



Tackling the emerging threat of antifungal resistance to human health

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Abstract | Invasive fungal infections pose an important threat to public health and are an under-recognized component of antimicrobial resistance, an emerging crisis worldwide. Across a period of profound global environmental change and expanding at-risk populations, human-infecting pathogenic fungi are evolving resistance to all licensed systemic antifungal drugs. In this Review, we highlight the main mechanisms of antifungal resistance and explore the similarities and differences between bacterial and fungal resistance to antimicrobial control. We discuss the research and innovation topics that are needed for risk reduction strategies aimed at minimizing the emergence of resistance in pathogenic fungi. These topics include links between the environment and One Health, surveillance, diagnostics, routes of transmission, novel therapeutics and methods to mitigate hotspots for fungal adaptation. We emphasize the global efforts required to steward our existing antifungal armamentarium, and to direct the research and development of future therapies and interventions.

Antifungal tolerance

A characteristic of drug-susceptible genotypes to grow slowly at or above inhibitory drug concentrations. Characteristically, only a proportion of cells manifest tolerance.

Antifungal resistance

Defined as the ability to grow at antifungal drug concentrations above a defined antifungal susceptibility break point, normally (but not exclusively) owing to a defined causal molecular change following adaptation to drug exposure. It is expressed as a minimum inhibitory concentration (MIC).

Fungi cause diverse diseases in humans, ranging from allergic syndromes to superficial, disfiguring and life-threatening invasive fungal diseases (IFDs), which together affect more than a billion people worldwide^{1,2}. Historically, treatment has relied heavily on just four classes of systemically acting antifungal drugs: the polyenes, azoles, echinocandins and the pyrimidine analogue 5-flucytosine³. However, fungi respond nimbly to chemical attack⁴ and treatment failure is a common outcome. This failure is attributable to an interplay between underlying host immune defects, antifungal drug properties (pharmacokinetics, pharmacodynamics and drug–drug interactions) and fungal characteristics including diverse cell morphologies, antifungal tolerance and antifungal resistance. Resistance to antifungal drugs is an emerging concern worldwide in both space and time⁴, including novel resistant variants of previously susceptible pathogens (for example, the ubiquitous mould *Aspergillus fumigatus*⁵) as well as entirely new emerging species that are resistant to multiple antifungal drugs (for example, the yeast *Candida auris*⁶). The increasing public health burden is now officially recognized with the listing of both of these pathogens on the urgent antimicrobial resistance (AMR) threat list published by the US CDC in 2019 (REF.⁷).

Traditionally, AMR programmes excluded antifungals because fungi have been widely neglected as a threat

to public health^{8,9}. Biological differences between fungal (eukaryotic) and bacterial (prokaryotic) pathogens also complicate the integration of fungi into existing AMR programmes. Yet the emerging problem of AMR is shared across the domains of life and many parallels exist between drug-resistant microorganisms (TABLE 1). The widespread use of broad-spectrum antibacterial antibiotics (for example, β -lactams, cephalosporins, carbapenems, quinolones and macrolides) profoundly impacts bacterial communities by purging susceptible genotypes in favour of those harbouring polymorphisms and genes conferring resistance, the fittest examples of which can go on to become globally widespread¹⁰. Although less well studied, aspects of this evolutionary process are mirrored across the fungal kingdom, and all pathogenic fungi can acquire resistance through adaptation to drug selection pressure⁴.

Mechanistically, antifungal resistance is usually acquired due to changes that directly or indirectly affect the drug–target interaction. Causally, resistance may arise via genetic changes to the target binding site (for example, mutation of the genes encoding lanosterol demethylase for azoles or β -glucan synthase for echinocandins)³, via overexpression of the amount of target available and/or by altering the effective drug concentration (via elevated drug efflux activity for intracellular

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Minimum inhibitory concentration

(MIC). The lowest concentration of an antifungal drug that inhibits fungal growth and, in the context of defined susceptibility break points, defines resistance.

Fungicides

Antifungal compounds used in the environment to inhibit fungal growth; widely used in agriculture, horticulture and timber industries as well as components of antifouling agents and paints.

Saprotrophic decay

Heterotrophic nutrition provided by extracellular digestion of organic matter in the environment.

drugs such as azoles³, or inhibition of prodrug activation for flucytosine¹¹). Generalized fungal resistance mechanisms are shown in FIG. 1 and BOX 1. In contrast to antifungal resistance, antifungal tolerance refers to the ability of drug-susceptible cells to grow at drug concentrations above the minimum inhibitory concentration (MIC) and involves a wide range of general stress response and/or epigenetic pathways (reviewed in REF.¹²). Tolerance is most evident with fungistatic drugs, and has been measured and characterized most extensively in *Candida albicans* isolates treated with fluconazole. However, its clinical importance remains an open question.

Acquisition and emergence of antifungal drug resistance is fundamentally an evolutionary response to the selective pressure exerted by the drug. The likelihood of resistance emerging due to genetic changes is governed by the size of the population exposed to the selective pressure, the rate of cell doubling, the number of different pathways (physiological mechanisms and causal genetic changes) that confer resistance and the fitness costs associated with each of them. Importantly, antifungal drug resistance may originate in the host or in the environment. On one hand, in vivo resistance evolves de novo in individuals during antifungal therapy and causes treatment failure for a spectrum of pathogenic fungi spanning moulds¹³ and yeasts¹⁴. This is highly relevant for diverse *Candida* yeasts that are leading causes of nosocomial bloodstream infections and show widespread emergence of resistance to antifungals^{15,16}. For instance, emergence of azole resistance in *C. albicans* during prolonged fluconazole therapy for oral candidiasis in individuals infected with HIV was well documented¹⁷.

This phenomenon is not restricted to azole antifungals as progressive loss of echinocandin activity has also been reported during prolonged caspofungin therapy for *C. albicans* oesophagitis¹⁸. On the other hand, environmental resistance can emerge due to prior exposure of human pathogenic fungi to fungicides in nature⁵. Application of fungicides is dictated by the perennial need to defend intensively farmed animals and cultivations of solo, genetically homogeneous crops against fungal infections, as well as to preserve materials against saprotrophic decay by fungi. The environmental pressure of fungicides drives the evolution of resistance against all major classes of fungicides, including benzimidazoles, anilinoimidazoles, strobilurins, succinate dehydrogenase inhibitors and the sterol demethylation inhibitors (DMIs) including azoles⁴. Environmental resistance has not only necessitated the development of resistance management strategies and the breeding of more disease-resilient crops; it is also inextricably linked to the emergence of antifungal-resistant IFDs in humans as a consequence of the use of sterol 14 α -DMIs both in the environment and in the clinic^{4,5}. This emergence of drug-resistant fungi in nature and the clinic alongside expanding at-risk patient populations has prompted international funding bodies to add antifungal resistance to their research agendas. Of note, the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) consortium first included antifungal resistance in their Strategic Research and Innovation Agenda on Antimicrobial Resistance in April 2021. Their comprehensive One Health framework integrates six priority topics for addressing antifungal resistance: environment, transmission, surveillance, diagnostics, therapeutics and potential interventions¹⁹. In this Review, we focus on these priority areas with the goal of outlining current and future strategies alongside the key research that is needed to tackle the emerging public health issue of antifungal resistance.

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Environment–One Health links and emerging antifungal resistance

Opportunistic pathogenic fungi are commonly found within our close living environments, and many can produce abundant airborne spores. Consequently, humans are exposed daily to diverse environmental fungal pathogens as bioaerosols. Whereas most environmental fungi cause no noticeable pathophysiological events in healthy individuals, those with compromised health or immunity are susceptible to a spectrum of disease including superficial, allergic, chronic and life-threatening IFDs. Patient populations at risk of IFDs are currently expanding and (of note) include older people²⁰, those with immune systems compromised by HIV, cancer chemotherapy or transplant-necessitated immune suppression therapy, as well as those with severe viral infections such as influenza virus²¹ and COVID-19 (REFS^{22,23}). This latter group of patients has experienced surges in infection by groups of fungi, notably *Aspergillus* spp.²⁴, *Candida* spp., including *C. auris*²⁵, and in India the Mucoromycota species²⁶, which exhibit robust intrinsic and acquired resistance to antifungal treatments.

