



## Fungal infections 5

# The global problem of antifungal resistance: prevalence, mechanisms, and management

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All serious fungal infections need appropriate antifungal therapy for successful patient outcome. Only a few classes of antifungal drugs are available, so the emergence of resistance to single drug classes and now multidrug resistance greatly hampers patient management. Azole resistance among *Candida* and *Aspergillus* species is one of the greatest challenges to clinical success, followed by echinocandin and multidrug resistance among some *Candida* species, especially *Candida glabrata*. The spread of agriculturally derived azole-resistant *Aspergillus fumigatus* and emerging threats such as multidrug resistant *Candida auris* are also alarming. The molecular mechanisms that cause drug resistance are naturally occurring in less susceptible species and are acquired in strains of susceptible organisms. Drug resistance mechanisms include altered drug-target interactions, reduced cellular drug concentrations mediated by drug efflux transporters, and permeability barriers associated with biofilms. Although *C. auris* is inherently multidrug resistant, other strains typically develop resistance through stepwise selection of multiple drug-resistance mechanisms. Cellular stress induced by drug treatment promotes adaptation, which contributes to breakthrough resistance. Drug exposure also drives the emergence of resistance. An effective antifungal stewardship programme is essential to control drug resistance, and should incorporate rapid fungal diagnostics, therapeutic drug monitoring, and clinical intervention teams. The development of better diagnostic tools and strategies that allow targeted use of antifungals is essential to preserve drug effectiveness.

### Introduction

Fungal pathogens cause life-threatening invasive diseases (eg, fungaemia, meningitis, pneumonia), severe chronic conditions (eg, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis), and complex chronic respiratory conditions (eg, asthma, chronic obstructive pulmonary disease). These pathogens also cause recurrent infections, such as oral and vaginal candidiasis. Many invasive fungal infections (IFIs) are a consequence of underlying health conditions associated with immunosuppression.<sup>1</sup> Generally, these infections are associated with high mortality, and successful clinical outcome requires early diagnosis and effective antifungal therapy. Yet, antifungal options are few, with chemical classes for invasive disease treatment limited to azoles, echinocandins, polyenes, and flucytosine.<sup>2</sup>

Over the past decade, the development of less toxic drugs, which can be applied safely in a range of patients with various conditions, has contributed to the expansion of antifungal use for prophylaxis, and empirical and directed therapy, which has in turn led to increased drug resistance. The use of medically related antifungal drugs in agriculture has resulted in environmental reservoirs for some drug-resistant pathogens.<sup>3,4</sup> The emergence of drug resistance to any one drug class severely limits therapy because so few treatment options are available. Multidrug resistance can eliminate treatment options entirely, which has a devastating effect on patient outcome. In this Series paper, the effect of antifungal resistance on patient care and the role of antimicrobial stewardship programmes in the prevention of antifungal resistance are discussed.

### Factors driving emergence of antifungal resistance

Therapeutic failure occurs when a patient either fails to respond or no longer responds to a drug administered at a standard dose. Various host, drug, and microbial factors contribute to such failures—eg, patients with a compromised immune system are more likely to fail to respond to therapy because the antifungal drug is

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### Key messages

- Antifungal drug resistance can occur with all drug classes, and involves acquired resistance in susceptible strains and selection of inherently less susceptible species
- Azole resistance is primarily caused by target modification in *Aspergillus* species, and by overexpression of drug efflux pumps and modification of the drug target in *Candida* species
- Agricultural fungicides drive acquired drug resistance in *Aspergillus* species, and these resistant strains are spreading globally
- *Candida glabrata* can acquire resistance to azoles and echinocandins as single drug classes, as well as multidrug resistance involving all major drug classes
- Echinocandin resistance is uncommon, but is conferred by hot spot aminoacid substitutions in glucan synthase
- Biofilms confer all or partial resistance to most drug classes
- Genetic factors, such as global transcription factors, DNA repair, and chromosomal abnormalities help to induce drug-resistant phenotypes
- The occurrence of cryptic species, which are often drug resistant, is increasing.
- Emerging species that are resistant to all antifungal classes (eg, *Candida auris*) are increasingly reported
- Robust antifungal stewardship programmes that integrate rapid diagnostics, therapeutic drug monitoring, and clinical intervention teams are required to overcome drug resistance in the clinic

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without the assistance of a robust immune response in the fight against the infection.<sup>5</sup> Indwelling catheters, artificial heart valves, and other implanted devices contribute to refractory infections because these surfaces are often colonised with drug-impermeable biofilms. Although drug penetration at sites of infection is poorly understood, drug delivery within the host is known to contribute to therapeutic failure—eg, an intra-abdominal abscess can seed drug resistance because fungi are exposed to suboptimal concentrations of drug.<sup>6</sup> Among patients with chronic infections, poor compliance with drug regimens might contribute to suboptimal drug exposure. Drug exposure in the form of prophylaxis, repeated, or long-term therapy is associated with the emergence of resistance. Similarly, exposure to agricultural fungicides with identical molecular targets to those of systemic antifungals has seeded environmental reservoirs of resistant organisms. Microbiological drug resistance can be either primary (intrinsic) or secondary (acquired). Primary drug resistance is found naturally among some fungi without previous exposure, and often involves the same mechanism as that which causes acquired resistance, although unknown mechanisms can also be implicated. The molecular mechanisms that confer antifungal resistance are known for all major drug classes, and are addressed in this Series paper, but the complex biological factors that promote these mechanisms are only starting to be elucidated.<sup>7</sup>

