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Antifungal Drug Resistance – Its Importance In Oral Cavity

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Abstract: In the era where antibacterial resistance and emergence of superbugs is a prime topic of concern, antifungal drug resistance is being slightly overlooked, mainly due to less incidence of fungal infections when compared to bacterial infections. But, the growing antifungal resistance in yeasts constitutes to a significant therapeutic problem, which might require the earliest attention. This review concises about the mechanisms of antifungal resistance, their role in dentistry and its future perspective.

Keywords: Resistance, candida, antifungal

1. INTRODUCTION

Fungal infections are ranked among the top 10 opportunistic infections in most of the febrile patients with an underlying disease. They are also the major cause of mortality among these patients. Only a few fungi, which are pathogenic to humans, has the potential to infect a healthy host.1 most others invade and infect only when the host's defense mechanism becomes less efficient in immune-compromised patients like patients undergoing organ transplant, hiv patients, patients under cancer chemotherapy, etc.2 after the discovery and vast usage of newer antifungal agents in 1990s, the overall mortality of patients with fungal infections have drastically reduced.3 however the evolving resistance among fungi to many antifungal drugs have created a new challenge in treating and managing such patients.

1.1 Resistance And Tolerance

A microbe is said to be clinically resistant when it does not respond to the normal therapeutic dose of a drug; Or if the microbial growth regresses only when abnormally higher doses of a drug is administered.[4] Resistance is when a pathogen is able to withstand the effects of a drug which is usually known to be harmful to it.[5] Some can be intrinsically resistant to certain antimicrobials which is PRIMARY RESISTANCE. Some others can develop resistance to a drug gradually during the course of a therapy which is SECONDARY RESISTANCE.[2] A third category of resistance is known, called CLINICAL RESISTANCE, where the organism responsible for the disease is fully susceptible to the drug under laboratory testing, but does not repond clinically[6]. On the other hand, Tolerance is the diminished response to a drug by the host, which is also a result of repeated drug usage.[5] Drug tolerance in other words is a condition that occurs when body cells gets used to a drug so much, that it no longer responds to them, requiring an alteration in the treatment regimen.[7] Drug tolerance is also exhibited by certain parasites and microorganisms, especially when they reside in the host for a longer time. Studies done in malarial parasites have demonstrated tolerance to antimalarial drugs.[8] Extensive research on Mycobacterium species have shown drug tolerance for most of the antitubercular drugs. Both invivo and invitro study models have established that majority of microbes are destroyed within the first few days of treatment. After this the rate of killing drops, leaving behind a population of drug tolerant species.[9]

1.2 Resistance And Tolerance Among Fungi

Initially multidrug resistance in fungi was demonstrated with many studies done on Sacharomyces cerevisiae, which is a non-pathogenic yeast in humans. In this fungi, mutations were seen in the PDR1 gene on chromosome VII. Pdr1p is a transcriptional regulator which was found to have major influences on the multidrug resistant phenotypes of cells thereby regulating numerous proteins to block the toxic action of drugs on this fungi. It is thus considered as a reliable determinant of multidrug resistance in this species.[10] After this, numerous studies were conducted on common fungi which were pathogenic to human such as Candida sp., Aspergillus fumigatus etc., and similar resistance mechanisms were demonstrated. Resistance is usually known to occur in chronic oropharyngeal candidiasis treated with fluconazole, since other forms of fungal infections are less frequent or acute form of disease.[6] After the HIV pandemic, oropharyngeal candidiasis was established as an important opportunistic fungal infection associated with AIDS. Also the immunodeficient and comorbid condition of the patients lead to easy and rapid development of resistance and tolerance, especially with the Azole group.[6] Fluconazole being a potent antifungal for prophylaxis and therapy in HIV patients, is being used with restriction due to this reason.[11]

1.3 Antifungal Drugs

Antifungal drugs are broadly classified into 5 groups based on their mechanism of action.[3,12]

GROUP		DRUGS	MECHANISM OF ACTION
Polyenes		Nystatin Amphoterecin B - Deoxycholate form - Liposomal AmB - AmB Lipid complex	Through hydrophobic interactions with ergosterol in the cell membrane, and forming pores. This leads to potassium efflux, eventually causing cell death.
Azoles	Imidazole	Ketoconazole miconazole clotrimazole econazole	They inhibit C14-α sterol demethylase thereby impairing the ergosterol synthesis. This leads to accumulation of sterol precursors and reduction of ergosterol which disrupts the cell membrane integrity.
	Triazole	Fluconazole Itraconazole Voriconazole Posaconazole	
Allylamines		Terbinafine Naftifine	Inhibits ergosterol synthesis by inhibiting the enzyme squalene epoxidase
Echinocandins		Caspofungin Micafungin Anidulafungin	Inhibits the synthesis of β 1,3-glucan, a fungal cell wall polysaccharide, thereby disrupting the cell wall
Others		Griseofulvin	Disrupts the mitotic spindle thereby inhibiting fungal mitosis through interaction with the polymerized spindles
		Flucytosine	Fungal cytosine permease and cytosine deaminase, respectively imports and converts this pyrimidine analogue to flurouracil, which impairs the nucleic acid synthesis and ultimately interferes with protein synthesis

1.4 Mechanisms Of Developing Antifungal Resistance:[13,14]

The fungi acquires resistance through any of the following mechanisms.

