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The Pharmacological Implications of Antifungal Drugs-Review

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ABSTRACT

There are many fungicides used against many fungal pathogens. Fungal pathogens like Histoplasm capsulatum , Cryptococcus neoformans,Candida, Epidermophyton all these fungal pathogens causes diseases like meningitis, lung abscess, skin disorders etc. The major antibiotics used for these disease and their diagnosis pattern are discussed.

KEYWORDS*: Histoplasm capsulatum, Cryptococcus neoformans, Candida, Epidermophyton, fungal pathogens, meningitis, lung abscess, skin disorders*

INTRODUCTION

Fungal species belonging to *Candida*, *Aspergillus*, the *Cryptococcus* and *Pneumocystis* genera are the most clinically relevant pathogens causing the IFIs . Unlike the numerous classes of antibiotics availed to treat bacterial infections, antifungals are limited in number and belong to three main classes, including azoles (the fluconazole, itraconazole, voriconazole, posaconazole, etc.), echinocandins (the caspofungin, micafungin, and anidulafungin), and polyenes, such as amphotericin B(AMB). The Azoles bind to *Erg11* in the *Candida* and *Cyp51A* in *Aspergillus* species and interrupt the production of ergosterol, a critical sterol component of the fungal cell membranes, while echinocandins target the catalytic subunit of β-1,3-D-glucan synthase, encoded by the *FKS* genes and interfere with β-1,3-D-glucan production, a major and pivotal cell wall structural component (Selmecki et al.,2008).

Fungicides

Lastly, polyenes bind to ergosterol in the cell membrane and the cause cell death through the

formation of large pores on the cell membrane, which paves to interruption of osmotic pressure. Either antifungals can be the fungicidal, where the antifungal agent causes fungal cell death, or fungistatic, where the antifungal drug arrests the cell proliferation but does not kill the fungal cell, such as fluconazole against *Candida* and the echinocandins against *Aspergillus* species. Currently, mold-active triazoles, encompassing voriconazole, posaconazole, and isavuconazole, and echinocandins, especially the micafungin and caspofungin, are recommended first-line antifungals availed to treat invasive *Aspergillus* and *Candida* infections, respectively. Polyenes are availed with caution due to the potential for nephrotoxicity and hepatotoxicity and are availed more commonly to treat refractory *Candida* and *Aspergillus* infestations (Selmecki et al., 2006). Echinocandins are insensitive against *Cryptococcus neoformans*.

Therefore, cryptococcal infection treatment often involves amphotericin B, 5-fluorocytosine, and the fluconazole. Azoles are not the drug of choice for the treatment of *Pneumocystis* pneumonia (PCP), and th ealternative treatments include dapsone plus trimethoprim, clindamycin plus primaquine, atovaquone, the pentamidine, or caspofungin.

Moreover, chemoprophylaxis and treatment with the trimethoprim–sulfamethoxazole (TMP–SMX) have been associated with a significant reduction of the mortality rate among HIV-infected and the non-HIV-infected immunocompromised host suffering from PCP. Considering the involvement of the host- and drug-related factors affecting the antifungal therapeutic failure, the emergence of the resistance to one antifungal agent can be devastating and severely limit the number of the antifungals available to treat IFIs (Slaven et al.,2002) .

Antifungal Drugs: Classes and Modes of Action

Currently, only four classes of the systemic antifungal treatments are used in clinical practice: azoles, echinocandins, the polyenes, and pyrimidines (Posteraro et al., 2003). Azole antifungal drugs, first reported in the late 1960s, work by inhibiting the synthesis of the ergosterol, an essential component of fungal cell membranes. This results in aggrandized permeability of the fungal cell, leading to its destruction (Fig-1).

