The global prevalence of fungal diseases has escalated in the last several decades. Currently, it is estimated that fungi infect 1.7 billion people annually and result in 1.5 million deaths every year\(^1\). Deaths due to fungal infections are increasing, with mortality often exceeding 50\%, further increasing to 100\% if treatment is delayed\(^1\). Despite these staggering figures, the contribution of fungal infections to the global burden of disease remains under-recognised.

In Australia, over a 5-year period fungal infections cost Australia an estimated $583 million\(^2\). The median cost for one invasive fungal disease (IFD) is AU$30 957, increasing to AU$80 291 if the patient is admitted to an intensive care unit\(^3\). Treatment of fungal infections poses significant challenges due to the small number of safe and effective antifungal drugs available and emerging antifungal drug resistance. Resistance to every class of antifungal drugs has been described and for some drug classes is extremely common\(^4,5\).

More than 90\% of all reported fungal deaths result from species that belong to one of three genera: Candida, Aspergillus and Cryptococcus\(^1\). Candida species normally live on the epithelial surfaces of the host and are common in immunocompromised patients who have undergone invasive procedures and can form biofilms on medical devices\(^1\). Candida albicans is the most prevalent species causing disease, followed by C. glabrata, C. tropicalis, C. parapsilosis and C. krusei (recently renamed C. acidothermophilum)\(^5\). Candida auris is an emerging fungal pathogen that has intrinsic multi-drug resistance. Since first emerging in 2009, C. auris has spread across five continents, including Australia, and resulted in a number of nosocomial outbreaks\(^6,7\).

Cryptococcosis, caused by Cryptococcus neoformans and Cryptococcus gattii, is the most common invasive fungal disease globally, at one point with an estimated >1M life-threatening infections per year\(^1\). C. neoformans, isolated from soil or bird excrement, has a worldwide distribution and predominantly causes infections in immunocompromised individuals. C. gattii is isolated from eucalyptus and other trees, so has a more restricted distribution, and causes infections largely in immunocompetent individuals\(^8\).

Aspergillus species commonly cause invasive pulmonary aspergillosis in neutropenic patients, transplant recipients and in patients on immunosuppression (e.g. corticosteroids). Aspergillus species are found worldwide and are common in the environment resulting in continuous lung exposure\(^1\). The most common species are in the A. fumigatus complex, but A. flavus, A. niger, A. terreus and A. nidulans also cause infections in humans.

**Antifungal drugs and emerging resistance**

There are four main classes of antifungal drugs: polyenes, azoles, allylamines and echinocandins (Figure 1, Table 1). Polyenes (e.g.
amphotericin B) bind ergosterol in the cell membrane and induce pore formation. Azoles (e.g. fluconazole, voriconazole) and allylamines (e.g. terbinafine) inhibit the enzymes lanosterol 14 α-demethylase (ERG11p/cyp51) and squalene epoxidase, respectively, which are needed for ergosterol biosynthesis in the fungal cell membrane. Echinocandins (e.g. caspofungin) inhibit the synthesis of glucan in the fungal cell wall. Echinocandins have few side-effects but are poorly absorbed and have limited efficacy (e.g. none against C. neoformans)\(^\text{22}\). Closely related species can exhibit differences in intrinsic susceptibility. For example, 98% of C. albicans isolates are susceptible to fluconazole whereas only 9% of C. krusei are susceptible\(^\text{1}\). Resistance to every family of antifungal drugs has been described and is at least partially responsible for the poor outcomes and high morbidity seen in patients with IFD. Resistance to azoles is common and increasing\(^\text{5}\).

The prevalence of antifungal resistance in Candida is not clear, mainly because of the differences in the levels of intrinsic susceptibilities between Candida species. Primary resistance to fluconazole is rare for C. albicans (1.4%), C. parapsilosis (3.6%) and C. tropicalis (4.1%) but species such as C. krusei are intrinsically resistant to fluconazole (78.3%)\(^\text{13}\). In a recent United States retrospective study acquired fluconazole resistance was reported in 19% of C. albicans infections\(^\text{13}\). At present, resistance to azoles is uncommon in Australia but may be increasing; a recent review of candidemia in Australia showed 16.7% of C. tropicalis isolates are resistant to both fluconazole and voriconazole\(^\text{23}\). A major concern is the emergence of infections caused by Candida species other than C. albicans, such as C. auris that has intrinsic multi-drug resistance. Currently C. auris is not established in Australia: the few Australian cases have come about through people being infected overseas (Victoria State Government, August 2018)\(^\text{7}\). Vigilance is warranted towards preventing this new multi-drug resistant pathogen from establishing a foothold in Australia.

A recent systematic review of resistance in Cryptococcus species from 1988–2017 revealed 10.6% of clinical isolates are fluconazole resistant and this rises to 24.1% in patients with relapsed cryptococcal infection\(^\text{24}\).

Table 1. Targets of antifungal drugs and resistance mechanisms.