### Assessment of antifungal resistance

Microbiological drug resistance is associated with reduced susceptibility to an antifungal agent. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) have developed a standardised in-vitro antifungal susceptibility testing method for yeasts and moulds, whereby the minimum inhibitory concentration is measured and referenced to a clinical breakpoint. Although there are drug-specific and species-specific breakpoints for most common pathogens<sup>8–10</sup> there are none for rare yeasts and moulds. In the absence of breakpoints for rare fungi, epidemiological cutoff values help to define the upper limit of the wild-type population.<sup>11</sup> Although epidemiological cutoff values have been broadly established,<sup>12,13</sup> antifungal susceptibility testing can be problematic because some drugs (eg, caspofungin) and species (eg, *Candida glabrata*) behave unreliably and cannot be relied upon for standardised testing.<sup>14</sup>

Although there are differences in the CLSI and EUCAST methods to determine minimum inhibitory concentrations, which result in different breakpoints, both methods are grounded in pharmacodynamic responses in animal models and patients. Importantly, there is not an absolute association between in-vitro minimum inhibitory concentration and clinical response. To address the problem of response variability, drug resistance is assessed as high probability of treatment

success, uncertain effect of treatment, or high probability of treatment failure. Antifungal susceptibility testing identifies fungal strains that probably cause treatment failure, and provides an index for trends in changing susceptibility or new drug resistance mechanisms. Drug susceptibility testing is routinely used to assess yeasts, but is not formally recommended for the assessment of *Aspergillus* species by the Infectious Diseases Society of America.

Of course, the application of antifungal susceptibility testing varies with clinical setting (eg, those with a high probability of resistance), individual patient risk factors, and response to therapy. In specialist laboratories, the EUCAST and CLSI reference methods are mostly used for yeast susceptibility testing, whereas routine microbiology laboratories rely on commercial techniques, such as Etest<sup>15</sup> and Sensititre YeastOne,<sup>13</sup> or automated systems, like Vitek.<sup>16</sup> For *Candida* spp, the results of these tests are generally in agreement with those obtained by reference methods.

### Azole resistance

Azole compounds (eg, fluconazole, voriconazole, posaconazole) target the cytochrome P450 enzyme sterol 14 $\alpha$ -demethylase, which converts lanosterol to ergosterol, and is encoded by *ERG11* in yeast and *Cyp51* in moulds. Inhibition of 14 $\alpha$ -demethylase is fungistatic in yeasts and fungicidal in moulds. Triazoles are recommended for the treatment of aspergillosis, and are widely used for the treatment of candidiasis.<sup>17</sup> Epidemiological studies report substantial azole resistance among *Candida* and *Aspergillus* species,<sup>18,19</sup> whereas azole resistance among *Cryptococcus* species remains low.<sup>20</sup>

Generally, drug resistance has emerged through the development of acquired resistance and an epidemiological shift towards inherently less susceptible species.<sup>21</sup> High rates of azole resistance in *C glabrata* and intrinsic azole resistance of *Candida krusei* are well known. The ARTEMIS Antifungal Surveillance Program reported an increase in *C glabrata* as the cause of invasive candidiasis, from 18% in 1992–2001 to 25% in 2001–07, and an increase in fluconazole resistance, from 9% to 14%, over the same periods.<sup>22</sup> In 2013, among 1846 clinical isolates from 31 countries, 11.9% of *C glabrata* and 11.6% of *Candida tropicalis*<sup>23</sup> were fluconazole resistant. Similarly, the CANDIPOP study reported an increase in candidiasis-causing *C glabrata* (8% in 2002–03 vs 13.2% in 2010–11).<sup>24</sup>

Azole resistance among *Aspergillus* species reflects an increase in drug use for prophylactic and long-term treatment regimens, and acquired azole resistance was reported in patients who had undergone long-term treatment with azoles.<sup>19</sup> Azole-based fungicides for crop protection<sup>25</sup> are the most widely used class of agricultural fungicide, accounting for more than 20% of some European markets.<sup>26</sup> Although global surveillance studies reveal 3.2% of *Aspergillus fumigatus* isolates are

resistant to one or more azoles,<sup>27</sup> some regions show higher resistance than others and this variability might reflect disparities in microbiological procedures for *A fumigatus* isolation and resistance detection.<sup>28</sup> The role of azoles as environmental factors that drive resistance is under investigation by the ECDC European Environment and Epidemiology (E3) Network.