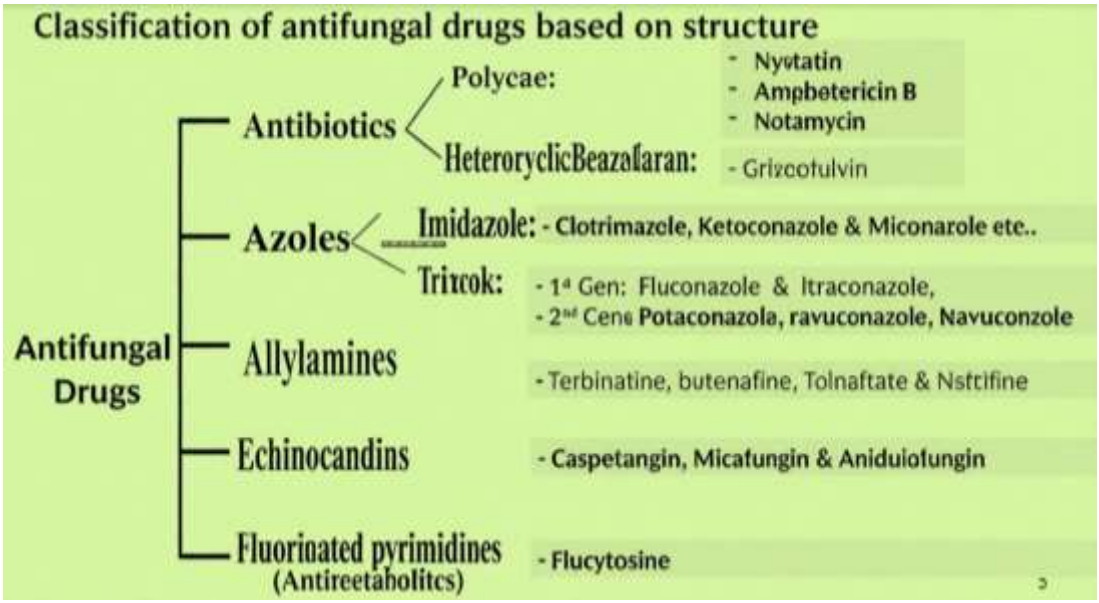

Figure: 1

Azoles are availed to treat a variety of fungal infections, including dermatophytosis, candidiasis, and the aspergillosis. There are several classes of azole antifungal drugs , including

(i) imidazoles, such as clotrimazole, the econazole, and miconazole, which are commonly used to treat skin and the nail infections,

(ii) triazoles, such as fluconazole, itraconazole, and voriconazole, which are availed to treat systemic fungal infections and

(iii) allylamines, such as terbinafine, which is availed to treat dermatophyte infections.

Ergosterol acts as a control mechanism for the fluidity, the asymmetry, and overall stability of the cell membrane in fungal cells. For the cell membrane to mainly maintain its integrity, the incorporated sterols must not have a C-4 methyl group. The Azoles primarily affect the heme protein Cyp51 (Erg11), which biocatalyzes the cytochrome P-450-dependent removal of a methyl group from the lanosterol through the process of 14α-demethylation within the ergosterol biosynthesis pathway. The Azole drugs inhibit the enzyme reaction by non-competitive reversible interaction with the heme and prevent accessing of the protons to the active site. When the 14α-demethylase is inhibited, the levels of the ergosterol decrease, and the levels of its precursors, such as the lanosterol, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol, increase, paving to structural and functional changes in the plasma membrane(Morschhäuser et al.,2007) .

The neo gene drive approach is based on the CRISPR-Cas9 system, in which a DNA-cutting Cas9 bio enzyme is targeted to two regions that flank a gene in haploid C. albicans fungi by two so-called guide RNAs (gRNAs). After the targeted gene sequences has been cut out, an engineered gene drive cassette expressing all the Cas9 and gRNA components is inserted in its place. When the two haploid fungi are mated to form diploid offspring, the gene drive will also substitute the gene's counterpart in the other chromosome, effectively precisely deleting the original versions from the organism entirely. By applying their gene deletion approach, the team was able to identify 2/4 combinations of the genes that act synergistically in defying certain drugs, or in triggering the biofilm formation. "For example, deleting either the two efflux pump-encoding genes CDR1 and the CDR2, or TPO3 and CDR2 together, rendered C. albicans highly sensitive to the fluconazole and other antifungal drugs, suggesting that targeting two mechanisms at the same time could aid overcome drug resistance."

