ADDRESSING THE GLOBAL THREAT OF RISING ANTI-FUNGAL RESISTANCE: MECHANISTIC INSIGHTS AND MANAGEMENT CHALLENGES

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ABSTRACT

Fungi are integral to natural ecosystems and maintaining soil health. Fungi play an important role in ecological balance through nutrient recycling and environmental sustainability, yet certain species are pathogenic and capable of causing a wide range of human diseases. In humans, fungal infections can vary from superficial infections similar as athlete's foot to potentially fatal systemic infections. Fungal infections are raising global health issue, especially among immunocompromised cases. Recent estimates suggest that approximately 65 million invasive fungal infections occur annually, resulting in nearly 3.8 million deaths worldwide. This review provides a comprehensive overview of fungi, including their types of causative organisms and pathogenic potential such as *Aspergillus*, *Candida*, *Cryptococcus* and various dimorphic fungi are highlighted along with the complications associated with the infections they cause. It also discusses the increasing incidence of opportunistic infections, the challenges in managing fungal infection and the rising prevalence of antifungal-resistant pathogens. Special emphasis is given to the discussion of burden of resistance development on antifungal classes of drugs such as azole, polyenes and echinocandin fungal species. The mechanisms underlying in antifungal resistance, including intrinsic and acquired resistance were also depicted in the article.

Keywords: Fungal infections, Antifungal resistance, Drug-resistant fungi, Mechanism of resistance.

INTRODUCTION

Fungi are eukaryotic creatures that can be unicellular or multicellular and can be found in all types of habitats. There are many different types of fungi, from microscopic yeasts and moulds to macroscopic fungi like mushrooms. (1) Although fungi are not harmful to humans, under some circumstances they can cause illness. Spores, which can be breathed or taken up by direct contact, are released when fungi reproduce. Fungal infections are most likely to harm the skin, nails, or lungs in general. They can enter the skin, impact your organs, and result in a systemic infection that affects your entire body. (2) Fungal diseases, also referred to as mycoses, are different from most bacterial infections because they usually develop as chronic conditions and cause slow progression leading to gradual harm to the host. Fungal infection related morbidity rates are a significant health concern. (3) In recent years, the rising number of immunocompromised patients, the increased use of broad-spectrum antibiotics, invasive medical procedures, and environmental changes, the frequency of fungal infections has dramatically grown in recent years. Therefore, enhancing fungal disease diagnosis, prevention, and treatment approaches requires an understanding of the biology of fungi, their pathogenic mechanisms, and the reasons causing antifungal resistance.

FUNGI (1,2)

Fungi are eukaryotic organisms with cellular structures similar to those of humans and plants, distinguishing them significantly from bacteria. They exist in diverse forms, ranging from microscopic yeasts to large multicellular organisms, eg., mushrooms. Most fungi are saprophytes, meaning they decompose organic matter and recycle nutrients essential for new growth. Although fungi are widespread and varied ranging from yeast cells less than 5 μm in diameter to fruiting bodies up to 1 meter tall and colonial networks spanning over 800 square meters. only a small fraction are pathogenic to humans. Diseases caused by fungi include aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, mucormycosis, paracoccidioidomycosis and sporotrichosis. Historically, fungi were inaccurately classified as nonphotosynthetic plants, but this view is now considered phylogenetically incorrect. Fungi are neither plants nor animals; they form their own distinct biological kingdom, reflecting their unique evolutionary lineage and ecological roles.

Types of Fungi (3,4)

Fungi kingdom is classified into five classes, they are,

- Chytridiomycota
- Zygomycota
- Glomeromycota
- Ascomycota
- Basidiomycota

Chytridiomycota: Typically asexual, chytrids are creatures that create spores using flagella, which are tiny appendages that simulate tails. It burrows under the skin of frogs, causing fungal infections. They are true fungi.

zygomycota: The majority of zygomycota are terrestrial. They are conjugated fungi. They grow on human bodies and cause problems. Eg. *Rhizopus stolonifer* (bread mould).

Glomeromycota: They are Soil hosts. In order to give the plant nutrition, the fungi take sugar from the plant and then dissolve minerals in the soil. Additionally, this fungus reproduces asexually.

Ascomycota: Ascomycota are plant and animal pathogens, including those which can infect people. They were called as sac fungi and which cause diseases like ringworm, athlete's foot and ergotism, which can cause vomiting, convulsions and hallucinations, occasionally even demise.

Basidiomycota: Basidiomycota are club fungi. It produce fruiting bodies that contain basidia in the form of clubs. Spores are stored in the basidia. Eg., Mushrooms. The basidiomycota includes smuts and rusts, which are important plant pathogens and also toadstools, shelf fungi stacked on tree trunks. Most edible fungi belong to the Phylum. However, some basidiomycetes produce deadly toxins. Eg., *Cryptococcus neoformans* causes severe respiratory illness.

Table 1: Fungal Disease Burden in India as per 2022 Data (5)

Condition	Incidence Rate
Overall fungal illness prevalence	4.1% of population
Chronic pulmonary aspergillosis	1.74 million
Severe asthma with fungal sensitisation	1.36 million
Recurrent vulvovaginal candidiasis	24.3 million
Allergic bronchopulmonary aspergillosis	2.0 million
Chronic fungal rhinosinusitis	1.52 million
Invasive aspergillosis	250,900 cases/year
Mucormycosis	195,000 cases/year
Esophageal candidiasis in HIV	266,600 cases/year
Candidemia	188,000 cases/year
Fungal keratitis	1,017,100 cases/year
Cryptococcal meningitis	11,500 cases/year
Pneumocystis pneumonia	58,400 cases/year
Less prevalent infections	Mycetoma, Talaromycosis, Chromoblastomycosis, Histoplasmosis

