Mem Inst Oswaldo Cruz* 2004; **99**: 425–31. https://doi.org/10.1590/S0074-02762004000400014

60 White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev* 1998; **11**: 382–402. https://doi.org/10.1128/CMR.11.2.382

61 Chavanet P, Lopez J, Grappin M *et al*. Cross-sectional study of the susceptibility of *Candida* isolates to antifungal drugs and *in vivo-in vitro* correlation in HIV-infected patients. *AIDS* 1994; **8**: 945–50. https://doi.org/10.1097/00002030-199407000-00011

62 Hajjeh RA, Sofair AN, Harrison LH *et al*. Incidence of bloodstream infections due to *Candida* species and *in vitro* susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. *J Clin Microbiol* 2004; **42**: 1519–27. https://doi.org/10.1128/JCM.42.4.1519-1527.2004

63 Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; **20**: 133–63. https://doi.org/10.1128/CMR.00029-06

64 Assress HA, Selvarajan R, Nyoni H *et al*. Antifungal azoles and azole resistance in the environment: current status and future perspectives—a review. *Rev Environ Sci Biotechnol* 2021; **20**: 1011–41. https://doi.org/10.1007/s11157-021-09594-w

65 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Third Edition: M27–A3*. 2008.

66 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Fourth Informational Supplement: M27–S4*. 2012.

67 NCLS. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidial-Forming Filamentous Fungi: Approved Standard: M38–A*. 2002.

68 Chryssanthou E, Cuenca-Estrella M. Comparison of the EUCASTAFST broth dilution method with the CLSI reference broth dilution method (M38–A) for susceptibility testing of posaconazole and voriconazole against Aspergillus spp. *Clin Microbiol Infect* 2006; **12**: 901–4. https://doi.org/10.1111/j.1469-0691.2006.01419.x

69 Espinel-Ingroff A, Barchiesi F, Cuenca-Estrella M *et al*. International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of Candida spp. to fluconazole, itraconazole, posaconazole, and voriconazole. *J Clin Microbiol* 2005; **43**: 3884–9. https://doi.org/10.1128/JCM.43.8.3884-3889.2005

70 Rex JH, Pfaller MA. Has antifungal susceptibility testing come of age? *Clin Infect Dis* 2002; **35**: 982–9. https://doi.org/10.1086/342384

71 Miranda-Cadena K, Marcos-Arias C, Mateo E *et al*. Prevalence and antifungal susceptibility profiles of *Candida glabrata*, *Candida parapsilosis* and their close-related species in oral candidiasis. *Arch Oral Biol* 2018; **95**: 100–7. https://doi.org/10.1016/j.archoralbio.2018.07.017

72 Kuriyama T, Williams DW, Bagg J *et al*. *In vitro* susceptibility of oral *Candida* to seven antifungal agents. *Oral Microbiol Immunol* 2005; **20**: 349–53. https://doi.org/10.1111/j.1399-302X.2005.00236.x

73 Yu S-Y, Zhang L, Chen S *et al*. *Candida* isolates causing refractory or recurrent oropharyngeal candidiasis in 11 hospitals in China. *Infect Drug Resist* 2019; **12**: 865–75. https://doi.org/10.2147/IDR.S199359

74 Hamza OJM, Matee MIN, Moshi MJ *et al*. Species distribution and *in vitro* antifungal susceptibility of oral yeast isolates from Tanzanian HIV-infected patients with primary and recurrent oropharyngeal candidiasis. *BMC Microbiol* 2008; **8**: 135. https://doi.org/10.1186/1471-2180-8-135

75 Goldman M, Cloud GA, Smedema M *et al*. Does long-term itraconazole prophylaxis result in *in vitro* azole resistance in mucosal *Candida albicans* isolates from persons with advanced human immunodeficiency virus infection? The National Institute of Allergy and Infectious Diseases Mycoses study group. *Antimicrob Agents Chemother* 2000; **44**: 1585–7. https://doi.org/10.1128/AAC.44.6.1585-1587.2000

76 Santhanam J, Yahaya N, Aziz MN. Species distribution and antifungal susceptibility patterns of *Candida* species: is low susceptibility to itraconazole a trend in Malaysia? *Med J Malaysia* 2013; **68**: 343–7.