In biofilm formation assays, researchers also found that loss of the ALS3 adhesion factor gene synergizes with the loss of the several other adhesion factor genes, which makes it a highly interconnected hub of the biofilm adhesion and an interesting candidate to further explore." The study offers new inroads into understanding the difficult territory of the C. albicans pathogenesis and drug resistance (Coste et al.,2006). "One can now get a much better handle on how genetic networks that underlie the virulence of the C. albicans are organized, see how they respond to specific environmental and drug perturbations, and thereby uncover neo vulnerabilities, that in the future may lead to new drug targets and the combination therapies."

Moreover, our gene drive array platform can be a blueprint for the similar approaches in other fungal pathogens, such as the newly emerging species Candida auris, which is highly drug resistant and has already been marked as a threat by the centers for the Disease Control and Prevention." "This symbiotic collaboration between the faculty leaders of two of the Wyss Institute's Enabling Technology Platforms, Jim Collins and the George Church, led to important new insights into the biology of this infectious fungal pathogens and how it develops resistance, in addition to the 3/4 opening an entirely new path for design of more effective antifungal therapies (Poikonen et al.,2010)."

STANDARD ASSAYS OF ANTIFUNGAL RESISTANCE

The Clinical laboratory definitions of resistance are based on assays performed according to stringent criteria. The Resistance is measured and quantified using three types of assays: disk diffusion assays, the broth micro dilution assay or e-test® strips. Those tests are performed usually after 24h of growth (Lindberg et al., 2019). The Disk diffusion assays are performed by spreading fungal cells on agar medium and then placing a filter disk containing a main standard concentration of drug on the plate; the fungal growth produces a lawn outside the zone of inhibition. Images of the plates are analyzed availing *diskImageR*, a software program that averages pixel intensity along the 72 radii emanating from the disk and estimates the average pixel intensity corresponding to the cell density as a function of distance from the disk.

The level of susceptibility is measured as the average radius (RAD), which mainly corresponds to the point where growth is inhibited by 20%, 50% or 80%. The minimum inhibitory concentration (MIC) is precisely inversely correlated with the RAD. Shown are disk diffusion assays for two isolates with the similar MIC levels and different tolerance levels, as determined from the degree of growth within the region of growth inhibition detected visually on the plates and calculated availing *diskImageR* (as described below).

Broth micro dilution assays are mainly performed by diluting a standard number of cells in microtiter wells containing drug in aggrnadizing concentrations (usually 2-fold increments) and propagating them (Hesstvedt et al., 2015). The lowest concentration that inhibits growth by about 50% or more is generally considered the MIC (bio indicated by the yellow line and boxed wells); the Clinical and the Laboratory Standards Institute (CSLI) suggests visual reading of the plates, and the European Committee on the Antimicrobial Susceptibility Testing (EUCAST) recommends measuring optical density. The E-test strips ® contain a gradient of drug and include markings that correspond to the drug concentration. The MIC is mainly determined from the position of the edge of the zone of inhibition (indicated by the arrow) after the 24h of growth.

The Clinical breakpoints refer to MIC values above which isolates are considered resistant. The Specific clinical breakpoints for fluconazole, voriconazole and the echinocandins have been mainly established for *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, the

Candida krusei, *Candida tropicalis* and *Candida guilliermondii*. No amphotericin B breakpoints are mainly available for any organisms. Based on the clinical breakpoints, the interpretive guidelines are established by CLSI or EUCAST for these organisms and classify the strains as sensitive, resistant or intermediate(Leroy et al.,2016).