Other multidrug-resistant species—eg, *Fusarium* spp,<sup>29</sup> *Scedosporium* spp,<sup>30</sup> and mucorales<sup>31</sup>—are also commonly encountered. The rise of multidrug resistance in the *Candida haemulonii* complex<sup>32</sup> and *Candida auris* is alarming, with high rates of mortality and therapeutic failure reported worldwide for the latter.<sup>33,34</sup>

### Mechanisms of azole resistance in yeasts

Azole resistance among *Candida* spp involves several well defined mechanisms, including upregulation of drug transporters, overexpression or alteration of the drug target, and cellular changes caused, in some cases, by non-target effects induced by stress responses<sup>35</sup> (table). These mechanisms can occur either alone or concurrently in a single isolate, and can produce additive effects or lead to cross-resistance among azole drugs. The induction of efflux pumps, which decrease drug concentration inside the cell, is the most common mechanism of drug resistance. Drug pumps are encoded by genes of the ATP-binding cassette (ABC) superfamily or the major facilitator superfamily (MFS). The transcription factors regulating the expression of these pumps, Tac1<sup>36</sup> and Mrr1<sup>37</sup> in *Candida albicans* and CgPdr1 in *C glabrata*,<sup>38</sup> are well characterised. Numerous point mutations in *ERG11* have been reported in response to fluconazole. Some aminoacid substitutions cause structural changes in the active site of the demethylase—eg, aminoacid substitutions in *Saccharomyces cerevisiae* cause reduced target affinity and, thus, azole resistance.<sup>39</sup> Overexpression of *ERG11*, due to mutations in the transcription factor UPC2 of *C albicans*, also confers azole resistance.<sup>40</sup> Similarly, azole resistance can be due to loss of function of the sterol Δ5,6-desaturase gene (*ERG3*).<sup>41</sup>

Yeasts show high genomic plasticity, especially among *Cryptococcus neoformans* and *C albicans*. This plasticity results in loss of heterozygosity, increased chromosomal copy number, aneuploidy, or isochromosome formation, which affect the expression of the azole target or drug pumps, or both, and cause azole resistance.<sup>42</sup>

Biofilm formation on epithelial surfaces, teeth, or artificial devices, including heart valves and indwelling catheters, is another important drug-resistance mechanism that either resists drug action or promotes microbial resistance due to other mechanisms (eg, drug pumps).<sup>43</sup> The biofilm effectively reduces the concentration of the drug by trapping it in a glucan-rich matrix polymer.<sup>44</sup> Mature biofilms show complex architecture with heterogeneous cell types enmeshed in

	Azole	Echinocandin	Polyene
Target site modification	Yes	Yes	..
Target abundance	Yes	..	Yes
Target site overexpression	Yes	..	..
Drug pump upregulation	Yes	..	..
Biofilm formation	Yes	Yes	Yes
Non-target effects	Yes	Yes	Yes
Most <i>Candida</i> spp and <i>Aspergillus</i> spp covered			

**Table: Major categories of mechanisms of resistance by drug class**

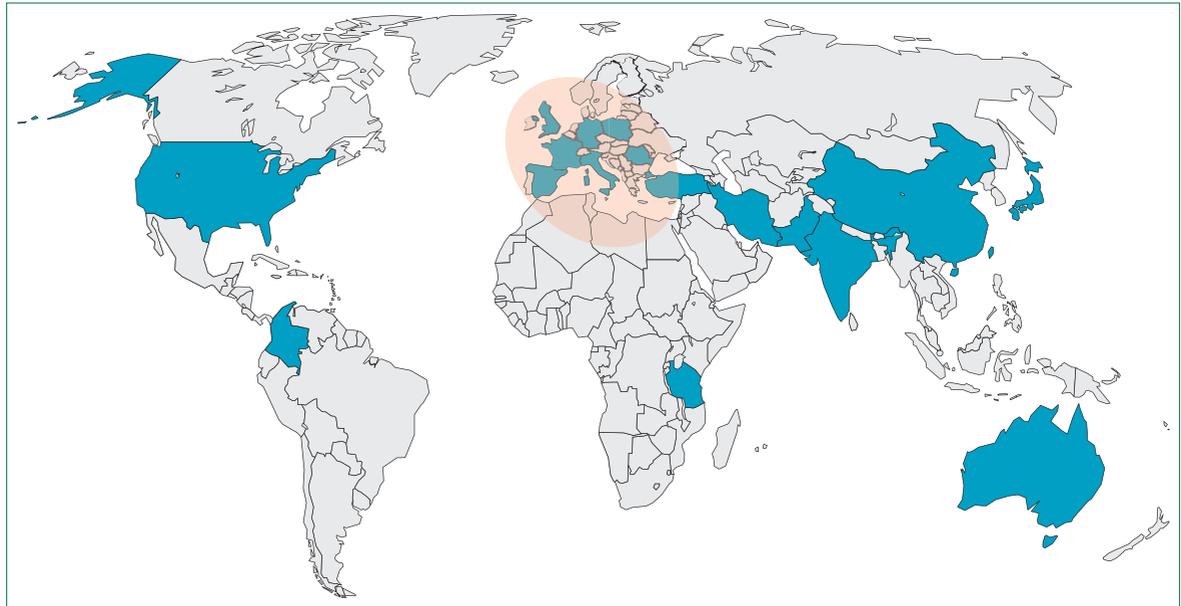
extracellular matrix. Disruption of this process by genetic or chemical modulation of the β-1,3-glucan synthase decreases drug sequestration in the matrix, rendering biofilms susceptible to antifungal agents.<sup>45</sup> Overall, biofilm formation is a universal mechanism that affects azole and other systemic antifungal drug classes.

