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ANTIFUNGAL DRUG RESISTANCE IN *CANDIDA* SP. – A REVIEW

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ABSTRACT

Antifungal drug resistance is problematic as initial diagnosis of systemic fungal infection may be delayed and the choice of antifungal drugs remains limited due to the emergence of resistant fungal species. Four classes of antifungal drugs are most widely used in clinical practice to treat systemic fungal infections and there are four main antifungal drug targets in *C.albicans.* Antifungal drug resistance can be divided into two categories: clinical resistance and *in vitro* resistance. Mechanisms of antifungal resistance are either primary or secondary, and are related to intrinsic or acquired characteristics of the fungal pathogen that interfere with the antifungal mechanism of the drug. Resistance can also occur when environmental factors lead to replacement of a susceptible species with a resistant one. Most of the established antifungal resistance mechanisms are due to genetic mutation – usually point mutations in genes encoding drug targets or enzymes in metabolic pathways, or transcription factors leading to gene over expression. Such mutations are stable, take time to be acquired, and can be considered as long-term stress responses. Antifungal resistance has consequences associated with poor clinical outcome during antifungal treatment and prophylaxis. Strategies to overcome antifungal drug resistance include the synthesis of new drugs with better antifungal activity. In addition, antifungal-control programmes to avoid extensive and inappropriate use of antifungal may be needed. Early identification of *Candida* sp. from clinical specimens and standard antifungal drug susceptibility testing would be an effective approach for controlling outbreaks caused by *C.albicans* and Non albicans *Candida* species. Application of standard techniques, guidelines for the use of antifungal agents and control measures for predisposing factors may reduce the risk of drug-resistant *Candida* infections.

KEYWORDS: Antifungal drug, drug targets, drug resistance, *Candida* sp.

1. INTRODUCTION

Antifungal drug resistance is a major phenomenon that has a critical impact on human health. In the 1990's fluconazole treatment failure emerged due to the development of resistance by *Candida albicans*.[1] Both *C.albicans* as well as non-*albicans Candida* species (NAC) have developed mechanisms to resist antifungal drugs. [2] Antifungal drug resistance is problematic as initial diagnosis of systemic fungal infection may be delayed and there are only a few antifungal drugs available. The choice of antifungal drugs remains limited due to the emergence of resistant fungal species.[3]

2. ANTIFUNGAL DRUGS

Only four classes of antifungal drugs are most widely used in clinical practice to treat systemic fungal infections: fluoro pyrimidine analogs, polyenes, azoles and echinocandins. Several other classes are used only as topical agents due to their poor efficacy and severe adverse effects when administered systemically.[4]

2.1. Fluoropyrimidines

Fluoropyrimidines, of which only 5-fluorocytosine (5-FC) and 5-fluorouracil (5-FU) are widely used in human medicine, are structural analogs of the DNA nucleotide cytosine. 5-FC was synthesized in 1957 by Duschinsky *et al*., and it is generally accepted that it possesses activity against *Candida.* The use of 5-FC in clinical practice is decreasing because of the increase in occurrence of resistance to this drug among fungal pathogens.[4]

2.2. Polyenes

The polyenes are generating considerable interest as a class of antifungal drugs that target membranes containing ergosterol. These drugs include amphotericin B, which is active against most species of pathogenic fungi. It is the drug of choice for the treatment of systemic candidiasis and is thought to be the "gold standard" for therapy. However, isolates resistant to this drug also have been reported.[5,6]

2.3. Azoles

Azoles are antifungal agents categorized into imidazoles and triazoles. Imidazole compounds (miconazole, clotrimazole and ketoconazole) consist of a five membered ring structure containing two nitrogen atoms and the triazoles (fluconazole, itraconaole) contain three nitrogen atoms.[4]

Fluconazole became available for use by clinicians in 1990 and has many advantages over the use of imidazoles. A remarkable feature of fluconazole is, it is highly hydro soluble and can be easily injected intravenously. It is completely absorbed through the gastrointestinal tract, and it diffuses throughout the body, including cerebrospinal fluid.[4] Another principal characteristic of fluconazole is that it is free of adverse effects and it is available in both intravenous and oral formulations. Because of the low toxicity and ready distribution into body fluids, fluconazole has been used in the treatment of both superficial and systemic fungal infections.[7] Fluconazole is suitable for the treatment of superficial candidiasis (oropharyngal, esophageal, or vaginal), disseminated candidiasis and cutaneous candidiasis. Fluconazole was considered to be the gold-standard treatment of fungal infections during the 1990s. Unfortunately, the over prescription of this drug for prophylaxis or treatment would have led to an increase in resistance to azole drugs.[4]