Molecular epidemiological studies have repeatedly shown that many fungal diseases are acquired from

Table 1 | Comparing drivers of bacterial and fungal antimicrobial resistance

Comparison	Bacterial resistance	Fungal resistance
Differences	Low-fidelity species boundaries drive widespread horizontal and vertical gene transfers, via both heterologous and homologous recombination	High-fidelity species boundaries dictated by pre and post-zygotic reproductive barriers; homologous recombination predominates
	Species boundaries are porous to gene transfer by MGEs, allowing widespread HGT amongst species; hitch-hiking of ARGs occurs upon MGEs — bacteriophages, plasmids, transposable elements and gene cassettes — comprising the bacterial 'resistome'	No evidence to date of antifungal resistance genes and alleles undergoing HGT among species, and no evidence of a pan-kingdom fungal 'resistome'; limited hybridization and rare HGT of MGEs such as homing endonuclease genes occurs, but not on the scale seen in bacteria
	Environmental and commensal bacteria comprise the main reservoir of ARGs that are available to potentially pathogenic species via HGT; therefore, in most cases, resistance does not evolve de novo for each species–drug combination	Environmental and commensal fungal species regularly cause disease, but AMR genes and alleles are constrained within species boundaries; therefore, resistance to antifungals needs to evolve de novo for each species–drug combination, which imposes limits on evolutionary rates
	Haploid core genome consisting of a single circular chromosome and housekeeping genes; extrachromosomal accessory genome on plasmids constitutes a 'pan-genome' of variable size containing the majority of ARGs	Haploid, diploid and multinucleated cells with each nucleus containing multiple chromosomes and with complex AMR determinants (see FIG. 1); extrachromosomal elements occur but are rare and have not been linked to AMR
	Zoonotic human infections by AMR-rich pathogens such as <i>Campylobacter</i> spp., <i>Salmonella</i> spp., <i>Escherichia coli</i> , <i>Enterococcus</i> spp. and <i>Staphylococcus aureus</i>	Animal reservoir of antifungal AMR currently unknown; zoonotic transmission occurs (for example, sporotrichosis and dermatophytosis), so theoretically possible
	Multiplicity of cidal drugs but extensive use of bacteriostatic drugs in settings of functioning host immunity to achieve full effect; tolerant bacterial subpopulations apply only to cidal drug use and arise via slowing of essential processes; quiescent persister subpopulations derived from epigenetically mediated tolerance remain dormant and metabolically inactive; higher tolerance in bacterial isolates is associated with longer lag phase growth	Paucity of cidal drugs and extensive use of fungistatic agents for prolonged periods in settings of immune dysfunction, promoting acquisition of drug tolerance; tolerant fungal subpopulations apply only to fungistatic drugs and arise through altered thresholds in stress responses and only indirectly, often via epigenetic or physiological changes that affect the ability to grow in the presence of a drug; tolerance may involve altered rates of drug efflux or uptake that can indirectly affect target–drug interactions; fungal isolates with higher tolerance levels have shorter lag phase growth
	Antibiotic resistance has been a target of international study for 30+ years with systematic surveillance and reference laboratories	Antifungal resistance only recognized in the 1990s with little organized surveillance and a paucity of reference laboratories
Similarities	Active global spread of AMR through travel and trade; clonal expansion for ARG-bearing lineages (for example, <i>S. aureus</i> clone EMRSA-15 (ST22) SCCmec); local passive spread in air (for example, in concentrated animal feed operations)	Active spread through nosocomial transmission and travel (for example, fluconazole-resistant <i>Candida auris</i> clades I–IV); long-range passive spread in air (for example, triazole-resistant <i>Aspergillus fumigatus</i>)
	Gut, mucosal and skin commensal carriage in Gram-negative and Gram-positive bacteria leading to local nosocomial health-care outbreaks as well as global spread of AMR	Gut, mucosal and epithelial commensal carriage in <i>Candida</i> spp. or dermatophyte fungi leading to local nosocomial health-care outbreaks as well as global spread of fungal AMR; however, less well defined
	Subtherapeutic levels or inadequate exposure to antibiotics can drive resistance emergence (for example, β -lactams); need for therapeutic drug monitoring to optimize pharmacokinetic and pharmacodynamic targets as an integral part of antibiotic stewardship	Subtherapeutic concentrations or inadequate exposure to antifungal drugs can drive resistance emergence (for example, azoles); need for therapeutic drug monitoring to optimize pharmacokinetics and pharmacodynamics as an integral part of antifungal stewardship
	Large-scale use of antibiotics in agriculture and livestock (for example, livestock-associated methicillin-resistant <i>S. aureus</i>) is causing a rapid increase in bacterial AMR	Large-scale use of fungicides in agriculture (for example, azole-resistant <i>A. fumigatus</i>) is causing a rapid increase in fungal AMR, some being clinically relevant
	Gene amplifications resulting in copy number variation can confer resistance to antibiotics	Aneuploid chromosomes and copy number variation can confer resistance or tolerance to stresses including antifungal drugs

The AMR, antimicrobial resistance; ARG, antimicrobial resistance gene; HGT, horizontal gene transfer; MGE, mobile genetic element.

our near environments; this is especially true for IFDs caused by *Coccidioides* spp.²⁷, *A. fumigatus*^{28–30} and *Cryptococcus* spp.³¹. The intimate relationship between environmental populations of fungi and ensuing exposures to antifungals means that emerging environmental resistance is likely to affect the clinical management of fungal infections. In the agricultural setting, phytopathogenic fungi continually evolve resistance to the array of fungicides deployed against them. This rapid adaptation necessitates a continuous cycle of development as agribusinesses synthesize variants of existing fungicides or

develop novel chemistries to thwart the accumulation of resistance^{4,32}. However, as with licensed medical antifungals, agricultural fungicides used in agriculture have broad-spectrum activity across the fungal kingdom. As such, resistance arises not only in the crop pathogens per se but also in other environmental fungi that include potential human fungal pathogens.

The One Health implications of the widespread use of broad-spectrum agricultural fungicides have been most closely studied for the DMI azoles, where these compounds (for example, difenoconazole, epoxiconazole,

propiconazole and tebuconazole) are not only structurally similar to the first-line medical triazoles (isavuconazole, itraconazole, posaconazole and voriconazole) but are used in increasing quantities worldwide. Azole fungicide usage in the United States has increased by more than 400% to ~3,000 metric tons per year from 2006 to 2016 (REF.³³). China uses ten times more (~30,000 metric tons per year)³⁴ with similar patterns repeated in the European Union³⁵. The degradation half-life of azole fungicides is long, ranging from 47 days for tebuconazole to up to 120 days for epoxiconazole. Given their annual global use, substantial azole persistence in the environment is expected and has the potential to

promote resistance or tolerance in opportunistic fungi. Worldwide increases in azole-resistant human fungal pathogens have been charted, both environmentally and clinically, since azoles were widely introduced in the 1980s (REF.⁴) and represent a ‘smoking gun’ linking agricultural fungicide use to burgeoning resistance in the clinic.

Dual use of azoles in the environment and clinic.

Potential eco-evolutionary links between environmental and clinical resistance have been widely explored for *A. fumigatus*, following initial reports of azole-resistant *A. fumigatus* occurring in the environment and in