Common fungal pathogens and challenges

Humans inhale between 1,000 and 10 billion fungal spores daily, exposing them to a wide range of potential infections depending on the type of fungus and the individual's immune status. Fungal infections can be superficial, affecting the skin, nails and hair. Eg., ringworm, athlete's foot and dandruff. (1) A small number of fungal species are responsible for the majority of deaths related to fungal infections, including *Candida albicans*, *Aspergillus*, *Blastomyces*, *Coccidioides*, *Cryptococcus neoformans*, *Histoplasma* and *Pneumocystis jirovecii*. (6)These infections often begin when microscopic spores, commonly found in soil and air, come into contact with the skin or are inhaled into the lungs, although most spores do not lead to disease unless the host is compromised. (7)

1. *Aspergillus*

Aspergillus is a globally widespread genus of mold with hundreds of species found both indoors and outdoors, especially in damp environments like compost, wet buildings and air conditioners. While exposure to its spores is common, Aspergillus primarily affects individuals with weakened immune systems, lung damage or severe allergies. The term *aspergillosis* refers to a group of diseases caused by this fungus, including invasive aspergillosis, chronic pulmonary aspergillosis, aspergilloma and allergic aspergillosis. Invasive aspergillosis, often seen in severely immunocompromised patients, mainly targets the lungs and has a high mortality rate of 25% to 90%. Chronic pulmonary aspergillosis affects those with pre-existing lung conditions and may present as fungal cavities or aspergillomas. Allergic aspergillosis results from strong immune reactions and can severely worsen respiratory conditions like asthma and cystic fibrosis. *A. fumigatus* is the most common species causing human disease, though *A. niger*, *A. flavus* and *A. terreus* are also pathogenic. Beyond human health, Aspergillus can infect plants, animals and birds. It produce toxins that lead to food spoilage or tumor formation. (8)

2. *Candida*

Candida is a genus of yeast with around 20 species capable of infecting humans. These yeasts normally reside on mucous membranes, skin and gut but can overgrow and cause candidiasis, when the immune system is weakened or beneficial bacteria are disrupted. (9) Common forms include oral thrush, especially in HIV/AIDS patients and vaginal yeast infections**,** which affect about 75% of women at least once, often triggered by pregnancy, diabetes, antibiotics or immunosuppressive treatments. (10) Candida can also cause invasive infections like candidemia, a serious bloodstream infection. Five species namely *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* are account for 95% of infections. (11) *C. auris* is particularly dangerous due to its drug resistance, difficulty in detection and high mortality rate (~30%). Both healthy and immunocompromised individuals can be affected, depending on the species and site of infection. (12)

3. *Cryptococcus*

Cryptococcosis is a serious fungal infection primarily caused by *Cryptococcus neoformans* and *Cryptococcus gattii*, affecting the lungs and central nervous system. *C. neoformans* is

widespread in the environment and infects humans through inhalation of spores or dried yeast cells, (13) especially in individuals with weakened immune systems, such as those with HIV/AIDS. (14)While antiretroviral therapy has reduced its prevalence in some regions, areas with limited healthcare access still face high infection rates. Each year, cryptococcal meningitis leads to approximately 220000 cases and over 180000 deaths globally, making Cryptococcus the leading cause of adult meningitis. (15) *C. gattii* is less common but poses a risk to those with lung conditions or compromised immunity, and is typically found in tropical and subtropical soils near certain trees. (16)

4. Dimorphic fungi (*Histoplasma, Coccidioides, Blastomyces*)

Dimorphic fungi exist in two forms: filamentous molds in the environment and spherical yeasts at body temperature. (13) Key human pathogens include *Histoplasma capsulatum* and *Coccidioides immitis/posadasii*, which can infect even healthy individuals through inhalation of spores. The body typically responds by forming granulomas into the lungs. *Coccidioides* causes coccidioidomycosis, endemic to certain regions, (17)while *Histoplasma* thrives in bird and bat guano, (13) with infections triggered when these habitats are disturbed. (17) Most cases are asymptomatic or mild, but some individuals experience flu-like symptoms or pneumonia and a few require lifelong antifungal treatment. In HIV/AIDS patients, histoplasmosis has a high mortality rate of around 30%, especially where antiretroviral therapy is limited. (18)

5. *Pneumocystis*

A fungus called *Pneumocystis jirovecii* can cause severe pneumonia in people with impaired immune systems. (19) It has evolved together with humans and to live in healthy people's lungs without triggering any visible signs. The fungus can be transmitted from person to person through the air by healthy individuals. Prior to the HIV/AIDS pandemic, pneumocystis pneumonia was uncommon, but in the 1980s, it rapidly emerged as a characteristic of AIDS, affecting over 75% of those who have the disease. (20) Pneumocystis pneumonia is still a common opportunistic infection in areas with limited resources and its prevalence has been rising among patients who are not HIV-positive, such as those who have lung disease, autoimmune or inflammatory diseases, blood or lymphatic system cancers, or transplant recipients. (19)

6. Mucormycetes

Mucormycosis(mucormycetes) is a rare but potentially fatal fungal infection caused by environmental molds commonly found in soil and decaying organic matter. It primarily affects individuals with weakened immunity. (13) The infection varies by entry point, pulmonary mucormycosis occurs from inhaling spores, often in cancer or transplant patients. Rhinocerebral mucormycosis affects diabetics and can spread from sinuses to the brain. Intestinal mucormycosis is seen in infants after ingesting spores. cutaneous mucormycosis, the most common in healthy individuals, arises from skin injuries. The fungus can spread via the bloodstream to organs like the brain, heart, spleen and skin. (20)