### Mechanisms of azole resistance in moulds

Secondary resistance in *A fumigatus* is a worldwide concern. The most common mechanism of azole resistance in *A fumigatus* involves modification of *Cyp51A* and its promoter (TR<sub>34</sub>/Leu98His; TR<sub>46</sub>/Tyr121Phe/Thr289Ala), which has been found in environmental and clinical isolates from 22 countries<sup>27,46,47</sup> (figure 1). This mechanism is thought to be related to the extensive use of fungicides in agriculture.<sup>48</sup> Other proven mechanisms of resistance in *A fumigatus* are *Cyp51A* aminoacid substitutions at Gly54, Gly138, Met220, Gly448;<sup>19,49</sup> in *Aspergillus flavus*<sup>50</sup> and *Aspergillus terreus*<sup>51</sup> point mutations in *Cyp51* have been described. A 53-bp tandem repeat in the promoter region of *A fumigatus*, which confers drug resistance to itraconazole and voriconazole, and high minimum inhibitory concentrations to posaconazole, has been reported in the environment<sup>52</sup> and in a clinical strain.<sup>53</sup> Nowadays, many resistant isolates do not have aminoacid substitutions in *Cyp51A*, and alternative mechanisms underlie the development of drug resistance.<sup>54</sup> Potential mechanisms include upregulation of ABC and MSF transporter genes,<sup>54</sup> *HapE* modification,<sup>55</sup> and *Cyp51B* overexpression.<sup>56</sup> Biofilm formation is also a resistance factor for *Aspergillus* species, which form extensive exopolysaccharide matrices.<sup>57</sup>

### Azole resistance among cryptic species

Taxonomic studies in aspergillus have identified new cryptic species that are almost morphologically indistinguishable by classic identification methods. The most common *Aspergillus* species—ie, *A fumigatus*, *A flavus*, *A terreus*, and *Aspergillus niger*—are species complexes, with several cryptic species. The prevalence of cryptic species is poorly investigated, but studies from the USA and Spain report between 10% and 15% of total aspergillosis are caused by cryptic species.<sup>58</sup> Many species show high minimum inhibitory concentrations



**Figure 1: Countries reporting azole-resistant isolates of *Aspergillus fumigatus* with either TR<sub>34</sub>/L98H or TR<sub>46</sub>/Y121F/T289A modifications**  
Countries where mechanistic resistance is found are shown in blue. The region of highest burden of resistance is marked by the shaded oval (adapted from Verweij et al<sup>67</sup>).

	Amphotericin B	Itraconazole	Voriconazole	Posaconazole
<i>Aspergillus lentulus</i>	Red	Red	Red	Grey
<i>Aspergillus fumigatiaffinis</i>	Red	Red	Red	Grey
<i>Aspergillus udagawae</i>	Grey	White	Grey	White
<i>Aspergillus viridinutans</i>	White	Red	Red	Red
<i>Aspergillus pseudofischeri</i>	White	Red	Red	Red
<i>Aspergillus hirsutiae</i>	White	White	White	White
<i>Aspergillus calidoustus</i>	Red	Red	Red	Red
<i>Fusarium solani</i>	Grey	Red	Red	Red
<i>Fusarium</i> spp	Grey	Grey	Grey	Grey
<i>Scedosporium apiospermum</i> complex	Grey	Red	Red	Red
<i>Lomentospora prolificans</i>	Red	Red	Red	Red
Mucorales	Grey	Grey	Red	Grey

**Figure 2: Antifungal resistance in less common fungal species**  
Red=species resistant to drug. White=species susceptible to drug. Grey=species has intermediate or low minimum inhibitory concentration to drug.

See Online for appendix

to antifungal drugs<sup>58</sup> (figure 2) and are associated with poor outcomes; therefore, the lack of breakpoints and scarcity of epidemiological cutoff values for most of these organisms is of concern.