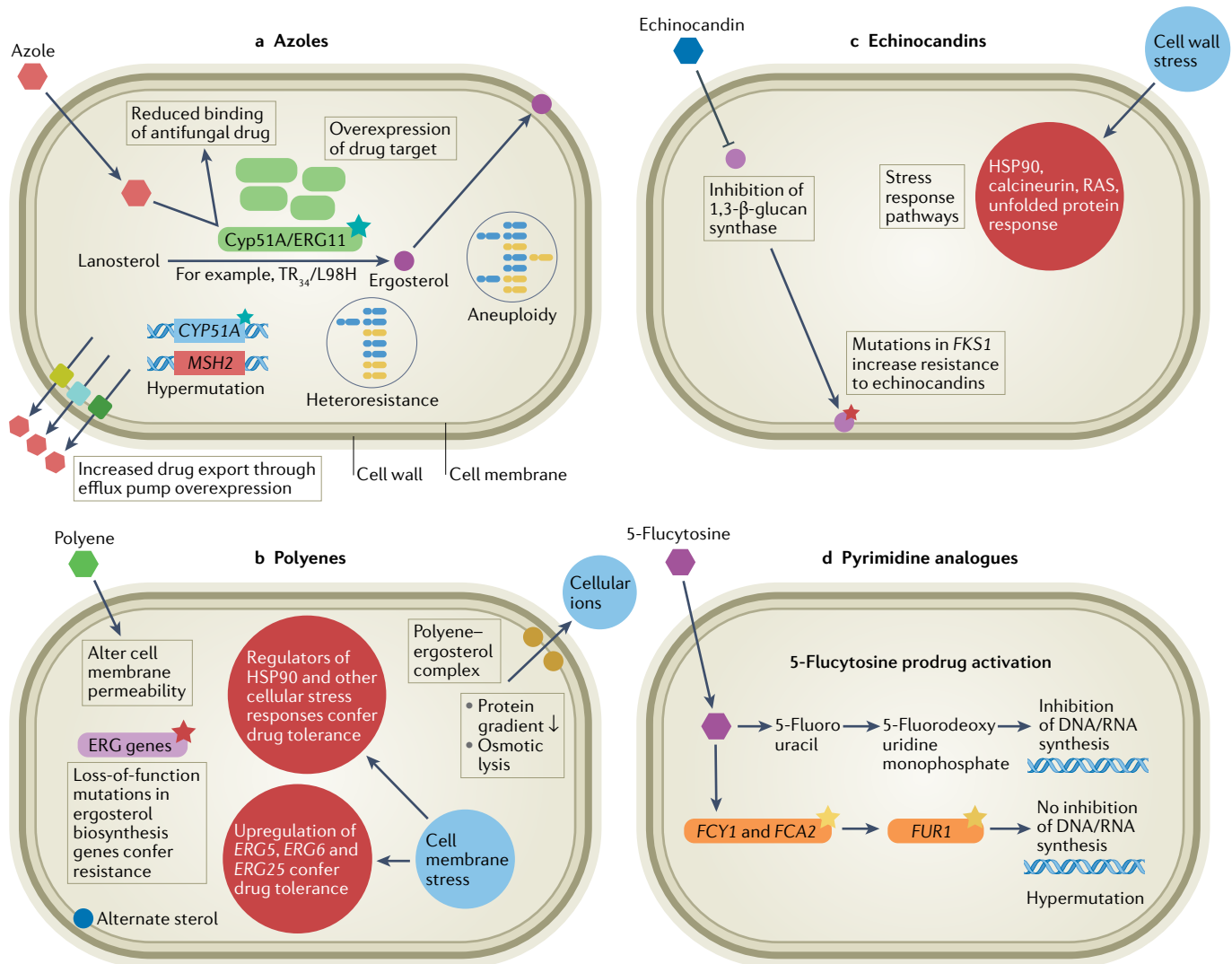


Fig. 1 | Major routes to acquiring antifungal drug resistance and/or tolerance in key invasive human fungal pathogens. Routes to acquiring antifungal drug resistance and/or tolerance vary depending on the mode of action (MOA). **a** | Azole drug resistance is primarily due to increased efflux of the drug from the fungal cell (particularly in *Candida* spp.) and modifications to the sterol biosynthesis pathway caused by point mutations and promoter insertions in *CYP51A* (*Aspergillus fumigatus*). In other fungal species, such as *Cryptococcus neoformans*, overexpression of the drug target and efflux pumps caused by chromosomal aneuploidy and hypermutation is common. **b** | Polyenes alter cell membrane permeability by forming a complex with ergosterol, and resistance is caused by loss-of-function mutations in ergosterol biosynthesis genes (particularly in *Aspergillus* and

Candida spp.). In *Candida albicans* in particular, double loss of *ERG3* confers resistance. However, drug tolerance is common, via upregulation of *ERG5*, *ERG6* and *ERG25* in *C. albicans*. **c** | Cell membrane stress can also impact regulators of HSP90, conferring drug tolerance. Echinocandins inhibit 1,3-β-D-glucan synthase (*FKS1*), and mutations in this gene cause resistance in *Candida* and *Fusarium* spp. Echinocandin exposure can also lead to cell wall stress through inhibition of β-glucan synthase, with indirect downstream activation of Ca^{2+} /calcineurin or HSP90/mTOR pathways, which are involved in drug tolerance. **d** | Pyrimidine analogues such as 5-flucytosine inhibit DNA and RNA synthesis. Resistance can arise via point mutations in the target gene *FCY1*, and is common in *Candida* spp. Hypermutation in *Cryptococcus* spp. is also known to cause resistance to this drug class. TR, tandem repeat.

Box 1 | Molecular mechanisms of drug resistance and tolerance

Antifungal resistance is defined as the ability to grow at antifungal drug concentrations that arrest growth and/or kill most isolates of that species. Some species have intrinsic resistance to some antifungals, due to ineffective binding to drug targets and/or efflux activities observed in all members of a given species. For example, all *Aspergillus* spp., *Candida krusei* and most *Candida auris* isolates are intrinsically resistant to fluconazole, and many environmental moulds (for example, Mucoromycota, *Lomentospora* spp. and *Fusarium* spp.) are resistant to azoles. Acquired resistance refers to the acquisition of resistance mechanisms that enable the fungal cells to grow at higher antifungal drug concentrations than members of the wild-type population. Antifungal tolerance, also termed trailing growth or heteroresistance¹³³, is the ability of a subpopulation of cells from a susceptible isolate to grow, albeit slowly, in the presence of drug concentrations above established minimum inhibitory concentrations (MICs)¹². Tolerance is thought to arise through genetic, physiological or epigenetic adaptation to the drug, with genetic background affecting the potential to exhibit tolerant growth. The terms ‘antifungal resistance’ and ‘antifungal tolerance’ often appear interchangeably (yet mistakenly) in the literature. The definition of antifungal tolerance differs from that of antibacterial tolerance and persistence, in which almost all cells or very rare cells, respectively, survive bactericidal drug treatment through transient metabolic quiescence of different durations¹³⁴.

Classes of mutations that can confer drug resistance and are common to fungi and bacteria (TABLE 1) include point mutations (~10⁻⁶ to 10⁻⁸ per cell per generation), gene duplications and transposon insertions (~10⁻³ to 10⁻⁴ per cell per generation). Unlike bacteria, fungi are often multinucleate and/or multicellular and carry multiple chromosomes. Such genomic organization provides enhanced opportunities for genetic changes fuelling adaptations and the emergence of resistance (FIG. 1). For example, clinically relevant resistance and/or tolerance to azoles can evolve through different classes of mutations, including whole chromosome and segmental aneuploidies^{135–138}. Loss of heterozygosity in diploid organisms can increase resistance or tolerance¹³⁹ with drug stress selecting for different loss of heterozygosity events. Occurrences of antifungal resistance also may be due to hypermutator fungal lineages in *Candida glabrata*¹⁴⁰ and in *Cryptococcus* spp.¹⁴¹, although the degree to which these specific mutations are responsible for elevated mutation rates remains elusive¹⁴². The known mechanistic drivers of fungal hypermutator status converge upon DNA mismatch repair mechanisms, primarily through *MSH2* mutations arising either via rapid in-host adaptation to drug exposure⁸⁵ or occurring amongst natural lineages of pathogenic fungi^{141,142}. Unlike bacterial hypermutator lineages, which often suffer significant fitness deficits, fungal hypermutator lineages incur only modest fitness costs^{85,140,143}.

Levels of azole tolerance vary widely between fungal genotypes isolated from different individuals, likely due to the considerable diversity of genome-wide single-nucleotide polymorphisms (SNPs) between isolates. During antifungal exposure, changes in drug tolerance arise at higher frequencies than changes in resistance levels¹⁴⁴. Presumably, the number of pathways that, when mutated, result in tolerance is larger than the number of genes that directly influence drug resistance. Under selection, it is likely that mutations conferring increased tolerance also increase rates of resistance. As in bacteria^{145,146}, this may be driven by increases in the effective size of cell populations with the potential to acquire and fix resistance mutations.

In contrast to azoles, which are generally fungistatic and often administered long term, resistance to polyenes such as amphotericin B emerges relatively infrequently and is rarely seen in the clinic¹⁴⁷. This is probably because amphotericin B binds to ergosterol, which, unlike a protein target, is not genetically encoded. When polyene resistance does arise, it appears to be due to modulation of the cell membrane composition through depletion or replacement of ergosterol¹³.

Phenotypic heterogeneity may alter antifungal susceptibility. For instance, biofilm formation, a sessile physiological state of multimorphic cells, is a non-genetic route to resistance and/or tolerance. Fungal cells in biofilms produce an extracellular matrix, which acts as a drug sink, reducing the effective drug concentration for cells within the biofilm¹⁴⁸. In addition, epigenetic states that are maintained by transiently heritable processes, such as chromatin modifications, may affect drug resistance and/or tolerance. This is exemplified by inhibitors of histone deacetylases that alter antifungal drug responses in vitro when mutated^{149,150}.

Intrinsic resistance

Species of fungi that have not obviously evolved resistance in response to drug pressure.

Acquired resistance

Species of fungi that have evolved resistance in response to drug pressure.

Aneuploidies

Increase in the numbers of copies of chromosomes, often resulting in phenotypic changes to drug resistance and/or tolerance profiles.

Hypermutator

Genotypes that manifest accelerated mutation rates because of mutations to genes involved in nucleic acid repair mechanisms.

Fungistatic

Exposure to a chemical that halts the growth of, but does not kill, the fungus.

patients with no prior history of antifungal treatment³⁶. Ecological ‘hotspots’ have been postulated, whereby biotic and abiotic conditions would converge, permitting growth of the fungus in contact with sub-MIC azole concentrations and, thereby, generating conditions that are suitable for adaptation to drug pressure⁵ (FIG. 2). Support for this hypothesis comes from studies of environments that support high growth rates of *A. fumigatus* in the presence of agricultural DMIs; these environments include both home and industrial composters³⁷, urban environments³⁸ and greenhouses³⁹.