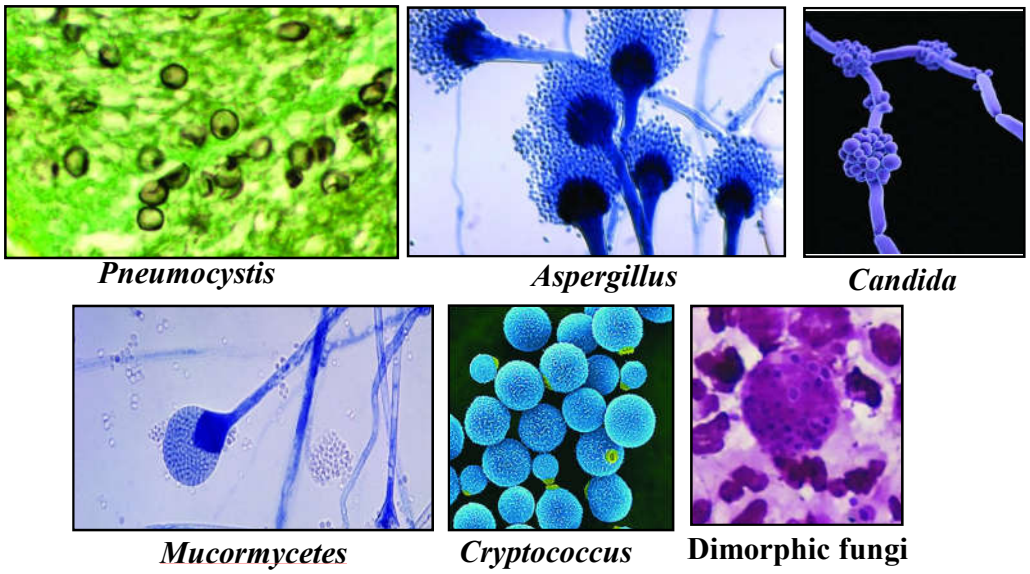

Fig.1: Fungal species which causes life threating diseases (1)

An Overview of Fungal Infections (2,21)

The location and severity of fungal diseases are used to classify them:

- **Cutaneous Infections/Superficial Infections**: Cutaneous infections, also known as superficial infections, are typically brought on by dermatophytes (such as Trichophyton, Microsporum and Epidermophyton species) and impact the skin, hair and nails. Around the world, particularly in tropical areas, diseases including onychomycosis, ringworm and athlete's foot are common. Although superficial infections are usually not serious, they can cause an enormous amount of pain and social humiliation.
- **Systemic Infections**: These can be fatal and affect internal organs. Aspergillosis, cryptococcosis and candidiasis are common systemic mycoses that mostly affect those with weakened immune systems. High lethality can result in these infections. It affects the skin as well as organs like the brain, liver, eyes and lungs. They usually affect those with compromised immune systems.
- **Localized fungal infections**: Localised fungal infections affect a single body area commonly the mouth, vagina, skin, or nails and can occur in both healthy and immunocompromised individuals. They often occur when the microbiome becomes disrupted, reducing the beneficial bacteria that usually keep fungi like *Candida* under control. Antibiotic use can eliminate these bacteria, allowing fungal overgrowth and causes mild symptoms. These infections typically resolve once microbial balance is restored.

Table.2: Types of fungal infections and causative organisms.

Sl No.	Fungal Infections	Typical causative organisms (22–25)
1.	Aspergillosis	*Aspergillus fumigatus, A. flavus*
2.	Blastomycosis	*Blastomyces dermatitidis*
3.	Candidiasis	*Candida albicans, C. glabrata, C. krusei, C. parasilosis, C. tropicalis, C. auris*
4.	Chromoblastomycosis (Chromomycosis)	*Cladosporium carrionii, Phialophora verrucosa, Fonsecaea pedrosoi*
5.	Coccidioidomycosis	*Coccidioides imitis, C. posadasii*
6.	Cryptococcosis	*Cryptococcus neoformans, C. gattii*
7.	Dermatophytosis (Tinea)	*Microsporum spp., Epidermophytum spp., Trichophyton spp.*
8.	Fusariosis	*Fusarium oxysporum, F. proliferatum, F. verticillioides*
9.	Histoplasmosis	*Histoplasma capsulatum*
10.	Mucormycosis (Zygomycosis)	*Mucor spp., Rhizopus spp.*
11.	Paracoccidioidomycosis	*Paracoccidioides brasiliensis*
12.	Pneumocystis pneumonia	*Pneumocystis jirovecii(P.carinii)*
13.	Sporotrichosis	*Sporothrix schenckii*
14.	Tinea (Pityriasis) Versicolor	*Malassezia furfur* (*Pityrosporum orbiculare), M. globosa*

How Fungal infections Developing?

A. Opportunistic fungal infection: Infections with opportunistic fungi target an impaired immune system. As a result, they typically affect persons whose immune systems are compromised by illnesses like AIDS or by medicines that weakens the immune system. Opportunistic fungal infections happen all around the world. Opportunistic fungal infections are frequently lethal and can be extremely aggressive, rapidly spreading to other tissues. (2) Example: Aspergillosis, Candidiasis and Mucormycosis.

Risk Elements for Opportunistic Fungal Infections (28)

Immunosuppressive drug use, cancer chemotherapy, corticosteroids, drugs that prevent organ transplant rejection, including cyclosporine, methotrexate, azathioprine and tumor necrosis factor inhibitors leads to opportunistic fungal Infections

Disorders that causes Fungal infection:
Diabetes, Hodgkin lymphoma or other lymphomas, leukaemia, AIDS, burns(if severe) may cause fungal infection.

B. Primary fungal infection: People with healthy immune systems can get primary fungal infections, which can occasionally have serious consequences. People may wait months or years to seek medical attention because many primary fungal infections progress slowly. Fungal infections usually do not spread to deep organs in the body if the immune system is functioning normally. For example, Pneumonia may develop in the lungs as the initial indication of infection and these illnesses typically follow inhalation of fungal spores.(2) Eg., Histoplasmosis, Blastomycosis, Coccidioidomycosis, Paracoccidioidomycosis

Challenges in Managing Fungal Infections

- **Misdiagnosis and Difficulty in Diagnosis** (26)

  Fungal infections can be easily mistaken for other conditions, leading to delayed or incorrect treatment.