Some *A. fumigatus* complex species (eg, *Aspergillus lentulus*, *Aspergillus fumigatiaffinis*, *Aspergillus viridinutans*, and *Aspergillus pseudofischeri*) show high minimum inhibitory concentrations to azoles, and some to

amphotericin B. Azole resistance in the *A. niger* complex is isolate-dependent, but is more common in the cryptic species *Aspergillus tubingensis*.<sup>19</sup> Multidrug-resistant species, such as the *Aspergillus ustus* complex, show high minimum inhibitory concentrations to azoles and other drugs.<sup>39</sup>

Resistance to azoles occurs in other genera with diverse morphologies, such as *Fusarium* spp, *Scedosporium*<sup>60</sup> spp, and mucorales<sup>61</sup> (appendix). *Fusarium* species show resistance to all azoles and amphotericin B;<sup>62</sup> the *Scedosporium apiospermum* complex usually displays high minimum inhibitory concentrations to azoles and some strains also to echinocandins;<sup>60</sup> and *Lomentospora prolificans* (also known as *Scedosporium prolificans*) is a multidrug-resistant species with high minimum inhibitory concentrations to all drugs.<sup>63</sup> Mucorales are mostly resistant to itraconazole, and show high minimum inhibitory concentrations to voriconazole, leaving posaconazole as the only azole with activity against mucorales.<sup>61</sup>

### Echinocandin resistance

The echinocandin drugs anidulafungin, caspofungin, and micafungin are lipopeptides that target glucan synthase (a key enzyme involved in cell wall biosynthesis),<sup>64</sup> and are the recommended therapy for various patients with candidiasis.<sup>17</sup> An estimated 60% of candidaemia patients receive an echinocandin.<sup>65</sup> Echinocandin use has expanded in the past decade,<sup>66</sup> which has increased the potential for the emergence of antimicrobial resistance. The echinocandins are highly active against most *Candida* species, but are less active against *Candida parapsilosis* (*Candida parapsilosis* sensu

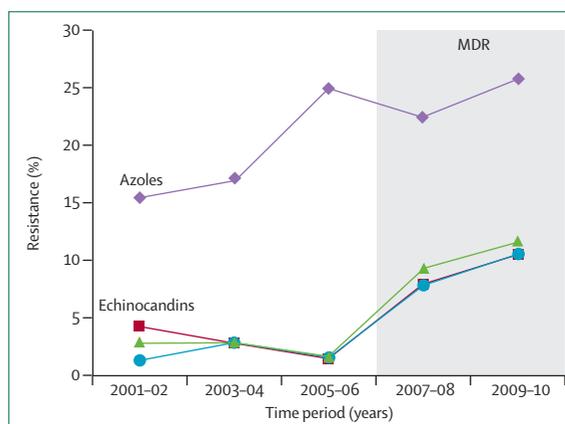
stricto, *Candida orthopsilosis*, and *Candida metapsilosis*) and *Candida guilliermondii*,<sup>67</sup> and are inactive against *Cryptococcus*, *Trichosporon*, and *Rhodotorula* species. Breakthrough infections with less susceptible species are reported with some yeasts and moulds,<sup>68</sup> and multidrug resistance involving different antifungal drug classes, including azole, echinocandins, and polyenes, has been reported for *Aspergillus calidoustus*, *A. lentulus*, *Fusarium* spp, *Scedosporium* spp, and some *Candida* species following therapy.<sup>58,68,69</sup>

EUCAST and CLSI have established drug-specific and species-specific breakpoints for most echinocandins,<sup>9,10</sup> and acquired drug resistance has been reported for the major *Candida* species.<sup>70</sup> In large-scale surveillance studies, the overall prevalence of *C. albicans* resistance is less than 1% and resistance among most susceptible *Candida* species is at or below this value.<sup>67</sup> The exception is *C. glabrata*: most epidemiological prevalence studies report antifungal resistance of 2–4% in this species.<sup>71</sup> In a population-based candidaemia surveillance study, the proportion of non-susceptible isolates increased from 4.2% in 2008 to 7.8% in 2014;<sup>72</sup> some institutional studies have reported higher rates, at close to or more than 10%.<sup>73,74</sup> Epidemiological studies typically report lower drug resistance because they focus on the first isolate of the infection, whereas institutional studies focus on all isolates. By contrast with North America, echinocandin resistance among *C. glabrata* is low (<1%) in Europe.<sup>75</sup> Whether this difference is because of strain types, clinical practice issues, or both is unclear. Generally, echinocandin resistance in susceptible *Candida* species arises after repeated or long-term exposure.<sup>76</sup> Alarmingly, an increase in echinocandin resistance among *C. glabrata* is now often accompanied by azole resistance, resulting in multidrug-resistant strains (figure 3).<sup>71,73</sup>

### Mechanisms of echinocandin resistance

The mechanism of echinocandin resistance in *Candida* species involves genetic acquisition of mutations in *FKS* genes, which encode the catalytic subunits of glucan synthase. Echinocandin resistance is associated with aminoacid substitutions in two narrow hot spot regions of Fks1 for all *Candida* species and Fks2 in *C. glabrata*.<sup>77</sup> This substitution is the only mechanism to produce clinical breakthrough infection during therapy. The echinocandins are not substrates for multidrug transporters,<sup>78</sup> and other mechanisms causing azole resistance are not cross-resistant with echinocandins.

