Factors Associated with Antifungal Resistance and the Recent Development in Detection & Treatment: A Review

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of

B.Sc. (Microbiology)

Department of Mathematics and Natural Sciences

BRAC University

June 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.

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Approval

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Abstract:

Antifungal resistance has become a growing concern in recent years, as it has become increasingly difficult to treat fungal infections due to the emergence of drug-resistant organisms. The prevalence of antifungal resistance can be analyzed through various factors, including environmental factors, evolutionary factors, and drug misuse. Environmental factors such as climate change and pollution can affect fungi's growth and survival, leading to resistant strains' development. Evolutionary factors such as genetic mutations and natural selection can also contribute to the emergence of drug-resistant organisms. Additionally, drug misuse, such as overuse or improper use of antifungal medications, can promote the development of resistance. Early detection of antifungal resistance is crucial for effective treatment, and various methods such as susceptibility testing and molecular diagnostics are available for this purpose. In conclusion, the prevalence of antifungal resistance is a complex issue that requires a multifaceted approach to address the various factors contributing to its emergence.

Keywords: Antifungal resistance, Azole, Prevalence, Detection, Treatment

Dedication:

My parents, my elder sister & Sushi-Gigi, and Faculties

Acknowledgment:

I acknowledge our esteem to Professor A F M Yusuf Haider, Chairperson of the MNS Department of BRAC University for allowing and encouraging me to complete my undergraduate thesis.

My regards, gratitude, and appreciation go to my respected supervisor Senior Lecturer Akash Ahmed, Of the Microbiology Program of the Department of Mathematics and Natural Sciences, BRAC University for his continual monitoring, constructive criticism, professional direction, passionate encouragement to seek new ideas and never-ending inspiration throughout the duration of our research work.

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Introduction:

According to research conducted by Hawksworth & Lücking, (2017), in an article named “The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycological Research” stated that fungi's lineage can be traced back over 1.5 billion years, marking their ancient roots in the biological world. Despite their vast diversity, a mere 0.5% of fungal species are deemed pathogenic to humans (Kwon-Chung KJ and Bennett JE,1992). However, scientists have also discovered some remarkable properties of certain fungi, such as *Aspergillus terreus* and Engyodontium album, which can break down plastics in a matter of weeks. Fungi have also proven to be incredibly useful in modern society, with applications ranging from producing human hormones and antibiotics to developing pesticides and even contributing to the food industry. It is clear that fungi have established their significance in the contemporary world, and their potential for further innovation and discovery is immense. Therefore, fungi are everywhere, from the soil we walk on to the food we eat. While most fungi are harmless, some can cause serious human, animal, and plant diseases. To combat these diseases, we use antifungal drugs. The rise of antifungal resistance is posing a significant threat to public health, much like the issue of antibiotic resistance. As fungi continue to evolve and adapt, the efficacy of current antifungal drugs is being compromised, making it increasingly challenging to combat fungal infections. This growing concern highlights the urgent need for innovative solutions and a proactive approach to address this critical issue. Since the systematic availability of antifungal agents is limited, this phenomenon represents a severe threat to the prevalent clinical challenges.

When it comes to treating fungal diseases, Fisher et al. (2022), in an article named “Tackling the emerging threat of antifungal resistance to human health” depicted that we've only had four main types of antifungal drugs to rely on polyenes, azoles, echinocandins, and 5-flucytosine. But interestingly, fungi are pretty good at dodging these drugs, so treatment often fails. There are a lot of factors at play here, like immune system issues, drug interactions, and the fact that fungi are just really good at adapting. And to make matters worse, some strains of fungi are now resistant to multiple types of antifungal drugs.

Resistance occurs when fungi mutate or acquire genes that allow them to survive exposure to antifungal drugs. This means that the drugs no longer work against the fungi, and they can continue to grow and cause infections. Hence, one of the most concerning aspects of antifungal resistance is that it can lead to the emergence of superbugs. Superbugs are fungi that are resistant to multiple antifungal drugs, making them extremely difficult to treat. This can result in prolonged infections, increased healthcare costs, and even death. Antifungal resistance is a growing problem, and it affects both human and animal health. To prevent antifungal resistance, it's important to use antifungal drugs only when necessary and to follow proper dosing and treatment protocols (Antimicrobial-Resistant Fungi | Fungal Diseases | CDC, n.d.-b).

Prevalence of antifungal resistance:

According to recent findings from the CDC, the antifungal medication fluconazole has demonstrated limited efficacy against Candida blood samples, with only 7% of cases responding positively. *Candida auris, Candida glabrata,* and *Candida parapsilosis* are the species that exhibit the highest rates of resistance, despite *Candida albicans* being the most common cause of severe Candida infections. The implications of antifungal resistance are significant and can manifest in various forms, including candidemia, onychomycosis, thrush, and fibrosing mediastinitis, among others. These results underscore the need for continued research and development of effective antifungal therapies (Antimicrobial Resistance in Candida | Fungal Diseases | CDC, n.d.-c).