Environmental triazole resistance in *A. fumigatus* is characterized by hallmark genetic changes involving expression-upregulating tandem repeats (TRs) in the promoter region of *CYP51A* that drive increased expression of the gene, accompanied by within-gene point mutations that alter the drug target (FIG. 1). The most commonly occurring resistance alleles, TR₃₄/L98H

and TR₄₆/Y121F/T289A, are associated with itraconazole and voriconazole resistance, respectively, both within and outside the clinic⁵, and are increasingly found worldwide⁴⁰. Molecular epidemiological methods uncovered numerous examples of paired resistant isolates, sourced from the environment and infected individuals, with statistically significant genetic identity implying the infection source was the resistant environmental isolate³⁰. Therefore, the topical question is no longer ‘does resistance in the environment affect patients?’. Rather, ‘where and why does resistance evolve?’ and ‘how does it disperse and what can be done to mitigate against it?’ have become crucial questions. The potential for global spread of triazole-resistant *A. fumigatus* through horticultural products, such as traded plant bulbs⁴¹, has been demonstrated and could be regulated. However, the dispersal of conidia on air currents is impossible to contain⁴². Moreover, although

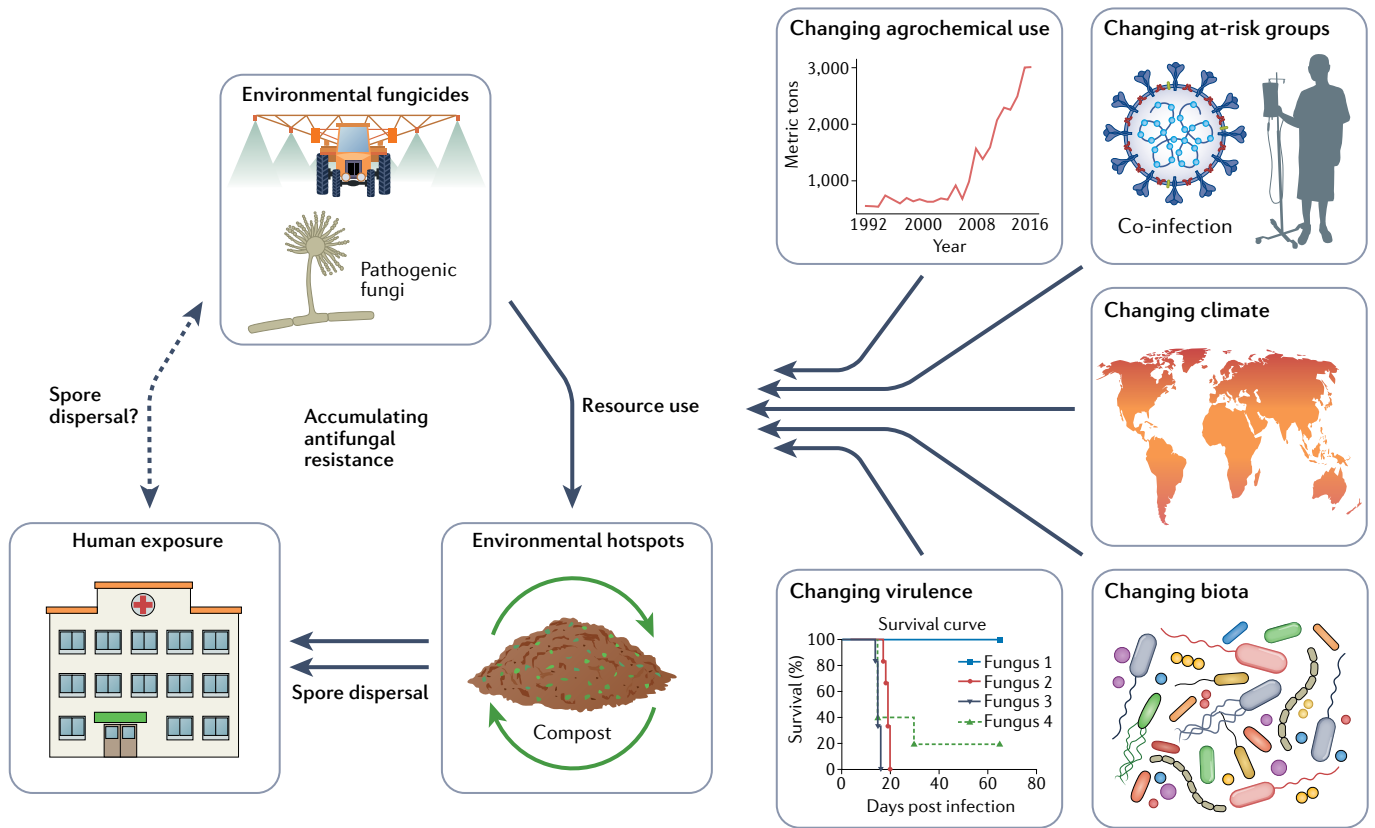


Fig. 2 | **Emerging antifungal resistance and environment–One Health drivers.** Fungi in the environment are exposed to broad-spectrum classes of antifungals that are also utilized as frontline antifungal treatments in the clinic. Ecological hotspots occur that can act as amplifiers of resistant genotypes. One example is green waste stockpiling and composting. Humans with invasive fungal diseases (IFDs) may also transmit resistant genotypes (for instance in nosocomial outbreaks); however, the extent to which humans and other animals contribute to the presence of antifungal resistance in the environment remains unknown. Multiple extrinsic factors exist that are expected to influence the incidence of antifungal resistance. These include changing patterns of fungicide use in the environment and in waste management³³; changing at-risk human host groups including viral infections such as COVID-19; changing climates that may alter the geographical range of fungi and adaptive landscape for resistance⁵⁰ as well as providing novel routes for infection (for example, natural disasters); changing biotic interactions that may include xenobiotic chemicals that are analogues to antifungals; and changing virulence of the fungi themselves owing to intrinsic genetic change or synergies with combinations of the above drivers⁴⁷.

humans are not widely considered as an ecologically relevant source of azole-resistant *A. fumigatus*, the potential for certain groups of patients to acquire and to shed azole-resistant pathogens in health-care settings means that they cannot be excluded as a source of drug-resistant inoculum⁴³ (FIG. 2).

Species-wide impacts of resistance in changing environments. The selection imposed by environmental fungicides likely has widespread effects upon the population genetic structures of human fungal pathogens and their genetically encoded phenotypic traits. The emergence of the TR₃₄/L98H resistance-associated trait in *A. fumigatus* is associated with the escalating frequency of specific azole-resistant clones that carry this allele. However, scans across the genome of *A. fumigatus* have shown that azole selection leads to selective sweeps that operate across multiple genomic regions, and upon specific genetic backgrounds³⁰. Accordingly, adaptation to fungicides in the environment may result in

phenotypic changes beyond those encoded by the resistance mechanism. One example concerns the hypothesis that azole resistance can also drive adaptation of *A. fumigatus* to infection-related stress and virulence^{44,45}. Sterol biosynthesis (the molecular target of the azoles), iron homeostasis and oxygen sensing are inextricably linked, as the production of ergosterol employs many iron-dependent enzymes and is highly oxygen-dependent⁴⁶. As the host environment is both iron and oxygen limiting, any changes in the genome of *A. fumigatus* that increase azole resistance by enhancing iron uptake and adaptation to hypoxia have the potential to concurrently promote heightened virulence, a hypothesis that should be tested. Similarly, adaptation by *Cryptococcus gattii* to the broad-spectrum fungicide benomyl was linked to cross-resistance to fluconazole and increased virulence in mice, a phenotype that was attributed to *MDR1* efflux pump overexpression⁴⁷. In another example, the higher average temperatures expected under climate change scenarios may affect the

emergence of antifungal resistance. Fungi respond to temperature by regulating cell membrane lipid composition, for example, by modulating ergosterol biosynthetic pathways⁴⁸, which in turn alters antifungal resistance indirectly. The frequency of azole-resistant *A. fumigatus* is elevated in high-temperature environments such as composts⁵, greenhouses³⁹ and tropical countries⁴⁹, suggesting that synergistic interactions between temperature and antifungal resistance do occur. Further investigations, however, are needed to establish the directionality and significance of these interactions⁵⁰. In parallel, synergies between temperature (thermal adaptation to warming climates) and fungicide exposure have been invoked to explain the rapid worldwide emergence of multidrug-resistant *C. auris* in humans, following its discovery in 2009 (REF.⁵¹).