Aminoacid substitutions in hot spots produce high minimum inhibitory concentrations and decrease the sensitivity of glucan synthase to drug by 50–3000-fold.<sup>79</sup> The most prominent Fks1 aminoacid substitutions occur at Ser-641 or Ser-645, and account for more than 90% of resistance in *C. albicans*.<sup>70</sup> The phenotypes associated with antifungal resistance have been supported by pharmacodynamic studies.<sup>80,81</sup>



**Figure 3:** Parallel rise in azole and echinocandin resistance in *Candida glabrata* bloodstream isolates over a 10-year period, showing emergence of multidrug-resistant strains

The grey-shaded box shows the time of emergence of substantial multidrug resistance. The three echinocandin-class drugs are shown: red, anidulafungin; green, casposfungin; blue, micafungin (adapted from Alexander et al<sup>73</sup>). MDR=multidrug resistance.

Similar mutations confer echinocandin resistance in other *Candida* species. In *C. glabrata*, aminoacid substitutions in both Fks1 and Fks2 occur, but are more common in Fks2.<sup>82</sup> Antifungal resistance mediated by Fks is associated with diminished clinical outcome,<sup>83,84</sup> and the presence of an *FKS* mutation is better than minimum inhibitory concentration alone in the prediction of clinical response.<sup>83</sup> A polymorphism at Pro-649 in hot spot 1 of the *C. parapsilosis* complex and at Met-633 and Ala-634 in *C. guilliermondii* induces reduced in-vitro antifungal susceptibility.<sup>85</sup> The clinical importance of intrinsic, reduced drug susceptibility is unclear because fungal infections are often successfully treated;<sup>86</sup> however, the value of this characteristic might vary with patient population, particularly in cases when strains are intermediate-susceptible or resistant.<sup>87</sup>

### Microbial factors and clinical reservoirs driving echinocandin resistance

Echinocandin action causes cell stress, which induces many adaptive responses that can render cells somewhat drug tolerant. The adaptive mechanisms include the heat-shock protein 90 (HSP90), the cell wall integrity pathway, the high osmolarity glycerol (HOG) pathway, and chitin biosynthesis.<sup>35</sup> Drug-tolerant fungal strains produce high minimum inhibitory concentrations in vitro, but they are not resistant because they do not cause clinical failure. This combination of tolerance and resistance occurs because glucan synthase remains sensitive to drug, and treated cells are attenuated. Paradoxical growth at high drug concentrations is also associated with compensatory responses in chitin biosynthesis.<sup>88</sup> These adaptive responses stabilise cells in the presence of drug, which allows them time to breakthrough by forming stable *FKS* mutations. The

development of drug resistance in *C glabrata* might be influenced by its biology because nearly 50% of clinical isolates display a hypermutator phenotype resulting from defects in the DNA repair machinery.<sup>89</sup> This mechanism appears most important for development of multidrug resistance.

Host reservoirs can be important sources of resistance—eg, the gastrointestinal tract is one such reservoir for *Candida* species, where they form a mixed biofilm. Drug penetration of the glucan matrix of the biofilm is irregular,<sup>90</sup> with varying concentrations of drug exposure promoting the emergence of drug resistance. Intra-abdominal candidiasis is a high-burden infection, which creates a reservoir that promotes resistance because of poor drug penetration.<sup>91</sup> In the presence of drug, resistant cells proliferate and cause systemic infection. *FKS* resistance is associated with long-term drug exposure, repeated drug exposure, or both. Because total drug exposure is an important driver of resistance, increased drug exposure from prophylaxis, empirical, and directed therapy requires antifungal stewardship to help to prevent the emergence of resistance.

