Antifungal drug resistance: an update

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ABSTRACT
The number of antifungal classes is small, and resistance is becoming a much more frequent problem. Much greater emphasis needs to be placed on susceptibility testing and antifungal stewardship. Such efforts demonstrably improve survival and overall clinical outcomes. Positively diagnosing a fungal infection with laboratory markers often allows antibacterial therapy to be stopped (ie, anti-tuberculous therapy in chronic pulmonary aspergillosis or antibiotics other than cotrimoxazole in Pneumocystis pneumonia), contributing to antimicrobial resistance control generally. Non-culture based diagnostics for fungal disease are transformational in terms of sensitivity and speed, but only occasionally identify antifungal resistance.

Systemic antifungal therapy is crucial for survival and reduction in morbidity of a wide range of fungal infections — some invasive (ie, invasive aspergillosis and candidaemia), some chronic (ie, chronic pulmonary aspergillosis and mycetoma), some allergic (ie, fungal asthma) and a very large number that are superficial (ie, oral or vaginal candidiasis, tinea capitis or corporis (ringworm)). The current systemic antifungal drugs included on the WHO Essential Medicines List are shown in table 1.1

Antifungal resistance may be intrinsic or acquired.2 Genus or species identification often reveals intrinsic resistance such as fluconazole resistance in Candida krusei, amphotericin B resistance in Aspergillus terreus and echinocandin resistance in Cryptococcus species. A tendency for higher rates of acquired resistance is also revealed by species identification, such as fluconazole resistance in Candida glabrata or azole resistance in Aspergillus fumigatus. So fungal identification is critical to good treatment decisions. A guideline on the therapy of rare mould infections has recently been published by the European Confederation of Medical Mycology which addressed therapy for intrinsically resistant mould fungi.3

However, the majority of antifungal resistance problems are acquired, meaning that the majority of strains from that species are susceptible, but some are not and have acquired resistance. The mechanisms of acquired resistance are many, and research continues to uncover more; many are combinations of changes leading to resistance. For azole resistance in Candida, the most common mechanisms are efflux (ie, increased export of drug from inside the fungal cell to the exterior), with less common causes related to chromosomal (or part chromosomal) duplication or target site mutation.4 In A. fumigatus, the most common cause is target site mutation, often combined with increased copy number of the target gene (CYP51A).5 Occasional strains of A. fumigatus have increased efflux of azoles, additional copies of a CYP51B protein and several other mechanisms conferring resistance. In Cryptococcus neoformans, chromosomal deletions (aneploidy or disomy) account for most fluconazole resistance.6 7 Different mechanisms account for amphotericin B, fluycytosine, echinocandin and terbinafine resistance.

RESISTANCE IN DERMATOPHYES
Tinea capitis, cruris, corporis and pedis are common infections across the world, affecting ~1 billion people. They are caused by a variety of filamentous fungi including Trichophyton, Microsporum and Epidermophyton species. Tinea capitis is especially common in children in Africa, affecting an estimated 138 million.8 Microsporum canis infections are more difficult to treat and are refractory to terbinafine.9

In recent years, increasing resistance to terbinafine in Trichophyton interdigitale is increasingly recognised, especially in India. Multiple strains of T. interdigitale causing tinea corporis or tinea cruris are terbinafine resistant. For example, Khurana and colleagues analysed 64 strains from patients and 39 (61%) had elevated minimum inhibitory concentrations (MICs) to terbinafine (MIC >1 mg/L).10 The European Committee on Antimicrobial Susceptibility Testing Antifungal Susceptibility Testing Subcommittee (EUCAST AFST) has determined an epidemiological MIC cut-off (ECOFF) of 0.125 mg/L for T. interdigitale and 0.03 mg/L for Trichophyton rubrum.11 Common substitutions found in the target gene squalene epoxidase (ErgA) were usually associated with MICs of >32 mg/L, with others at 4 and 8 mg/L. All isolates were resistant to fluconazole in vitro but fortunately almost all are susceptible to itraconazole. Higher doses of itraconazole (ie, 400 mg daily) give response rates of ~65%. The recent emergence of resistance is notionally attributed to over the counter medication, incomplete courses of therapy and combined steroid, antifungal and antibacterial creams with incomplete coverage and low exposures.12 The spread of resistance to other countries is documented. This resistant grouping of dermatophytes have been renamed as Trichophyton indolitae.13

