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Understanding antifungal resistance: Challenges, mechanisms, and strategies to combat rising threats

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Abstract
Fungal infections have become a major global health problem, especially among immunocompromised patients. The limited number of antifungal drug classes and the growing resistance among fungal pathogens makes treatment increasingly difficult. This review discusses the different mechanisms that fungi use to resist antifungal drugs, such as target site mutations, drug efflux, and stress responses. It also highlights clinical and environmental factors contributing to resistance, including agricultural practices. Finally, it explores current strategies to address antifungal resistance, including stewardship programs, combination therapies, and the urgent need for new antifungal drugs. A better understanding of these issues is critical for improving outcomes and managing the growing threat of antifungal resistance.

Keywords: Fungal infection, Antifungal resistance, Resistance mechanisms, drug efflux, antifungal stewardship, combination therapy

Introduction
Fungal infections were largely overlooked in the 20th century; conversely, the bacterial infections which were a huge problem and cause a lot of deaths. Later then in 1960s antibiotic drugs were improving while a dramatic increase in fungal infections was noticeable [1]. Antibacterial therapies are considerably more numerous as opposed to antifungal agents which are few in number and are almost three families, such as azoles, echinocandins and polyenes [2]. Globally, over 100 million and nearly 200 million severe cases of fungal infections occur each year, reaching nearly two million deaths annually. terrifyingly, these numbers are on a continuous increase along with the developments in the society in all aspects of life during the last ages that have influenced the outbreak of fungal infections, in addition to that, the long-time administration of antifungal agents as prophylaxis therapy led to rising of (multi)drug-resistant fungi especially in high-risk patients, such as immunocompromised patient [2, 3].
Antifungal drugs such as azoles have a tendency to attach to ergosterol 11 gene in *Candida* and *cytochrome p51A* in *Aspergillus* species and interfere with the formation of the most vital constituent of the cell wall which is ergosterol, on the other hand echinocandins attack the catalytic subunit of β-1,3-D-glucan synthase, encoded by "*FKS* genes" and interrupt with β-1,3-D-glucan production process, a critical structural component in the call wall [2]. Finally, Polyene antifungal agents exert their effect by binding to ergosterol in the fungal cell membrane, leading to cell lysis [2]. A significant concern is the global resistance to traditional antifungal agents [4]. Drug resistance constitutes a dynamic phenotype that allows fungi to endure exposure to detrimental chemical substances. This may result from several physiological strategies employed by fungi to avert cell death. [2]. A comprehensive understanding of the mechanisms underlying resistance, including genetic, molecular, and environmental factors, is essential to developing innovative therapeutic strategies. This study aims to bridge existing knowledge gaps by systematically analyzing the molecular and physiological mechanisms underlying antifungal resistance and evaluating potential strategies for overcoming this growing threat.

Antifungal Drug Resistance
Globally, resistance to conventional antifungals is a huge dilemma [5] to the point that Antifungal drugs exhibit minimal or no efficacy against certain fungi. They possess inherent resistance to more than one antibiotic. This phenomenon is referred to as inherent or intrinsic resistance. Numerous factors contribute to the ability of fungal species to become resistant to routine medications, referred to as acquired resistance. Acquired antifungal resistance has emerged in clinically relevant fungi due to the routine and prophylactic administration of antifungal agents. These factors have been associated with alteration or excessive expression of drug targets, elevated levels of multidrug transporters, and triggering stress response cascade as adaptive processes contributing to antifungal drug resistance [6]. Although resistance to polyenes is remains uncommon, resistance to azoles and echinocandins is Long-standing resistance.

1. Molecular Mechanisms Underlying Antifungal Resistance
Microorganisms utilize many essential mechanisms to overcome the fungicidal or fungistatic effects of all antifungal classes: 1- Lowering the intracellular concentration of the drug in the fungus; 2- decreasing its attraction to the binding site; and 3- modifying metabolism to mitigate the agent's effects. Multiple resistance mechanisms are often associated with specific highly resistant clinical isolates obtained from patients undergoing prolonged treatment. The advancement of novel mechanisms has led to an increase in resistance to antifungal medication [7].

1.1. Resistance to Azoles
Extensive research has established that pathogenic fungi develop azole resistance through multiple well-characterized molecular mechanisms.

