Antifungal resistance: Emerging mechanisms and implications (Review)

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Received March 5, 2025; Accepted June 11, 2025

DOI: 10.3892/mmr.2025.13612

Abstract. Antifungal resistance is a growing concern in clinical medicine, driven by the increasing incidence of fungal infections and the limited arsenal of effective antifungal drugs. This resistance is achieved by intrinsic mechanisms, such as ineffective drug-target binding, high efflux pump activity and unique cell wall and membrane composition, as well as acquired mechanisms, including genetic mutations, gene duplication, transposon insertions, aneuploidies and loss of heterozygosity. Antifungal tolerance, characterized by subpopulations of fungal cells that persist and proliferate even at high drug concentrations, complicates treatment. The present review aimed to examine the genetic, physiological and epigenetic factors that contribute to antifungal resistance and tolerance. Understanding these mechanisms may enable the development of novel antifungal therapies and effective diagnostic strategies to combat the increasing threat of resistant fungal infection. Advanced diagnostic tools and combination therapies are key for managing resistant infections and ongoing research into these mechanisms may enhance the ability to mitigate antifungal resistance.

Contents

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Key words: antifungal, resistance, epigenetic, infectious disease

1. Introduction

Antifungal resistance is an increasingly prominent issue in clinical medicine, primarily because of the rising incidence of fungal infection and limited arsenal of effective antifungal drugs. For example, the estimated global burden of invasive aspergillosis has risen from 250,000 in 2012 to 2.1 million in 2021, with only four classes of systemic antifungal are available-polyenes, azoles, echinocandins, and pyrimidine analogues (1-3). In 2020, diseases caused by fungi affected >1 billion individuals worldwide with 1.7 million death, comparable with the mortality caused by tuberculosis or human immunodeficiency virus (HIV) infection (1-4). Antifungal resistance is defined as the ability of fungal species to proliferate even in the presence of antifungal drug concentrations that would typically inhibit or kill the majority of isolates (4). Antifungal resistance leads to failure of treatment, prolonged infection and increased healthcare costs and morbidity and mortality rates (4). The prevalence of severe fungal infection has increased owing to the increasing population of immunocompromised individuals, such as those with diabetes, advanced cancer, HIV and old age (5).

Resistance in fungi can be intrinsic or acquired. Intrinsic resistance is a natural characteristic of certain fungal species, where antifungal agents are inherently ineffective due to pre-existing genetic traits (6) (for example, the intrinsic resistance of *Cryptococcus* species to echinocandins) (7). Species such as *Aspergillus* pluralis (spp)., *Pichia kudriavzevii* (formerly *Candida krusei*), *Nakaseomyces glabratus*

(formerly *C. glabrata*) and certain strains of *C. auris* exhibit intrinsic resistance to fluconazole without prior exposure to the drug (8,9). By contrast, acquired resistance arises through genetic mutations and adaptations in response to antifungal exposure, allowing previously susceptible fungi to survive under drug pressure (2).

Antifungal tolerance further compounds the complexity of antifungal resistance. Antifungal tolerance involves a subpopulation of fungal cells that persist and proliferate slowly at drug concentrations higher than the minimum inhibitory concentration (MIC) (6). This tolerance can lead to treatment failure and is often mistakenly equated with resistance in the literature, although it represents a distinct adaptive strategy (10-12).

The present review summarizes current antifungal resistance and tolerance mechanisms, highlighting the genetic, physiological and epigenetic factors involved. Examining classes of mutations and their roles in the development of resistance may demonstrate how fungi adapt to antifungal pressures. Additionally, the present review aimed to explore the specific challenges and clinical implications of different types of resistance, particularly in the context of diagnosis and therapeutic limitations.

While previous reviews have addressed either genetic alterations, such as point mutations and aneuploidy (13-15), or epigenetic modulation through chromatin remodelling and histone modification (16,17), to the best of our knowledge, an integrated approach has not been performed. The present review aims to present a unified framework of both genetic and epigenetic drivers of antifungal resistance. Furthermore, the present review aimed to offer a translational perspective by linking these mechanistic insights with therapeutic strategies, including the use of histone deacetylase (HDAC) inhibitors, antifungal-loaded nanoparticles and stewardship approaches that aims to optimize treatment selection, dose and duration to minimize resistance. Understanding these mechanisms in an integrated manner is key for developing novel antifungal therapy and devising effective strategies to combat the increasing threat of drug-resistant fungal infection.

2. Intrinsic resistance

In fungi, intrinsic resistance refers to the innate ability of certain species to withstand the effects of specific antifungal agents without prior exposure or adaptation (18,19). This form of resistance is encoded within the genetic framework, making the species naturally less susceptible or entirely resistant to antifungal drugs (18,19). Several mechanisms underly intrinsic resistance (Table I).

Ineffective drug-target binding affinity. A key intrinsic resistance mechanism is the ineffective binding of antifungal drugs to targets. For example, variations in the structure of the enzyme sterol 14α-demethylases (SDMs) in certain fungi result in decreased binding affinity for azole antifungals, rendering these drugs ineffective (20). SDM is a key enzyme in fungal ergosterol biosynthesis. SDM catalyses the conversion of lanosterol or eburicol to a 14α-demethylated product (20). The binding of azole antifungals to SDM depletes ergosterol and decreases the integrity of the lipid bilayer of the fungal membrane (20). Certain amino acid (AA) substitutions in the ligand-binding pocket of SDMs decrease the binding of azole antifungals. For example, two natural AA substitutions, F129 and V291, in *Mucormycota* SDMs cause intrinsic resistance to short-tail azoles such as fluconazole and voriconazole (21). The T301I substitution in the SDM of *A. fumigatus* causes intrinsic resistance to fluconazole (22).

Efflux pump activity. Efflux pump activity is a key mechanism of intrinsic resistance. Drugs need to reach a certain level in their cellular targets. Intrinsically resistant fungi exhibit high efflux pump activity, which actively expels antifungal agents from the cell, decreasing their intracellular concentrations and diminishing their efficacy (23). A common efflux system in fungi involves the ATP-binding cassette (ABC) superfamily and major facilitator superfamily (MFS) (23). Fungi express multiple ABCs that are key to their physiology, particularly for the expulsion of toxic metabolites. For example, *N. glabratus* has 18 ABC-encoding genomes and *A. fumigatus* has 50 genomes (24,25). In *C. auris*, four efflux pump encoding genes, Subsequent to Nucleotide Q21 (*SNQ21),* Subsequent to Nucleotide Q22 (*SNQ22),* Multidrug Resistance 1 *(MDR1)* and Candida Drug Resistance 1 (*CDR1)*, contribute to fluconazole resistance (26).

Cell wall and membrane composition changes. Fungal cell wall and membrane composition serve key roles in intrinsic resistance. For example, variations in sterol content affect the binding and efficacy of polyene antifungals such as amphotericin B. Environmental molds such as those from *Mucoromycota* and *Lomentospora* spp. have unique cell wall structures that limit the penetration and action of azole antifungals, thereby contributing to their intrinsic resistance (21). Mucoromycota phyla such as *Rhizopus* and *Mucor* species have alternative sterol synthesis pathways (21). Duplication of the gene encoding FK506-sensitivity gene (FKS) synthetase, which synthesizes the cell wall component (1,3)-d-glucan, is responsible for the intrinsic resistance of *Mucor circinelloides* to micafungin (27). *Fusarium* species also exhibit resistance to several antifungal agents, including azoles, because of their unique cell wall structures, which impede drug penetration, in addition to their robust efflux pump systems (28).