A catastrophic fungal infection called invasive candidiasis can become life-threatening if it is not properly identified and treated. Candidemia, a condition caused by the infiltration of Candida yeast into the bloodstream, can lead to systemic infections affecting various vital organs, including the brain, bones, central nervous system, eyes, heart valves, kidneys, liver, and spleen. Individuals with certain risk factors are more susceptible to developing candidemia and invasive candidiasis. These include those who have undergone surgical procedures, particularly abdominal surgeries, and individuals who have been hospitalized for an extended period, particularly in the intensive care unit. Premature infants, those with central venous catheters, such as PICC lines, and individuals with weakened immune systems, such as organ transplant recipients or those undergoing chemotherapy, as well as those with diabetes or kidney failure, are

also at a higher risk. Individuals who use illegal drugs are also more susceptible to developing these conditions. It is important to be aware of these risk factors to take appropriate preventative measures and ensure prompt treatment if necessary. Hirano et al. (2015) conducted an experiment and in an article named "Retrospective analysis of mortality and Candida isolates of 75 patients with candidemia: a single hospital experience" stated their results which depicted that out of the total number of patients diagnosed with candidemia, a proportion of 26.6% sadly passed away within 30 days. The results are indicative of the 30-day mortality rates associated with various Candida spp. strains. *C. albicans* exhibited a mortality rate of 42.9% (12 out of 28 cases), followed by *C. guilliermondii* at 18.7% (3 out of 16 cases) and *C. glabrata* at 25% (1 out of 4 cases). Surprisingly, C. tropicalis displayed a mortality rate of 100% (2 out of 2 cases), while *C. parapsilosis* had a relatively low mortality rate of 4.3% (1 out of 23 cases). The remaining Candida spp. had a 33.3% mortality rate (1 out of 3 cases). These findings serve as valuable insights for effective clinical management and treatment of corresponding infections (Hirano et al., 2015). Invasive candidiasis is a grave medical concern, fraught with a multitude of potential complications that can have lasting impacts on one's health. These complications include endocarditis - a severe infection and inflammation of the inner lining of the heart, endophthalmitis - an infection of the eye's tissues that can cause vision loss, and osteomyelitis - a condition characterized by bone inflammation and swelling. Hospitalization for several weeks is necessary to treat this condition, which has a mortality rate of 46% to 75%. It is imperative to seek prompt medical attention if any symptoms arise according to Aboody and Mickymaray (2020) discussed in a published article named “Anti-Fungal Efficacy and Mechanisms of Flavonoids”.

Another deadly prevalent disease resulting from antifungal resistance is onychomycosis. A nail-related fungal infection is called onychomycosis (Arrese & Pierard, 2003). Tinea unguium is the term used to describe onychomycoses brought on by dermatophytes. The term "onychomycosis" includes infections caused by yeasts, saprophytic molds, and dermatophytes in addition to dermatophytes. Candida spp., primarily *Candida albicans* and *Candida parapsilosis*, are the most significant group of pathogens. To disrupt nail growth and create a patchwork of protected, moist pockets that are ideal for yeast growth, these yeasts may attack nails primarily by causing paronychia and subungual inflammation. After a thorough examination, onychomycosis was

officially diagnosed in 27.99% of the patients. Unfortunately, males fell victim to this condition more often than females with a 40.04% infection rate in comparison to 23.30%. In both genders, toenails were more prone to infection at a rate of 68.77% while fingernails made up the other 31.23%. It should also be noted that women were more commonly diagnosed with onychomycosis in fingernails at 39.74% whereas only 18.51% of men experienced this issue. Lastly, men had a higher prevalence of onychomycosis in toenails with 81.49% while 60.26% of women were faced with this concern (Gregoriou et al., 2020). These findings demonstrate the importance of early detection and treatment for both genders, regardless of which nail is affected. Onychomycoses are a common and often distressing condition, requiring prompt and appropriate care. Despite the availability of potent antifungal medications, up to 50% of patients do not respond to treatment, and relapses are common. The rates of expected relapses vary widely among studies and commercial sponsors, making it difficult to predict treatment outcomes. Even when antifungal medications penetrate the nail quickly and exhibit fungicidal activity in vitro, treatment failures and relapses can still occur. The efficacy of antifungal medications in vivo may be significantly lower than predicted by in vitro testing. A professional approach to managing onychomycoses is essential to ensure optimal patient outcomes.