Itraconazole is hydrophobic and is more toxic than fluconazole. Itraconazole is only recommended for the treatment of onychomycosis or superficial infections.[4]

Fluconazole and itraconazole are still not the perfect antifungals, since they have some drug interactions with drugs that are used in chemotherapy or with AIDS treatment. These interactions can result in a decrease in azole concentration or even to an increase in toxicity.

Recently, new generation triazoles have been developed like voriconazole and posaconazole and were approved in 2002 and 2006, respectively. Their side effects and drug interactions are similar to those with fluconazole and itraconazole. Fungal isolates resistant to classical triazoles are also cross resistant to new generation triazoles.[4]

2.4. Echinocandins

These drugs are poorly absorbed in the gastrointestinal tract and are only used intravenously. These molecules become important because they possess low toxicity and are slowly degraded, and a daily injection is sufficient, and on contrary to other antifungals, interactions between echinocandins and other drugs are rare. Echinocandins are a good alternative against fungal infections; therefore, caspofungin is used for the treatment of candidemia and invasive candidiasis.[4]

3. THE ANTIFUNGAL DRUG TARGETS IN *C.albicans*

There are four main antifungal drug targets in *C.albicans*. The fluorinated pyrimidine analogue 5-fluorocytosine (5-FC) causes RNA synthesis and interferes with DNA replication. The polyenes insert into lipid bilayers, bind to sterols, and form pores. These pores disrupt plasma membrane integrity which is fungicidal for *C.albicans*. The azole antifungals interfere with sterol biosynthesis. They inhibit the cytochrome P450 14a-lanosterol demethylase, encoded by the ERG11 gene, which is part of the ergosterol biosynthetic pathway.[8] Modifications in the ergosterol pathway can lead to resistance to other related drugs also.[9] The echinocandins interfere with cell wall biosynthesis by inhibiting (1, 3)-D-b-glucan synthase and are fungicidal for *C.albicans*.[8] This causes the formation of a defective cell wall associated with cellular instability and lysis in Yeasts.[10]

4. ANTIFUNGAL DRUG RESISTANCE

4.1. Classification

Antifungal drug resistance can be divided into two categories: clinical resistance and *in vitro* resistance. Clinical resistance is the lack of clinical response to the antifungal agent used, due to low levels of the drug in serum or tissues. *In vitro* resistance can be subdivided into primary resistance and secondary resistance. Primary resistance is also known as innate resistance and occurs when the organism is naturally resistant to the antifungal agent. On the other hand, secondary or acquired resistance is described when the isolate becomes resistant to the antifungal agent.[11]

4.2. Mechanisms of antifungal drug resistance

Mechanisms of antifungal resistance are either primary or secondary, and are related to intrinsic or acquired characteristics of the fungal pathogen that interfere with the antifungal mechanism of the drug. Resistance can also occur when environmental factors lead to replacement of a susceptible species with a resistant one.[10]

Fungi develop mechanisms to resist antifungal classes based on three major mechanisms, namely, (i) reducing the accumulation of the drug within the fungal cell, (ii) decreasing the affinity of the drug to its target, and (iii) modifications of metabolism to counter balance the drug effect. Phenotypic switching and biofilm formation are other phenotypic responses that can increase drug resistance.[4]

Most of the established antifungal resistance mechanisms are due to genetic mutation – usually point mutations in genes encoding drug targets or enzymes in metabolic pathways, or transcription factors leading to gene over expression. Such mutations are stable, take time to be acquired, and can be considered as long-term stress responses.[4]