The intrinsic resistance of fungal species highlights the need to develop novel antifungal agents that bypass the resistance mechanisms inherent to these fungi. Additionally, the accurate and timely identification of intrinsically resistant fungal species is key for effective treatment, emphasizing the need for advanced diagnostic tools to identify resistance profiles. Some therapeutic strategies can be employed using alternative drugs or combining them with efflux pump inhibitors or other antifungals that target cell walls.

3. Antifungal tolerance

Tolerance is a phenomenon distinct from resistance (Table II). Antifungal tolerance refers to the ability of a subpopulation of fungal cells to survive and proliferate in the presence of antifungal agents at concentrations exceeding the established MIC, without exhibiting permanent resistance mechanisms. This tolerance is hypothesized to arise through genetic, physiological or epigenetic adaptations to drugs (29). By contrast, antifungal

Table I. Characteristics and implications of mechanisms of intrinsic resistance.

Resistance mechanism	Mechanism	Genetic basis	Examples	Drug resistance	Therapeutic challenges	Clinical implications	Treatment strategy
Ineffective drug target binding	Mutation or natural variation in drug target protein	Mutation affecting drug target sites	T3011 substitution in the SDM of *Aspergillus fumigatus*; *Pichia kudriavzevii*; *C. auris*	Azoles (such as fluconazole and voriconazole)	Developing drugs that effectively bind altered targets	Decreased efficacy of standard antifungal drugs	Alternative drugs; higher dose
Increased efflux pump activity	Upregulation of efflux pump genes	Upregulation of efflux genes	SNQ21/22, MDR1 and CDR1 upregulation in *C. auris*	Multiple classes; azoles	Overcoming high efflux activity via combination therapy	Multidrug resistance, requiring combination therapy	Efflux pump inhibitor; combination therapy
Altered cell wall/membrane composition	Altered cell wall/membrane composition/ structure	Gene variations affecting cell wall/membrane synthesis	Altered sterol composition in *Mucoromycota*; *Aspergillus* spp.	Azoles; polyenes	Developing agents that penetrate/ disrupt the cell wall/membrane	Increased difficulty in treating biofilm-associated infection because of decreased drug binding or uptake	Combination therapy; drugs targeting the cell wall/membrane

SDM, sterol 14α-demethylase; spp, species pluralis

resistance involves heritable genomic changes that confer a permanent inability to be effectively treated by antifungal agents, typically manifesting as increased MICs (30-32).

However, in practice, tolerance and resistance are not always distinguishable. The MIC within the same fungal species can vary and only strains with considerably different MICs have been recognized as resistant in several studies (33-35). Clinically, patients with infection caused by tolerant strains may initially respond to antifungal therapy but experience a resurgence of symptoms or persistent infection despite appropriate drug levels. This is typically due to the survival of a subpopulation of tolerant cells that can proliferate when drug concentrations fall below the effective levels (12,32,36). By contrast, patients infected with resistant strains typically do not respond to standard antifungal treatments from the outset, as these strains possess inherent mechanisms that allow them to survive at therapeutic concentrations (32,36). Therefore, resistant strains often require higher doses or combination therapy, which can increase the risks of drug toxicity and side effects. Clinicians may need to switch to second-line or less commonly used antifungal agents that may be less effective or have more adverse effects. However, tolerance may require different treatment approaches compared with resistance, such as prolonging the treatment duration to ensure that the surviving fungal cells are eradicated without switching to a second-line treatment.

Genetic analysis and time-kill assays may identify specific mutations associated with resistance, whereas tolerance may not be associated with stable genetic changes but rather transient stress responses (30,37). For example, the presence of mutations in genes associated with drug targets or efflux pumps may indicate resistance, whereas the expression of stress response genes may suggest tolerance (32). In time-kill assays, fungal cells are exposed to antifungal agents over time, and viability is assessed at intervals. A characteristic of tolerance is that cells may initially survive at high drug concentrations but eventually succumb to treatment, whereas resistant strains continue to proliferate despite the presence of the drug (12,38). This method can reveal the dynamics of fungal survival and proliferation in response to antifungal exposure, which may differentiate between transient tolerance and stable resistance.

4. Acquired resistance

Acquired resistance in fungi refers to the development of resistance mechanisms that enable previously susceptible fungal species to proliferate despite the presence of antifungal drug concentrations that would typically inhibit or kill them. Unlike intrinsic resistance, which is a natural trait of certain species, acquired resistance results from genetic mutations and adaptations that occur in response to antifungal exposure. This resistance poses a considerable challenge in the clinical setting, leading to treatment failure. The development of acquired resistance in fungi involves genetic mechanisms, including point mutations, gene duplication, transposon insertions, aneuploidy and loss of heterozygosity (LOH) (39). In addition to genetic mechanisms, epigenetic mechanisms and phenotypical resistance are also involved. These mechanisms can alter drug targets, enhance drug efflux or modify cellular pathways to decrease the efficacy of antifungal agents (39).

Table II. Characteristics of intrinsic and acquired resistance and antifungal tolerance.

Antifungal mechanism	Definition	Genetic basis	Occurrence	Mechanism	Examples	Clinical impact	Adaptation speed	Fitness cost	Diagnostic challenges	Management
Intrinsic resistance	Natural ability to resist antifungal agents without prior exposure	Encoded in the genome	All members of a species	Ineffective drug binding, enhanced efflux pump activity, unique cell wall/membrane composition	*C. krusei* resistance to fluconazole and *Aspergillus* spp and resistance to fluconazole	Limits effective treatment options	Inherent	Low	Requires species identification	Alternative antifungal agents
Tolerance	Ability of a cell subpopulation to grow slowly at drug concentrations >MIC	Arises through potentially unstable genetic, physiological or epigenetic modification	Subpopulations of susceptible isolates	Transient or non-stable genetic mutations along with epigenetic changes and physiological adaptations	*C. albicans* subpopulations proliferate at high fluconazole concentrations	Persistent infection	Relatively fast under drug pressure	Low, allowing sub-populations to persist	Requires identification of tolerant isolate sub-populations	Adjusted treatment regimen/dose/duration
Acquired resistance	Development of resistance in response to antifungal exposure	Result of genetic mutation and adaptation	Cells exposure to antifungal drugs	Point mutation, gene duplication, transposon insertion, aneuploidy, loss of heterozygosity	*C. albicans* mutations in *ERG11* and *FKS* genes and *Nakaseomyces glabratus* hypermutator lineages	Treatment failure, require alternative therapy	Develops with drug exposure	Varied, typically modest	Requires detection of resistance mutations	Combination therapy, novel antifungal agents

MIC, minimum inhibitory concentration; FKS, FK506-sensitivity gene. Fitness cost refers to the reduced growth rate or survival of resistant fungal strains in the absence of antifungal pressure.