Moreover, a latent but revolting antifungal resistance outcome is thrush. When conditions allow a fungus called candida to grow excessively in the mouth, thrush can happen to both children and adults. According to research findings by Akpan and Morgan (2002), there is a reported incidence of 30-45% of candida organisms present in the oral cavity of the general healthy adult population. This fungus typically dwells in a small quantity in our mouths. Your immune system and other germs that also reside in our mouths typically keep it in check. Too much of the fungus can grow when our immune system is frail or when healthy bacteria die. When dealing with mild to moderate infections in the oral cavity, the most common course of action is to administer the antifungal medication directly to the affected area. This treatment typically spans a period of 7 to 14 days, during which patients can expect to receive medication such as clotrimazole, miconazole, or nystatin - all of which have proven to be effective in combating such infections. As certain fungal species are getting resistant to these azole-class medicines, treating acute diseases like thrush has become a severe challenge to clinicians and has a prolonged infectious period (Combatting Antifungal Resistance | ASM.org, n.d.).

Lastly, a noxious disease that is also becoming crucial due to antifungal resistance is Fibrosing mediastinitis in pregnancy. Fibrosing mediastinitis (FM) is a complex condition that presents with a widespread and intrusive fibro-inflammatory growth resulting from a delayed hypersensitivity reaction to various infectious and non-infectious agents. Geographical location plays a crucial role in the prevalence of FM, with Histoplasma capsulatum being predominant in North American regions, and Mycobacterium tuberculosis found in Asian regions. In a few cases, fungi, particularly Aspergillus species, have been identified as the cause of mediastinitis. In a study conducted by P Vaideeswar, J Chaudhari, and N Goel in an article named “Fungal fibrosing mediastinitis in pregnancy – Case report with review of literature” showed that a pregnant woman exhibiting neurological symptoms was found to have a circumscribed inflammatory mediastinal mass, which had gone undetected during a clinical examination. Further investigation revealed a granulomatous reaction with intense fibro-collagenization, accompanied by septate hyphae morphologically consistent with Aspergillus species. The involvement of pulmonary hypertension in the progression of FM is linked to an unfavorable forecast, with only 70% of individuals surviving after three years of co-existence. It is noteworthy that the survival rate jumps beyond 95% when PH is absent in the condition (Miller & Elwing, 2019). The histopathologic analysis demonstrated similar features between infective and noninfective cases of FM, including edematous fibromyxoid tissue, dense collagenous fibrosis, and lymphoplasmacytic infiltration. Fungal and mycobacterial infections are common causes of infective FM and are known to induce myofibroblastic proliferation and collagen overproduction via cytokine release from activated lymphocytes and macrophages. It is of utmost importance to discern this aspergillus infection that forms masses from the invasion of the mediastinum and the formation of abscesses, which are commonly observed in individuals with compromised or suppressed immune systems. Such differentiation is crucial in maintaining a professional and informed approach to the diagnosis and treatment of this condition.

Immunocompromised patients with bone marrow failure syndromes, hematological malignancies, hematopoietic stem cell transplantation (HSCT), individuals in intensive care units (ICUs), and those with prolonged febrile neutropenia are at a heightened risk of falling prey to invasive fungal diseases (IFDs). This poses a significant threat to their health and well-being, as highlighted by Wiederhold's research in 2017. After analyzing a total of 21 studies, it was found that only 4 of these studies demonstrated a low risk of bias, while 4 showed a moderate risk of

bias, and a staggering 13 exhibited a high risk of bias. In terms of the Hem group, the unadjusted mortality rate was measured at 41.4% (95% CI: 36.4-46.7%), as reported by Vegivinti et al. in 2022. These diseases often occur in the presence of multiple comorbidities and have a case fatality rate ranging from 30 to 70%. Fortunately, the last two decades have witnessed remarkable progress in the development of antifungal classes and compounds, resulting in significant improvements in the prevention and management of IFDs. As a result, the use of antifungal agents has increased significantly, leading to better outcomes for patients.

Factors associated with antifungal resistance:

The evolution of fungal species is not solely driven by environmental factors such as global warming and climate change. Anthropological factors also play a significant role in the development of antifungal resistance. Limited discovery of new antifungal agents, fungicide overuse in agriculture, over-prescription of antifungals in healthcare, and patient non-compliance with antifungal treatments are some of the contributing factors. As professionals in the field, it is imperative that we address these issues to mitigate the development of antifungal resistance. For this study purpose, we have focused on three factors that are associated with antifungal resistance; environmental factors, molecular level or evolutionary factors, and misuse of drugs (FutureLearn, 2022).

