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ANTIFUNGAL RESISTANCE: WHERE DO WE STAND?

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ABSTRACT
Antifungal resistance continues to evolve and complicate patient management. The development of resistance is becoming apparent despite advances in preventive, diagnostic and therapeutic interventions. Fungal pathogens use several mechanisms to evade inhibiting action of antifungal agents. An endeavour to delineate the mechanisms, causes, diagnosis and strategies to combat antifungal resistance has been made in the present article.

KEYWORDS: Azoles, Resistance, Echinocandins, Tolerance, Biofims.

INTRODUCTION

Development of resistance to various antifungal drugs has become a matter of concern specially in high risk patients. There is emergence of resistant flora to more resistant species.[1] Invasive fungal infections have been found to cause significant mortality and morbidity in immunocompromised patients.[2] Candida species cause 96% of all opportunistic fungal infections.[3,4] Relapse are common in dermatophytosis, particularly onychomycosis which can be attributed to acquisition of resistance. Antifungal resistance to dermatophytes is not common inspite of its high incidence, difficult and long term treatment in contrast to candidiasis and aspergillosis in which numerous species resistant to various antifungal agents have been found.[5] It is important to understand the mechanism of antifungal resistance for appropriate management of patients with fungal resistance.

DEFINITION OF RESISTANCE

Primary antifungal resistance (intrinsic): It is defined as resistance inherently present among certain group of fungus to some antifungal drugs.[4] Fungal species showing primary antifungal resistance are shown in table no.1.

Table no.1: Fungal organisms showing primary resistance:[6]

Fluconazole	Candida krusei, C. glabrata, C. dubliniensis, Aspergillus species
Amphotericin B	C. lubitaniae, Fusarium , Aspergillus species
Flucytosine	C. albicans, Aspergillus sp. , Cryptococcus neoformans

Secondary resistance (acquired): It is the emergence of resistance in previously susceptible strains after exposure to antifungal drugs.[4]

Clinical resistance: It is defined as the failure of therapy to eradicate the infection or relapse of disease despite proper antifungal therapy with specific in vitro activity against the pathogen.[7]

Antifungal drug tolerance: It is the ability of fungal organism to exist in presence of drug at growth inhibiting concentration.[8]

Dermatophytosis is a common prevalent infection of keratinized tissue like skin, hair and nail caused by dermatophytes. Among the 3 genera Epidermophyton, Microsporum and Trichophyton, Trichophyton species are T. rubrum, T. mentagrophytes and T. tonsurans. Trichophyton rubrum, among all dermatophytes, is responsible for 69.5% of all superficial dermatophytic infections like onychomycosis and tinea pedis.[5,9]

Among yeasts and moulds, Candida and Aspergillus are responsible for most of the serious invasive infections. Candida species responsible for opportunistic infection are C. albicans, C. glabrata, C.parapsilosis, C. tropicalis, C. krusei. Candidemia is 4th major cause of bloodstream infection with a mortality rate of 35-55%.[4]

The mechanism of antifungal drug resistance in non dermatophtes and dermatophytes are listed in table no. 2.

Table no.2: Mechanism of drug resistance[1-3,8,10-16]