Acquired resistance in fungi has clinical implications, particularly for the treatment of invasive fungal infection. The emergence of drug-resistant strains can lead to treatment failure, prolonged infection and increased healthcare costs (39). The development of acquired resistance can lead to the failure of standard antifungal therapy, necessitating alternative agents that may be less effective or have a greater number of adverse side effects (39). Infection caused by resistant fungal strains is associated with high morbidity and mortality rates because of the limited effectiveness of available antifungal agents (39,40). For example, a national-scale multi-centre cohort study in the Netherlands revealed that infection by azole-resistant *A. fumigatus* increased mortality by 20-30% compared with azole-susceptible infection (40).

5. Genetic mechanisms of acquired resistance

Antifungal resistance is a complex and multifaceted phenomenon driven by genetic mechanisms that enable organisms to survive and proliferate despite antifungal treatments. These mechanisms include a range of genetic alterations such as point mutations, gene duplications, transposon insertion, aneuploidy, LOH and hypermutator lineages. Each mechanism contributes to resistance by modifying specific genes or regulatory pathways, enhancing the ability of fungi to withstand antifungal agents and complicating diagnosis and treatment (Table III).

Point mutations. Point mutations are a key genetic mechanism through which fungi acquire resistance to antifungal agents. Point mutations occur at a rate of $1x10^{-6}$-$1x10^{-8}$/cell/generation and involve changes in a single nucleotide base pair within the DNA sequence (41,42). Such alterations lead to modifications in the structure or function of the target protein, thereby diminishing the efficacy of antifungal drugs. Point mutations have clinical implications as they often result in resistance to commonly used antifungal agents, complicating the management of fungal infection.

Point mutations can result in changes in the genetic code, including missense, nonsense and silent mutations. Missense mutations, which result in the substitution of one AA with another in the protein product, alter the structure of the target enzyme or protein, thereby affecting its interaction with antifungal drugs (43,44). Nonsense mutations introduce a premature stop codon into the DNA sequence, producing a truncated and often non-functional protein (43,44). Although silent mutations do not change the AA sequence of a protein, they affect protein folding or stability, potentially influencing drug susceptibility (43,44).

Point mutations confer resistance to antifungal agents in clinically relevant fungal species. One example is the *ERG11* gene in *C. albicans* (Fig. 1A). The *ERG11* gene encodes 14α-SDM, an enzyme targeted by azole antifungals such as fluconazole (45). Point mutations in *ERG11* lead to AA substitutions that reduce its binding affinity for azole drugs, thereby conferring drug resistance (45). For example, the Y132H mutation in *ERG11*, which changes a tyrosine to a histidine at position 132, alters the structure of the enzyme and decreases drug binding, resulting in fluconazole resistance (45). Other point mutations in the *ERG11* gene of *C. albicans* (including *K143Q, Y205E, A255V, E260V, N435V, G472R* and *D502E*) decrease the ability of fluconazole and voriconazole to inhibit the drug target (45).

Similarly, mutations in the *FKS* genes, which encode subunits of β-1,3-D-glucan synthase, lead to decreased susceptibility to echinocandin antifungals. In *N. glabratus*, the S629P mutation in *FKS2*, in which serine is replaced by proline at position 629, affects the interaction of the enzyme with the drug, thus conferring resistance to echinocandins (46). In *A. fumigatus*, mutations, such as S678P, in *FKS1* confer resistance by altering the drug-binding site of the enzyme (47). In *C. albicans*, mutations in the gene encoding FKS synthetase, the target of echinocandins, decrease susceptibility to these antifungal agents (48). Another example is the point mutation in the *CYP51A* gene of *A. fumigatus*. Point mutations in *CYP51A* lead to azole resistance, particularly to itraconazole (49). The *G54E* mutation, which changes glycine to glutamic acid at position 54, markedly decreases the efficacy of itraconazole and other azoles by altering enzyme structure (49).

Among resistance mechanisms, point mutations that target genes, particularly ERG11 and FKS1/FKS2, are the most clinically relevant. These mutations directly impair drug-target binding, resulting in resistance (46,47). Their genomic stability ensures resistance persists in the absence of antifungal pressure. Clinically, such mutations are among the most frequently observed in treatment-refractory infection and are often associated with elevated MICs that render standard antifungal regimens ineffective (45-48,50,51). Consequently, infection caused by fungi harbouring these mutations is harder to treat and requires alternative or combination therapies, highlighting the serious clinical impact.

Gene duplication and transposon insertions. Gene duplication and transposon insertion are key genetic mechanisms that contribute to antifungal resistance. These alterations lead to the increased expression of resistance genes or disruption of regulatory pathways that control drug susceptibility, thereby enhancing the ability of fungi to survive in the presence of antifungal agents. These mechanisms occur at a relatively high frequency of $1x10^{-3}$-$1x10^{-4}$/cell/generation, making them notable drivers of resistance in fungal populations (41,42).

Gene duplication involves the creation of one or more copies of a gene within the genome, leading to the upregulation of genes that confer resistance to antifungal agents. Increased gene expression can enhance the production of proteins that degrade the antifungal drug, modify the drug target or expel the drug from the cell, thereby decreasing its efficacy. A notable example of gene duplication is the *MDR1* gene in *C. albicans* and *C. dubliniensis* (52,53). The *MDR1* gene encodes the MFS transporter involved in drug efflux. Duplication of the *MDR1* gene leads to the upregulation of the efflux pump, increasing the expulsion of azole antifungals such as fluconazole from the cell, thereby conferring resistance (52,53). Clinical isolates of *C. albicans* with duplicated *MDR1* genes exhibit high levels of resistance to fluconazole, the most commonly used antifungal (52,53). Similarly, duplication of the *ERG11* gene in *N. glabratus* (Fig. 1B), which encodes 14α-SDM, results in overproduction of the enzyme and is associated with resistance to multiple azole antifungals, making infections difficult to treat (54). An increased amount of the target enzyme can

Table III. Features and implications of genetic mechanisms of antifungal resistance.