1.1.1. Modification of target sites or excessive expression

Azoles are the primary pharmacological agents employed in the treatment of invasive fungal infections in developing countries. Acquired antifungal resistance is largely caused by changes in medication targets. Resistance to azole antifungals may arise from various changes to the genes encoding lanosterol 14α-demethylase, specifically ergosterol gene11 in yeasts and cytochrome P51 in moulds. Alterations to the enzyme's heme molecular can inhibit azole affinity, while the excessive expression of ergosterol gene11 or cytochrome P51 can diminish azole susceptibility by increasing the level of the target, necessitating higher drug concentrations for effective inhibition [8].

As in 2010, more than 150 amino acid substitutions in the ergesterol gene11 had been identified, each resulting in different genetic implications [9]. Numerous single substitutions are synonymous and do not affect gene function. Antifungal resistance is not an inherent consequence of non-synonymous single-nucleotide changes in Candida strains. Using restriction fragment length polymorphism (RFLP) research, White *et al*. (2002) found that D116E and E266D were the two most common ERG11 alterations in a single sample set [10]. Different azole drugs on the market now have different structures, including different chain lengths. Therefore, changes that affect how well they work might differ appropriately. Point mutations K128T and Y132H in ERG11, for example, may affect the binding and penetration capabilities of fluconazole and voriconazole molecules in the target active site. Resistance can also be conferred by mutations in alternate gene sequences; one such mutation is G464S, which affects haem coordination because it is close to a crucial cysteine residue [11]. These mutations exhibit differing binding inefficiencies for posaconazole and itraconazole treatments, indicating that these two antifungals possess additional critical interaction sites within the Erg11 protein [11]. The extended chains incorporated into the posaconazole and itraconazole molecules may enhance the contact points necessary for stabilizing drug–protein binding, even in the presence of mutations that could affect this interaction [12]. Mutations in the genes or promoters encoding lanosterol 14α-demethylase, specifically CYP51, represent the most common way that fungi resist the azole in Aspergillus supp [13]. The extensive agricultural application of azole antifungals is thought to have facilitated the propagation of this mechanism across various isolates [14].

Up-to-date study investigated the related mechanisms underlying resistance to fluconazole by analyzing sterol composition, fluconazole accumulation, and the inhibition of 14α-demethylase by fluconazole in two clinical strains of Candida krusei (which exhibit intrinsic resistance to fluconazole) and a sensitive isolate of Candida albicans [15] .No notable variation in sterol content between Candida krusei and Candida albicans were observed, with ergosterol being the predominant sterol in mentioned above species. Research on cell extracts demonstrated that the fluconazole concentration necessary to inhibit ergosterol synthesis by nearly 50% was nearly 20-50-fold in Candida krusei compared to Candida albicans, indicating a variation in the bonding of the target enzyme between the two species [15]. A comparison of fluconazole accumulation by Candida albicans and Candida krusei revealed that fluconazole accumulation during the initial 60 minutes was comparable across all study strains. Analysis after 90 minutes of incubation indicated that Candida krusei accumulated nearly 65% less fluconazole compared to Candida. Albicans suggest that active efflux contributes to the fluconazole resistance observed in these Candida krusei strains [16]. An increase in resistance to azole antifungals can be attributed to the overexpression of 14α-demethylase [17]. Compared the pretreatment isolate to an azole-resistant C. glabrata strain, we found that the latter had a higher ergosterol concentration. A decrease in sensitivity to azoles and amphotericin B occurred simultaneously with this increase. The resistant strain was found to have an overexpressed enzyme known as microsomal P-450, which was thought to be responsible for the increased ergosterol synthesis. Resistant strains had active efflux of fluconazole, as their intracellular concentration was 1.5 to 3 times lower than pretreatment isolates; nevertheless, resistant strains retained amounts of itraconazole that were like pretreatment isolates [17]. It has been found that the cross-resistance to these two triazoles was caused by higher P-450 levels. Overexpression of the target enzyme contributes minimally to clinical resistance to azoles, as indicated by the limited availability of isolates showing excessive production of 14α-demethylase, the fact that this event has only been observed in Candida glabrata, and the discovery of other resistance mechanisms that could be present in the same strain.