Drugs	Mechanism of resistance in non dermatophytes	Mechanism of resistance in dermatophytes
1.**Azoles** Mechanism of action (MOA): Inhibition of cytochrome P450 14α- lanosterol demethyalse by binding to its heme domain	1.Increase in drug efflux: a) CDR 1-5 genes b) Major facilitator superfamily transporters 2.Target alteration: a) Target mutation: mutation in gene ERG11 (codes for lanosterol demethylase) b) Target expression deregulation: transient upregulation of ERG 11 gene leading to increase in target enzyme c) CYP51A & 51B mutations 3.Metabolism modification: Ergosterol biosynthesis pathway alteration through inactivation of ERG3 gene (via point mutation) leads to inactivation of C5 sterol desaturase (responsible for production of toxic methylated products). Alternate pathway produces 14 α methylfecosterol (non toxic) which replaces ergosterol and supports fungal cell growth 4.Alteration of drug influx: alteration in plasma membrane composition leads to decreased drug intake 5.Biofilm formation: constitutes a physical barrier and prevents efficient penetration of azoles	1.Increase in drug efflux: a)TruMDR1, TruMDR2, ABC transporters 2.Adaptation to stress
2.**Allylamines** MOA: Inhibition of squalene epoxidase	1.Modification of target enzyme by mutation in gene (ERG1P) coding the enzyme squalene epoxidase 2.Increase of drug efflux: genes encoding membrane transport proteins CDR1, AGP2 and HOL3	1. Modification of target enzyme by mutation in gene (ERG1P) coding the enzyme squalene epoxidase 2.Increase of drug efflux: TruMDR2, ABC transporters 3.Adaptation to stress 4.Overexpression of salicylate monooxygenase
3.**Polyenes (Amphotericin B, Natamycin, Nystatin)** MOA: Ergosterol binding and destabilizer of cell membrane functions	1.Target site alteration: a)Mutation of ERG3 (accumulation of other non toxic sterols) 2.Increased catalase activity 3.Alteration of drug	1.Increase of the drug efflux: a)Multidrug transporters of the ABC family 2.Adaptation to stress
4. **Echinocandins** MOA: Inhibits 1,3 b-D glucan synthase leading to disruption of 1,3 b-D glucan, component of fungal cell wall	1.Target site alteration a)Point mutation in hot spot region of genes Fks1 and Fks2 2.Activation of salvage pathway for chitin synthesis	
5. **5-Fluorocytosine** MOA: Inhibition of nucleic acid synthesis	1.Defect in cytosine permease coded by FCY2 2.Point mutation in FCY1 gene that codes for cytosine deaminase 3.Deregulation of pyrimidine biosynthetic pathway	

MECHANISM OF RESISTANCE TO AZOLES IN NON DERMATOPHYTES[1-3,10-18]

1.Increase in drug efflux

a) CDR 1-5 genes: Overexpression of candida drug resistance (CDR 1-5) genes is a recognized mechanism of drug resistance to azoles in candida. CDR gene products are responsible for energy dependent drug efflux which leads to decreased intracellular accumulation of fluconazole in resistant strains.

b) MFS (Major facilitator superfamily) transporters: Upregulation of this protein transporter encoded by MDR-1 (multidrug resistance-1) gene is one of the mechanisms of resistance in candida.

2. Target alteration

a) Target mutation: Azoles inhibit lanosterol 14 α demethylase (coded by ERG11gene) and cause ergosterol synthesis inhibition. Point mutation in ERG11 leads to alteration in binding site on target enzyme so that azoles can't bind (table 3).

b) Dysregulation of target expression: Overexpression of ERG 11 gene leads to increased synthesis of lanosterol 14 α demethylase which leads to increased ergosterol synthesis in fungi.

c) CYP51A & 51B mutations: In aspergillus fumigates, CYP51A and CYP51B encode for 14 alpha demethylase and mutation of these genes are responsible for resistance in these species.

3. Metabolic modification: In azole susceptible strains, accumulation of toxic product 14 methyl,3-6-diol leads to fungistatic effect but in resistant strains mutation in ERG 3 gene leads to altered Δ 5,6 desaturase resulting in accumulation of 14 methylfecosterol which is an alternate to ergosterol in fungal cell membrane and sustain fungal cell growth.

4. Alteration of drug influx: Changes in the composition of plasma membrane (variation in lipid content) cause decreased permeability of azoles. This leads to decrease in intracellular concentration of azoles causing resistance.

5. Biofilm formation: Biofilms, a polysaccharide rich extracellular matrix, form a physical barrier through which antifungal drugs cannot penetrate easily. Biofilms are resistant to all currently available antifungals except echinocandins and lipid formulations of amphotericin. Many fungal species like candida, cryptococcus and aspergillus are associated with biofilm associated infections. Biofilms are major cause of resistant fungal infection in immunocompromised patients with foreign material like catheters, pacemakers and heart valves etc.