Resistance mechanism	Definition	Genetic change	Mechanism	Impact on genes	Development rate	Examples	Effect on drug target	Diagnostic challenges	Management strategy	Fitness cost
Point mutation	Single nucleotide changes in DNA sequence	Altered base pairs	Missense, nonsense or silent mutation	Altered sequence and function	Moderate	*ERG11* mutations in *C. albicans* (azole resistance)	Alters target site to decrease drug binding	Detecting mutations	Alternative drugs targeting different pathways	Typically low to moderate
Gene duplication	Increased gene copy number	Increased gene dosage; overexpression of specific genes	Gene amplification	Upregulation of resistance-related genes	Rapid	Overexpression of *ERG11* or efflux pump genes in *Candida albicans*	Increases target protein or efflux pump levels	Detecting copy number variants	Combination therapy or targeting regulatory pathways	Low to moderate
Transposon insertion	Insertion of mobile genetic elements into the genome	Disruption or activation of genes by transposable elements	Transposition	Disruption or dysregulation of target/ regulatory genes	Rapid	*CNL1* transposon in *Cryptococcus neoformans* causing 5-flucytosine resistance	Alters expression of drug target or efflux pumps	Mapping insertion sites; gene expression profiling	Combination therapy to overcome adaptive resistance	Low to moderate
Aneuploidy	Gain or loss of chromosomes/ segments	Large-scale chromosomal alteration	Chromosome mis-segregation during mitosis	Gene upregulation on affected chromosome	Rapid	Chromosome 5 aneuploidy in *C. albicans* (fluconazole resistance)	Elevates expression of drug targets or resistance-associated genes due to increased chromosome copy number	Karyotyping for chromosomal changes is challenging due to its limited resolution, technical complexity and difficulty detecting subtle or transient chromosomal changes in fungi.	Combination therapy and novel antifungal agents	Varied
Loss of heterozygosity	Loss of one allele, resulting in homozygosity	Unmasking of recessive mutations	Mitotic recombination, gene conversion, deletion	Reveals mutations by dominant alleles	Moderate	*ERG11* and *TAC1* in *C. albicans* (azole resistance)	Unmasks mutations affecting drug targets or efflux pumps	Detection of loss of heterozygosity	Combination therapy, monitoring	Varied; typically moderate

Table III. Continued.

Resistance mechanism	Definition	Genetic change	Mechanism	Impact on genes	Development rate	Examples	Effect on drug target	Diagnostic challenges	Management strategy	Fitness cost
Hypermutator lineage	Elevated mutation rate due to DNA repair defects	Widespread genetic instability	Mutated DNA repair genes (such as MSH2)	Increase mutation rate across the genome	Rapid	*MSH2* mutations in *C. glabrata* increases resistance to azoles, echinocandins and polyenes	Widespread changes may affect multiple pathways	Identifying hypermutator phenotypes	Alternative treatments to bypass resistance mechanisms	Typically low

MSH2, MutS Homolog 2; TAC1, transcriptional activator of efflux pump CDR gene 1 .

sequester the drug, decreasing its inhibitory effect and leading to drug resistance.

Transposon insertions involve the movement of transposable elements or 'jumping genes', within the genome. These elements insert themselves into various genomic locations, disrupting genes or regulatory regions and leading to altered gene expression. Transposon insertions can enhance the expression of resistance genes or disrupt the genes involved in susceptibility to antifungal agents.

The clinical and public health importance of transposon insertions in antifungal resistance has been observed in *C. neoformans*, which causes 15% of HIV-associated mortalities worldwide (55). The standard treatment for *C. neoformans* combines rapamycin and FK506 (Fig. 1C) (56). Sequencing of drug target genes revealed *C. neoformans* LINE-1-like element (*CNL1*) transposon insertions in an isolate of *C. neoformans* are resistant to rapamycin and FK506 (56). Cnl1 transposon insertion can cause resistance to 5-flucytosine, which is the standard therapy for cryptococcal meningitis and encephalitis (56).

Aneuploidy. Aneuploidies, defined as the gain or loss of entire chromosomes or large chromosomal segments, are key contributors to antifungal resistance due to their ability to cause widespread changes in gene expression (57,58). Unlike point mutations or gene duplication, these large-scale genomic alterations can upregulate resistance genes or disrupt cell pathways, thereby enhancing fungal survival under drug pressure. Fungi exhibit high genomic plasticity, making aneuploidy a relatively frequent adaptive mechanism. It arises from errors in chromosomal segregation during cell division and typically results in increased expression of drug targets, efflux pumps or other resistance-associated genes (59). This enables rapid and reversible resistance during antifungal treatment.

One of the most studied examples of aneuploidy in antifungal resistance involves *C. albicans* (60-66). In *C. albicans*, fluconazole resistance is frequently associated with the formation of an isochromosome consisting of the left arm of chromosome 5, which contains the ERG11 gene (target of fluconazole) and transcriptional activator of efflux pump CDR gene 1 (*TAC1*) (60-62). This chromosomal alteration increases resistance by enhancing drug target abundance and efflux activity. Similarly, in *C. neoformans*, duplication of chromosome 1 is associated with fluconazole resistance through upregulation of ERG11 and azole fungal resistance 1 (*AFR1*), particularly under antifungal stress conditions (Fig. 1D) (67,68).

Aneuploidy in fungal pathogens has notable clinical implications, as it enables rapid gene expression changes that confer drug resistance and complicate treatment. This can lead to treatment failure, prolonged infection and the need for combination or novel antifungal therapy (69). Rapid acquisition of aneuploidy under antifungal pressure allows fungi to adapt quickly, increasing resistance risk during therapy. Therefore, advanced diagnostic techniques, such as karyotyping, comparative genomic hybridization and next-generation sequencing, are key for detecting aneuploidy, guiding treatment and monitoring resistance emergence.

Aneuploidy also helps fungi to mitigate decreased growth and survival due to the acquisition of antifungal resistance.