<table>
<thead>
<tr>
<th>Antifungal drug</th>
<th>Cellular target and mechanism</th>
<th>Resistance mechanism/genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoles (e.g. fluconazole, itraconazole)</td>
<td>Lanosterol 14 α-demethylase, to inhibit ergosterol synthesis</td>
<td>Heteroresistance (aneuploidy of regions containing ERG11 and drug efflux genes TAC1 or AFR1)(^\text{9–11}). point mutations in ERG11(^\text{13–16}). Increased expression of ERG11(^\text{13,16–18}). Increased azole efflux through increased expression of pumps: MDR1, PDR1, CDR1, CDR2 and AFR1(^\text{4,19}).</td>
</tr>
<tr>
<td>Allylamines (e.g. terbinafine)</td>
<td>Squalene epoxidase, to inhibit ergosterol synthesis</td>
<td>Point mutations in ERG1(^\text{20}).</td>
</tr>
<tr>
<td>Polynes (e.g. amphotericin B)</td>
<td>Binds ergosterol and induces pore formation in the cell membrane</td>
<td>Mutation in ERG2, 8-7 isomerase(^\text{21}).</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>1,3-β-D-glucan synthase, to inhibit glucans required for the cell wall</td>
<td>Mutations in FKS1 and FKS2 encoding subunits of 1,3-β-D-glucan synthase(^\text{5,6}).</td>
</tr>
</tbody>
</table>
The first few instances of azole resistance in *A. fumigatus* were reported in the USA and Europe in the late 1980s and 90s in association with long-term antifungal treatment (extensively reviewed by Meis et al.\(^{25}\)). Since then, the prevalence of resistant isolates has increased and has been found in patients who have not received azole treatment. The acquisition of resistance in patients who have not received azole treatment has led to the hypothesis that resistance can also be gained from the agricultural use of azoles\(^{17}\). In 1999, the prevalence of azole-resistant *A. fumigatus* in The Netherlands was 12.8% but current incidence is 20% and resistance has now been reported in 11 different countries\(^{5,26}\).

**Molecular mechanisms of antifungal drug resistance**

The emergence of antifungal drug resistance can occur due to changes in the genome\(^{9-11}\). On exposure to azole antifungal drugs, fungal pathogens can undergo a process termed heteroresistance in which rapid, yet reversible, resistance is conferred by the development of one or more aneuploidies (large scale chromosomal rearrangements) (reviewed by Morrow and Fraser\(^{12}\)). Cells return to normal ploidy when the azole is removed, as the aneuploidies result in strains with reduced fitness both in culture and in the host\(^{10}\). In *C. albicans*, the most commonly occurring aneuploidy is an isochromosome of the left arm of chromosome 5, i(5L). This region contains the *ERG11* gene encoding the target of fluconazole, as well as the *TAC1* gene encoding the transcriptional activator of drug efflux genes.\(^{9}\) In *C. neoformans*, chromosome 1, containing *ERG11* and the azole transporter *AFR1*, is duplicated in response to increasing concentrations of fluconazole, followed by the successive duplication of chromosomes 4, 10 and 14\(^{10}\).

Resistance to azole drugs in *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *C. neoformans* and *A. fumigatus* can also be acquired by single nucleotide mutations in the *ERG11* gene (cyt51) encoding the target enzyme 14-α-demethylase.\(^{14,15}\) Mutations that lead to overexpression of the efflux pumps encoded by *MDR* (major facilitator superfamily pumps) in *C. albicans* and *C. parapsilosis* or *CDR* genes (ATP-binding cassette pumps) also confer resistance in *C. albicans*, *C. glabrata* and *C. krusei*.\(^{14}\) Mutations in *C. glabrata* *PDR1*, a transcription factor regulating the expression of drug efflux pumps, also confers resistance to fluconazole.\(^{19}\) *C. auris* clinical isolates possess acquired mutations in *ERG11* (azole resistance), *FKS1* (echinocandin resistance) and *FUR1* (5-flucytosine resistance)\(^{27}\). *Candida* species become resistant to echinocandins due to mutations in the *FKS* genes encoding the two subunits of the target enzyme 1,3-β-D-glucan synthase, specifically *FKSI* in *C. albicans, C. krusei* and *C. tropicalis* and both *FKSI* and *FKS2* in *C. glabrata*\(^{3}\).

In *A. fumigatus*, the most common mechanism of azole resistance occurring within a host involves mutations in the *cyp51A* (*ERG11*) gene, which prevent the azole from binding to the heme molecule.\(^{16,25}\) In contrast, *A. fumigatus* resistant clinical strains acquired from the environment most commonly possess a duplication of a 34 bp tandem repeat in the promoter region of *cyp51A*, combined with a specific substitution that causes overexpression of the gene.\(^{5,18}\) These mutations confer multi-drug resistance and have been found to be correlated with exposure to agricultural azoles in the environment.\(^{16,17}\).