MECHANISM OF RESISTANCE TO AZOLES IN DERMATOPHYTES

1. Increase in drug efflux: TruMDR1,TruMDR2 are the genes encoding efflux pumps like ATP binding cassette transporters which are responsible for azole resistance in T.rubrum.

2. Adaptation to stress: Various genes and signal transduction pathways have been identified through which fungi respond to stress conditions leading to their survival in adverse stimulus.

MECHANISM OF RESISTANCE TO ALLYLAMINES

1. Modification of target enzymes: Allylamines bind to squalene epoxidase enzyme encoded by ERG1P gene resulting in accumulation of squalene which disturbs the cellular organization and membrane permeability causing cell death. ERG1P gene mutation results in altered binding of allylamine to its target, squalene epoxidase causing resistance as mentioned in (table 3). This mechanism of resistance has been identified in both dermatophytes and non dermatophytes.

2. Increase in drug efflux: Various genes coding for membrane transporter proteins like CDR1, AGP2, HOL3 in non dermatophytes and TruMDR2 and ABC transporters in dermatophytes are implicated in resistance by decreasing intracellular concentration of allylamines.

3. Stress adaptation: This mechanism of resistance remains same as in azoles and is well studied in dermatophytes specially T. rubrum.

MECHANISM OF RESISTANCE IN POLYENES

Amphotericin forms pores in fungal plasma membrane causing disruption of proton gradient through leakage of potassium ions. ERG 3 gene mutation leads to decreased ergosterol content in plasma membrane and accumulation of other sterols. Thus polyenes have low affinity for plasma membrane due to lack of binding site (ergosterol) in resistant strains. Moreover resistance is also mediated by increase catalase activity which in turn leads to decreased susceptibility to oxidative damage.

MECHANISM OF RESISTANCE IN ECHINOCANDINS:

Echinocandins act by inhibition of 1,3 β-glucan synthase leading to disrupted synthesis of 1,3 β-glucan, a main component of fungal cell wall. FKS1 and 2 genes encode for catalytic subunit of 1,3 β-glucan synthase enzyme. Point mutation involving FKS1and 2 cause resistance by alteration of β-glucan synthase.

MECHANISM OF RESISTANCE TO 5-FLUOROCYTOSINE (5-FC)

5-FC is transported into fungal cell by cytosine permease (encoded by FCY2) and gets converted to 5-FU by cytosine deaminase (encoded by FCY1). 5-FU is then converted into 5- fluorouridine monophosphate by uracil phosphoribosyl transferase (encoded by FUR1) which then inhibits DNA and protein synthesis as shown in table 3. Point mutation in FUR1, FCY1 and FCY2 genes confer resistance to 5-FC.

Table no.3: Drug resistance at genes and enzymes level [14,15,18]

Chemical group	Antifungal drug	Target pathway	Metabolic intermediates	Enzyme	Gene involved in resistance
Allylamines	Terbinafine	Ergosterol synthesis	Squalene ↓ 2,3Oxidosqualene	Squalene epoxidase	Altered enzyme due to ERG1mutation so that drug can't bind
Azoles	Fluconazole, Itraconazole, Voriconazole, Posaconazole	Ergoserol synthesis	Lanosterol ↓ (a) ↓ Zymosterol ↓ ↓ Episterol ↓ (b) ↓ Egrosterol	(a) lanosterol 14 α demethylase (b) Δ 5,6 Desaturase	(a) Altered enzyme due to ERG11mutation so that drug can't bind (b) Altered enzyme due to ERG3 mutation →alternate sterol synthesis and fungal growth
Polyenes	AmphotericinB , Nystatin	Ergosterol (membrane function)	Various metabolites of ergosterol synthesis	Δ8,7 Isomerase	Lack of enzyme due to ERG3 mutation →↓ergosterol
Pyrimidines	5-Flucytosine	DNA and RNA synthesis	(a) 5-FC entry into cell 5-FC ↓ (b) 5-FU ↓ (c) 5-Fluorouridine monophosphate	(a) Cytosine permease (b) Cytosine deaminase (c) Uracil phosphoribosyl transferase	(a) FCY2 gene point mutation (b) FCY1gene point mutation (c) FUR1gene point mutation
Echinocandins	Capsofungin, Micafungin, Anidulafungin	Cell wall (Glucan) synthesis	1,3 β-Glucan	1,3 β-Glucan synthase	FKS1and 2

Clinical resistance can be attributed to combination of factors linked to host, fungal organism or antifungal agent. It helps in individualizing the treatment strategies depending upon clinical condition.[7] Various factors responsible for antifungal resistance are listed in table 4.