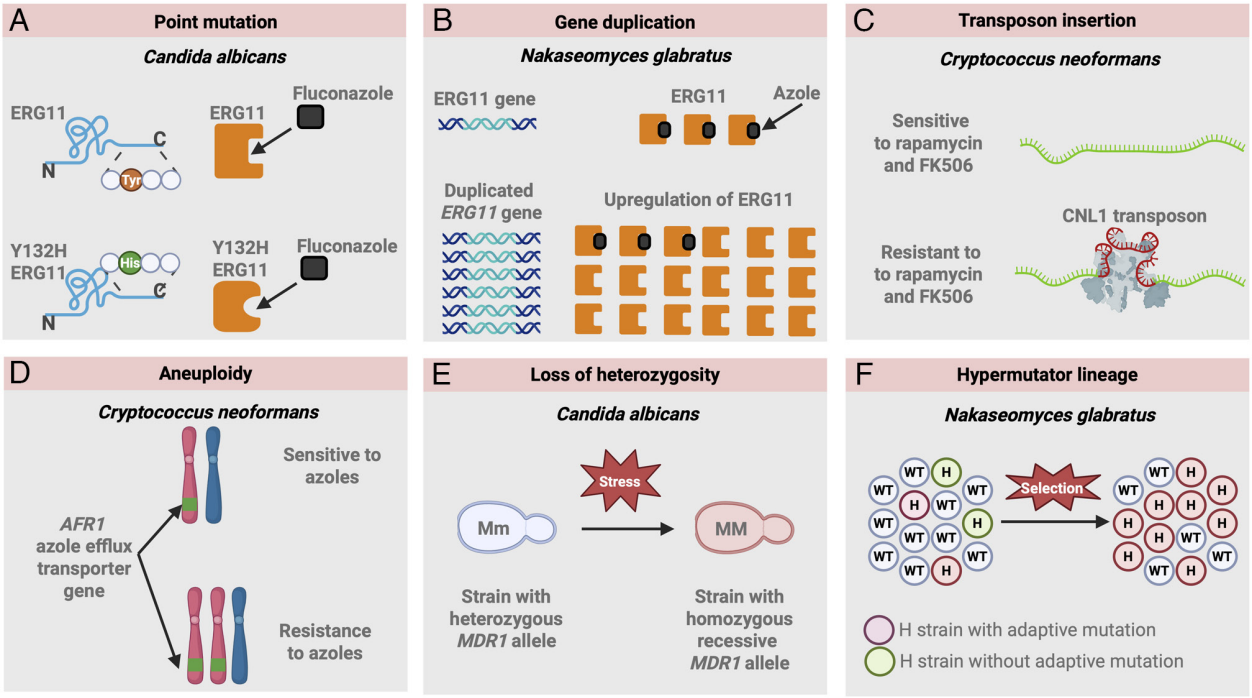

Figure 1. Genetic mechanisms of acquired antifungal resistance in pathogenic fungi. (A) Point mutation in *Candida albicans*. A single nucleotide substitution in the ERG11 gene (such as Y132H) leads to amino acid changes in lanosterol 14α-demethylase, decreasing azole binding and conferring fluconazole resistance. (B) Gene duplication in *Nakaseomyces glabratus*. Duplication of resistance-related genes, such as ERG11, leads to gene upregulation and increased tolerance to azoles. (C) Transposon insertion in *Cryptococcus neoformans*. Insertion of mobile genetic elements such as CNL1 transposon disrupts pathways involved in calcineurin signalling, contributing to resistance against calcineurin inhibitors (for example, FK506 and rapamycin). (D) LOH in *Candida albicans*. LOH converts a heterozygous MDR1 allele into a homozygous recessive state, unmasking resistance-conferring mutations and leading to azole resistance. (E) Aneuploidy in *Candida neoformans*. Duplication of chromosomes (such as those carrying AFR1, an azole efflux transporter gene) under antifungal stress leads to increased drug efflux and resistance. (F) Hypermutator lineage in *Nakaseomyces glabratus*. Defects in DNA repair machinery result in elevated mutation rates. Some hypermutator strains acquire adaptive mutations that confer resistance, while others do not. H, hypermutator; WT, wild-type; LOH, loss of heterozygosity; CNL1, *Cryptococcus neoformans* LINE-1-like element; MDR, Multidrug Resistance; AFR, azole fungal resistance. This figure was created using Biorender: https://BioRender.com/or7ka94"

Resistant strains frequently exhibit slower proliferation rates compared with wild-type strains under drug-free conditions. This has been observed, for example, in *C. albicans* with *ERG11* mutations, where the resistant strains demonstrate decreased proliferation rates and virulence in the absence of azole or echinocandin exposure (70). However, in certain cases, compensatory mutations or genomic adaptations through aneuploidy can partially or fully restore fitness, allowing resistant strains to proliferate at rates comparable with wild-type strains even without antifungal agents (63,65). For example, *C. albicans* strains exhibit distinct transcriptional profiles that lead to altered proliferation and oxidative balance relative to euploid strains (65). Acquisition of aneuploidy in *C. albicans* can lead to increased fitness during the evolution of antifungal drug resistance. Specifically, the formation of an isochromosome 5L, which amplifies the *ERG11* and *TAC1* genes, confers fluconazole resistance while also enhancing proliferation rates in the absence of the drug (63).

LOH. LOH is a key genetic mechanism underlying antifungal resistance. LOH refers to mitotic recombination in which one allele at a specific locus is lost, converting heterozygous into homozygous loci (71). Compared with other resistance mechanisms, LOH may occur at higher frequencies under antifungal pressure, particularly in diploid fungi, facilitating rapid adaptation by unmasking recessive resistance-conferring mutations (72). The baseline rates of LOH is $1x10^{-6}$-$1x10^{-7}$/cell division in *C. albicans*, but these rates can increase ≥285-fold, under antifungal stress such as fluconazole exposure, reaching frequencies of $1x10^{-3}$-$1x10^{-4}$ (72). These elevated rates are markedly higher compared with those of point mutations (~$1x10^{-9}$) and suggest that LOH is a key driver of rapid adaptation and antifungal resistance in diploid fungal pathogens (72). This genetic alteration can unmask the recessive mutations that confer resistance and enhance the ability of an organism to survive in the presence of antifungal agents. LOH can occur through numerous mechanisms, including mitotic recombination, gene conversion and chromosomal deletion (71). This process is particularly relevant in diploid organisms where heterozygous loci are common.

In diploid fungi, LOH can lead to the unmasking of resistance-conferring mutations that were hidden by a dominant wild-type allele. When a diploid organism loses one copy of a gene, the remaining allele is expressed (73). An organism exhibits a resistant phenotype if the retained allele carries a resistance mutation (73). This allows fungi to adapt quickly to antifungal pressures through advantageous mutations.

An example of the effect of LOH on antifungal resistance is the *MDR1* gene in *C. albicans* (Fig. 1E). The *MDR1* gene encodes an MFS transporter involved in drug efflux (74). LOH can lead to the loss of the wild-type allele and retention of a mutant *MDR1* allele, resulting in overexpression of

the efflux pump and enhanced resistance to azole antifungals (74). Similarly, the LOH in the Δ5,6-desaturase (*ERG3P*) gene in *C. albicans*, involved in the ergosterol biosynthesis pathway, causes resistance to amphotericin B (75). Mutations in *ERG3* alter the cell membrane composition and LOH can unmask these mutations, leading to decreased susceptibility to amphotericin B and posing treatment challenges (75). Another example is the LOH-mating-type-like (MTL) locus, which causes resistance to azole antifungal agents (76). Single nucleotide polymorphism (SNP) analysis in azole-resistant *C. albicans* isolates revealed that replacing an MTL locus with a duplicate of the other locus results in two homozygous copies of the MTL locus (76).

LOH of fungal pathogens has clinical implications. LOH can lead to rapid and substantial changes in gene expression and unmasking of resistance mutations that complicate treatment strategies. Advanced diagnostic tools such as comparative genomic hybridization and next-generation sequencing are key for identifying LOH events in clinical isolates, guiding appropriate antifungal therapy and monitoring the emergence of resistance. SNP and Southern blot analyses can also be used to detect common LOH.

Hypermutator lineage. Hypermutator lineages are a notable genetic mechanism contributing to antifungal resistance. Elevated mutation rates characterize these lineages due to defects in DNA repair mechanisms, particularly those involved in mismatch repair (MMR) (77). The increased mutation frequency enhances the genetic diversity within a fungal population, providing a larger pool of mutations from which resistance-conferring changes can arise (77). This mechanism is particularly concerning in clinical settings, as it can lead to the rapid development of resistance to multiple antifungal agents, complicating treatment.

One of the well-documented examples of the hypermutator lineage involves *N. glabratus* (Fig. 1F) (78-81). Mutations in MutS Homolog 2 (*MSH2*) gene, a key component of the MMR system, lead to hypermutator phenotypes (78,79). The *MSH2* gene is responsible for recognizing and initiating the repair of mismatched nucleotides during DNA replication (78,79). Clinical isolates of *N. glabratus* with *MSH2* mutations exhibit elevated mutation rates that rapidly lead to resistance to multiple antifungal agents, including azoles, echinocandins and polyenes (78,79). These hypermutator strains are challenging to treat because of their ability to quickly adapt to antifungal pressures.