- **Limited Awareness and Education** (26)

  Public awareness about fungal diseases and their prevention is often lacking.

- **Rise in Drug Resistant Strains** (27)

  Some fungal pathogens, like Candida auris are becoming increasingly resistant to antifungal medications, posing a significant challenge in treatment.

- **Environmental Sources and HAIs** (28)

  Fungal infections can be acquired from the environment or in healthcare settings (HAIs), particularly in vulnerable populations.

- **Limited Treatment Options** (29)

  For some fungal infections, especially in certain geographical regions, treatment options are limited and mortality rates can be high, even with appropriate therapy.

- **Climate Change** (26)

  Climate change can alter the patterns of fungal diseases, potentially leading to new outbreaks and increased incidence.
  In Tamil Nadu, specific fungal infections like Aspergillosis and Candidiasis are prevalent and the challenges in diagnosis and treatment are amplified by factors like the warm and humid climate, which favors fungal growth and the high prevalence of immunocompromising conditions like diabetes and HIV.

Table 3- Antifungal Drug Classification (30–32)

Sl No.	Name of the Inhibitors	Mechanism of action	Drugs
1.	**Inhibitors of Ergosterol Synthesis**	Ergosterol is the main sterol in fungal cell membranes (like cholesterol in humans). Disrupting it weakens the membrane.	
	a) Inhibit Ergosterol Biosynthesis (14α-demethylase inhibitors)	Inhibit fungal cytochrome P450 enzyme lanosterol 14α-demethylase which prevents conversion of lanosterol to ergosterol	Ketoconazole, Miconazole, Clotrimazole, Econazole, Fluconazole, Itraconazole, Voriconazole, Posaconazole, Isavuconazole, Lanoconazole, Luliconazole, oxiconazole, amorolfine
	b) Directly Bind to Ergosterol (Membrane Disruptors)	Bind to ergosterol and form pores in the membrane were leakage of cellular contents.	Amphotericin B, amphotericin B deoxycholate, liposomal amphotericin B, amphotericin B lipid complex, Nystatin, Natamycin.
	c) Inhibit Squalene Epoxidase	Block squalene epoxidase were accumulation of toxic squalene and decreased ergosterol synthesis	Terbinafine, Naftifine , Tolnaftate
2.	**Inhibitors of Cell Wall Synthesis**	Fungal cell walls contain β-(1,3)-D-glucan, Chitin absent in human cells, making this a selective target	
	a) Inhibit β-(1,3)-D-glucan synthase	Inhibit β-(1,3)-D-glucan synthase which weak cell wall intergrity osmotic lysis takes place	Caspofungin, Micafungin, Anidulafungin
	b) Inhibit chitin synthase	Inhibit chitin synthase which is essential for synthesis of chitin plays a crucial component of the fungal cell wall	Nikkomycin Z
3.	**Inhibitors of Nucleic Acid Synthesis**	Converts inside fungal cells to 5-fluorouracil (5-FU) which will inhibits thymidylate synthase and then disrupts DNA & RNA synthesis	Flucytosine (5-FC)

4.	**Inhibitors of Microtubule Function**	Binds to fungal tubulin which inhibits mitotic spindle formation and arrests cell division (mainly in dermatophytes).	Griseofulvin
5.	**Miscellaneous**	a) Chelates metal ions needed for fungal enzyme activity	Ciclopirox
		b) Fungistatic	Zinc pyrithione

Rising Antifungal Resistance

The effectiveness of traditional antifungal medicines is progressively undermined by the rise of resistant fungus strains. Drug target changes, efflux pump overexpression and biofilm formation are examples of resistance mechanisms. Particularly, echinocandin resistance in *Candida glabrata* and azole resistance have been recorded worldwide.(33)

Candida dubliniensis and ***Candida albicans*** emerged as the causes of azole-resistant mucosal candidiasis during the early HIV epidemic. Whereas fungal infections in humans have increased due to the HIV epidemic, cancer and immunomodulating medications, novel human-disease-causing fungi still have not evolved. We are currently seeing the rise of drug-resistant progenitors of common, well-known fungus, including Aspergillus fumigatus, as well as the introduction of novel fungal species, like ***Candida auris*** and ***Trichophyton indotineae***. (34)The three fungi ***C. auris***, ***A. fumigatus***, and ***T. indotineae*** have two traits in common. They are all presently spreading over the world and have a high rate of acquired resistance. (33)

Antifungal Resistance: There are two types of antifungal resistance, acquired resistance and intrinsic resistance. When a fungus species wild form spontaneously demonstrates resistance to antifungal substances, this is known as intrinsic resistance. On the other hand, acquired resistance occurs when a fungus species that is typically sensitive to antifungal drugs has developed mutations or epigenetic modifications that provide resistance. (35,36)

Intrinsic antifungal resistance

The occurrence of intrinsic resistance to antifungals is widespread. Echinocandin resistance are *Fusarium*, *Mucormycetes* and *Basidiomycetes* are intrinsically resistant because β-1,3-glucan, the drug's target is minimally present in their cell walls. (37) Fluconazole resistance in *Candida krusei* species naturally resists fluconazole due to a unique amino acid sequence in its lanosterol deacetylase, preventing drug binding and allowing ergosterol synthesis to continue. (38,39)

Acquired antifungal resistance

Mutations to target protein binding sites that block the antifungal's ability to bind are the most prevalent types of acquired resistance. **Target site mutations**: Common in azole-resistant *Aspergillus* and *Candida* species. These mutations prevent antifungal binding. **Overexpression of target proteins**: Caused by transcription factor changes, reducing drug effectiveness. **Efflux pump activation**: Especially in *Candida glabrata*, transcription factor changes increase pumps that expel antifungals before they act. **Genomic changes**: Polyploidy and chromosomal duplication can amplify target protein production.(40)**Cell wall/membrane alterations**: Some fungi modify their structure to compensate for lost target protein function, though this is less understood. (41)