1.4.1. Mutations in the target site

Mutations can occur in the target site of the fungal pathogen leading to conformational changes of the target site which might decrease affinity towards the target or its overexpression. It is distinctly seen in resistance towards the azole group of antifungals. Mostly point mutations are seen in the gene ERG11 which encodes for the target enzyme 14- demethylase, where there is replacement of Arginine for Lysine at position 467 (R467K). Another point mutation was observed (T315A) where Threonine was replaced with Alanine at position 315.[15]

1.4.2. Target site overexpression

Sometimes there is gene amplification, increased transcription rate, or decreased degradation of the gene product occurring in ERG11. This leads to overproduction and intracellular accumulation of high concentrates of the target enzyme. Thus the normal dosage of drug is no longer effective.[16]

1.4.3. Efflux pump overexpression

There is upregulation of various efflux pump such as adenosine triphosphate binding cassette (ABC) transporters or major facilitator superfamily (MFS) pumps which reduces the accumulation of drug within the cell. There are numerous transporter proteins under these two groups which decreases the effective drug concentrations. The ABC transporters use ATP hydrolysis for drug efflux. However, the MFS transporters are transmembrane proteins, and use the electrochemical proton-motive force for the same.[17]

1.4.4. Poor drug metabolization

This mechanism is observed in 5-flucytosine which is taken in as a prodrug in its inactive form. Then it is converted to its active drug metabolite 5-fluorouridine by the enzyme cytosine deaminase. Mutations in this enzyme has been observed which leads to formation of resistance in the drug.[17]

1.4.5. Genomic plasticity:

This can result in chromosome arm duplication further leading to conformational changes and target site overexpression

ANTIFUNGAL GROUP	MECHANISM OF RESISTANCE
Polyenes	- Regulation of stress response pathways - Absence of target site
Azoles	- Target site conformational changes - Target site overexpression - Efflux pump overexpression - Genomic plasticity
Allylamines	Unknown
Echinocandins	- Regulation of stress response pathways - Target site conformational changes
Pyrimidine analogues	- Target site overexpression - Efflux pump over expression - Inadequate conversion of prodrug

1.5 Factors Influencing The Developing Resistance:[13]

Numerous factors are involved in the evolution of resistant strains of fungi. These can be broadly categorized into environmental and drug related causes.

1.5.1. *Environmental factors*

Antifungal agents are not only used in medicine but also in agriculture to prevent crops from fungal decay. Numerous studies and reports have quoted Azole resistance in environmental isolates with specific target mutations and were able to identify the same mutation in patient samples, who were not at all exposed to Azole antifungal therapy before, suggesting that the resistance have been acquired solely from these environmental isolates.[17]

The following are some of the environmental factors which are responsible for the issue

- Global dispersal of resistant strains
- Aerial spore dispersal
- Host population density and susceptibility
- Population growth and urbanization
- Movement of people

1.5.2 *Drug related factors*

The limited groups of antifungal drugs available for treatment leaves the healthcare professional with no other options for therapy, if resistance sets in. However it becomes more challenging in cases of debilitated patients and also with emerging multidrug resistant strains. The drug related factors responsible for resistance are enlisted below

- Lack of chemical diversity
- Long courses of prophylactic therapy
- Empirical treatment with the same drug
- Lacking knowledge with antifungal combinations
- Concomitant use of antibiotics along with antifungals
- Immunocompromised patients and aggressive cancer therapies

Studies have demonstrated that administration of broad spectrum antibacterials along with antifungals increases the probability of developing resistance in the fungi. Antibiotics tend to wipe out the gut flora which leads to colonization of candida in the gut and further causing dissemination into blood. Also certain antibiotics have the potential to directly induce resistance in some fungi.[18]