In *C. neoformans*, hypermutator lineages are associated with mutations in the *MSH2* and MutL Homolog 1 genes, which are involved in the MMR pathway. These mutations result in defective repair systems and increased genetic variability (56,82). Hypermutator strains of *C. neoformans* develop resistance to antifungal agents, such as fluconazole and amphotericin B, more rapidly than non-hypermutator strains (56,82). This rapid adaptation poses challenges to the management of cryptococcal infection, particularly in immunocompromised patients.

Although less commonly reported than *C. neoformans* and *N. glabratus*, hypermutator phenotypes have also been observed in *A. fumigatus* (77,79,83). Mutations in the DNA repair gene *MSH* lead to increased mutation rates and the rapid emergence of resistance (83). A study of environmental and clinical isolates of *A. fumigatus* revealed that hypermutator strains quickly develop resistance to azole antifungals, which are commonly used to treat aspergillosis (83). Additionally, genetic instability caused by *MSH* mutations increases virulence (83). The presence of these strains in clinical settings underscores the need for monitoring and development of alternative therapeutic strategies.

The emergence of hypermutator lineages of fungal pathogens has key clinical implications. These strains rapidly develop resistance to multiple antifungal agents, making infection more difficult to treat and increasing the risk of treatment failure (56,78,79,82,83). The elevated mutation rates in hypermutator lineages lead to a high degree of genetic variability, which can result in the selection of resistance-conferring mutations under antifungal pressure (56,78,79,82,83).

6. Phenotypical heterogeneity and resistance

Phenotypical heterogeneity refers to the presence of different phenotypes in genetically identical populations of fungal cells. This diversity can affect the susceptibility of fungi to antifungal agents and contribute to the development and persistence of antifungal resistance (10,84-86). Phenotypical heterogeneity allows a subset of the fungal population to survive under adverse conditions such as antifungal treatment, thereby promoting the survival and adaptation of the species (87-90).

Biofilm formation. Phenotypical heterogeneity in fungi arises through numerous mechanisms. A notable example is biofilm formation. Fungal cells in biofilms exist in a sessile, multicellular state and produce an extracellular matrix that protects them from antifungal agents (85,86). This matrix serves as a physical barrier that reduces the effective concentration of the drug reaching fungal cells within the biofilm (85,86). Biofilms are problematic in clinical settings because they are associated with chronic infection and are difficult to eradicate (85,86). Species such as *C. albicans* can form biofilms on medical devices such as catheters and prosthetic valves, leading to persistent infection (85,86).

Persister fungi. Another mechanism underlying phenotypical heterogeneity involves persister cells. Within a fungal population, a small fraction of cells enter a dormant state, becoming persister cells (10,84). These cells are highly tolerant of antifungal treatment because their reduced metabolic activity makes them less susceptible to drugs targeting active cell processes (10,84). Persister cells do not carry genetic mutations that confer resistance and their tolerance arises from a reversible phenotype (10,84). This makes them challenging as they can survive treatment and later repopulate once the antifungal pressure is removed. Persisters have been extensively studied in *Candida* spp. and *Cryptococcus* spp (84,87,88,91). Studies have reported the occurrence of persistent *C. neoformans* in clinical isolates from patients with cryptococcal meningitis and pneumonia treated with amphotericin B, a standard treatment for cryptococcosis (87,88).

Stress response activation. Fungi activate stress response pathways that alter their phenotype and increase tolerance to

antifungal agents. Adaptive responses to stress in human hosts are key for pathogen survival and virulence. For example, the heat shock response lead to the upregulation of chaperone proteins that help the cell cope with the damage caused by antifungal drugs (89). Another example is *A. fumigatus*, when they are exposed to reactive oxygen species (ROS) produced by immune cells such as macrophages and neutrophils. Analysis of the transcriptomes of *A. fumigatus* revealed that ROS exposure induces the expression of catalases and superoxide dismutases for survival in an oxidative environment (92). In *C. albicans*, the activation of the calcineurin pathway in response to azole treatment increases tolerance to the drug, allowing cells to survive otherwise lethal conditions (90).

7. Epigenetic mechanisms of resistance

Unlike genetic mutations, which alter DNA sequences, epigenetic changes modify gene expression without altering the underlying genetic code. These modifications can be reversible and are influenced by environmental factors, allowing fungi to adapt to antifungal agents rapidly. Understanding these mechanisms is key for the development of novel strategies to combat fungal resistance. Epigenetic changes in fungi primarily involve modifications of histones and DNA methylation, which affect chromatin structure and gene expression. These processes regulate the expression of genes involved in antifungal resistance, including those encoding efflux pumps, drug targets and stress response elements (Table IV).

Histone modification in fungi. Histones are proteins around which DNA is wrapped, forming chromatin. Post-translational histone modification, such as acetylation, methylation, phosphorylation and ubiquitination, influences chromatin structure and gene expression (17,93). Histone acetylation, typically associated with gene activation, is catalysed by histone acetyltransferases and reversed by HDACs (17,93). HDAC inhibitors alter the antifungal resistance by altering the expression of resistance genes.

Histone modifications are among the most studied epigenetic mechanisms in fungi and are linked to antifungal drug resistance, biofilm formation and virulence regulation (94-99). There are seven genes of *HDAC* in *C. neoformans*, two of which serve key roles in resistance and virulence. Histone Deacetylase 1 (*HDA1*) and *HDAC* genes are required for the survival of *C. neoformans* in a cultured macrophage model and a mouse model of infection (94,95). Fluconazole works synergistically with HDAC inhibitors in an *in vitro* infection model of *C. neoformans* (96). This highlights the fact that HDAC genes modulate the survival and susceptibility of *C. neoformans* to the antifungal agent.

In *C. albicans*, three classes of HDAC have been identified: class I, which include proteins similar to yeast Reduced Potassium Dependency 3 (*RPD3*); class II which consist of proteins related to HDA1; and class III which are NAD^+-dependent. SET domain protein 3 (SET3) and Histone Deacetylase of the Rpd3S Complex 2 (HOS2) are HDAC genes that are clinically important in *C. albicans*, particularly for promoting biofilm formation (97). The deletion of *SET3* and *HOS2* decreases biofilm formation by *C. albicans* (97). *SET3C* complex interacts with master regulator genes of biofilms, such as Negative Regulator Gene 1 (*NRG1*), Biofilm Regulator Gene 1 (*BRG1*), TEA/ATTS domain-containing transcription factor 1 (*TEC1*), Non-Disjunction and Telomere Defect 80 (*NDT80*) and Regulator of Biofilm 1 (*ROB1*) (98). *HDA1* and *RPD3* increase during the early phase of azole resistance in *C. albicans* and decrease during the later phase (99). HDAC inhibitors increase the sensitivity of *C. albicans* to azole antifungals by altering the expression of stress response and drug resistance genes (99).

DNA methylation. Although less characterized in fungi compared with higher eukaryotes, DNA methylation serves a key role in transcriptional repression of antifungal target genes and may contribute to stable yet reversible drug resistance. DNA methylation involves the addition of a methyl group to cytosine residues in DNA, typically leading to gene suppression. Although DNA methylation has been well studied in higher eukaryotes, its role in fungi is less clear (100-103). DNA methylation affects the expression of the genes involved in antifungal resistance (93). Changes in methylation patterns can lead to the silencing or activation of genes that help the fungus survive in the presence of antifungal drugs (93).