Table no. 4: Factors determining clinical resistance to antifungal drugs: [2,6,15,17,19,20]

1.Host factors	2.Factors related to pathogen	3.Factors related to antifungal agent	4.Other factors
State of immunosupression	High load of fungus at beginning of treatment	Relative low drug concentration	Delay in diagnosis
Site of infection	Strain attainment of high virulence	Empirical drug therapy	Wrong diagnosis
Compliance of patient		Drug characteristics: pharmacokinetics and pharmacodynamics	Mixed infection
		Improper selection of antifungal drugs	
		Prolonged drug therapy	

HOST FACTORS

1. State of immunsupression: Immunosupression status as a result of acquired immunodeficiency syndrome, cytotoxic drugs, bone marrow transplantation, neutropenia, diabetes mellitus and malnutrition predisposes to dermatophytic and nondermatophytic (invasive aspergillosis and candidiasis) infections and

adversely affects the treatment outcome resulting in treatment failure.[2]

2. Site of infection: It helps in determining treatment outcome as onychomycosis is often intractable and needs prolonged therapy and relapse are quite common. There are certain sites where fungal eradication is difficult to achieve eg. CNS infection, endovascular infection, infection associated with foreign material (catheters, stents, prosthetic valves) with biofilm formation and deep tissue abscesses/necrotic tissue with poor vascular supply.[2,19] Site of infection also helps in choosing appropriate therapy, for example good penetration of fluconazole makes it a better choice for treating cryptococcal meningitis.[2]

3. Compliance of patients: Poor compliance in patients can be attributed to therapy related causes such as frequent dosing schedules, prolonged duration of treatment, side effects of drugs, costly medications, poor doctor-patient communication and lack of instructions given to patients. According to a review, patients on self administered drugs showed poor adherence to treatment as they used less than half of prescribed medications.[21]

FACTORS RELATED TO PATHOGEN

1. High load of fungus at initiation of treatment: Garey et al[22] and Morrell[23] et al found that early treatment of fungal infection with low burden of organisms is associated with less treatment failures. Antifungal drugs sometimes cannot overcome high fungal load.[2]

2. Attainment of virulent strains: It has been found to be associated with bad prognosis. Patients with less virulent strain can be treated successfully, for example less virulent C. parapsilosis was treated with echinocandins successfully.[2]

FACTORS RELATED TO ANTIFUNGAL AGENTS

1. Relatively low drug concentration: If drug concentration achieved is relatively low, it leads to development of resistance to that particular drug. For example, T. rubrum has been shown to develop resistance to terbinafine, itraconazole and amorolfine when propagated in media containing subinhibitory drug concentration.[12]

2. Empirical drug therapy: For invasive infections, patients are usually started on empirical therapeutic approach based on clinical and non specific findings, but this has been found to increase the probability of resistance.[4] However empirical/prophylactic therapy in early course of disease with lower burden of pathogen is predicted to decrease the number of treatment failures.[2]

3. Pharmacokinetics and pharmacodynamics of antifungal drugs: Drug toxicities (eg.hepatitis in azoles and nephrotoxicity in polyenes), drug interactions resulting in lowering of antifungal drug levels and poor drug absorption can contribute to resistance. All azoles inhibit CYP3A4. Itraconazole and posaconazole strongly inhibit CYP3A4 than fluconazole and voriconazole. Fluconazole and voriconazle also inhibit CYP2C9 and CYP2C19. Drug interactions depend on potency of antifungal drug inhibiting that CYP isoform which metabolizes the coadministered drug.[19,24]

Interaction between antifungal drugs and coadministered drug has been shown in table no. 5.