Much remains to be learned about the genetic architecture and fitness landscapes of fungi following their adaptation to agrochemicals and how this impacts their interplay with other aspects of environmental change (FIG. 2). Thus, One Health solutions that address antifungal resistance must span site-specific local (for example, green waste composting containing chemical residues from agriculture) and global (for example, biosecurity in trade and changing climate) scales^{40,52}. The evolution of resistance may cause wider phenotypic changes including elevated virulence, either as a direct consequence of the initial mutations or as secondary adaptation to the azole-rich environment found in patients, or in agricultural settings. Changes in fitness may ultimately affect their persistence after azole application has ceased, and future research should include assessing the 'background' frequency of resistance genotypes in sample sites where azoles have been discontinued, or have never been applied. These complex eco-evolutionary scenarios heighten the necessity of understanding the One Health consequences of antifungal resistance on fungal pathogens, their ecology and the outcome of our exposures to such organisms: this understanding requires heightened surveillance.

Diagnostics and surveillance

Identifying antifungal resistance. The identification of antifungal resistance (and tolerance) has relied on susceptibility testing of cultured microorganisms, identifying MICs for specific antimicrobials that, when compared with clinical break points, define susceptibility or resistance. Several methods are available for antifungal susceptibility testing: broth microdilution, disk diffusion, azole agar screening, gradient diffusion and the use of rapid automated instruments⁵³. The Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) organizations establish standards for performing susceptibility testing and determine clinical 'break points' for effectively treating infections. However, standardized CLSI and EUCAST broth microdilution reference methods — the gold standard for antifungal susceptibility testing — are labour-intensive, time-consuming and performed infrequently in most clinical laboratories. In addition, they require mycological culture from clinical specimens,

which limits sensitivity and does not detect unculturable *Pneumocystis jirovecii*^{54–56}. Clinical break points have only been defined for the main antifungal agents for the most common species (for example, *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *A. fumigatus*) and there is an over-reliance on these as proxy break points for less studied species. Considerable variation between EUCAST and CLSI break points further complicates comparisons⁵⁷. The application of break points relies on accurate species-level identification; this has improved for yeasts with the increasing use of MALDI-TOF mass spectrometry systems, but for moulds is still dependent on local database content⁵⁸. Direct detection of antifungal resistance with the MALDI-TOF platform for yeasts⁵⁹ and moulds⁶⁰ is an exciting new direction; however, MALDI-TOF is too costly for many centres (thereby complicating international resistance surveillance initiatives) and reliance on culturing increases the time to diagnosis.

Resistance detection, transmission and surveillance.

Molecular diagnostic approaches have been proven, but underutilized, capacity to identify genetic markers potentially associated with antifungal resistance and to also recognize fungal species that are intrinsically resistant (reviewed in REF.⁶¹). Their sensitivity allows direct application to clinical specimens, avoiding the need for culture and improving turnaround times. Species of the *Aspergillus fumigati* complex, such as *Aspergillus lentulus* and *Aspergillus felis*, that are difficult to differentiate using conventional methods and have potentially higher MIC values to azole antifungals can be identified by real-time PCR⁶². Resistant *Candida* spp., such as *C. auris*, *C. glabrata* and *Candida krusei*, can be detected and differentiated by PCR, potentially aiding infection control and patient management⁶³. The utilization of fully automated molecular platforms (T2 Biosystems or Becton Dickinson Max) provide rapid testing systems requiring minimal specialist training comparable with the Cepheid GeneXpert platform for detecting multidrug-resistant tuberculosis. However, the range of this potential near-patient test must be expanded to include detection of mutations associated with resistance in generally susceptible fungal species.

Direct sequencing of genes encoding drug target proteins (for example, *CYP51A* in *A. fumigatus* or *ERG11* in *Candida* spp.) was commonly used to identify potential resistance-associated mutations⁶¹. Subsequently, and based on the high prevalence of common mutations (for example, TR₃₄/L98H and TR₄₆/Y121F/T289A in *A. fumigatus* and dihydropteroate synthase mutations in *P. jirovecii*), commercial real-time PCR assays were launched^{64,65} and their diagnostic use is increasing owing to the high sensitivity and specificity of PCR-based approaches. With azole resistance in *Candida* spp. associated with a wide range of mechanisms and subsequent mutations, development of real-time PCR approaches are limited. DNA sequencing remains the best option for identifying the mutations associated with azole resistance, limiting clinical application, particularly direct sample testing⁶⁶. Sequencing of *ERG11* and *FKS1* genes in *C. auris* strains with resistance to

Antifungal susceptibility testing

An in vitro measure of susceptibility and resistance to the drug concentrations required to inhibit fungal growth, measured by the minimum inhibitory concentration (MIC).

Loop-mediated isothermal amplification
Enzymatic nucleic acid amplification at a single temperature.

azoles and echinocandins has identified associated hotspots and specific mutations permitting the development of rapid molecular tests⁶⁷. A small number of *FKS1* gene mutations are associated with the majority of echinocandin resistance in *Candida* spp. and PCR assays have been developed⁶⁸. Currently, there are no commercial PCR tests to detect mutations associated with antifungal resistance in yeasts, and to improve diagnosis it is essential that this be recognized through enhanced commercial development and regulatory body support.

Resistance detection is being facilitated by technical and computational advances. Examples here

include integrating thermocycler-free DNA amplification by loop-mediated isothermal amplification onto lab-on-a-chip platforms with silicon-chip detectors and cloud connectivity to allow future point-of-care resistance detection⁶⁹, or newly developed pyrosequencing techniques⁷⁰. The implementation of whole-genome sequencing (WGS) holds great promise for exploring the biological basis of gene mutations more fully. Routine implementation of WGS for bacterial pathogen identification, resistance allele detection and identifying pathways of transmission is becoming commonplace. Beyond the detection of resistance alleles⁷¹, a major advantage of WGS is the ability to reconstruct the evolutionary trajectories of AMR variants across time and space¹⁰. However, in contrast to antibacterial resistance, a standardized WGS typing method is not widely used for fungi because of their larger genome sizes, frequent sexual recombination and the lack of standardized bioinformatic pipelines. Improved knowledge of antifungal resistance determinants and species genomes would support the transition to a WGS-powered understanding of fungal AMR for several human fungal pathogens⁷². Towards this goal, the development of rapid genomic analysis has been key to understanding the international⁷³ and local-scale⁷⁴ transmission of *C. auris* including the emergence of multidrug-resistant variants. Unculturable fungi present a challenge, and more targeted methods are needed. For instance, a successful consensus multilocus sequence typing scheme for *P. jirovecii*⁷⁵ enables antifungal resistance marker analysis⁷⁶. For *Aspergillus* spp., more knowledge of resistance mechanisms is required as many resistant isolates do not carry the few known resistance-associated alleles⁷⁷. Nonetheless, WGS is increasingly being used to trace transmission of AMR in *A. fumigatus* for known polymorphisms³⁰. Improvements in the ability of point-of-care WGS devices such as nanopore sequencers are accelerating our ability to detect antifungal resistance mutations and will likely transform our ability to understand pathways of nosocomial transmission in outbreak settings⁷⁴ (FIG. 3).