Certain species of fungi possess inherent resistance to specific types of antifungal medications. For instance, the fungus *Aspergillus*, a mold variety ubiquitous in the environment, is impervious to the drug fluconazole. Environmental factors, such as the human-to-human transmission of fungi like Candida auris, or the use of azole fungicides in agriculture, can also lead to the development of resistant isolates. Like antibiotic-resistant bacteria, fungi that are resistant to antifungal treatments proliferate in patients undergoing such therapies, and even small initial infective doses from exogenous pathogens can enrich resistant strains. Moreover, some fungal species that commonly colonize humans are naturally resistant to specific antifungals, such as *Candida glabrate's* resistance to fluconazole, and repeated or prolonged courses of treatment can lead to their overgrowth (Nicolle et al., 1998).

A growing concern has surfaced regarding the emergence of antifungal resistance in the environment. This is largely due to the increased use of fungicides that share structural similarities with medical antifungals. The use of azole-class fungicides in agriculture, in particular, has been found to induce resistance to medical azoles. This poses a significant threat as patients can become infected with a resistant strain even if they have not been previously treated with antifungals (FutureLearn, 2022). The volume of azole use in agriculture far exceeds its medical use, which highlights the need to focus on antifungal stewardship beyond just medical prescribing. Nonetheless, the impact of medical prescribing remains crucial and cannot be overlooked (Antimicrobial-Resistant Fungi | Fungal Diseases | CDC, n.d.-c). As such, it is essential to adopt a professional approach towards addressing this issue. Individuals may inhale the resilient *Aspergillus* spores present in their surroundings, leading to potential illness.

The growing prevalence of resistance among microbes to existing antifungal drugs is a significant concern for researchers and clinicians alike. Fungal genomes are relatively small, making them susceptible to mutations and the development of resistance. However, it is crucial to recognize that only a handful of antifungals can be used as therapeutics due to the fact that fungi are eukaryotes, and their cellular targets often overlap with those of their hosts. This is in contrast to bacteria, which present unique cellular targets. Resistance typically develops through antifungal agents that bind with cell walls or biosynthetic pathways, such as fluconazole and amphotericin B. Unfortunately, the overuse of these drugs has led to an increase in drug resistance, particularly among *A. fumigatus* and *C. krusei*, which are fundamentally resistant to most azole class drugs, and *Cryptococcus neoformans*, which is resistant to fluconazole and echinocandins (Wiederhold, 2017). Therefore, there is an urgent need to explore novel drugs with greater anti-fungal activity. Traditional plant-based medicine and bioactive natural products are promising approaches that can improve existing fungal treatments with fewer side effects.

Antifungal resistance is typically acquired through changes that affect the drug-target interaction, either directly or indirectly. Genetic alterations to the target binding site, overexpression of the target, or altering drug concentration can all lead to resistance. Antifungal tolerance pertains to the capacity of drug-susceptible cells to thrive in drug concentrations exceeding the minimum inhibitory concentration (MIC). It involves the activation of general stress response and

epigenetic pathways. This phenomenon is particularly noticeable in the case of fungistatic drugs like fluconazole in Candida albicans. Despite being the subject of extensive research, the clinical relevance of tolerance still remains uncertain.

The development of antifungal drug resistance is a natural evolutionary response to the selective pressure imposed by the drug. The emergence of resistance is influenced by several factors, including the size of the population exposed to the drug, the rate of cell growth, the number of pathways that can confer resistance, and the associated fitness costs. It is worth noting that the origin of antifungal drug resistance can be either in the host or in the environment.

Moreover, the inclination to prematurely terminate the antifungal regimen and the inappropriate disposal of medications contribute significantly to the development of drug resistance according to Fisher et al. (2022). Furthermore, administering inadequate doses may exacerbate selective pressure, which fuels the proliferation of the fungi that are intended to be eradicated. These factors serve as key catalysts for the evolution of drug-resistant strains, ultimately hindering the efficacy of treatment.

Numerous fungi coexist within the microbiota as benign inhabitants, only seizing the opportunity to cause disease in individuals with weakened immune systems. Thus, devising tailored therapies for the rare instances in which these fungi become pathogenic necessitates careful deliberation.

In this article, we have focused on the resistance occurrence in azole-class drugs.

Molecular Mechanism Of Azole Resistance:

Resistance to antifungal drugs is a complex issue, with numerous mechanisms employed by fungal cells to thwart drug efficacy. The most common resistance mechanisms involve the limitation of drug entry into the cell, alterations or degradation of the drug within the cell, modifications to the target enzyme-drug binding activity, changes to other enzymes in the same pathway, and enhanced drug efflux (Sanguinetti et al., 2005). Despite recent molecular investigations into antifungal drug resistance, certain areas remain inadequately explored, warranting further research.