Susceptibility testing of dermatophyte fungi is not usually done in Europe, but is now necessary in patients who are unresponsive to terbinafine. Multicentre validated methodology studies have been conducted by the EUCAST group.14 National surveillance for dermatophyte resistance is required in countries with extensive links to the Indian subcontinent, probably using a sentinel site approach, preferably including clinical outcome data.
Short report

Table 1  Essential antifungal agents as assessed by the WHO. Access and antifungal price by country is visible here: https://www.gaffi.org/antifungal-drug-maps/

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Route(s)</th>
<th>Primary indications</th>
<th>Resistance concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griseofulvin</td>
<td>Oral</td>
<td>Tinea corporis and capitis</td>
<td>Some clinical resistance described</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>Oral, IV</td>
<td>Mucosal candidiasis, prophylaxis in leukaemia, HSCT and intensive care, treatment and maintenance therapy for cryptococcosis</td>
<td>All moulds, including Aspergillus resistant. Lower response rates for endemic mycoses such as histoplasmosis. All Candida auris and Candida krusei strains resistant—some other species less susceptible or resistant</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>IV and topical</td>
<td>Invasive candidiasis and cryptococcal meningitis, endemic fungal infections. Empiric therapy in febrile neutropenia. Lower response rate for invasive aspergillosis than azoles</td>
<td>Aspergillus terreus and nidulans resistant. Some strains of Candida auris resistant. Several intrinsically resistant fungi</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>Oral, IV*</td>
<td>Cryptococcal meningitis, neonatal candidiasis and Candida endocarditis and endophthalmitis, other rare fungal infections</td>
<td>Low levels of resistance in Candida and Cryptococcus. Aspergilli and most moulds and endemic fungal resistant</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>Oral, IV*</td>
<td>All skin infections, all forms of aspergillosis, endemic fungal infections, mucosal candidiasis, prophylaxis in leukaemia</td>
<td>Rising problems with resistance in Aspergillus fumigatus, flavus and niger. Some cross resistance with fluconazole in Candida</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Oral, IV</td>
<td>Invasive and chronic aspergillosis, some rare moulds</td>
<td>Some azole cross resistance in Aspergillus. Mucorales intrinsically resistant</td>
</tr>
<tr>
<td>Natamycin 5%</td>
<td>Topical, eye</td>
<td>Fungal keratitis</td>
<td>Most effective agent, but some rarer fungi resistant, probably</td>
</tr>
<tr>
<td>Echinocandins (micafungin, caspofungin, anidulafungin)</td>
<td>IV</td>
<td>Candidaemia, invasive candidiasis, invasive and chronic pulmonary aspergillosis, prophylaxis</td>
<td>Most effective agent for most Candida infections, notably the majority of Candida auris strains. Less effective than azoles for aspergillosis.</td>
</tr>
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*Many countries only have oral HSCT, haematopoetic stem cell transplant; IV, intravenous.

TRIAZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS

A. fumigatus is the most common cause globally of invasive, chronic and allergic aspergillosis, collectively affecting ~10 million people. The only oral class of antifungal agents active against Aspergillus species is the triazole group—itraconazole and voriconazole (both WHO Essential Medicines) and posaconazole and isavuconazole. In 2007, alarming reports from Manchester and Nijmegen described increasingazole resistance in A. fumigatus.15 Broadly, two circumstances led to the growing resistance problem. First, in the environment strains highly resistant to azole and triazoles emerged rapidly as a consequence of the widespread use of azole fungicides (about 1/3 of all fungicide use). Such strains are characterised by two principal genetic signatures (TR/ L98H and TR/ Y121F/ T289A).16 Second, in patients on long-term therapy, strains acquire a variety of resistance mechanisms including target site mutations, increased target copy number, efflux and other mechanisms still being described.17 18 Most isolates that are resistant are resistant to at least two triazoles and most are pan-azole resistant. Resistance has been seen in every continent except Antarctica. Resistance rates in Europe vary from ~1–20%, with higher rates in northern Europe.19 In Yunnan province in China, cultures from greenhouses found ~80% of A. fumigatus resistant to at least one medical triazole drug, with >30% showing cross-resistance to both itraconazole and voriconazole.20 In southern Vietnam, azole resistance rates in A. fumigatus are about 90%.21 Strains of Aspergillus flavus may also be azole resistant, with a recent report from Vietnam finding ~50% of environmental strains to be multi-azole resistant and 85% resistant to itraconazole.22 These were linked to aquaculture, a new association.

Susceptibility testing of Aspergillus species is now well established and available in most developed countries. Aspergillus niger strains are always resistant to itraconazole and isavuconazole, and Aspergillus terreus and nidulans to amphotericin B. It is generally recommended to susceptibility test all strains grown in patients taking antifungal therapy (not including fluconazole), as resistance is suggested by a positive culture on therapy, and preferably all strains in whom therapy is planned, whether from invasive, chronic or allergic aspergillosis.22 While susceptibility testing can be slow, direct PCR detection of resistance is possible, particularly for the mutations found in the environment.24–26 These data can all be part of antifungal stewardship which needs more emphasis in hospital practice. It would be helpful if the antifungal resistance programme run by the WHO Global Antimicrobial Resistance Surveillance System (GLASS) included surveillance for itraconazole and voriconazole resistance in Aspergillus.27

Patients with triazole resistance fail therapy.17 A recent cohort study showed that the mortality in voriconazole-resistant invasive aspergillosis was 20–30% higher than in patients with voriconazole-susceptible disease,16 indicating that the major advances in survival of patients through azole-based therapy are completely lost in resistant cases.

Alternative treatments for pan-azole resistance are all intravenous—amphotericin B and either micafungin or caspofungin (no data for anidulafungin). These agents are 15–20% less effective than azoles for invasive aspergillosis and are troublesome to administer long term. Some data are published on long term usage for chronic pulmonary aspergillosis.28 29 There are several new agents in clinical development for aspergillosis that may address this problem, including rezafungin (once weekly IV),30 ibrexafungerp (oral),31 olorofim (IV and oral)32 and fosmanogipix (IV and oral).33

To help retain the medically important azoles, reduction of usage of these triazole fungicides in the environment is required.16 This could be a voluntary withdrawal from some or all fungicide market segments. New antifungal drugs with novel chemistries and modes of action in clinical development and/or commercially launched after regulatory approval should never be used as fungicides in agriculture. The authorisation procedure for new fungicides should include testing for activity against non-target fungi such as A. fumigatus, that are known to cause infections in humans.

MULTI-DRUG RESISTANT CANDIDA GLABRATA AND CANDIDA AURIS

The optimal therapy of invasive candidiasis and candidaemia relies on an intravenous echinocandin (caspofungin, micafungin

and anidulafungin) with the exception of a less susceptible or resistance species such as Candida parapsilosis complex, Candida guilliermondii (rare) and Candida famata (rare). In these cases amphotericin B or fluconazole are used, occasionally augmented with fluconazole combination therapy. Unfortunately C. glabrata is poorly responsive to fluconazole and is often associated with urinary tract infection (which is itself very common in hospitalised, catheterised patients). Echinocandin therapy is usually successful for systemic C. glabrata infection, but none of the echinocandin drugs are excreted into the urine. C. glabrata therapy is usually successful for systemic candidaemia in India (n=918 strains), C. auris caused 8.2% and C. glabrata 7.1%. Multi-drug resistant (MDR) Candida species were found in 1.9%.