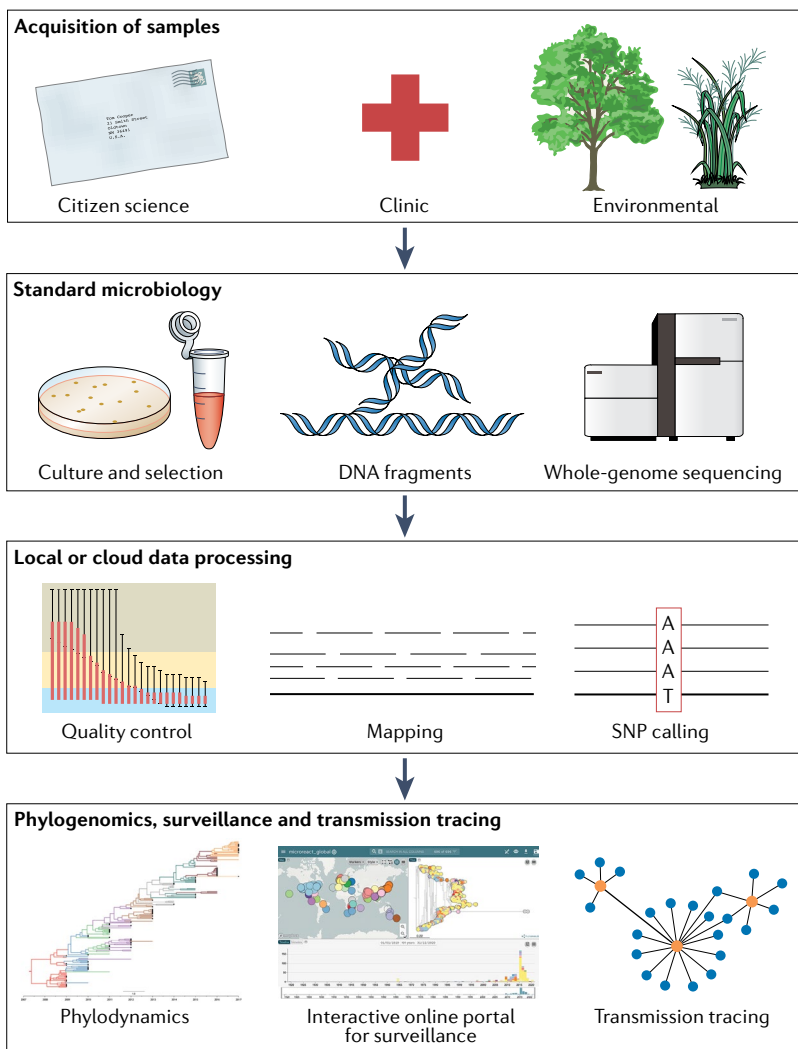


Fig. 3 | Resistance detection, tracking and surveillance. Fungal samples can be acquired from the clinic or environment, including engaging with the public as ‘citizen scientists’¹⁴². Traditional, established microbiology methods can culture and select isolates from these samples, ready for extraction of genomic DNA. These DNA fragments are used to generate a sequencing library for whole-genome sequencing (WGS). There are many sequencing platforms available, generating both long-read and short-read sequence data. Raw sequence data need to be quality controlled prior to mapping against a reference genome, either locally or using cloud computing. Calling high-confidence single-nucleotide polymorphisms (SNPs) can help infer alleles associated with drug resistance and their evolutionary histories. Phylodynamic inference and building interactive online portals (such as Nextstrain¹³¹ or Microreact¹³²) that are available to researchers and clinicians alike enable tracing of transmission events.

Towards global surveillance of antifungal resistance. Public health agencies have instigated systematic surveillance for bacterial AMR in many countries and have appointed reference laboratories to liaise with routine medical microbiological laboratories. Large international surveillance studies, led by the US CDC and the European Centre for Disease Prevention and Control, monitor the spread of antibiotic-resistant bacteria and broadcast early warning signals. However, fungi have, hitherto, been excluded from most AMR surveillance programmes. In 2018, the WHO (World Health Organization) launched a pilot *Candida* surveillance scheme to gather retrospective data on antifungal resistance for invasive *Candida* isolates; this was recently formally included in the Global Antimicrobial Resistance Surveillance System (GLASS) programme (BOX 2). The Emerging Infections Program of the CDC currently conducts active population-based surveillance in ten state health departments in the United States, monitoring epidemiological trends in candidaemia. Globally, the

Box 2 | Priorities for optimizing antifungal resistance surveillance

Existing antifungal surveillance

- SENTRY Antimicrobial Surveillance Program
- WHO (World Health Organization) Global Antimicrobial Resistance Surveillance System (GLASS): *Candida* spp.
- Ad hoc national and local-scale antifungal surveillance

Future antifungal surveillance priorities

- Develop and adapt tools suitable for use in low- and middle-income countries, and build capacity for their use
- Increase availability of rapid and simple antifungal resistance screening techniques suitable for local clinical laboratories
- Appoint National Reference Laboratories to monitor antifungal resistance
- Increase fundamental research to identify molecular mechanisms of antifungal resistance and associated diagnostic markers
- Develop standardized clinical research databases to link in vitro and in vivo resistance to clinical outcomes
- Generate globally accessible genomic antifungal resistance databases for priority human fungal pathogens
- Implement standardized and linked antifungal resistance surveillance networks at national and international levels together with international and harmonized definitions and data types
- Build accessible sample biorepositories and metadata to accelerate academic and industry collaboration to develop resistance diagnostics
- Extend human antifungal resistance surveillance to veterinary, wildlife and environmental samples within a 'One Health' paradigm

SENTRY Antimicrobial Surveillance Program has at least 427 participating centres⁷⁸ and antifungal resistance data are collected both indirectly (via blood culture surveillance) and directly. Unfortunately, relatively few centres contribute fungal pathogen data. Apart from these broader and more systematic surveillance programmes, nationwide surveillance data for *Candida* spp. are available from several countries such as Australia, Scotland, Finland, Iceland, Norway, Sweden, the United Kingdom and Denmark⁷⁹. Nevertheless, surveillance of other fungal species is rare with most published data restricted to azole-resistant *A. fumigatus*^{80,81}.

The rising rates of antifungal resistance and rapid global emergence of new multidrug-resistant species such as *C. auris*⁸² make it imperative to include fungal infections into existing national and international surveillance programmes. Despite the detection of azole-resistant genotypes of *A. fumigatus* worldwide, in most clinical settings its presence is not tested for and there are few studies exploring its association with clinical failure. Notably, as a 'call to arms', the WHO is currently defining a fungal pathogen priority list⁸³ in line with its bacterial counterpart, a major step likely to trigger research and innovation in the field. A current high priority is the need to implement standardized surveillance through the collection of basic clinical and epidemiological data. This is because improved surveillance will further increase understanding of the evolution and transmission of fungal AMR alongside helping to implement modern genomic surveillance methodologies. In tandem, there is an urgent need for collaborative networks that include research, clinical and industry partners to undertake multicentre studies; these networks

will also require access to shared biorepositories that collate validated samples alongside metadata, and that can distribute these rapidly and equitably when needed. Locally, accurate fungal species identification, simple resistance screening methodologies and MIC testing should be empowered at clinical laboratories in both high-resource and resource-limited countries, where there is a need for capacity building of clinical mycological expertise. When resistant isolates are identified locally, confirmation by reference laboratories in combination with the collection of essential clinical and epidemiological data will facilitate the downstream development of policy recommendations and control strategies.

Therapeutic approaches for tackling antifungal resistance

For commensal organisms, antifungal drug resistance can be acquired through drug exposure in treated individuals. For example, echinocandin resistance is more common in individuals previously treated with echinocandins⁸⁴, and azole-resistant genotypes of *Cryptococcus neoformans*⁸⁵ and *A. fumigatus*¹³ develop during long courses of treatment. For antifungal drugs to be effective, they must reach the site of infection. Each individual antifungal drug has vastly different absorption, distribution, metabolism and excretion (pharmacokinetic) properties, and even more pronounced are the differences amongst drugs in their tissue-specific penetration. Persistently low, or transiently high, drug concentrations may accelerate the evolution of resistance. However, using overly high doses of drugs carries an attendant risk of toxicity. For these reasons, regular therapeutic drug monitoring is required to optimize the dosage to maximize therapeutic potential, and to minimize the evolution of resistance whilst minimizing adverse reactions. Tissue-specific pharmacokinetics are largely unknown, although physiologically based modelling approaches have begun to shed some light on this issue^{86–89}. Real-world studies are increasingly using therapeutic drug monitoring to explore pharmacokinetics across clinical cohorts, for example monitoring of individuals with cystic fibrosis has demonstrated a high prevalence of subtherapeutic levels of azoles alongside a high probability (>20%) of developing a resistant infection after 2 years⁹⁰. For these reasons, better implementation of therapeutic drug monitoring through antifungal stewardship programmes is needed in susceptible patient cohorts. In tandem, the informed application of drug combinations may circumvent drug resistance. For example, micafungin inhibits several human and fungal efflux pumps, and thus when combined with drugs such as azoles may enhance their intracellular retention and efficacy.

Future studies will need to identify the likelihood with which resistance and tolerance mechanisms emerge. Pharmacometric approaches allow the simulation of model predictions⁹¹, and, for example, the hollow fibre model uses available pharmacokinetic data to mimic the human pharmacokinetics of antimicrobials⁹². Moreover, drug delivery at the site of infection remains a challenge due to extensive necrosis resulting in poor

Therapeutic drug monitoring

The pharmacological practice of measuring drug concentrations at specific intervals in order to optimize individual dosage regimens.

outcomes. For diseases where drug penetration at the site of infection is poor, improved pharmacodynamic models are needed to optimize dosing regimens and prevent treatment failure. Together, twinned pharmacokinetic/pharmacodynamic approaches could facilitate integrative, dynamic studies of the interplay between (unbound) drug concentrations, pathogen growth and kill kinetics in order to identify conditions that minimize the evolution of antifungal resistance *in situ*⁹³.