MEASURING ANTIFUNGAL TOLERANCE

Tolerance is quantified availing either disk diffusion assays or broth micro dilution assays, generally after 48h of growth. In the disk diffusion assays, *diskImageR* calculates the fraction of growth (FoG) inside zone of inhibition for a given RAD (usually at 20% or 50% inhibition) as the pixel intensity in the area under the Strains with similar levels of the susceptibility (determined as MIC/RAD) often exhibit different levels of tolerance. Note that the tolerant growth is often similar in density, measured as a similar pixel intensity, over some distance from the disk. Broth micro dilution assays analysed at 48h also garner a tolerance metric, termed the supra-MIC growth (SMG). SMG is quantified as the average growth per well (measured as the optical density), for all drug concentrations above the MIC, normalized to growth in the absence of the drug. The E-test strip® assays provide a visual approximation of tolerance as the proportion of growth within the zone of the inhibition relative to growth outside it. Solid media assays enable the visualization of the subpopulation growth, evident as individual colonies, as well as the effect of drug concentration (Papadimitriou-Olivgeris et al.,2019).

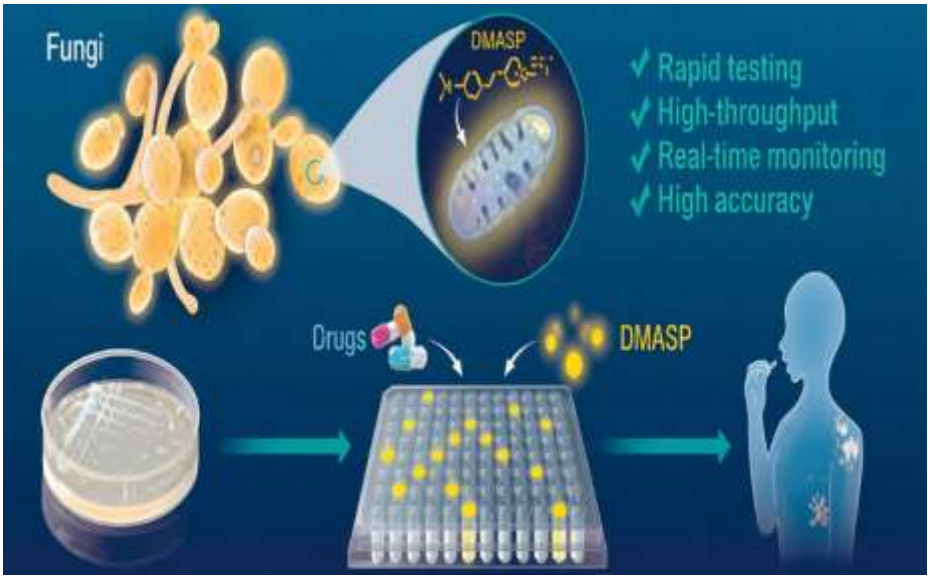

Figure: 2

The terms antifungal drug resistance and the tolerance are frequently used as synonyms in the literature (Fig-2). However, a more nuanced understanding of the fungal response to the drugs may help explain the poor outcomes for the infections caused by apparently susceptible isolates (that is, isolates that have a susceptible MIC at 24h, the standard endpoints for MIC measurements for *Candida* species) (Dimopoulos et al.,2009).

Antifungal tolerance as the ability of a drug-susceptible fungal strain to grow in the presence of an antifungal drug at the concentrations above the MIC. The Subpopulations of tolerant cells grow slowly in drug concentrations above the MIC, which is usually visually evident after time periods much longer than the standard 24 h for the MIC measurements. In the same isogenic population, a second subpopulation of cells does not grow in the drug or its extremely slow growth is quiet not detectable following 48 h in the drug, researchers assessed that antifungal tolerance is a property of pathogenic fungi that can be distinguished from the resistance using the same *in vitro* assay that can quantify both susceptibility [G] (measured as the MIC at 24 h) and growth properties at 48 h (the fraction of growth [G] or the supra-MIC growth [G]) (Puig-Asensio et al,2014).