Emerging fungal threats (42)

New fungal dangers are another cause for concern. *Candida auris* is a prime example of an emerging pathogen and worldwide health concern, according to the CDC. *C. auris* is frequently resistant to all of the antifungal medications that are frequently used to treat fungal infections and its risk factors are identical to those for other Candida infections. Likewise, the healthcare sector is left without oral medications to treat *aspergillosis* because to the global expansion of pan-azole resistance in *Aspergillus fumigatus.*

Emerging drug-resistant molecules

Azoles, polyenes, echinocandines, pyrimidine analouges, allyamines, thiocarbamates and morpholines are the main classes of antifungal drugs used in clinical applications. These compounds have antifungal properties which are caused by either direct contact to inhibit cell membrane or suppression of ergosterol production. The main component of the fungal cell membrane is ergosterol. (43) Fungal pathogens develop resistance through several mechanisms such as mutations in target enzymes, overexpression of efflux pumps and alteration in ergosterol biosynthesis pathways. These mechanisms reduce drug susceptibility and limit the effectiveness of antifungal therapies.

1. AZOLES(21)

Azoles constitute the largest class of antifungal agents commonly used against fungal infections. Among the most well-known examples of the earliest imidazole-based azoles to be made are miconazole (MCZ), clotrimazole (CLT), econazole (ECO), ketoconazole (KTC), tioconazole (TIO) and sulconazole (SUL). Later, triazole-based drugs with broader mechanisms of action were developed, including itraconazole and fluconazole. The most recently approved imidazole, luliconazole, is applied topically to treat infections caused by dermatophytes. Triazoles are used to treat both systemic and mucosal infections, while imidazoles are mostly used to treat fungal infections of the mucosa. Azoles act by causing the fungal cell membrane to become unstable. The ERG11 gene encodes 14-lanosterol demethylase, which transforms lanosterol into ergosterol in the fungal cell membrane. The active site of this enzyme contains an iron protoporphyrin unit. Azoles prevent the formation of ergosterol because they bind to iron. When ergosterol production is blocked, 14-methyl sterols can build up and change the stability, permeability and function of the membrane and the associated enzymes.

Mechanisms of Resistance to Azoles(44)

Azoles work by inhibiting 14α-demethylase, an enzyme crucial for ergosterol synthesis, which is essential for fungal cell membranes. Resistance arises not by modifying the azole drug itself, but by altering the target enzyme or limiting the drug's access to the enzyme**.**

(a) Modification of the target 14α-demethylase

1. Resistance can result from changes in the quality (affinity) or quantity (expression level) of the enzyme 14α-demethylase.
2. A study comparing that *C. krusei* (intrinsically resistant to fluconazole) and *C. albicans* (fluconazole-susceptible), which found,
    - No difference in sterol composition (ergosterol was major sterol in both).
    - However, the amount of fluconazole needed to inhibit ergosterol synthesis by 50% was 24 to 46 times higher in *C. krusei*, indicating the enzyme in *C. krusei* has lower affinity for fluconazole.

(b) Reduced Access to the Target Efflux Mechanisms

1. Initially, fluconazole accumulated similarly in both species.
2. But inducing drug again to enzymes, *C. krusei* showed 60% less accumulation than *C. albicans*, suggesting that C. krusei actively pumps out the drug, reducing its intracellular concentration.
3. This points to an active efflux mechanism, which is another contributor to resistance.

C. krusei displays dual resistance mechanisms: Low-affinity of 14α-demethylase (enzyme target alteration) and Active drug efflux (reduced intracellular drug levels). These mechanisms work together to produce significant fluconazole resistance.

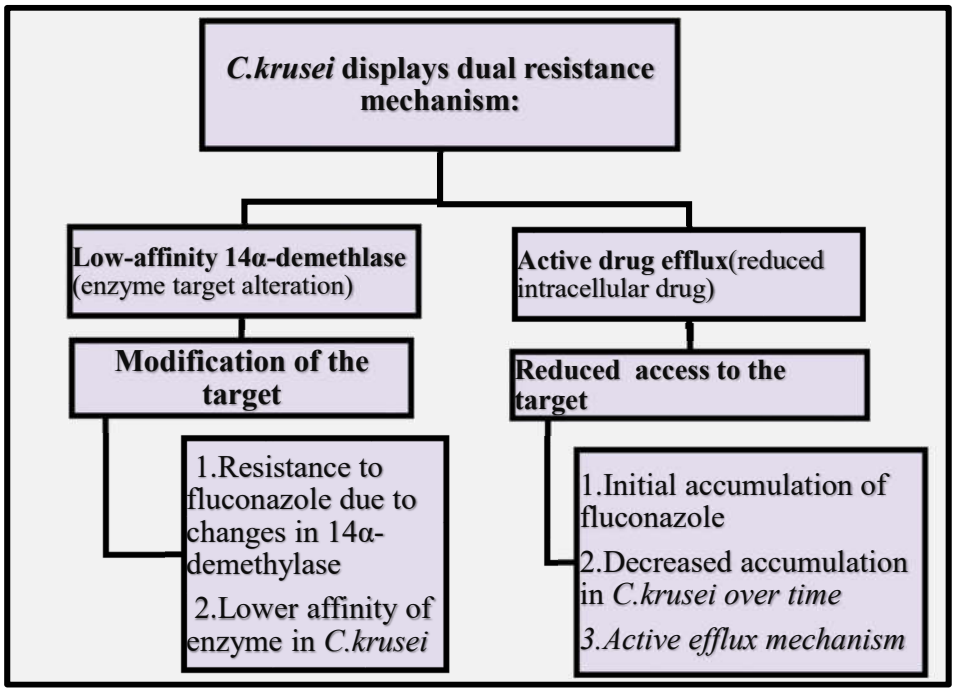

Fig.2: Flowchart of Resistance mechanism of Azoles

2. POLYENES. (21)

Polyenes are amphipathic organic compounds that are macrolides, such amphotericin B (AMB), which was initially identified from Streptomyces. Polyenes bound to ergosterol which creating holes in the plasma membrane. Loss of ionic equilibrium and weakened membrane integrity leads to cell death. Amphotericin B, nystatin and natamycin are the three primary polyenes. While natamycin and nystatin are recommended for topical infections due to their limited absorption, AMB is very effective against Cryptococcus, Candida and Aspergillus species in systemic invasive fungal infections. Because of their serious side effects, drugs like AMB are often used for invasive mycoses despite the lack of resistance.