In comparison to resistance to azoles, almost nothing is known about the mechanisms that give rise to resistance to polyenes in fungi. Strains naturally resistant to amphotericin B exist but resistance can also develop on treatment. In *Candida* species, acquisition of resistance to amphotericin B can occur through mutations of some of the *ERG* genes of the ergosterol biosynthesis pathway, although most resistant strains have only been characterised by biochemical analysis of membrane sterol composition. The only *C. neoformans* amphotericin B resistant isolate characterised had a mutation in *ERG2*, encoding sterol 8-7 isomerase.\(^{21}\)

**The role of mutation rate in accelerating the emergence of resistance**

Recent studies in *C. glabrata* and *C. neoformans* have revealed that a proportion of clinical isolates possess an elevated mutation rate (a ‘mutator’ phenotype), which can contribute to the rapid emergence of spontaneous antifungal drug resistance via increasing the opportunity for selectively advantageous mutations to occur\(^{26,29}\). Initial studies showed that 55% of *C. glabrata* clinical isolates contain non-synonymous variation in the *MSH2* gene, which encodes a component of mismatch DNA repair.\(^{28}\) The presence of non-synonymous variation in *MSH2* correlated with multi-drug resistance.\(^{28}\) *C. glabrata* clinical isolates possessing non-synonymous variation in *MSH2* have now been detected in clinical populations in many parts of the world with varying prevalence (North America 55%; India 69%; France 44%; Korea 65%).\(^{30-32}\) However, there is not always an obvious correlation with drug resistance. In addition, an assessment of mutation rate was not performed in these studies, leading to the criticism that the variation in *MSH2* may not result in a true mutator phenotype. A recent new green fluorescent protein (GFP) reporter coupled with Fluorescence Activated Cell Sorting (FACS) technique has been developed to test mutation rates in *C. glabrata* clinical isolates strains with different *MSH2* alleles.\(^{33}\) An elevated rate was not observed for isolates with...
the MSH2^{22-23Glu239Ph} alleric variant suggesting that not all non-synonymous variation in MSH2 results in a true mutator phenotype. Clinical isolates of *C. neoformans* with non-synonymous variation in *MSH2* have also been identified. These isolates exhibit a mutator phenotype and an increase in the emergence of spontaneous fluconazole and amphotericin B resistance. Deletion of *MSH2* in both *C. glabrata* and *C. neoformans* results in high levels of spontaneous resistance to multiple types of antifungals.

**Conclusions**

Resistance to antifungal drugs is clearly becoming an important clinical issue that will escalate in the future unless new classes of antifungals are developed. Early treatment strategies such as prophylaxis or extensive, long-term use of antifungals to avoid relapse frequently selects for drug resistance, as does environmental exposure possibly to agricultural azoles and over the counter use (e.g. fluconazole pessaries for vaginal thrush). An improved understanding of factors influencing the emergence of, and mechanisms of, resistance is required to develop effective future treatment strategies.

**Conflicts of interest**

Dr Morrissey has been a member of advisory boards for, received investigator-initiated grants from, and given lectures for Gilead Sciences, and Merck, Sharp and Dohme. All funds received are administered by Alfred Health/Monash University.

**Acknowledgements**

This research did not receive any specific funding.

**References**


**Biographies**

**Dr Kylie Boyce** is an expert in the molecular genetics of pathogenic fungi. Her research at RMIT University focuses on how pathogenic fungi interact with, and adapt to, the human host. She has recently been investigating how DNA repair and mutation rate contribute to the microevolution of fungal pathogens and their ability to rapidly generate spontaneous antifungal drug resistance.

**Dr Orla Morrissey** is an Infectious Diseases Physician at Alfred Health and Senior Lecturer in the Department of Infectious Diseases at Monash University, Melbourne. Dr Morrissey is a lead clinician within the Immunocompromised Host Consult Service at Alfred Health and co-chair of the Australia and New Zealand Mycoses Interest Group. Dr Morrissey is active in the research sphere: determining the epidemiology of a variety of opportunistic infections; determining *Aspergillus* virulence factors and examining inflammatory responses to *Aspergillus*.

**Dr Alexander Idnurm** is an expert in human and agricultural pathogenic fungi at Melbourne University. His research is focused on how fungi respond to their environment to change physiology and development. His research encompasses the genetic and molecular biology analyses of a number of different fungal species, providing an ability to take comparative approaches across the fungal kingdom. A recent focus has been on how quickly fungi change during their encounters with hosts, as this microevolution has ramifications for the emergence of antifungal drug resistance.

**Prof Ian Macreadie** is a molecular microbiologist who works with yeast as a model for studying Alzheimer’s disease. He also studies the effects of biochemicals and drugs on yeast, as well as studying the drug resistance of yeast. He teaches Industrial Microbiology at RMIT University and leads students to learn about how the gut microbiota of Australian animals aids their survival.