77 Quereda C, Polanco AM, Giner C *et al*. Correlation between *in vitro* resistance to fluconazole and clinical outcome of oropharyngeal

candidiasis in HIV-infected patients. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 30–7. https://doi.org/10.1007/BF01586182

78 Kamikawa Y, Mori Y, Nagayama T *et al*. Frequency of clinically isolated strains of oral *Candida* species at Kagoshima University Hospital, Japan, and their susceptibility to antifungal drugs in 2006–2007 and 2012–2013. *BMC Oral Health* 2014; **14**: 14. https://doi.org/10.1186/1472-6831-14-14

79 Asmundsdottir LR, Erlendsdottir H, Gottfredsson M. Nationwide study of candidemia, antifungal use, and antifungal drug resistance in Iceland, 2000 to 2011. *J Clin Microbiol* 2013; **51**: 841–8. https://doi.org/10.1128/JCM.02566-12

80 Moretti ML, Trabasso P, Lyra L *et al*. Is the incidence of candidemia caused by *Candida glabrata* increasing in Brazil? Five-year surveillance of *Candida* bloodstream infection in a university reference hospital in southeast Brazil. *Med Mycol* 2013; **51**: 225–30. https://doi.org/10.3109/13693786.2012.708107

81 Aslani N, Abastabar M, Hedayati MT *et al*. Molecular identification and antifungal susceptibility testing of *Candida* species isolated from dental plaques. *J Mycol Med* 2018; **28**: 433–6. https://doi.org/10.1016/j.mycmed.2018.05.006

82 Bilal H, Hou B, Shafiq M *et al*. Antifungal susceptibility pattern of Candida isolated from cutaneous candidiasis patients in eastern Guangdong region: a retrospective study of the past 10 years. *Front Microbiol* 2022; **13**: 981181. https://doi.org/10.3389/fmicb.2022.981181

83 Blignaut E, Messer S, Hollis RJ *et al*. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. *Diagn Microbiol Infect Dis* 2002; **44**: 169–74. https://doi.org/10.1016/S0732-8893(02)00440-6

84 De-la-Torre J, Ortiz-Samperio ME, Marcos-Arias C *et al*. *In vitro* antifungal susceptibility of oral *Candida* isolates from patients suffering from caries and chronic periodontitis. *Mycopathologia* 2017; **182**: 471–85. https://doi.org/10.1007/s11046-017-0112-1

85 Lomeli-Martinez SM, González-Hernández LA, Villanueva JFA *et al*. *In vitro* azole antifungals susceptibility of *Candida* spp. Isolates from HIV-infected patients with periodontitis. *Med Mycol* 2022; **32**: 101294. https://doi.org/10.1016/j.mycmed.2022.101294

86 Manfredi M, McCullough MJ, Polonelli L *et al*. *In vitro* antifungal susceptibility to six antifungal agents of 229 Candida isolates from patients with diabetes mellitus. *Oral Microbiol Immunol* 2006; **21**: 177–82. https://doi.org/10.1111/j.1399-302X.2006.00274.x

87 Taghipour S, Rezaei-Matehkolaei A, Mahmoudabadi AZ. Antifungal susceptibility profiles of *Candida* species isolated from Ahvaz Jundishapur educational hospitals. *J Microbiol* 2018; **11**: e78851. https://doi.org/10.5812/jjm.78851

88 Tantivitayakul P, Panpradit N, Maudcheingka T *et al*. Genotyping of *Candida albicans* and *Candida dubliniensis* by 25S rDNA analysis shows association with virulence attributes in oral candidiasis. *Arch Oral Biol* 2019; **97**: 18–24. https://doi.org/10.1016/j.archoralbio.2018.10.006

89 Waltimo TMT, Ørstavik D, Meurman JH *et al*. *In vitro* susceptibility of *Candida albicans* isolates from apical and marginal periodontitis to common antifungal agents. *Oral Microbiol Immunol* 2000; **15**: 245–8. https://doi.org/10.1034/j.1399-302x.2000.150406.x