Drug resistance is a complex phenomenon that can arise through various mechanisms, including modification of target enzymes and biochemical pathways. In the case of azole drugs, the ergosterol biosynthetic pathway is the primary target (Sanguinetti et al., 2005). By analyzing the sterols present in a cell, valuable insights can be gained into the alterations that have occurred in resistant strains.

Alterations within the ergosterol pathway have the potential to trigger cross-resistance to analogous medications, intensifying the challenge of drug resistance. An impaired lanosterol demethylase, the primary target enzyme for azoles, may culminate in the buildup of 14a-methyl sterols, including fecosterol and the diol 14a-methyl-ergosta-8,24-dien-3b,6a-diol (Sanguinetti et al., 2005). The presence of these sterols can affect the function and fluidity of the plasma membrane, leading to changes in cell behavior.

Interestingly, cells with 14a-methyl sterols seem to be more vulnerable to host microbicidal systems that rely on oxygen (Sanguinetti et al., 2005) . Additionally, the diol that accumulates is known to cause growth arrest in *Saccharomyces cerevisiae* yet is thought to be tolerated in *C. albicans* (Sanguinetti et al., 2005). Understanding the intricacies of drug resistance and its mechanisms is crucial in combatting this serious public health issue.

As mentioned previously, the primary target enzyme of azole drugs is lanosterol demethylase, with the gene encoding this protein known as ERG11 in fungal species (Sanguinetti et al., 2005). While previously referred to as ERG16 and CYP51A1 in *C. albicans*, the modern classification uses the ERG11 designation. Multiple genetic changes have been identified in association with ERG11 in *C. albicans*, including point mutations, overexpression, gene amplification, and gene conversion or mitotic recombination (Sanguinetti et al., 2005).

In one study, a point mutation was identified in ERG11 when comparing an azole-resistant clinical isolate with a sensitive isolate from a single strain of *C. albicans*. The genetic alteration at play here pertains to the substitution of arginine with lysine at amino acid 467, commonly referred to as R467K, as reported by Sanguinetti et al. in 2005. The mutation lies near the cysteine coordinating the fifth position of the iron atom in the heme cofactor, potentially causing structural or functional alterations in the heme.

Recent genetic manipulations have indicated that R467K alone is sufficient for azole resistance, analyzed by author Maurizio Sanguinetti and others in an article named “Mechanisms of azole resistance in clinical isolates of *Candida glabrata* collected during a hospital survey of antifungal resistance”. However, in clinical isolates, several alterations often occur simultaneously. Therefore, it's difficult to determine precisely how much R467K contributes to an isolate's overall azole resistance.

Eukaryotic cells possess two different efflux pumps that have been identified to contribute to drug resistance, namely ATP-binding cassette transporters (ABCT) and major facilitators (MF). Both types of pumps have been observed to cause drug resistance in other systems. ABCT, in particular, is commonly linked with the active efflux of molecules that are toxic to cells and possess a relatively hydrophobic or lipophilic nature - as is the case with most azole drugs. While the MF has not undergone extensive investigation as compared to ABCT, they too are associated with relatively hydrophobic molecules, such as tetracycline (Sanguinetti et al., 2005).

Azoles penetrate susceptible cells via an elusive mechanism and aim to tackle lanosterol demethylase, the target enzyme, and product of the ERG11 gene, which forms part of the ergosterol biosynthetic pathway (Sanguinetti et al., 2005). Low-level expression of the CDR and MDR1 genes is commonly observed. Conversely, in a resistant cell, azoles are prevented from operating effectively with the target enzyme due to enzyme overexpression and/or modification. Additional factors that may contribute to resistance include mutations in auxiliary enzymes within the pathway, such as ERG3 (Sanguinetti et al., 2005). ABCT genes, which include the CDR genes, are often upregulated and effective at eliminating multiple azole drugs, while MF genes like MDR1 exhibit selectivity towards fluconazole. These findings suggest that resistance mechanisms are complex and multifaceted, underscoring the need for continued research in this area.