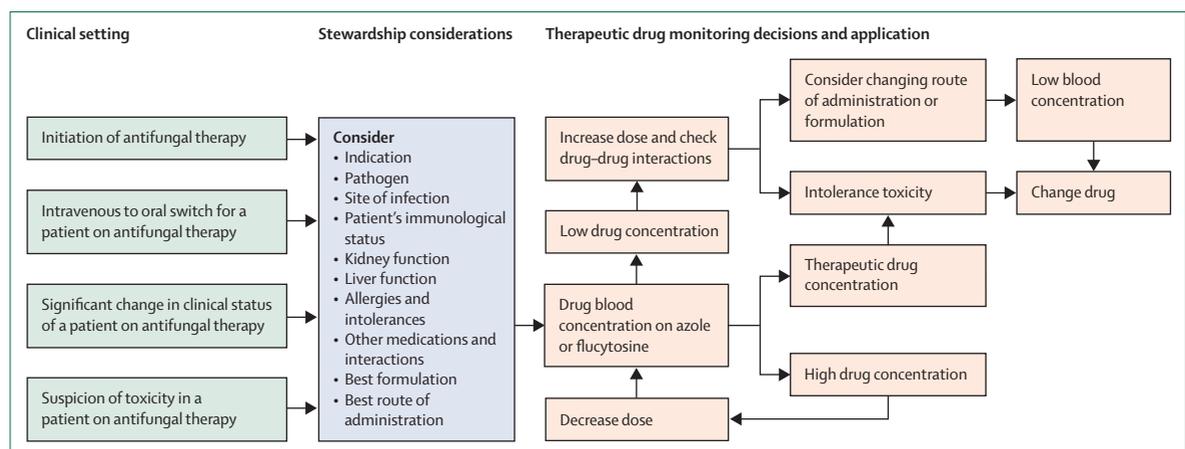
### Polyene resistance

The polyenes are the oldest antifungal drug class, and include amphotericin B and nystatin. Amphotericin B was first approved in 1957 for the treatment of life-threatening IFIs. Polyene drugs bind ergosterol, a fungal-specific sterol, in the plasma membrane of fungi, which causes the formation of concentration-dependent channels that kill cells by allowing ions and other cellular components to escape. In extramembranous aggregates, amphotericin B is suggested to kill cells by extracting ergosterol from lipid bilayers.<sup>92</sup>

For generations, amphotericin B was the primary therapy for a range of IFIs, including invasive aspergillosis, cryptococcosis, blastomycosis, candidaemia, coccidioidomycosis, histoplasmosis, and mucormycosis;

however, renal toxicity relegated it to a second-line therapy. Although lipid formulations of amphotericin B have lower toxicities than the original formulation, their high cost prevents their wider use. Amphotericin B is usually fungicidal, and resistance typically involves selection for inherently less susceptible species during therapy; acquired resistance in susceptible species is rare. Amphotericin B-resistant organisms include *Scedosporium* spp, *Fusarium* spp, *Trichosporon* spp, *Sporothrix schenckii*,<sup>93</sup> *Aspergillus nidulans*, *A terreus*, *A flavus*, *A calidoustus*,<sup>94</sup> and *A lentulus*.<sup>68</sup> Breakthrough infections with acquired resistance to amphotericin B have been reported for *C albicans*,<sup>95</sup> *C glabrata*,<sup>96</sup> *Candida rugosa*,<sup>97</sup> *Candida lusitanae*,<sup>98</sup> and *C tropicalis*<sup>99</sup> (figure 2). Polyene resistance among *Aspergillus* species has increased in the past decade, with only 11.5% of *A fumigatus* isolates inhibited at the EUCAST breakpoint of 1 µg/mL in some settings.<sup>100</sup> Acquired resistance to polyenes among *Cryptococcus* and *Candida* species is rare, although reports of high minimum inhibitory concentration to amphotericin B, or poor therapeutic outcome, or both, have been reported for *C albicans*, *C krusei*, *C rugosa*,<sup>97</sup> *C lusitanae*,<sup>98</sup> and *C glabrata*.<sup>96</sup> Furthermore, there are reports of treatment failure associated with high minimum inhibitory concentration to amphotericin B in *C neoformans*<sup>101</sup> and in multidrug-resistant *C auris*.<sup>34</sup>

The mechanism of resistance to amphotericin B involves a reduction in ergosterol content in the cell membrane. Treatment with an azole antifungal that lowers cellular sterol concentrations can confer polyene resistance.<sup>102</sup> Acquired resistance to amphotericin B has been studied genetically in *Saccharomyces* and *Candida* species, and strains with mutations affecting sterol biosynthesis—specifically, defects in *ERG1*, *ERG2*, *ERG3*, *ERG4*, *ERG6*, and *ERG11*—were shown to confer a range of resistance to the polyenes.<sup>96</sup> In *C neoformans*, a defective C8-isomerase and diminished sterol content



**Figure 4: Antifungal stewardship considerations and the use of therapeutic drug monitoring as a tool in antifungal stewardship programmes** Therapeutic drug monitoring of itraconazole and voriconazole is required, and of posaconazole or isavuconazole, or both, is recommended.

render cells less susceptible to amphotericin B.<sup>103</sup> In *A fumigatus*, the role of sterol depletion in polyene resistance is less clear.

### Antifungal stewardship

The aim of an antimicrobial stewardship programme is to preserve the future effectiveness of antimicrobials and improve patient outcome; thus, the selection of the optimal antimicrobial drug regimen, dose, route of administration, and duration of therapy are key.<sup>104</sup> The main tools in antimicrobial stewardship are guidelines for empirical therapy and diagnostic tests that reliably and safely guide therapy. An antifungal stewardship plan builds on the joint commitment of medical professionals engaged in patient care to adhere to the guidelines and follow a self-directed educated approach, by including a multidisciplinary antimicrobial stewardship team to oversee the programme.<sup>105</sup> In many centres, antimicrobial stewardship tools are not readily available because of poor access to diagnostic tests with long turn-around times. Guidelines that only cover the most common fungal infections and patient settings, combined with a generally poor understanding of fungal infections outside of specialist clinical teams, adds to the challenge of successful antimicrobial stewardship.<sup>106</sup> Newer drugs with lower toxicities than drugs such as amphotericin B have increased empirical and prophylactic use of antifungals in many patient groups, which affects antifungal resistance and is also financially unsustainable.<sup>107</sup>