90 de Aguiar Cordeiro R, Reis AT, Lima XTV *et al*. Malassezia spp. and *Candida* spp. from patients with psoriasis exhibit reduced susceptibility to antifungals. *Braz J Microbiol* 2023; **54**: 169–77. https://doi.org/10.1007/s42770-022-00883-2

91 de Assis Santos D, de Carvalho Araújo RA, Kohler LM *et al*. Molecular typing and antifungal susceptibility of *Trichophyton rubrum* isolates from patients with onychomycosis pre- and post-treatment. *Int J Antimicrob Agents* 2007; **29**: 563–9. https://doi.org/10.1016/j.ijantimicag.2006.09.028

92 James JE, Santhanam J, Lee MC *et al*. *In vitro* antifungal susceptibility of *Neoscytalidium dimidiatum* clinical isolates from Malaysia. *Mycopathologica* 2017; **182**: 305–13. https://doi.org/10.1007/s11046-016-0085-5

93 Takahashi H, Oyama N, Tanaka I *et al*. Preventive effects of topical washing with miconazole nitrate containing soap to diaper candidiasis in hospitalized elderly patients: a prospective, double-blind, placebo-controlled study. *J Dermatol* 2017; **44**: 760–6. https://doi.org/10.1111/1346-8138.13781

94 Sonego B, Corio A, Mazzoletti V *et al*. *Trichophyton indotineae*, an emerging drug-resistant dermatophyte: a review of the treatment options. *J Clin Med* 2024; **13**: 3558. https://doi.org/10.3390/jcm13123558

95 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. https://doi.org/10.3390/jof6040207

96 Gebremedhin S, Dorocka-Bobkowska B, Prylinski M *et al*. Miconazole activity against Candida biofilms developed on acrylic discs. *J Physiol Pharmacol* 2014; **65**: 593–600.

97 Kean R, Rajendran R, Haggarty J *et al*. *Candida albicans* mycofilms support *Staphylococcus aureus* colonization and enhances miconazole resistance in dual-species interactions. *Front Microbiol* 2017; **8**: 258. https://doi.org/10.3389/fmicb.2017.00258

98 Lamfon H, Al-Karaawi Z, McCullough M *et al*. Composition of *in vitro* denture plaque biofilms and susceptibility to antifungals. *FEMS Microbiol Lett* 2005; **242**: 345–51. https://doi.org/10.1016/j.femsle.2004.11.032

99 Lamfon H, Porter SR, McCullough M *et al*. Susceptibility of *Candida albicans* biofilms grown in a constant depth film fermentor to chlorhexidine, fluconazole and miconazole: a longitudinal study. *J Antimicrob Chemother* 2004; **53**: 383–5. https://doi.org/10.1093/jac/dkh071

100 Rautemaa R, Richardson M, Pfaller M *et al*. Decreased susceptibility of *Candida albicans* to azole antifungals: a complication of long-term treatment in autoimmune polyendocrinopathy-candidiasisectodermal dystrophy (APECED) patients. *J Antimicrob Chemother* 2007; **60**: 889–92. https://doi.org/10.1093/jac/dkm299

101 Rautemaa R, Richardson M, Pfaller M *et al*. Reduction of fluconazole susceptibility of *Candida albicans* in APECED patients due to long-term use of ketoconazole and miconazole. *Scand J Infect Dis* 2008; **40**: 904–7. https://doi.org/10.1080/00365540802275853

102 Blanco D, van Rossem K. A prospective two-year assessment of miconazole resistance in *Candida* spp. with repeated treatment with 0.25% miconazole nitrate ointment in neonates and infants with moderate to severe diaper dermatitis complicated by cutaneous candidiasis. *Ped Dermatol* 2013; **30**: 717–24. https://doi.org/10.1111/pde.12107

103 Isham N, Ghannoum MA. Antifungal activity of miconazole against recent *Candida* strains. *Mycoses* 2009; **53**: 434–7. https://doi.org/10.1111/j.1439-0507.2009.01728.x