In *C. neoformans*, DNA methylation patterns are associated with the resistance to antifungal agents. The DNA methylation in *C. neoformans* is mediated by the maintenance DNA methyltransferase Dnmt5, which catalyses the deposition of 5-methylcytosine primarily at centromeric and repetitive regions (104). Although this organism lacks a canonical *de novo* methyltransferase, Dnmt5 maintains heritable methylation patterns across generations (104). Disruption of Dnmt5 causes global hypomethylation, chromosomal instability and altered expression of stress-responsive genes (104). These epigenetic changes may indirectly modulate the expression of genes associated with antifungal resistance, including those encoding efflux pumps and cell wall remodelling proteins. While the spectrum of fungal DNA methyltransferases remains to be characterized, emerging evidence suggests that epigenetic silencing via methylation may contribute to reversible antifungal tolerance (93,104,105).

Chromatin remodelling. Chromatin remodelling allows dynamic control of gene expression in response to antifungal stress, enabling fungi to adjust resistance pathways without permanent genetic mutations. Chromatin remodelling complexes alter the structure of chromatin, making certain regions of the genome more or less accessible to transcription factors and other regulatory proteins. These complexes facilitate the expression of resistance genes by repositioning nucleosomes and exposing regulatory regions of the DNA (17,93). In *C. albicans,* the chromatin remodelling Switch/sucrose non-fermenting complex 5 (SNF5) subunit is key for tolerance to azoles and amphotericin B under hypoxic stress (106). A study revealed that hypoxia potentiates the antifungal effects of azoles and amphotericin B in *C. albicans* (106). Remodelling complexes reposition nucleosomes to expose or hide regulatory regions, thereby influencing the expression of resistance genes.

8. Diagnostic and treatment implications

Diagnostic approaches for resistance detection. Identification of antifungal resistance driven by genetic mechanisms

Table IV. Characteristics and implications of antifungal resistance caused by epigenetic mechanisms.

Epigenetic mechanism	Definition	Function	Types of modification	Enzyme involvement	Effect on chromatin	Reversibility	Role in resistance	Examples	Detection method	Therapeutic target	Treatment implications	Associated resistance gene roles
Histone modification	Post-translational modification of histone protein	Regulation of gene expression via chromatin accessibility	Acetylation, methylation, phosphorylation, ubiquitination	Histone acetyltransferase, HDAC, histone MT, histone demethylase	Relaxation via acetylation; condensation via methylation	Reversible, dynamic	Alters expression of resistance genes (such as upregulating efflux pumps)	HDAC inhibition in *C. albicans* affects drug resistance gene expression	Chromatin immunoprecipitation, western blotting	HDAC inhibitors to modulate resistance gene expression	Modulation can sensitize fungi to antifungal agents	Drug efflux, stress response
DNA methylation	Addition of methyl groups to DNA, typically at cytosine residues	Regulation of gene expression via DNA accessibility	Methylation of cytosine residues	DNMT	Condensation, leading to gene suppression	Reversible, stable	Silences or activates resistance genes	Methylation patterns in *Cryptococcus neoformans* influence antifungal resistance	Bisulfite sequencing, methylation-specific PCR	DNMT inhibitors to alter methylation pattern	Altering methylation status may reverse resistance	Drug target modification, efflux
Chromatin remodelling	Dynamic alteration of chromatin structure	Regulation of gene expression via nucleosome repositioning	Nucleosome sliding, eviction or restructuring	Chromatin remodelling complexes (SWI/SNF, ISWI)	Alters nucleosome position to alter DNA accessibility	Reversible, dynamic	Exposes or hides regulation regions of resistance genes	Chromatin remodelling in *C. albicans* affects azole and amphotericin B resistance	Nucleosome positioning assay (ATAC-sequencing)	Remodelling complexes to alter gene accessibility	Targeting remodelling can change expression of resistance genes	Metabolic pathways, efflux pump

HDAC, histone deacetylase; SWI/SNF, switch/sucrose non-fermenting; ISWI, imitation switch/sucrose nonfermenting; DNMT, DNA methyltransferase. Fitness cost refers to the reduced growth rate or survival of resistant fungal strains in the absence of antifungal pressure.

presents diagnostic challenges. The diversity of genetic alterations requires comprehensive and precise diagnostic tools. Traditional methods such as culture-based assays and susceptibility testing may not detect specific genetic changes that confer resistance. Advanced molecular techniques, such as PCR and next-generation and whole-genome sequencing, are essential for detecting specific genetic mutations and alterations (107,108). Techniques such as chromatin immunoprecipitation and bisulfite sequencing are necessary to identify epigenetic changes such as histone modification and DNA methylation patterns that contribute to resistance (109,110). However, these advanced diagnostic tools are expensive and not readily available in all clinical settings, particularly in resource-limited regions. Ensuring accessibility and affordability of these technologies is key for their widespread implementation. Additionally, the time required to perform and analyze advanced diagnostic tests can delay the initiation of appropriate antifungal therapy, potentially worsening patient outcomes. Therefore, rapid diagnostic methods are required to identify resistance mechanisms.

Challenges in antifungal drug development. Development of novel antifungal drugs is challenging due to several interrelated scientific, clinical and economic barriers. Fungi are eukaryotic organisms, sharing key processes with human cells. This limits the availability of pathogen-specific targets and raises the risk of host toxicity (111). Clinically, antifungal drug development must address diverse fungal species, several of which form biofilms, reside in immune-privileged or poorly perfused sites (such as the central nervous system and eyes) and demonstrate intrinsic or acquired resistance to existing therapies (112). Economically, antifungal infections represent a smaller market share than bacterial or viral disease, resulting in lower commercial incentive for pharmaceutical investment (113). In addition, the regulatory pathways for antifungal agents are complex and complicated by the need for robust efficacy data in severely ill and immunocompromised populations, where trial enrolment and design are difficult.

Clinical consequences of resistance mechanisms. Genetic and epigenetic resistance mechanisms complicate treatment of fungal infection. Fungi with hypermutator phenotypes or multiple resistance mechanisms may exhibit resistance to several classes of antifungal drug, thereby limiting the treatment options. This necessitates the use of combination therapy or novel antifungal agents to manage infections effectively. Moreover, epigenetic changes and phenotypical heterogeneity can lead to dynamic and reversible resistance, thereby complicating treatment strategies (17,93). Fungal cells may revert to a resistant state following an initial response to therapy, leading to the recurrence of infection. Higher doses of antifungal agents or prolonged treatment required to overcome resistance can increase the risk of toxicity and adverse side effects (114,115). Balancing efficacy and safety is a key consideration in antifungal therapy.

Strategies to overcome antifungal resistance. Numerous strategies can be used to address the clinical challenges posed by genetic and epigenetic resistance mechanisms. Combinations of antifungal agents with different mechanisms of action can help overcome resistance by simultaneously targeting multiple pathways (116-118). For example, combining azoles with echinocandins or vorinostats may be more effective against biofilm-associated infections than azoles alone (119,120). Continued research and development of antifungal drugs with novel targets are essential to expand the arsenal against resistant fungal strains. Agents that bypass existing resistance mechanisms or target epigenetic modifications hold promise as effective treatments. Developing drugs that modulate epigenetic changes, such as histone deacetylase or DNA methylation inhibitors, can sensitize resistant fungal cells to existing antifungal agents. These approaches offer novel avenues for overcoming resistance.