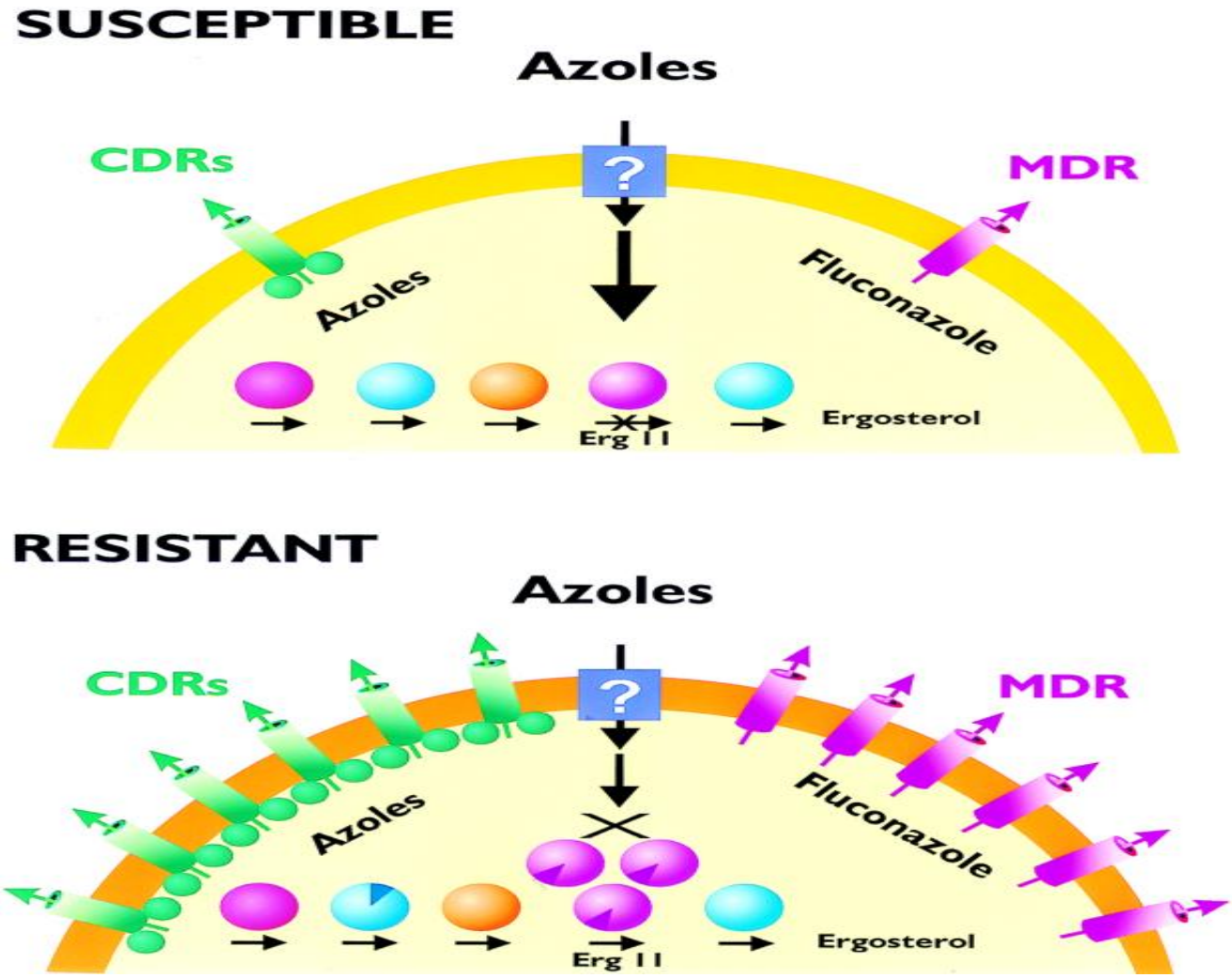

Figure 1 | Molecular mechanism of azole resistance; Source: White, T. C., Marr, K. A., & Bowden, R. A. (1998b). Clinical, Cellular, and Molecular Factors That Contribute to Antifungal Drug Resistance. Clinical Microbiology Reviews, 11(2), 382–402. https://doi.org/10.1128/cmr.11.2.382

The figure intends to elaborate on the fact that the molecular mechanisms of azole resistance are complex and multifaceted. In susceptible cells, azole drugs enter through an unknown mechanism, potentially passive diffusion. These drugs then inhibit Erg11, which is responsible for ergosterol formation. Low levels of efflux pumps, including ABCT CDR proteins and MF MDR proteins, are expressed in these cells. However, in resistant cells, the azoles still enter through an unknown mechanism, but the drugs are less effective against Erg11 for two reasons. Firstly, point mutations have modified the enzyme, and secondly, the enzyme is overexpressed.

Modifications in other enzymes also contribute to azole resistance, and the sterol components of the plasma membrane are altered as well. Furthermore, these resistant cells overexpress the CDR genes (ABCT) and MDR (MF), which remove the azoles from the cell. The CDR genes are effective against many azole drugs, while MDR seems to be specific for fluconazole. Understanding the molecular mechanisms of azole resistance is essential for the development of more effective antifungal therapies.

Resistance detection and surveillance:

The identification of antifungal resistance has long been based on susceptibility testing utilizing cultured microorganisms. The process entails determining the Minimum Inhibitory Concentration (MIC) of specific antimicrobials and contrasting them with clinical breakpoints to ascertain susceptibility or resistance (Yamin et al. 2022). A range of techniques are available for conducting this type of testing, including broth microdilution, disk diffusion, azole agar screening, gradient diffusion, and the utilization of rapid automated instruments.