104 Ghannoum MA, Herbert J, Isham N. Repeated exposure of *Candida* spp. to miconazole demonstrates no development of resistance. *Mycoses* 2010; **54**: e175–7. https://doi.org/10.1111/j.1439-0507.2010.01867.x

105 Bremenkamp RM, Caris AR, Jorge AOC *et al*. Prevalence and antifungal resistance profile of *Candida* spp. oral isolates from patients with type 1 and 2 diabetes mellitus. *Arch Oral Biol* 2011; **56**: 549–55. https://doi.org/10.1016/j.archoralbio.2010.11.018

106 Cartledge JD, Midgley J, Gazzard BG. Non-albicans oral candidosis in HIV-positive patients. *J Antimicrob Chemother* 1999; **43**: 419–22. https://doi.org/10.1093/jac/43.3.419

107 Davies A, Brailsford S, Broadley K *et al*. Resistance amongst yeasts isolated from the oral cavities of patients with advanced cancer. *Pall Med* 2002; **16**: 527–31. https://doi.org/10.1191/0269216302pm583oa

108 Tamai IA, Pakbin B, Fasaei BN. Genetic diversity and antifungal susceptibility of *Candida albicans* isolates from Iranian HIV-infected patients with oral candidiasis. *BMC Res Notes* 2021; **14**: 93. https://doi.org/10.1186/s13104-021-05498-8

109 Tumbarello M, Caldarola G, Tacconelli E *et al*. Analysis of the risk factors associated with the emergence of azole resistant oral candidosis in the course of HIV infection. *J Antimicrob Chemother* 1996; **38**: 691–9. https://doi.org/10.1093/jac/38.4.691

110 Magaldi S, Mata S, Hartung C *et al*. *In vitro* susceptibility of 137 *Candida* sp. Isolates from HIV positive patients to several antifungal drugs. *Mycopathologia* 2000; **149**: 63–8. https://doi.org/10.1023/A:1007237711099

111 Milan EP, Burattini MN, Kallás EG *et al*. Azole resistance among oral *Candida* species isolates from AIDS patients under ketoconazole exposure. *Diagn Microbiol Infect Dis* 1998; **32**: 211–6. https://doi.org/10.1016/S0732-8893(98)00107-2

112 Quindós G, Carrillo-Muñoz AJ, Arévalo MP *et al*. *In vitro* susceptibility of *Candida dubliniensis* to current and new antifungal agents. *Chemotherapy* 2000; **46**: 395–401. https://doi.org/10.1159/000007320

113 Khan PA, Fatima N, Khan HM *et al*. Antifungal susceptibility pattern of *Candida* isolates: a comparison in H.I.V. positive and negative patients from tertiary care hospital of Northern India. *J Pure Appl Microbiol* 2021; **15**: 1230–5. https://doi.org/10.22207/JPAM.15.3.12

114 Chunchanur SK, Nadgir SD, Halesh LH *et al*. Detection and antifungal susceptibility testing of oral *Candida dubliniensis* from human immunodeficiency virus-infected patients. *Indian J Pathol Microbiol* 2009; **54**: 501–4. https://doi.org/10.4103/0377-4929.56138

115 Wu J, Guo H, Yi G *et al*. Prevalent drug resistance among oral yeasts from asymptomatic patients in Hainan, China. *Mycopathologia* 2014; **177**: 299–307. https://doi.org/10.1007/s11046-014-9747-3

116 Wu J, Gan C, Li J *et al*. Species diversity and antifungal susceptibilities of oral yeasts from patients with head and neck cancer. *Infect Drug Res* 2021; **14**: 2279–88. https://doi.org/10.2147/IDR.S316368

117 Heinic GS, Stevens DA, Greenspan D *et al*. Fluconazo.le-resistant Candida in AIDS patients. *Oral Surg Oral Med Oral Pathol* 1993; **76**: 711–6. https://doi.org/10.1016/0030-4220(93)90039-7

118 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts—Fourth Edition: M27*. 2017.