1.6 ROLE OF ANTIFUNGAL RESISTANCE IN DENTISTRY

The oral cavity lodges a diverse population of microorganisms including various bacteria, viruses and fungi. Though fungi contributes to a minor component to the oral microflora, it plays a significant part in causing oral diseases. Most of the fungi present in the mouth act as commensals and does not cause any harm in a healthy host. They become pathogenic under favorable conditions like immune suppression, improper oral hygiene, etc. Candida is the most common species colonizing the oral cavity and Oral candidiasis is the most common fungal disease occurring in the mouth which can be manifested in various forms.[19] Occurrence of other deep fungal infections involving the oral cavity and associated structures like Mucormycosis are also reported. Mostly polyenes and Azoles are useful in treating oral fungal infections. However, Echinocandins and other

parenteral drugs can be used for deep fungal infections with systemic involvement. Azoles are the major group which innately has less susceptibility to Candida species and their predominant mechanism of developing resistance is through overexpression of drug efflux pumps.[19] Oral fungi exhibits resistance individually as cells or when they coexist with other organisms in a biofilm. The resistance is stronger when occuring in biofilms, since the fungi communicate and interact through various chemicals and quorum sensing molecules.

1.7 ROLE IN CANDIDAL INFECTIONS

Candida albicans is the primary and a relatively common species isolated from a bloodstream candidal infection. The first gene isolated from Candida albicans, responsible for resistance was MDR1 gene which encodes for a group of proteins, which are members of major facilitator superfamily of membrane transporters. Later, two other genes encoding the homologues of these proteins were found namely CDR1 and CDR2. Lab studies have proved the over production of CDR1 and CDR2 transcripts in numerous drug resistant isolates[10]. Candida glabrata is the next most common and predominant species associated with fungemia. They can easily acquire resistance compared to C. albicans. Sacharomyces cerevisiae and Candida glabarata share similar pathways of multidrug resistance, with the gene involved being CgPDR1 encoding for the protein Cgpdr1p. Candida auris was identified in 2009. It is an emerging multidrug resistant yeast known to spread easily within hospitals. According to the CDC, it has appeared in four different strains across the globe at the same time and suspected to spread through international travel. This species is known to be resistant to all the groups of antifungal drugs making the treatment difficult[20]. Candida species are predominantly known to exhibit resistance against Azole group of drugs in multiple mechanisms.[6,19,21] Point mutations occur at the target site, causing overexpression of Erg11p gene and conformational changes in the target site. There is also overexpression of efflux pumps which prevents accumulation of the drug within the cell[19,21]. Candida cells also exhibit resistance properties when growing in a biofilm. The formation of a biofilm acts as a major challenge in treating fungal infections. Candida is known to secrete a QSM called farnesol continuously which inhibits hyphal formation at high cell concentrations and promotes dispersal of yeast cells to form new colonies.[19] There is increase in resistance towards Ketaconazole, Itraconazole, Fluconazole and Amphoterecin B. Some of the factors which facilitates and enhances resistance includes: the presence of extracellular materials that prevents antifungal penetration like carbohydrates, proteins, hexosamine and uronic acid; Presence of a subpopulation of resistant 'PERSISTER CELLS' which can remain viable even in the presence of an antimicrobial agent; Reduced synthesis of ergosterol by the fungal cells.[21]

1.8 HOW TO PREVENT OR TACKLE ANTIFUNGAL RESISTANCE – FUTURE PERSPECTIVE

Avoid dosages which are too low or very short courses of treatment. It is essential that proper treatment regimen should be followed for the treatment. Drugs should be administered at the higher end of the maximum tolerable dose. This could reduce the emergence of resistant fungi.[21] Once resistance is identified for a particular drug high doses can be administered in order to achieve the desired effect for treatment. Prudent use of antifungals are mandatory to prevent resistance. Treatment should be given appropriately only after susceptibility testing. Empirical therapy or irrational use should be avoided at the maximum. For invasive or deep fungal infections, surgery can be the first choice of treatment to reduce most of the microbial load, followed by antifungal therapy for maintainance. Limited groups of antifungal agents available for therapy is one of the reasons for developing resistance and dispersion of resistant strains. Discovery of newer generation antifungals with different targets might help to overcome this crisis.[15]. Certain studies also quote that use of herbal sources like lemon juice, lemon grass, Lawsone methyl ether extracted from Impatiens balsmina and Swertia calycina, etc are known to have potent antifungal and antimicrobial properties and can be used as an alternative for chemical therapeutics.[11] Role of probiotics in antifungal therapy has also been studied in order to tackle this situation and has proved to be successful.[11]

2. CONCLUSION

Thus antifungal resistance is an emerging crisis which should be carefully dealt with in order to minimize the evolution of more antifungal resistant strains, thereby making the treatment less challenging. Limited groups of antifungal drugs available, pose more threat, and multidrug resistance largely limit the treatment options. Antifungal resistance is of concern mainly for patients with invasive fungal infections. However, invention of newer novel antifungal agents and appropriate use of antifungals with proper protocols being followed might help to overcome this obstacle.

3. CONFLICT OF INTEREST

Conflict of interest declared none.

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