1.1.2. Drug Efflux

The activation of efflux pumps linked with cell membranes is a common resistance mechanism that allows for the development of multidrug resistance (MDR). There are two different drug efflux systems in fungi that control azole resistance: the ABC superfamily and the MFS superfamily. Two transmembrane span (TMS) domains and two cytoplasmic nucleotide-binding domains (NBD) enable ATP hydrolysis in ABC proteins, which are ATP-dependent transporters normally structured in a duplicated topology. Genomic analysis of fungal pathogens has uncovered a wide variety of ABC transporters with different topologies. Candida albicans is estimated to possess 28 ABC proteins, while Candida glabrata has around two-thirds of that quantity. A greater number of ABC proteins are present in A. fumigatus and Candida neoformans [18]. Numerous classes of fungal ABC transporters exist. [19]. The up regulation of CDR1 and CDR2 facilitates azole resistance by increased drug efflux and diminished azole accumulation [20].

1.2. Echinocandin Resistance

Echinocandins represent a very important class of antifungals, mostly in the treatment of invasive fungal infection [21]. Echinocandin antifungals work by blocking the enzyme 1,3-D-glucan synthase. This means that the fungal cell wall, which is essential for survival, produces less 1,3-D-glucan. For most azole-resistant Candida species, echinocandins continue to work since their mechanism is different from that of azole antifungals. Isolates of Candida glabrata show a marked increase in resistance to echinocandins [22].

1.2.1. Drug Target Alteration

Gene alterations affecting the therapeutic targets, FKS1 (present in Candida, Cryptococcus, and Aspergillus species) and FKS2 (unique to Candida glabrata), are a common

cause of resistance to echinocandin treatment [23]. The 1,3-β-glucan synthase complex catalytic subunit is encoded by the essential gene FKS1 in Candida albicans. The two FKS genes present in C. glabrata, on the other hand, are essential for the survival of the organism but have no functional purpose. One hundred and twenty-one anidulafungin-resistant lineages were generated in a recent study that used experimental evolution with Candida glabrata. All these strains exhibited non-synonymous mutations in FKS genes, with a higher frequency of mutations in FKS2 compared to FKS1[24].

1.2.2. Cellular changes

Another mechanism of echinocandin resistance is the increased production of chitin. In C. albicans and A. fumigatus, a primary mechanism is the compensatory overexpression of chitin, an essential polymer in the fungal cell wall [25]. Increased cell wall chitin levels in response to caspofungin can diminish antifungal efficacy at elevated concentrations, partially promoting growth through a process termed the paradoxical growth effect (or Eagle effect) [25]. The compensatory chitin response to caspofungin in C. albicans depends on the key stress response regulators PKC, calcineurin, and the high-osmolarity glycerol (HOG) MAP kinase [26].

Like glucan, chitin is a structural element of the fungal cell wall. The inhibition of 1,3-β-D-glucan synthase by an echinocandin results in diminished glucan synthesis, causing the organism to enhance chitin production, which is associated with decreased vulnerability to echinocandins.

1.3. Drug Resistance to Polyenes

Polyenes have been utilized for A prolonged period [27]. Resistance to polyenes remains uncommon in comparison to resistance against other antifungal agents [28]. This can be explained by its frequent association with significant fitness trade-offs [28]. Another suggestion suggests that, unlike most other antifungals, polyenes primarily target a key component of the cell membrane rather than a vital enzyme. Alterations in ergosterol content or the replacement of sterol intermediates represent the predominant mechanism of acquired polyene resistance [29]. Alterations in genes associated with the ergosterol production pathway (ergosterol gene) have been correlated with this mechanism. Mutations in many genes associated with ergosterol production, namely ergosterol gene 11, ergosterol gene 3, ergosterol gene 2, and ergosterol gene 6, provide a modified sterol profile that confers AmB resistance in Candida species. [30] The dysfunction of ergosterol genes 11 and 3 in C. albicans, which code for lanosterol 14α-demethylase and C-5 sterol desaturase, respectively, leads to the substitution of ergosterol with alternative sterols, such as lanosterol, eburicol, and 4,14-dimethyl-zymosterol in the membrane [31]. The formation of polyene resistance in Candida depends on Hsp90, highlighting the conserved function of this protein in the development of resistance to many antifungals [32]. The cellular stress response is a mechanism that enhances pathogen survival against diverse environmental disturbances and is essential in alleviating antifungal-induced stress. Hsp90 stabilizes several signal transducers that mediate antifungal-induced stress in Candida, Aspergillus, and Cryptococcus [33].