119 CLSI. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi—Third Edition: M38*. 2017.

120 Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs f antifungal agents for fermentative yeasts. *Clin Microbiol Infect* 2008; **14**: 398–405. https://doi.org/10.1111/j.1469-0691.2007.01935.x

121 CLSI. *Epidemiological cutoff Values for Antifungal Susceptibility Testing- Second Edition: M59*. 2018.

122 Metzger S, Hofmann H. Fluconazole-resistant *Candida* species from HIV-infected patients with recurrent *Candida* stomatitis: cross resistance to itraconazole and ketoconazole. *Mycoses* 1997; **40**(Suppl): 56–63. https://doi.org/10.1111/j.1439-0507.1997.tb00543.x

123 Chebil W, Haouas N, Eskes E *et al*. *In vitro* assessment of azole and amphotericin B susceptibilities of Malassezia spp. isolated from healthy and lesioned skin. *J Fungi* 2022; **8**: 959. https://doi.org/10.3390/jof8090959

124 Gupta AK, Kohli Y. Evaluation of *in vitro* resistance in patients with onychomycosis who fail antifungal therapy. *Dermatology* 2003; **207**: 375–80. https://doi.org/10.1159/000074118

125 Maurya VK, Kachhwaha D, Bora A *et al*. Determination of antifungal minimum inhibitory concentration and its clinical correlation among treatment failure cases of dermatophytosis. *J Family Med Prim Care* 2019; **8**: 2577–81. https://doi.org/10.4103/jfmpc.jfmpc_483_19

126 Korting HC, Ollert M, Abeck D. Results of German multicenter study of antimicrobial susceptibilities of *Trichophyton rubrum* and *Trichophyton mentagrophytes* strains causing *tinea unguium*. German Collaborative Dermatophyte Drug Susceptibility Study Group. *Antimicrob Agents Chemother* 1995; **39**: 1206–8. https://doi.org/10.1128/AAC.39.5.1206

127 Shaw D, Singh S, Dogra S *et al*. MIC and upper limit of wild-type distribution for 13 antifungal agents against a *Trichophyton mentagrophytes-Trichophyton interdigitale* complex of Indian origin. *Antimicrob Agents Chemother* 2020; **64**: e01964-19. https://doi.org/10.1128/AAC.01964-19

128 Kong X, Tang C, Singh A *et al*. Antifungal susceptibility and mutations in the squalene epoxidase gene in dermatophytes of the *Trichophyton mentagrophytes* species complex. *Antimicrob Agents Chemother* 2021; **65**: e00056-21. https://doi.org/10.1128/AAC.00056-21

129 Robertson MH, Rich P, Parker F *et al*. Ketoconazole in griseofulvin-resistant dermatophytosis. *J Am Acad Dermatol* 1982; **6**: 224–9. https://doi.org/10.1016/S0190-9622(82)70015-5

130 Ngo TMC, Ton Nu PA, Le CC *et al*. First detection of *Trichophyton indotineae* causing tinea corporis in Central Vietnam. *Med Mycol Case Rep* 2022; **36**: 37–41. https://doi.org/10.1016/j.mmcr.2022.05.004

131 Russo G, Toutous Trellu L, Fontao L *et al*. Towards an early clinical and biological resistance detection in dermatophytosis: about 2 cases of *Trichophyton indotineae*. *J Fungi (Basel)* 2023; **9**: 733. https://doi.org/10.3390/jof9070733

132 Khurana A, Agarwal A, Agrawal D *et al*. Multidrug resistant tinea corporis/cruris: response to voriconazole. *J Mycol Med* 2022; **32**: 101306. https://doi.org/10.1016/j.mycmed.2022.101306

133 Cervelatti EP, Fachin A, Ferreira-Nozawa M *et al*. Molecular cloning and characterization of a novel ABC transporter gene in the human pathogen *Trichophyton rubrum*. *Med Mycol* 2006; **44**: 141–7. https://doi.org/10.1080/13693780500220449

134 Pinjon E, Jackson CJ, Kelly SL *et al*. Reduced azole susceptibility in genotype 3 *Candida dubliniensis* isolates associated with increased Cd*CDR1* and Cd*CDR2* expression. *Antimicrob Agents Chemother* 2005; **49**: 1312–8. https://doi.org/10.1128/AAC.49.4.1312-1318.2005