Increasing rates of MDR C. glabrata have been documented in candidaemia studies comparing rates over time. The number of cases is probably underestimated, as only initial blood isolates are included in surveillance studies. One study demonstrated echinocandin resistance in 21.6% of C. glabrata isolates from patients exposed to echinocandins for 7 days or longer.

Resistant C. glabrata are also a significant issue for women with recurrent vulvovaginal candidiasis (rVVC) as intravenous therapy is inappropriate for these women and many become untreatable. At any one time, an estimated 138 million women suffer from rVVC and repetitive courses of local azoles (ie, clotrimazole) or oral fluconazole, almost certainly are directly linked to replacement of C. albicans with C. glabrata.

C. auris has caused outbreaks across the world and is now endemic in many countries including the USA (ie, New York), South Africa, Colombia and India to name a few examples. One outbreak occurred in a neonatal unit in Colombia. The vast majority of clinical strains of C. auris are resistant to fluconazole, and the proportion that are also echinocandin or amphotericin B resistant varies by study. A common mechanism of fluconazole resistance is one of several mutations in zinccluster transcription factor-encoding gene TAC1B which increases drug efflux via increased CDR1 expression. Almost all are susceptible to fluconazole but resistance emerges rapidly on therapy. A small proportion (3–10%) are pan-resistant and currently untreatable. Echinocandin prophylaxis is ineffective as it does not penetrate adequately to the skin surface where C. auris resides, and so breakthroughs of MDR C. auris are promoted by this practice.

Fluconazole resistant C. parapsilosis is also a problem and can cause outbreaks. One large study found widespread dissemination in South Africa, especially in private hospitals, and other outbreaks have been described in Turkey and Mexico.

All blood and other sterile site cultures of Candida should be identified to species level and susceptibility tested. Stewardship programmes should focus in part on stopping unnecessary antifungal therapy for suspected cases of candidiasis and for a positive culture which is not significant (notably respiratory samples). The use of rapid beta 1,3-glucan testing can be useful to allow the cessation of therapy as it has a high negative predictive value.

**ANTIFUNGAL STEWARDSHIP**

Several studies have convincingly shown that antifungal stewardship reduces mortality in the hospitalised patient. The basic elements of successful stewardship are: (1) comprehensive knowledge and continuous reference to the best clinical guidance on fungal disease management; (2) a primary focus on the best quality care, not on cost saving, as some expensive antifungals are the best choice (stopping unnecessary therapy is what saves money); (3) clinical experience to know when to infer likely results if not yet available or samples cannot or were not taken. One important tool to assist in top quality fungal disease stewardship is rapid diagnostic services, notably mycology results, but also imaging. Too often, turnaround time for the results takes days, and so empirical (and often wrong) choices need to be made. Another key tool is antifungal therapeutic drug monitoring (TDM), especially for voriconazole and itraconazole, but also fluconazole in neonates and patients with renal dysfunction. A third key tool is immediate access to drug interaction data—one online tool which is curated weekly provides this, and can be downloaded onto Android phones.

Outpatient antifungal stewardship relies on experienced clinicians seeing complex patients, and not assuming that ‘any old clinic’ will do. If long-term antifungal therapy is started, the follow-up requires those patients to see experienced clinicians for optimal care, including TDM if appropriate.

In many situations, if a fungal disease is diagnosed then antibacterial therapy can be stopped or drastically reduced. Good examples are a diagnosis of chronic pulmonary aspergillosis so that tuberculosis can be ruled out, Pneumocystis pneumonia when only cotrimoxazole is needed, candidaemia, fungal asthma and Aspergillus bronchitis when treatment with antifungals drastically reduces exacerbations and antibacterial prescriptions.

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**Competing interests**

The author and his family hold Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company. He acts or has recently acted as a consultant to Pulmox, Mumbiance, Zambon, Bioseren, Bright Angel Therapeutics, Ciplina and Metis. He sits on the DSMB for a SARS CoV2 vaccine trial. In the last 3 years, he has been paid for talks on behalf of Dynamiker, Hikma, Gilead, Merck, Mylan and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group.

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