1.4. Flucytosine Resistance

5-Flucytosine is characterized by its swift emergence of resistance; consequently, it is utilized exclusively in conjunction with AMB and triazoles. Primary and secondary resistance in clinically relevant Candida species has arisen due to modifications in the FCY2, FCY1, and FUR1 genes, which are responsible for the uptake and conversion of 5-FC [34]. The FCY2 gene encodes cytosine permease, an enzyme that facilitates the uptake of flucytosine into fungal cells. About 10% of flucytosine resistance in C. albicans is associated with reduced drug uptake resulting from mutations in this gene. These mutations lead to impaired cytosine permease activity, inhibiting the entry of flucytosine into the cell. FCY1 encodes the enzyme cytosine deaminase, responsible for the conversion of flucytosine to 5-fluorouracil. Point mutations in this gene restrict this conversion, resulting in the ineffectiveness of flucytosine. In C. glabrata, certain resistant mutants with FCY1 mutations exhibit growth failure in uridine-free, cytosine-supplemented medium, indicating inactive cytosine deaminase. FUR1 encodes uracil phosphoribosyltransferase, a critical enzyme for the conversion of 5-fluorouracil into its active nucleotide forms. Mutations in this gene lead to cross-resistance to 5-fluorouracil, indicating that the resistance mechanism operates downstream of cytosine deaminase [35, 36].

Challenges of antifungal resistance

Antifungal resistance poses a significant clinical challenge for clinicians treating invasive fungal infections, given the restricted range of systemically available anti-fungal agents.

Antifungal resistance: Contemporary trends and prospective strategies for mitigation. Additionally, the development of antifungal drugs presents challenges due to the genetic similarities between humans and fungi, which may result in toxicity concerns. For instance, it is estimated that Saccharomyces cerevisiae, a common brewer's yeast, possesses approximately 30% of proteins that are analogous to those found in humans, positioning it as one of the most closely related lower eukaryotes to humans. Some drugs and their doses may be detrimental to humans because of analogous enzymes and metabolic pathways [37]. Patients at the highest risk for developing antifungal resistance frequently present with multiple comorbidities, including immunosuppression, which may diminish the efficacy of therapy even when drug resistance is not present. Current medications may be constrained by drug–drug interactions and significant adverse effects or toxicities that hinder their extended use or dosage increase. A contributing factor to the complexities of antifungal resistance is the scarcity of novel agents, as no new antifungal classes have emerged in decades, with the most recent drugs being derivatives of existing ones [38]. The application of antifungal agents in agriculture is associated with the emergence of antifungal resistance, presenting a considerable challenge for public health and food security. Agricultural fungicides, especially azoles, are extensively utilized to safeguard crops against fungal diseases; however, they impose selective pressure on fungal populations, resulting in the emergence of resistant strains. Resistant strains like Aspergillus fumigatus can transmit to humans, diminishing the efficacy of clinical anti-fungal treatments. Research indicates that resistant fungal strains in agricultural settings exhibit genetic similarities to

those responsible for human infections, suggesting a direct correlation. The dual application of azoles in both agriculture and medicine has expedited the development of resistance, surpassing the pace of new antifungal compound discovery. Resolving this issue necessitates coordinated efforts utilizing a One Health approach, which integrates strategies across agriculture, healthcare, and environmental sectors to mitigate resistance and maintain the efficacy of existing antifungal agents.