135 Albertson GD, Niimi M, Cannin RD *et al*. Multiple efflux mechanisms are involved in *Candida albicans* fluconazole resistance. *Antimicrob Agents Chemother* 1996; **40**: 2835–41. https://doi.org/10.1128/AAC.40.12.2835

136 Kim M, Cho Y-J, Park M *et al*. Genomic tandem quadruplication is associated with ketoconazole resistance in *Malassezia pachydermatis*. *J Microbiol Biotechnol* 2018; **28**: 1937–45. https://doi.org/10.4014/jmb.1810.10019

137 Park M, Cho Y-J, Lee YW *et al*. Genomic multiplication and drug efflux influence ketoconazole resistance in *Malassezia restricta*. *Front Cell Infect Microbiol* 2020; **10**: 191. https://doi.org/10.3389/fcimb.2020.00191

138 Watamoto T, Samaranayake LP, Egusa H *et al*. Transcriptional regulation of drug-resistance genes in *Candida albicans* biofilms in response to antifungals. *J Med Microbiol* 2011; **60**: 1241–7. https://doi.org/10.1099/jmm.0.030692-0

139 Yin Y, Zhang H, Zhang Y *et al*. Fungal Zn(II)2Cys6 transcription factor ADS-1 regulates drug efflux and ergosterol metabolism under antifungal azole stress. *Antimicrob Agents Chemother* 2021; **65**: e01316-20. https://doi.org/10.1128/AAC.01316-20

140 Fox R, Neal KR, Leen CLS *et al*. Fluconazole resistant candida in AIDS. *J Infect* 1991; **22**: 201–4. https://doi.org/10.1016/0163-4453(91)91767-R

141 Ruhnke M, Eigler A, Tennagen I *et al*. Emergence of fluconazole-resistant strains of *Candida albicans* in patients with recurrent oropharyngeal candidosis and human immunodeficiency virus

infection. *J Clin Microbiol* 1994; **32**: 2092–8. https://doi.org/10.1128/jcm.32.9.2092-2098.1994

142 Vanden Bossche H, Marichal P, Odds FC. Molecular mechanisms of drug resistance in fungi. *Trends Microbiol* 1994; **2**: 393–400. https://doi.org/10.1016/0966-842X(94)90618-1

143 Heald AE, Cox GM, Schell WA *et al*. Oropharyngeal yeast flora and fluconazole resistance in HIV-infected patients receiving long-term continuous versus intermittent fluconazole therapy. *AIDS* 1996; **10**: 263–8. https://doi.org/10.1097/00002030-199603000-00004

144 Redding S, Smith J, Farinacci G *et al*. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by *in vitro* susceptibility testing and DNA subtype analysis. *Clin Infect Dis* 1994; **18**: 240–2. https://doi.org/10.1093/clinids/18.2.240

145 Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. *Antimicrob Agents Chemother* 1995; **39**: 1–8. https://doi.org/10.1128/AAC.39.1.1

146 Cartledge JD, Midgley J, Gazzard BG. Clinical response to ketoconazole of HIV-related oral candidosis is predicted by odds' relative growth method of susceptibility testing. *J Antimicrob Chemother* 1997; **40**: 117–9. https://doi.org/10.1093/jac/40.1.117

147 Johnson EM, Warnock DW, Luker J *et al*. Emergence of azole drug resistance in *Candida* species from HIV infected patients receiving prolonged fluconazole therapy for oral candidosis. *J Antimicrob Chemother* 1995; **35**: 103–14. https://doi.org/10.1093/jac/35.1.103

148 Vasquez JA, Arganoza MT, Boikov D *et al*. Stable phenotypic resistance of *Candida* species to amphotericin B conferred by preexposure to subinhibitory levels of azoles. *J Clin Microbiol* 1998; **36**: 2690–5. https://doi.org/10.1128/JCM.36.9.2690-2695.1998

149 Fichtenbaum CJ, Koletar S, Yiannoutsos C *et al*. Refractory mucosal candidiasis in advanced human immunodeficiency virus infection. *Clin Infect Dis* 2000; **30**: 749–56. https://doi.org/10.1086/313765