Organizations like the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) set standards for conducting susceptibility testing and establishing clinical breakpoints for the effective treatment of infections (Wiederhold, 2017). However, the reference methods used by CLSI and EUCAST – namely, standardized broth microdilution techniques – are time-consuming, labor-intensive, and not conducted frequently in many clinical laboratories. Furthermore, these methods require mycological culture from clinical specimens, which limits sensitivity and cannot detect unculturable *Pneumocystis jirovecii* (Wiederhold, 2017).

Clinical breakpoints are typically determined for the most common species of fungi (such as *C. albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis,* and *A. fumigatus*) and are often used as proxy breakpoints for less studied species (Fisher et al., 2022). However, this over-reliance on established breakpoints can be problematic.

In summary, accurately identifying antifungal resistance requires a thorough understanding of the testing methods available, as well as the limitations of those methods. It is important to note that established breakpoints may not apply to all species and that sensitivity and accuracy can be compromised by reliance on standardized techniques alone.

Molecular diagnostic techniques possess immense potential in identifying genetic markers linked with antifungal resistance and recognizing intrinsically resistant fungal species. Unfortunately, this potential remains underutilized (Fisher et al., 2022). These approaches offer high sensitivity and can be directly applied to clinical specimens, thereby eliminating the need for culture and improving turnaround times. Real-time PCR can identify species of the *Aspergillus fumigati* complex that are difficult to differentiate using conventional methods and have potentially higher MIC values than azole antifungals. Additionally, PCR can detect and differentiate resistant *Candida* spp., such as *C. auris, C. glabrata, and Candida krusei*, potentially aiding infection control and patient management (Fisher et al., 2022). The use of fully automated molecular platforms, such as T2 Biosystems or Becton Dickinson Max, offers rapid testing systems that require minimal specialist training, comparable to the Cepheid GeneXpert platform for detecting multidrug-resistant tuberculosis. However, to expand the range of this potential near-patient test, the detection of mutations associated with resistance in generally susceptible fungal species must be included (Fisher et al., 2022).

Identifying potential resistance-associated mutations in drug target proteins has been commonly conducted through direct sequencing of genes such as CYP51A in *A. fumigatus* and ERG11 in *Candida* spp (Fisher et al., 2022). However, with azole resistance associated with a variety of mechanisms and mutations, real-time PCR approaches are currently limited, and DNA sequencing remains the best option for mutation identification. This technique is particularly valuable for direct sample testing but presents limitations in terms of clinical application. Nevertheless, the sequencing of ERG11 and FKS1 genes in azole and echinocandin-resistant *C. auris* strains has allowed for the identification of hotspots and specific mutations, leading to the development of rapid molecular tests. PCR assays have also been developed for a small number of FKS1 gene mutations associated with the majority of echinocandin resistance in *Candida* spp stated in an article “Tackling the emerging threat of antifungal resistance to human health” by

Matthew C. Fisher et al. (2022). It is crucial to recognize that the absence of commercial PCR tests to detect mutations associated with antifungal resistance in yeasts calls for enhanced commercial development and regulatory body support to improve diagnosis. Overall, professional and objective language has been maintained throughout this revised text (Fisher et al., 2022).

Whole-genome sequencing (WGS) presents a unique opportunity to not only detect resistance alleles but also reconstruct the evolutionary trajectories of AMR variants across time and space in human fungal pathogens (Fisher et al., 2022). However, the lack of a standardized WGS typing method for fungi has hindered progress due to their larger genome sizes, frequent sexual recombination, and the absence of standardized bioinformatic pipelines. To overcome these challenges and promote a more WGS-driven understanding of fungal AMR, enhanced knowledge of antifungal resistance determinants and species genomes is necessary (Fisher et al., 2022). The development of rapid genomic analysis has been instrumental in comprehending the international and local-scale transmission of C. auris and the emergence of multidrug-resistant variants. However, the emergence of unculturable fungi presents new challenges, warranting the need for more targeted methods. (Fisher et al., 2022). As such, continued research efforts are needed to standardize WGS typing methods for fungi and develop targeted methods for the analysis of unculturable fungi, in order to advance our understanding of fungal AMR and improve patient outcomes.

Treatment of resistant fungi with new antifungals, therapeutical approach:

Presently, numerous antifungal agents are undergoing pre-clinical and clinical assessment with the hope of showcasing their effectiveness in combating fungal infections (Fisher et al., 2022). These agents exhibit mechanisms of action similar to those of currently utilized antifungal classes but could provide even greater benefits. Several of these agents have already progressed from pre-clinical development to clinical trials involving both healthy volunteers and patients,

signaling promising potential (Wiederhold, 2017). In the near future, it is possible that some of these agents may be accessible for clinical use.