Mechanism of Resistance to Polyenes (44)

Polyenes (e.g., amphotericin B, nystatin) target ergosterol in fungal membranes. Resistance is rare but occurs mainly in species like *Candida lusitaniae*, *C. glabrata* and *C. guilliermondii*.

Resistance Mechanisms:

1. **Altered Sterol Content:**
    - Reduced ergosterol levels or replacement with other sterols that bind poorly to polyenes.
    - May result from mutations in sterol biosynthesis enzymes.
    - Leads to less drug binding and lower membrane damage.
2. **Membrane or Sterol Changes:**
    - Structural modifications or reorientation/masking of ergosterol reduce polyene interaction.
3. **Growth Phase Effects:**
    - Stationary-phase cells are more resistant due to reduced cell wall turnover, limiting drug access.
4. **Genetic Mutations:**
    - Identified in *S. cerevisiae*, involving polyenes (pol1–pol5) linked to altered sterol production.
5. **No Efflux or Drug Modification:**
    - Polyenes act on the membrane surface, so efflux pumps and drug degradation are not major resistance pathways.

Resistance to polyenes mainly involves qualitative or quantitative changes in membrane sterols, especially ergosterol, reducing the drug's binding and effectiveness.

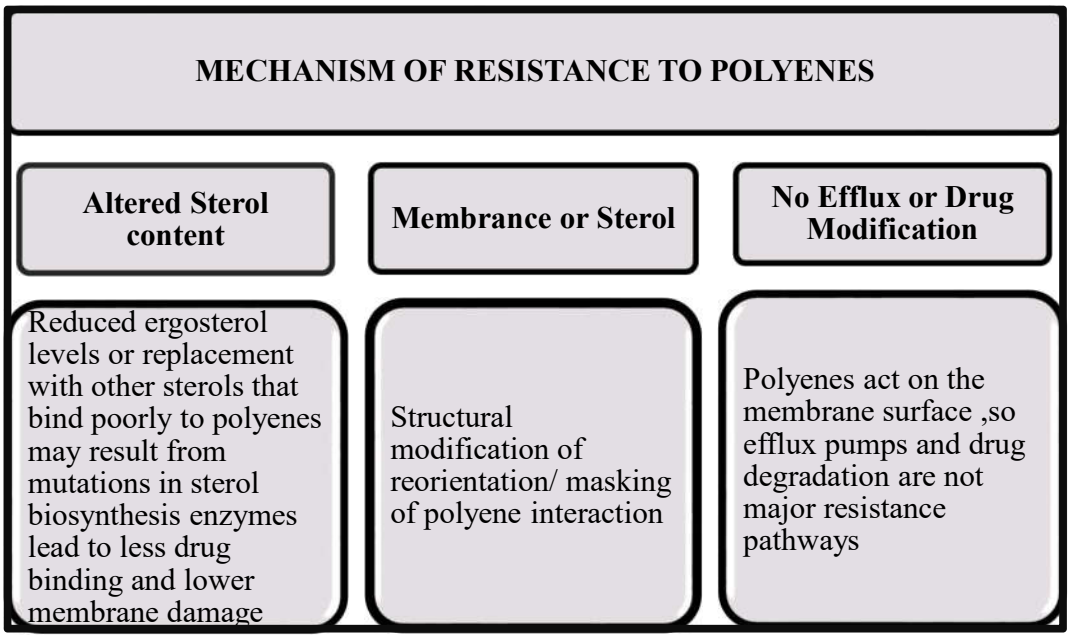

Fig.3: Flowchart of Resistance mechanism of Polyenes

3. **PYRIMIDINE ANALOGUES** (21)

Synthetic derivatives of the nucleotide cytosine 5-fluorocytosine (5-FC) and 5-fluorouracil, which are pyrimidine substitutes. Cytidine deaminase transforms the pyrimidine analogue 5-FC into 5-FU, which is then attached to DNA and RNA during their synthesis and impairs cellular activity by preventing either protein synthesis or DNA replication. These medication equivalents possess anti-cyanococcus and anti-candida qualities. 5-FC has a high bioavailability due to its rapid absorption.

Mechanism of Resistance to 5-Fluorocytosine (5-FC) (44)

Resistance to 5-FC occurs primarily due to loss of enzymes involved in its activation inside fungal cells:

1.**Loss of Cytosine Deaminase or UPRTase:**

- These enzymes convert 5-FC into 5-Flurouridine monophosphate (FUMP).
- Mutation or loss of either enzyme especially in *Candida albicans* and *Cryptococcus neoformans*) prevents drug activation, causing resistance.

2.Decreased Drug Uptake:

- It is caused by loss of cytosine permease activity.
- It has been seen in *S. cerevisiae* and *C. glabrata*, but not common in *C. albicans* or *C. neoformans*.

3.Gene Dosage Effect:

- Reduced UPRTase activity in heterozygotes leads to partial resistance, complete loss in homozygotes leads to full resistance.

5-FC resistance is mainly due to mutational loss of enzymes in the pyrimidine salvage pathway**,** which are not essential under normal conditions, allowing fungi to survive without activating the drug.