Nurturing new therapeutic directions. An obvious solution to the allied problems of limited classes of drugs that may be compromised by dual use is to accelerate drug development. However, this is not a solution that can be achieved rapidly as it takes around 5–7 years from first initiation in human trials to approval of a novel anti-infective^{94,95} and can cost hundreds of millions of dollars. Timescales and costs are much higher if early development costs are accounted for. For instance, the development programme for Cresemba (isavuconazole), developed by Basilea Pharmaceutica and, subsequently, Astellas Pharma and Pfizer, took 13 years and required circa US\$100 million of funding, with further downstream post-approval costs of circa US\$30 million. Although isavuconazole has a broader spectrum than voriconazole, including efficacy against the Mucorales, and was similarly effective in patients with invasive aspergillosis with fewer drug-related adverse events than voriconazole⁹⁶, the drug still shows cross-resistance to other azoles in both *Aspergillus* and *Candida* spp.⁹⁷. The drug discovery company F2G Ltd is developing olorofim, a new mode of action (MOA) antifungal that targets dihydroorotate dehydrogenase, which has required several rounds of investment totalling more than US\$213 million since their incorporation in 1998. The total time for F2G Ltd to identify the initial compound and develop their lead to phase II trials has been around 23 years. Although olorofim is not active against *Candida* spp., the drug shows promising activity against *Aspergillus* spp., including isolates with acquired azole resistance and other difficult to treat moulds such as *Lomentospora prolificans*⁹⁸. These examples highlight the investment and risk associated with identifying and developing a novel class of antifungal drug.

These high costs and protracted timescales have clear implications with respect to developing therapies to treat IFDs caused by antifungal-resistant species, most of which are relatively rare and are unlikely to provide a significant return on investment. Novel therapies to treat such diseases are likely to appear only as adjuncts of broad-spectrum antifungals that have been progressed primarily to treat more common fungal diseases. A key question then arises of what market size is sufficient to make an antifungal development project viable. One answer may lie with the development of the promising fungal cell wall chitin-synthase inhibitor Nikkomycin Z⁹⁹, which stalled after an apparently successful phase I trial¹⁰⁰. The developers, Valley Fever Solutions Inc., have to date been unable to secure investment to develop the compound further. This may well be related to the limited spectrum of activity

of Nikkomycin Z that is most active against relatively rare endemic mycoses such as *Coccidioides* spp., which in turn only have a patient population of circa 25,000 (REF.¹) and predicted peak sales of US\$130 million per annum. Even though a large proportion of these infections occur in the United States, investors have until now considered this market size to be too small even though Nikkomycin Z had support from governmental initiatives such as orphan drug designation and fast-track designation, and promising results in combination with other antifungals⁹⁹.

That the antifungal pipeline is experiencing a substantial boost suggests that the US\$13 billion global market for antifungals is encouraging the development of refined pre-existing compounds alongside new MOA antifungals that have a broad spectrum of activity. Of note, the Gwt1 inhibitor fosmanogepix (newly acquired by Pfizer), the (1 → 3)-β-D-glucan synthase inhibitor ibrexafungerp (Scynexis) and olorofim (F2G Ltd) are all new MOA antifungals that will open opportunities for treating azole-resistant or echinocandin-resistant pathogens (Supplementary Fig. 1). Other new MOA antifungals under development have intracellular targets, and thus are likely to be effective against isolates that are resistant to the existing drug classes.

In addition to novel drugs that are systemically given, new strategies for delivering antifungal drugs to the site of action are currently being explored. Opelconazole (Pulmocide), a reformulated azole drug administered by nebulization, has been evaluated for treating invasive aspergillosis in a phase I trial. Owing to the far higher drug concentrations that can be achieved in the lung, local application may overcome azole resistance in *A. fumigatus*. The useful life of an anti-infective relative to the potential rate of resistance emergence needs to be considered with the next generations of antifungals. Therefore, estimated evolutionary risks of resistance for new antifungals should be determined at the earliest possible stage of development, as has been advocated for antibacterial pipelines¹⁰¹. Chronic aspergillosis and acute candidiasis models or *in vitro* systems that better replicate the *in vivo* environment are recommended for monitoring the potential for the development of resistance *in vivo*, both for the target organism and for commensal fungi at the site of infection and distant body sites. Combining these *in vivo* models with pharmacokinetic/pharmacodynamic models could facilitate dosing studies estimating the likelihood of resistance emerging and minimizing the emergence of resistance, fungal persistence and tolerance.

Use of the same drug class in agriculture and medicine is a key driver for environmental drug resistance in *Aspergillus* spp. Removing azoles from agriculture is not trivial nor practical, as it would have a significant effect on global food production. Yet azole resistance in plant pathogens is emerging rapidly in agricultural settings. So what is the future of antifungal development with One Health in mind? Clearly, the development of fungicides for agriculture and antifungals for pharma needs to diverge⁴. In agriculture, this could be achieved by developing integrated disease management in crops, including ‘evolution-smart’ disease-resistant crops with mosaics

of pathogen resistance genes alongside, for instance, the development of species-specific novel antifungal treatments based on RNA interference¹⁰². Approaches that focus on targets that are crucial for pathogenicity in plants but are different to those in humans may also lead to diverging methods of controlling fungal pathogens. Towards this end, significant technological strides have been made to enable high-throughput identification of virulence determinants by combining functional genomics and next-generation sequencing^{103,104}. Undoubtedly, accelerated development of diverse, differentiated and ring-fenced antifungal pipelines for both agribusiness and pharma are not only the key to developing new fungicidal compounds but are also key to addressing evolving antifungal resistance in the coming years.

Current and future interventions

How can we stem the tide of emerging antifungal resistance? Integrating the ‘pillars’ of the JPIAMR and WHO initiatives will protect and augment our ability to treat

IFDs (FIG. 4). Currently available strategies to limit the evolution of human fungal pathogens to chemical control include boosting surveillance and antifungal stewardship programmes, both of which require improved diagnosis of IFDs and antifungal resistance; minimizing environmental–clinical dual usage of antifungals; and optimizing resilient combination therapies using existing licensed drugs. Future strategies to lessen the impact of antifungal resistance largely require treating at-risk individuals with novel antifungal compounds patented solely for clinical use. This ‘personalized medicine’ approach should include reducing the risk of acquired IFDs by addressing the weakened immunity that predisposes individuals to these diseases, by employing immunotherapies and/or vaccines against IFDs.

Widespread prophylactic and empiric prescribing of antifungals to treat suspected IFDs in individuals who are chronically at risk (for example, individuals with cystic fibrosis), those who are critically ill and patients with haemato-oncology remains a concern. Effective antifungal stewardship is required to optimize antifungal