Strategies to Combat Resistance

1. Antifungal Stewardship

The extensive availability of antifungal medications as over-the-counter options by pharmacists and drugstore personnel increases the risk of antifungal misuse and the development of resistance. The proportion of the immunosuppressed population has increased due to various factors, including anticancer therapy, organ transplantation, the use of immunosuppressants, and the prevalence of HIV-AIDS. Consequently, reliance on antifungals has risen [41]. The discovery of a novel antimicrobial agent necessitates extensive research over several years. The misuse of antimicrobials, including antifungals, has led to a significant increase in antifungal resistance, which has outstripped the discovery on novel agent. Antifungal resistance is increasingly a significant concern for clinicians. Antimicrobial stewardship is a coordinated initiative aimed at promoting the scientific and rational application of antimicrobials, minimizing the risk of drug resistance, and enhancing patient outcomes. Advanced antifungal stewardship via a multidisciplinary team in a pediatric and adult tertiary center in the UK.

The antifungal stewardship program should encompass guidelines for antifungal usage in patients necessitating empirical therapy. ii. Treatment informed by biochemical markers. iii. Treatment for patients exhibiting signs and symptoms of fungal infection enhances therapy appropriateness, facilitates targeted treatment, and diminishes resistance pressure [41, 4 9].

The fundamental components of effective stewardship include: [1] extensive knowledge and ongoing consultation of optimal clinical guidelines for fungal disease management; [2] prioritization of high-quality care over cost reduction, recognizing that certain costly antifungals may be the most appropriate choice, with cost savings achieved through the cessation of unnecessary therapies; [3] clinical expertise to deduce probable outcomes in the absence of available results or when samples have not been collected or were not obtained [50].

2. Combination Therapy

Combination therapy effectively addresses antifungal drug resistance and is primarily beneficial in prolonging the efficacy of present medications. Dual-drug therapy can enhance the efficacy of pathogen eradication, decrease pathogen number present in the infection site, and reduce the likelihood of developing acquired resistance mutations [42,48] Combination therapy Allows for dose reduction of the individual agent, shortens the duration of treatment, and mitigates drug side effect [42]. The combination of AmB and FU exemplifies effective combination therapy for meningitis caused by fungi and fungal infections caused by Candida [43] Combined regimens may be sequentially or paralleled. Sequential therapy represents that the combination is not given simultaneously. The initial drug is administered for a defined period and then the second drug is given subsequently. Parallel therapy represents combination of both drugs is given at the same time. There is no consideration when sequential therapy and parallel therapy are used. Parallel therapy is used when patient Combinations included 5-flucytosine combination therapies (24%), azoles plus echinocandins (36%), polyenes plus azoles (18%), polyenes plus echinocandins (16%) and other types of combination therapy (6%). Some regimens combined agents show a synergistic effect against resistant Candida species (i.e., azoles plus echinocandins; polyenes plus 5-flucytosine), or they were more potent rathar than monotherapy in lowering biofilm formation and increase the healing rate from infected areas (i.e., polyenes plus echinocandins [44].

3. Discovery of new Antifungal Agents

The creation of novel agents with antifungal activity is a crucial aspect in combating the emergence of antifungal resistance. Currently, the categories of antifungal drugs remain restricted in comparison to the antibiotics available for bacterial infections, and there has been no significant progress in the identification of new antifungal agents in recent years. The vast majority of newly approved antifungal drugs in the previous decade are updated formulations within the same class of current medications. Multiple medications in the late phases of discovery have innovative dosing regimens and modes of action to address this health threat [45]. Notably, fungal cells differ from human cells in both physical and molecular characteristics, presenting a difficulty in identifying novel agents that possess antifungal action while exhibiting low harm to human health. A promising method employed in recent years utilized nanoparticles or Nano formulations of clinically accessible antifungals [46,47].

Conclusion

Antifungal resistance poses a significant clinical challenge, particularly in the management of invasive mycoses in immunocompromised patients, where treatment options are already limited. Resistance mechanisms such as efflux pump overexpression, target site mutations undermine the efficacy of key antifungal classes, including azoles, echinocandins, and polyenes. This growing resistance has critical implications for pharmacotherapy, as it often necessitates the use of more toxic agents, higher drug dosages, or combination therapies, thereby increasing the risk of adverse drug reactions and drug–drug interactions. The limited pipeline of new antifungal drugs, combined with inadequate diagnostic tools to rapidly detect resistance patterns, further exacerbates treatment failures. Advancing antifungal pharmacotherapy will require integrated efforts to optimize drug design, improve susceptibility testing, and promote antifungal stewardship to preserve the utility of existing agent.

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