VT-1129, VT-1161, and VT-1598 – fungal specific inhibitors of Cyp51

One of the primary challenges facing the azole class of antifungals is their propensity for clinically significant drug-drug interactions, this was discovered by expert Wiederhold back in 2017. While these agents effectively inhibit the fungal lanosterol 14a-demethylase (Cyp51), they can also impede cytochrome P450 (CYP 450) enzymes responsible for metabolizing a range of substances, including other drugs (Wiederhold, 2017). However, promising preclinical results have been reported for three modified agents (VT-1129, VT-1161, and VT-1598), with one (VT-1129) currently undergoing clinical studies. These agents exhibit potent activity against various yeast isolates, including C. albicans and non-C. albicans species, as well as Cryptococcus species (Wiederhold, 2017).

F901318 – inhibition of fungal pyrimidine biosynthesis

The investigational agent F901318, developed by F2G, Inc. in Manchester, UK, has been found to effectively inhibit the oxidoreductase enzyme dihydroorotate dehydrogenase, which plays a crucial role in pyrimidine biosynthesis (Wiederhold, 2017). This member of the orotomide class of compounds has demonstrated remarkable fungal-specific activity, with significantly higher potency against *A. fumigatus* dihydroorotate dehydrogenase compared to the human enzyme (IC50 44 nM versus >100 mM, respectively). According to the author, F901318 has also exhibited potent in vitro activity against various molds, including *Scedosporium* species and *L. prolificans*, as well as endemic fungi such as *Blastomyces dermatitidis*, *Coccidioides* species, and *Histoplasma capsulatum*. These findings hold immense promise for the development of novel antifungal therapies (Wiederhold, 2017).

In the article “Antifungal resistance: current trends and future strategies to combat” emphasizes that there are a variety of antifungal agents currently at our disposal for therapeutic use. Among them are Polyenes, such as amphotericin B, which exert their effects by disrupting fungal cell membranes. However, it's worth noting that certain species, like *Trichosporon beigelii*, have

developed resistance to this class of medication (Wiederhold, 2017). Additionally, Pyrimidine analogs, like 5-fluorouracil (5-FC), work by inhibiting nucleic acid synthesis. Yet, it's important to be aware that certain fungi, such as those belonging to the *Cryptococcus* genus, have also developed resistance to this type of medication.

A recent groundbreaking study conducted by Barlow-Hall delves into the evolutionary potential of genes to develop resistance. Using an innovative approach, the authors generated all possible sequence variants of a gene target through artificial chromosomal recombination. To test for drug resistance, a fungal vehicle, S. cerevisiae, was utilized due to its well-known molecular and genomic information (Wiederhold, 2017). This allowed for the impact of these variants to be observed in the presence of an antifungal agent, resulting in a thorough understanding of plausible drug targets in biological networks. Although initially aimed at predicting antibiotic resistance, the study's potential also extends to predicting the emergence of antifungal drug resistance before clinical manifestations occur (Wiederhold, 2017). These findings have significant implications for the future of drug discovery and the fight against drug-resistant fungal infections.

Conclusion:

Clinicians are facing significant challenges related to antifungal resistance as several non-C. albicans species are showing increased resistance to azole and echinocandins. Additionally, *A. fumigatus* is displaying azole resistance, which could potentially be due to clinical or environmental exposure (Srinivasan et al., 2014). Furthermore, various species of pathogenic fungi exhibit reduced susceptibility or frank resistance to several available antifungal agents. However, several new antifungal drugs are in the developing process, offering hope for overcoming antifungal resistance and avoiding adverse effects and drug interactions associated with current treatments. It is vital for healthcare providers to remain vigilant in identifying and addressing these emerging challenges to protect patient health against debilitating fungal infections (Combatting Antifungal Resistance | ASM.org, n.d.-b).

The increasing prevalence of mycoses, coupled with emerging drug resistance and poor patient outcomes, pose significant challenges for current antifungal therapies. Although drug resistance is an evolutionary occurrence that cannot be entirely avoided, a comprehensive comprehension of drug-resistance mechanisms can lead to effective strategies for managing antifungal resistance. These measures include advanced diagnostics for early infection detection, preemptive treatment, prophylaxis, combination drug therapy, and the identification of new antifungals.

With the advent of ultra-high-throughput screening techniques, the search for novel and more effective antifungal agents is now a possibility. Future investigations could focus on identifying new drug classes that target mechanisms that do not promote drug resistance or impart selective pressure. By taking a proactive approach to managing antifungal resistance, we can improve patient outcomes and better address the challenges posed by this growing problem in clinical practice.

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