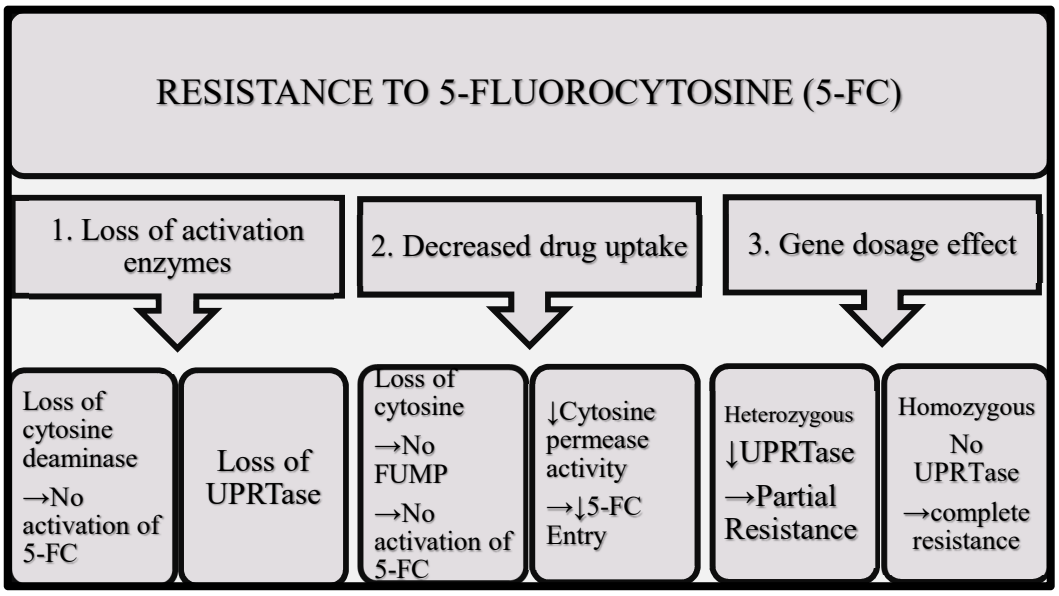

Fig.4: Flowchart of Resistance mechanism of 5-FC

4. Allylamine, Thiocarbamates and Morpholines (21)

Two antimycotics, allylamines and thiocarbamates, are very effective against dermatophytes but only modestly effective against yeasts. Allylamines and thiocarbamates may bind to the enzyme more easily because of their naphthalene moiety. They have had very little contact with the mammalian enzyme that produces cholesterol. Onychomycosis is treated with morpholine, amorolfine, a topical antifungal drug. The enzymes 7,8-isomerase and 14-reductase, which are involved in the synthesis of ergosterol, are blocked. While allylamines and thiocarbamates only interfere with the ERG1 gene, morpholines like fenpropimorph and amorolfine block the ERG24 and ERG2 genes of ergosterol production. Tolnaftate is one of the thiocarbamates, and terbinafine is one of the allylamines.

Mechanism of Resistance to Allylamines (44)

Allylamines (e.g., terbinafine) target squalene epoxidase, blocking ergosterol synthesis. Though clinical resistance is rare, some fungal strains show potential for resistance.

1. **Resistance Not Yet Common:**
    - Clinical resistance to allylamines is not widespread in human pathogenic fungi.

- However, cross-resistance has been observed in fluconazole-resistant *C. glabrata* strains.

2. **Efflux Pumps:**
   - CDR1, a known efflux pump, may expel terbinafine, reducing drug efficacy.
3. **Potential for Future Resistance:**
   - Resistance mechanisms may already exist and could emerge with increased drug use.
   - Allylamine resistance is currently uncommon, but may develop through efflux mechanisms or cross-resistance with other antifungals like azole.