Table no. 5: Interaction between antifungal drugs and coadministered drug

Antifungal agents	Coadministered agents	Interpretation
Fluconazole	Ibuprofen, omeprazole, warfarin, cyclophosphamide	Monitor for toxicity of these drugs
Itraconazole (ITZ)	Omeprazole Cyclophosphamide, diazepam	Avoid combination with ITZ capsules, use oral solution of ITZ Monitor toxicity of these drugs
Voriconazole	Ibuprofen, omeprazole, warfarin, rifampin, rifabutin, efavirenz, ritonavir, carbamazepine, phenytoin, barbiturates	Monitor toxicity of these drugs
Posaconazole	Cimetidine, rifabutin, phenytoin	Avoid concomitant use

4. Improper selection of antifungal drugs: It is a recognized cause of clinical resisance. Identifying the causative fungal pathogen and institution of specific antifungal agent in proper dosage, frequency and duration is important in overcoming fungal resistance.[17]

5. Prolonged therapy: It can lead to clinical resistance as both patient and physician tend to lose attention on long term drug administration.[21]

OTHER FACTORS

1. Delay in diagnosis: Delay in initiation of treatment is directly proportional to treatment failure and mortality. Garey et al found that delay in administration of fluconazole therapy in patients with candidemia resulted in significant mortality.[22]

2. Wrong diagnosis: Sometimes clinical picture of other conditions may mimic fungal infection leading to wrong diagnosis of fungal infection and unnecessary initiation

of antifungal therapy which may contribute to development of antifungal resistance. For example, antifungal agents prescribed for oral conditions like geographic tongue and recurrent aphthous stomatitis can lead to antifungal resistance. Atrophic plaques of oral candidiasis can be confused with nutritional deficiency and treated as such leading to incomplete resolution and recurrent infection.[20]Immune reconstitution inflammatory syndrome can occur in HIV patient during HAART administration who have invasive fungal infection. As IRIS is associated with features of inflammation, it can be confused with treatment failure.[19]

3. Mixed infections: Immunocompromised patients usually have fungal infection admixed with bacterial or viral infection and sometimes fungal infection with two or more fungal species, so treament of one pathogen gives a misinterpretation of treatment failure.[19]

DIAGNOSIS OF ANTIFUNGAL RESISTANCE

A rapid and correct evaluation of primary and secondary resistance is provided by molecular techniques in addition to various culture based, immunological and biochemical assays for detection of pathogen. These assays allow rapid species identification. Molecular probing techniques identify specific resistance mechanisms in azoles precisely. Various molecular techniques which provide rapid identification of infecting organism are real time PCR, NASBA (nucleic acid sequencing based analysis) along with high fidelity self reporting probes, hybridization melt profiles, microarrays and high throughput sequencing.[4]

MANAGEMENT OF CLINICAL RESISTANCE

An attempts should be made to treat the disease early in the course for better results.[17] Use of empirical therapy and optimal prophylactic antifungal agents in proper dosage and duration reduce the number of treatment failures.[7] Clinical practice guidelines should be updated based on epidemiology and evidence based studies. Use of specific and newer diagnostic tools can significantly reduce the chances of antifungal resistance.[7] Proper debridement/debulking surgery in necrotic tissues or abscesses ensures proper penetration of antifungal agents and hence manages antifungal resistance.[2] Removal of disease focus, for example, contaminated foreign objects like catheters, pacemakers and heart valves can reduce fungal load.[15] Management of underlying disease and improvement of immune functions can also help. Emphasis should be laid on development of vaccine which can improve immune response in immunocompromised individuals.[7] Monitoring drug toxicities, tolerability, drug interactions and improved drug delivery methods are known to reduce resistance.[15,19] There is a constant need for development of next generation of antifungal drug which should possess broad spectrum of fungicidal action.[7,25] Change of drug class is an easier strategy to manage clinical resistance.[7] Combination antifungal therapy can also be used.[7] Proper communication with patients, easy dosing schedules and better patient education improve compliance of patients and reduce clinical resistance.[21]

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