Numerous strategies are under investigation to address antifungal resistance. Combination therapy (such as azoles with echinocandins or HDAC inhibitors) can enhance efficacy and delay resistance emergence (121). Antifungal classes such as orotomides (for example, olorofim) and glycosylphosphatidylinositol (GPI)-anchor pathway inhibitors (such as fosmanogepix) have demonstrated activity against resistant strains in preclinical and clinical trials (122-124). Adjunctive immunotherapy, including IFN-γ and monoclonal antibodies, offers potential benefit in immunocompromised hosts (125). Antifungal stewardship programs also serve a key role in optimizing antifungal use and minimizing resistance pressure (126).

The identification of novel antifungal drug candidates is achieved through numerous complementary approaches. High-throughput screening of large chemical libraries has led to the discovery of olorofim, a dihydroorotate dehydrogenase inhibitor developed by screening agents targeting fungal pyrimidine biosynthesis, and is in phase III trials against invasive *A. fumigatus* infections (124,127). Drug repurposing has resulted in identifying sertraline (an antidepressant) with activity against *C. neoformans*, and tamoxifen, which targets calmodulin in *Cryptococcus spp* (128,129). *In silico* modelling and structure-based design have facilitated the development of manogepix (APX001A), which targets GPI-anchored wall transfer protein 1 (GWT1) and has demonstrated broad-spectrum antifungal activity (130). Natural product screening identified ibrexafungerp (SCY-078), a semi-synthetic derivative of enfumafungin, a triterpenoid glycoside (131). Ibrexafungerp inhibits β-1,3-glucan synthase and has been approved for vulvovaginal candidiasis by the U.S. Food and Drug Administration (FDA) in 2021 (131,132). Furthermore, structure-guided drug design based on fungal enzyme crystal structures has supported the optimization of such agents for potency and selectivity (131).

Improving the delivery of antifungal agents to the infection site can increase drug efficacy and decrease resistance. Strategies such as liposomal formulations, nanoparticles and localized delivery systems have been explored to enhance drug delivery (133,134). Developing rapid and accurate diagnostic tools is key for the timely identification of resistance mechanisms and guiding appropriate therapy. Point-of-care diagnostics and streamlined genomic assays help clinicians quickly adapt treatment strategies. By understanding and targeting the underlying mechanisms of resistance, clinicians may improve patient outcomes and effectively manage resistant fungal infection.

To overcome the limitations of conventional antifungal therapy, novel drug delivery systems have been developed to enhance targeted delivery and therapeutic efficacy. Liposomal formulations, such as liposomal amphotericin B, encapsulate antifungal agents within lipid bilayers, enhancing drug solubility, decreasing toxicity and enabling targeted delivery (133,135). A randomized controlled trial in 2022, conducted within resource-limited settings in Africa (Botswana, Malawi, South Africa, Uganda, and Zimbabwe) demonstrated that a single high-dose (10 mg/kg) liposomal amphotericin B regimen, combined with flucytosine and fluconazole, was non-inferior to the conventional 7-day amphotericin B regimen in treating HIV-associated cryptococcal meningitis (136). The simplified regimen markedly decreased adverse events, including nephrotoxicity and anaemia, while maintaining comparable survival outcomes (136). This highlights the clinical efficacy and improved safety profile of liposomal formulations in resource-limited settings, where the use of simplified regimen may decrease the treatment cost.

Nanoparticles can be engineered to respond to stimuli such as pH, temperature or specific enzymes, facilitating drug release at the site of infection, especially where biofilm is involved (134,137). A pre-clinical study demonstrated that ~100 nm diameter liposomal nanoparticles loaded with anidulafungin enhance the solubility and antifungal targeting (138). The formulations were tested *in vitro* against *C. albicans*, showing equivalent MICs to free drug for planktonic cells and superior efficacy in disrupting biofilms, decreasing fungal burden by ≥99% (138). *In vivo* evaluation using a *Galleria mellonella* infection model demonstrated notably improved survival rates (33-67%) in larvae treated with liposomal formulations compared with 25% in the free drug group, without causing haemolysis, indicating both efficacy and safety (138). A randomized clinical trial evaluated a topical gel containing fluconazole-loaded solid lipid nanoparticles (SLNs) for the treatment of pityriasis versicolor (139). The SLN-based gel demonstrated considerably increased clinical and mycological cure rates compared with conventional fluconazole gel, with enhanced drug retention and patient compliance (139). These findings support the clinical utility of nanoparticle-based topical delivery systems in improving antifungal treatment outcomes.

Localized delivery systems, including hydrogels and coated catheters, enable sustained and concentrated antifungal release at the infection site, particularly for biofilm- or device-related infections (140). In a comparative *in vitro* biofilm colonization model, central venous catheters (CVCs) and peripherally inserted central catheters impregnated with a novel chlorhexidine-minocycline-rifampin (CHX-M/R) combination were evaluated against resistant bacteria (methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, *Pseudomonas aeruginosa*) and fungi (*C. albicans, N. glabratus*) (140). The CHX-M/R-coated catheters completely inhibited biofilm colonization by all tested pathogens at all time points and revealed considerably superior efficacy compared with first-generation M/R catheters, CHX-silver sulfadiazine-treated CVC and uncoated controls (140). The aforementioned studies support the potential of CHX-M/R-coated catheters for sustained, broad-spectrum antimicrobial and antifungal protection, particularly in preventing biofilm-associated device infection.

Overall, the clinical implications of antifungal resistance driven by genetic and epigenetic mechanisms present notable diagnostic and therapeutic challenges. Addressing these challenges requires a multifaceted approach, including the development of advanced diagnostics, novel antifungal agents, combination therapy and strategies targeting epigenetic modifications.

9. Conclusion

Antifungal resistance is a complex and growing threat that enables fungal pathogens to evade treatment and persist in hostile environments. Antifungal resistance is not a static, mutation-driven process but a dynamic, multilayered phenomenon involving both stable genetic alterations and reversible epigenetic adaptations. The present review highlights key resistance mechanisms, including point mutations, aneuploidy, histone modification and chromatin remodelling and emphasizes their clinical consequences in limiting the efficacy of antifungal therapies. Addressing this challenge requires integrated solutions: rapid molecular diagnostics for early detection, development of novel antifungal agents such as olorofim and fosmanogepix, use of combination and epigenetic-targeting therapies and the implementation of antifungal stewardship programs to preserve drug efficacy. Translating mechanistic insights into diagnostic and therapeutic innovations is key to improve patient outcomes and contain the spread of resistance.

Acknowledgements

The authors would like to thank Ms Rahmati Putri Yaniafari (Nanyang Technological University, Singapore, Singapore) for providing access to several sources of information for this review.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

INK, MAE, TIS and YA conceived the study and wrote and revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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