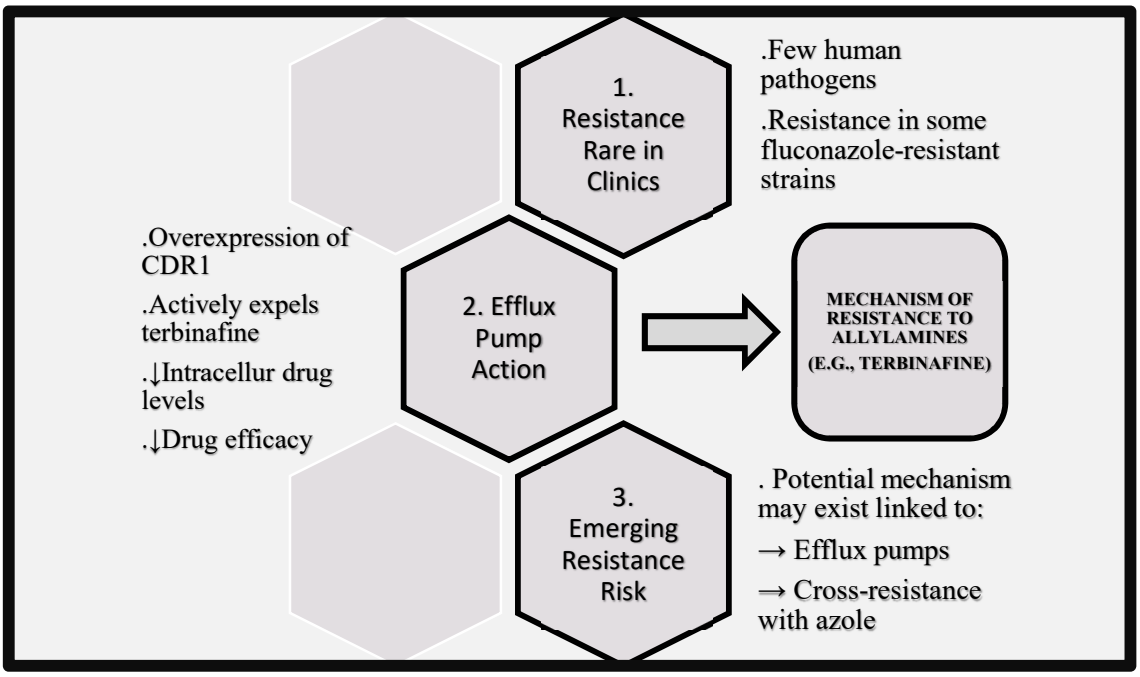

Fig.5: Flowchart of Resistance mechanism of Allylamines

5. ECHINOCANDINS (21)

A relatively new class of antifungal medications known as lipopeptide echinocandins functions as a non-competitive inhibitor of 1,3-D-glucan synthase, an enzyme necessary for the synthesis of glucan. Cell wall stress is caused by errors in the production of cell wall components, which affect the integrity of fungal cells. Consequently, cells treated with echinocandin exhibit thicker cell walls, reduced sterol levels, pseudohyphae formation, increased osmotic sensitivity and separation abnormalities. Echinocandins are usually non-toxic to mammalian cells because they operate on a cell wall assembly pathway that is unique to fungal cells.

Mechanism of Resistance to Glucan Synthesis Inhibitors (44)

Glucan synthesis inhibitors (e.g., echinocandins, like caspofungin) target the glucan synthase enzyme, essential for fungal cell wall synthesis.

Resistance Mechanisms (Based on Lab Studies):

1. **Mutation in FKS1 Gene**:
   - Major resistance mechanism.
   - Alters the glucan synthase target, reducing drug binding and efficacy.
2. **Other Genetic Mutations**:
   - GNS1 mutations cause low-level resistance.
   - FKS2 mutations do not cause resistance.
3. **Efflux or Uptake Mechanisms Not Involved**:
   - Since echinocandins do not enter the cell, resistance is not linked to drug uptake or efflux.
4. **No Cross-Resistance**:
   - Resistant strains remain susceptible to other antifungals like azoles, 5-FC, and amphotericin B.

Resistance to glucan synthesis inhibitors arises primarily through mutations in the FKS1 gene, with no cross-resistance to other antifungal classes observed.

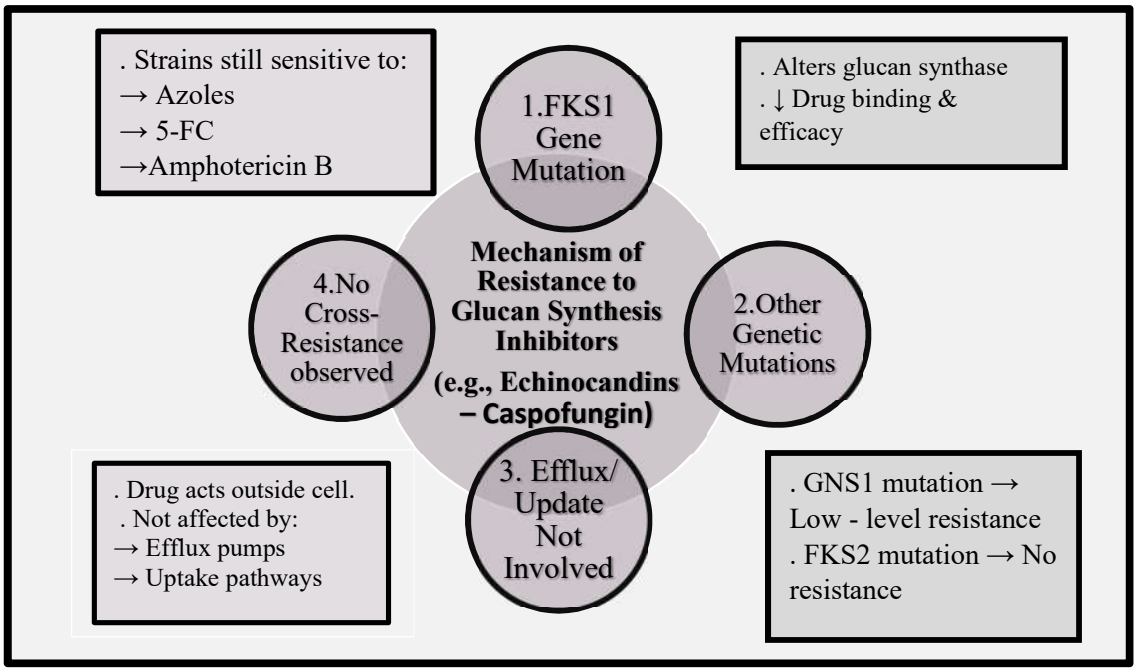

Fig.6: Flowchart of Resistance mechanism of Glucan synthesis inhibitors

Conclusion

Fungal infections are still a major global health concern, especially for people with weakened immune systems. The growing prevalence of infections brought on by serious pathogens including Candida, Aspergillus, Cryptococcus and other opportunistic fungus emphasises the critical need for better knowledge and treatment approaches. Even though there are already a number of antifungal medication classes available, such as azoles, polyenes, echinocandins and pyrimidine analogues the development of antifungal resistance is posing a growing danger to their efficacy. The effectiveness of current treatments is diminished by both intrinsic and acquired resistance mechanisms, such as drug target mutations, efflux pump overexpression and structural changes in fungal cell membranes or cell walls.

Treatment outcomes are further complicated by the global spread of multidrug-resistant organisms, especially Azole-resistant *Aspergillus fumigatus* and *Candida auris*. Thus, encouraging sensible antifungal therapy and reinforcing early diagnostic methods. To prevent resistance from developing, it is crucial to improve surveillance systems, encourage sensible antifungal use and increase early diagnostic techniques. To improve clinical care and reduce the rising burden of fungal illnesses globally, research on new antifungal drugs and other therapeutic approaches must continue.

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