In general, antifungal stewardship programmes are multifaceted and should be tailored for each institution and health-care system. The key elements of an antifungal stewardship plan typically include: provision of local guidelines and diagnostic tests that guide when therapy should start and stop; development of multidisciplinary care bundles; provision of bedside advice from infectious disease specialists, microbiology specialists, and the pharmacy team on dose, route of administration, and cessation of treatment; identification of prescriber knowledge gaps and education; and implementation of prescribing restrictions when close infectious diseases support is available.<sup>108</sup>

Various diagnostics-driven approaches are used to guide the timing of antifungal treatment. Although many diagnostic tests are not sensitive or fast enough to reduce empirical antifungal use, they might be useful for signalling early cessation of therapy when the result is negative. New serological or molecular diagnostic methods will probably be an essential part of future antifungal stewardship programmes.<sup>108</sup> Some biomarkers, including galactomannan,  $\beta$ -D-glucan, and aspergillus PCR, have a high negative predictive value for particular IFIs.<sup>109,110</sup> These tests can also be used to pause or stop antifungal therapy safely when the result is negative, but their efficient implementation requires the involvement of the antifungal stewardship team.<sup>111</sup> When

#### Search strategy and selection criteria

Preference was given to studies published within the past decade, with an emphasis on studies published within the past 3–5 years, although some older classic references were also included. PubMed and MEDLINE were used for searches of English-language journals using the terms: “azole resistance”, “echinocandin resistance”, “mechanism of azole and echinocandin resistance”, “multidrug resistance”, “epidemiology of resistance”, “antifungal susceptibility”, “cryptic resistant species”, and “antifungal stewardship”.

implemented accordingly, this diagnostic-driven approach can reduce the overall cost of stay substantially, without negatively affecting mortality.<sup>112</sup> Drug exposure can be controlled by the implementation of restrictions on electronic prescribing systems. However, IFIs typically develop in complex patients with various underlying conditions, and the establishment of safe rules for these restrictions can be difficult, if not impossible, in these patients.

Therapeutic drug monitoring is commonly used to tailor drug exposure to optimise treatment response and to minimise side-effects. Therapeutic drug monitoring is most relevant to triazole antifungals, which have varied and unpredictable pharmacokinetics—especially in severely ill adults and in children.<sup>113</sup> Factors such as food (eg, fats), proton pump inhibitors, gastric pH, mucosal health, and saturable absorption<sup>113</sup> influence drug exposure. Severely ill patient populations are at high risk of suboptimal drug exposure, and increased risk of the selection and overgrowth of resistant subpopulations.<sup>114</sup> There is no immediate application for therapeutic drug monitoring of echinocandins because they have more predictable pharmacokinetics; however, this should be revisited as echinocandin doses are increased, and new developmental drug candidates are expected to allow for higher and less frequent dosing. Overall, therapeutic drug monitoring can guide the choice between alternative agents, formulations, or routes of administration in cases where optimal drug concentrations cannot be achieved with one (figure 4). This tool is especially important when managing patients on long-term therapy and those at risk of developing resistance.

### Conclusion

Antifungal resistance is on the rise, and is an emerging threat to patient management and clinical success. The global spread of azole-resistant *Aspergillus* species and rise of multidrug-resistant *C glabrata* and *C auris* is particularly concerning. Although the mechanisms that confer drug resistance and the genetic factors that influence their emergence have been elucidated, the underlying biological factors are not yet well understood. The robust response of fungi to stress causes drug adaptation and tolerance, and is a step toward clinical breakthrough. Host reservoirs,

such as those in the gut or intra-abdominal abscesses, can restrict drug entry, which might seed resistance to antifungals. Finally, because drug exposure helps to drive resistance, drug stewardship is crucial in preventing resistance. Ultimately, there remains a need for new antifungal drugs against novel targets.

#### Contributors

DSP primarily contributed to the introduction, the polyene and echinocandin resistance sections, and the underlying resistance mechanisms section. AA-I primarily contributed to the azole resistance section, and the emerging resistance of cryptic species section. RR-R primarily contributed to the stewardship section. All authors edited the manuscript.

#### Declaration of interests

DSP reports grants from US National Institutes of Health and from Astellas Pharmaceuticals. He serves on scientific advisory boards for, and receives grant support from, Astellas, Cidara, Amplyx, Scynexis, and Matinas. DSP has an issued US patent concerning echinocandin resistance. RR-R reports personal fees from Gilead Sciences and Astellas. AA-I reports personal fees from Gilead Sciences and non-financial support from Pfizer.

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