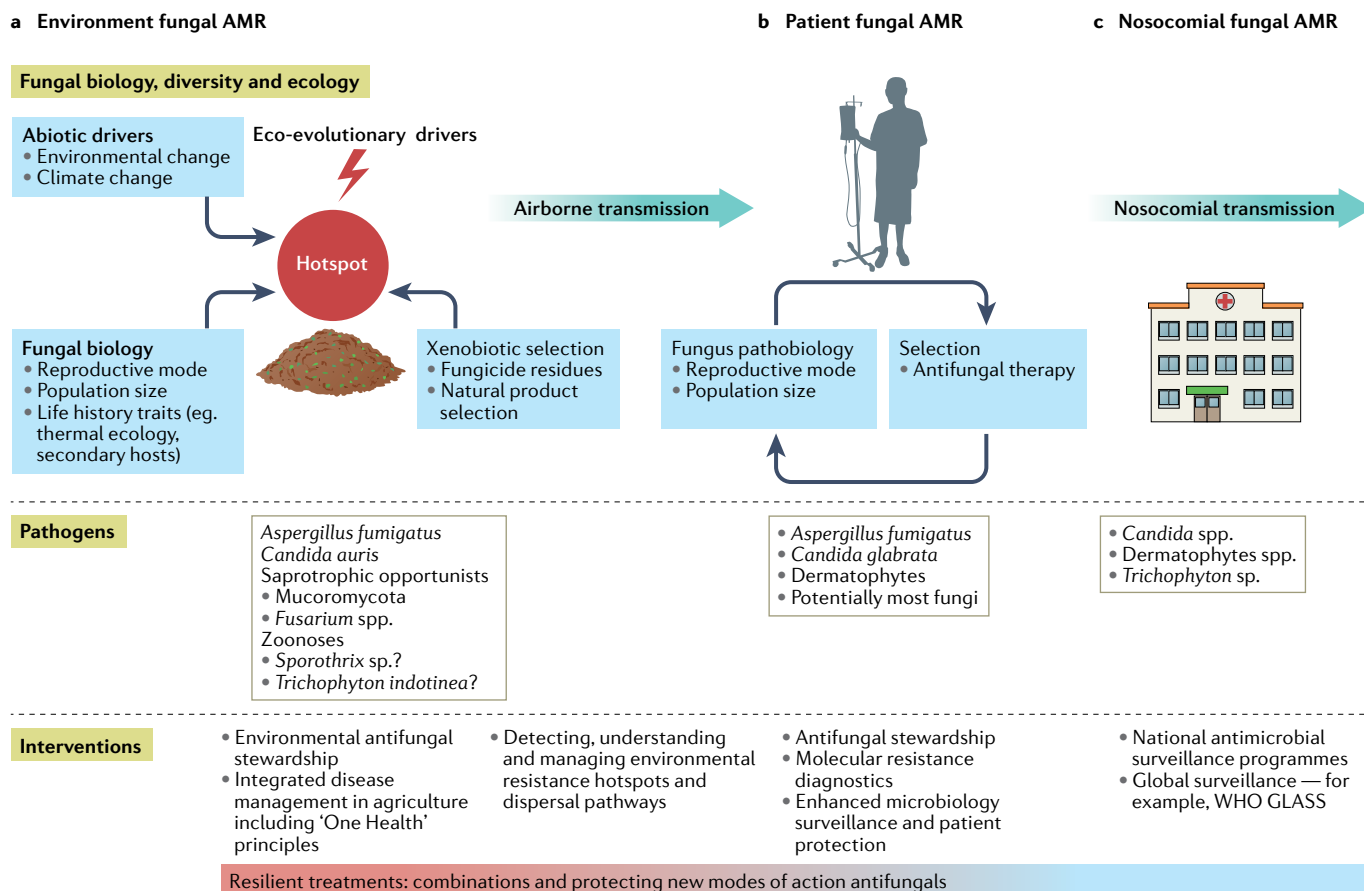


Fig. 4 | Interventions for invasive fungal infections within the landscape of antifungal resistance. Synoptic integrated One Health understanding is necessary to understand not only the complex multifactorial pathways that lead to the emergence of resistance across the fungal kingdom but also potential interventions to mitigate the rate of emergence. **a** | Complex biotic and abiotic interactions lead to occurrence of evolutionary hotspots for antimicrobial resistance (AMR) development in environmental opportunistic fungi requiring targeted interventions in the environment. **b,c** | Patient exposures to environmental AMR require enhanced methods

of detection with more focus on key fungal life-history factors (part **b**), and new and emerging drug-resistant fungal pathogens that have the potential for global nosocomial carriage and outbreaks in health-care settings require transnational surveillance (part **c**). A cross-cutting theme is the need for industry to separate development and use of agricultural fungicides from those antifungals that are used in the clinic to develop treatments that are resilient to the evolutionary forces at play in parts **a–c**. GLASS, Global Antimicrobial Resistance Surveillance System; WHO, World Health Organization.

use and to preserve the limited antifungal arsenal^{105,106}. This is especially relevant for fungal infections that are highly transmissible, such as *Candida* spp. and skin-infecting *Trichophyton* spp.¹⁰⁷.

In largely single-centre, historic cohort observational (non-randomized) studies, antifungal stewardship programmes have consistently demonstrated an improvement in measures such as timely and appropriate antifungal prescribing (guideline-driven), the use of diagnostics and drug monitoring as well as a reduction in antifungal consumption, reducing antifungal selective pressures and the development of resistance^{108–111}. Although such studies were not designed to demonstrate improved clinical outcomes, the absence of an adverse impact of antifungal stewardship implementation on the incidence of IFDs, length of hospital stay and in-hospital mortality are important findings¹¹². Antifungal stewardship is underpinned by access to timely and sensitive diagnostics, and although a review of various pre-emptive diagnostic versus empirical antifungal strategies confirmed the suitability of pre-emptive strategies, the optimal strategy and limits have not been defined¹¹³. Goals for future work include optimizing rapid diagnostic strategies for ‘early start–de-escalation–early stop’ antifungal strategies and better hospital infection control, as well as demonstrating the impact of antifungal stewardship on rates of antifungal resistance inpatient cohorts or the hospital environment.

Combination antimicrobial treatment is an established and effective strategy to prevent the development of secondary AMR for various bacterial and viral infections. The principle was established in the 1950s in the treatment of tuberculosis, and has been repeated, for example, for HIV treatment in the 1990s and for the treatment of hepatitis C virus more recently¹¹⁴. Combination therapies with amphotericin B plus flucytosine (or fluconazole plus flucytosine in settings where amphotericin B is not available) are the established standard of care in cryptococcosis¹¹⁵. Combining flucytosine and fluconazole can prevent the selection of fluconazole hetero-resistant fungal populations that occur in individuals with cryptococcal meningitis following initial treatment with fluconazole monotherapy¹¹⁵. In terms of primary, environmentally derived, antifungal resistance, combination treatment of patients may have a limited effect, but combinations could reduce treatment failure due to primary resistance and limit the development of secondary, clinical antifungal resistance. Combination treatments may be additive or synergistic in terms of antimicrobial efficacy, and further work is needed to further their potential in a wide range of life-threatening fungal infections.

For invasive aspergillosis, consistent in vitro and animal model data both suggest that combining azole and echinocandin classes increases fungal killing and improves survival^{116–118}. In a randomized clinical trial, mortality in those given this combination was 19% compared with 28% for those on azole monotherapy¹¹⁹; although the size of this study was limited, meaning the survival benefit did not reach conventional statistical

significance, the approach described is encouraging. Animal models suggest a role for combination therapy in azole-resistant invasive aspergillosis¹²⁰, but more work is needed to systematically explore combinations of established and new antifungal agents in experimental models and phase II clinical studies before moving to adequately powered phase III trials. In comparison with opportunistic fungal pathogens, *C. auris* can persist and spread within intensive care units and other health-care settings, leading to severe and intractable nosocomial outbreaks. Echinocandin monotherapy is commonly used to treat patients with *C. auris*, which is generally resistant to fluconazole. As this approach may facilitate the evolution and spread of multidrug-resistant isolates¹⁶, combination therapy strategies must be evaluated systematically to mitigate risk in this now globalized fungus.

Other approaches to protect existing antifungals include exploiting host-directed approaches to manage antifungal resistance. These include immunotherapy¹²¹, fungal vaccines¹²² and antibodies to fungal targets¹²³. Because IFDs are most common in immunocompromised hosts, host-directed immunotherapies, including recombinant cytokines, monoclonal antibodies and fungus-specific engineered T cells¹²¹, have been in development. The use of interferon- γ to prevent and treat invasive aspergillosis in patients with chronic granulomatous disease was the first successful host-directed antifungal immunotherapy¹²⁴. Since then, patient case series describing successful use of the TLR7 agonist imiquimod in chromoblastomycosis¹²⁵ and granulocyte-macrophage colony-stimulating factor (GM-CSF) therapy for central nervous system candidiasis associated with CARD9 deficiency¹²⁶ have been reported. These advances highlight the potential for host-directed approaches to lessen the pressure on antifungal drugs. Moreover, cell-based therapies, including dendritic cell transfer and chimeric antigen receptor (CAR) T cell therapy, have shown promising results in vitro¹²⁷ but require evaluation in clinical trials.

The combination of immunotherapeutics with conventional antifungal therapy also holds promise. Numerous candidate fungal vaccines have been studied in the preclinical setting¹²², but only the *C. albicans* recombinant Als3 protein vaccine has shown promising results in phase II clinical trials¹²⁸. Advancing antifungal vaccines will require overcoming several hurdles, especially the ubiquitous nature of fungi in the human holobiont¹²⁹, and the expected suboptimal immune response in those people most at risk for IFDs¹³⁰. Also showing promise are antibodies and fungal pattern recognition receptors that potentially target antifungal agents for pathogen delivery¹²³. Preclinical studies of dectin-2 coupled to liposomal amphotericin B have shown encouraging results in experimental pulmonary aspergillosis¹²³ and may help reduce antifungal toxicity in the host. However, although host-directed antifungal strategies, alone or in combination with conventional antifungals, hold immense promise, furthering and financing these novel strategies from the laboratory to clinical trials will be a significant challenge in the coming decade.

Conclusions

Challenges to a clinician's ability to manage drug-resistant IFDs today include the lack of access to sensitive and specific diagnostic tests, the lack of clinically calibrated antifungal susceptibility testing and a limited repertoire of antifungal drug classes. Furthermore, the breadth and diversity of the fungal kingdom ensures a bottomless reservoir of new pathogens, alongside endless supplies of variants of old enemies, that readily adapt and evolve when exposed to antifungal chemicals. The sheer ecological breadth of fungal species, with their unique and varied ecological trophisms, in rapidly changing environments means that human health will always be enmeshed with the complex ecology of fungal communities, whether

commensal or environmental. Similarly, our simultaneous need to control fungal disease in agricultural environments and the clinic means that integrated responses take these needs into consideration. Pathogenic fungi are widely vectored both actively and passively, such that tackling antifungal resistance both in the clinic and in the field requires a coordinated global response. The current lack of transnational support for networks, infrastructures, research funding and career development must be addressed through greater coordination between policy-makers, funding agencies and researchers, and include the producers and users of antifungals.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

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