Researchers further suggest that the question of whether tolerance may explain some of the discordance between the clinical outcome and clinical MIC measurements is worthy of the larger scale clinical studies. Many antimicrobial drugs like sulpha drugs inhibit the pathogenic activity (Dr. S. Sreeremya, 2024). The antimicrobial resistance study provides a clear delineation (S. Sreeremya, 2018).There are other classes of pathogens like parasites and helminths, which can be inhibited by potent drugs (Dr. S. Sreeremya, 2025).

RESISTANCE TO AZOLES

Azole antifungals inhibit the ergosterol biosynthetic pathway by targeting mainly the cytochrome P450-dependent enzyme lanosterol 14-α-demethylase, encoded by the Erg11 in yeasts, and Cyp51A/Cyp51B in the molds. Inhibiting this pathway disrupts the production of ergosterol and results in a bioaccumulation of toxic sterol intermediates that perturb membrane stability and also impede fungal growth (Tortorano et al.,2013) . One of the most prevalent mechanisms of azole resistance involves alteration or the overexpression of the drug target gene, *ERG11/cyp51A/cyp51B,* with *Candida* and the *Aspergillus* resistant isolates often having amino acid substitutions in regions close to the heme-binding site of the biocatalyst.

Furthermore, constitutive overexpression of the *ERG11* via gain-of-function mutations in the transcriptional activator Upc2 is commonly found in resistant isolates of the *C. albicans*. In other human fungal pathogens, the distinct sterol regulatory elements such as the transcription factor Sre1 in the *C. neoformans* and SrbA in *A. fumigatus,* have also been implicated in responses to the antifungal drugs and the virulence. Alterations in other components of the ergosterol biosynthetic pathway, such as loss of the function of the Δ-5,6-desaturase enzyme Erg3, can also enable azole resistance. The *ERG3* mutations pave to the depletion of the ergosterol and the accumulation of the alternative sterols, often resulting in cross-resistance to azoles and the polyenes. *ERG3*-mediated azole resistance intimately depends on the key stress response regulators such as the protein phosphatase calcineurin, the protein kinase Pkc1, the bio molecular chaperone Hsp90, and likely other regulators that remain to be identified. In fact, the bio functional genomic screens have identified several genes encompassing the diverse cellular processes important for mediating azole tolerance in both *C. albicans* and *C. neoformans* (Mirhendi et al., 2020).

Another common mechanism of the acquired azole resistance involves the upregulation of multidrug transporters. The ATP-binding cassette (ABC) transporters Cdr1 and the Cdr2, as well as the major facilitator the Mdr1 have all been implicated in clinical azole resistance of many *Candida* species. Upregulation of Mdr1 has been shown to simultaneously enable *C. albicans* azole resistance as well as the escape from intrinsic host defences through the efflux of antimicrobial peptides, such as histatin 5. The expression of the *CDR1* and *CDR2* is regulated by the transcription factor Tac1 in *C. albicans*, with the *TAC1* alleles harboring gain-of-function mutations readily identified in resistant isolates. Similarly, the mutations in the transcription factor gene *MRR1* pave to upregulation of Mdr1 in azole-resistant isolates of *C. albicans*. In *C. neoformans* and the *A. fumigatus*, the ABC transporters responsible for azole efflux are Afr1 and AtrF, respectively (Taj-Aldeen et al., 2014).

COCNLUSION

Many antifungal drugs like cotrimazole, amphotericin, caspafungin are the major drug categories used to inhibit the action of fungal pathogens.Genomic alterations that pave to an increased dosage of drug transporters provide an alternative route to enhance drug efflux. Fungal species are capable of the remarkable genomic plasticity in response to diverse the environmental stresses.

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