Resistance of clinical isolates to 5-FC often correlates with mutations in the enzyme uracil phospho ribosyl transferase. Within the *Candida* genus, 7% to 8% of clinical isolates are resistant to 5-FC and this frequency increases to 22% when only non *albicans Candida* species are considered.[4] Proposed mechanisms of polyene resistance include decreased access to the drug target due to altered membrane ergosterol content. Currently, yeast resistance to polyenes is very low.[10] Resistance of *C.albicans* to polyenes is rare and can be caused by a reduction in the amount of plasma membrane ergosterol, to which polyenes bind. First, increased activity of the enzymes of the ergosterol pathway (e.g., ERG11) reduces impact of the drug on its target. Second, increased efflux of the drug from cells (encoded by CDR1 and CDR2) or by the major facilitator super family efflux pump (encoded by MDR1). [12] Mutations in ERG3, which lower the concentration of ergosterol in the membrane, cause amphotericin B resistance and also confer resistance to azoles.[4] In *C.albicans,* the target of the azoles ERG11 is duplicated. Cross-resistance among azoles can be expected as the target of action on fungi is similar.[3] There are multiple mechanisms that can give rise to azole resistance in *C.albicans*. The drug target, ERG11p, can be over expressed or can develop point mutations that reduce fluconazole binding. Likewise, azole resistance can be caused by genetic rearrangement. The molecular mechanisms leading to azole resistance are divided into four categories (i) decrease in azole affinity for their target, (ii) increase in azole target copy number, (iii) alteration of ergosterol biosynthetic pathway after azoles action, and (iv) decrease in intracellular azole accumulation. Little clinical resistance to the echinocandins has been reported so far. This may be due to its limited use, or that resistance events are rare. Echinocandin-resistant *C.albicans* isolates have point mutations in (1, 3)-b-glucan synthase.[4] Resistance of *Candida* to echinocandins has been linked with point mutations in FKS1, the gene encoding for the 1, 3-_-D-glucan synthase. This resistance mechanism has been demonstrated in *C.albicans* and non-albicans *Candida* species, NAC.[10]

4.3. Consequences of Antifungal drug Resistance

Michael (2012) reported that antifungal resistance has consequences in terms of elevated MICs that are associated with poor clinical outcome during antifungal treatment and prophylaxis.[10]

4.4. Strategies to overcome Antifungal drug Resistance

According to Richard *et al*., (2007) strategies to overcome antifungal drug resistance include the synthesis of new drugs with better antifungal activity. Rapidly acting fungicidal drugs are better than fungi static drugs as they do not allow the selection of resistant strains. Extracellular targets are also better than intracellular targets as the intracellular action of drugs provides more options for drug inactivation. Combination therapy can be used to attack two different targets simultaneously with a low probability that resistance to both drugs will arise, or to attack a drug target and its resistance mechanism.[8]

In addition, in the opinion of Nagendra *et al*., (2007) antifungal-control programmes to avoid extensive and inappropriate use of antifungal agent may be needed. Appropriate use of antifungal agent may be critical to prevent the emergence of antifungal drug resistance. Another factor that could influence the emergence of resistance is the dose of drug used for antifungal therapy.[9]

It is proposed by Rajendra *et al.* (2010) that (1) rapid detection of candidiasis followed by a drug susceptibility test needs to be performed on the clinical isolates. (2) The removal of fungus-contaminated foreign objects can reduce the fungal burden. (3) Therapeutic application of fluconazole should be limited to selected high-risk patients to minimize the risk of emergence of azole-resistant strains of *Candida*. (4) Patients with recurrent candidiasis, but recently treated with fluconazole, should not be treated again with the same drug. (5) Treatment with novel antifungal agents such as second-generation triazole and echinocandins may be preferred in clinical management of hospitalized patients. (6) Increased awareness of risk factors for NAC species can provide guidance for appropriate choices of antifungal therapy. (7) The best way to improve antifungal drug therapy is to improve immunity of the host.[13]

5. CONCLUSION

Early identification of *Candida* sp. from clinical specimens and standard antifungal drug susceptibility testing would be an effective approach for controlling outbreaks caused by

C.albicans and NAC species. Application of standard techniques, guidelines for the use of antifungal agents and control measures for predisposing factors may reduce the risk of drug-resistant *Candida* infections. Studies in this area will contribute towards the identification of new targets for new antifungal agents against recently emerged *Candida sp*.

REFERENCES

1. Theodore C. White, Kieren A. Marr and Raleigh A. Bowden. Clinical, Cellular, and Molecular Factors That Contribute to Antifungal Drug Resistance. Clinical Microbiology Reviews, Apr, 1998; 11(2): 382–402.
2. Nagendra Nath Mishra, Tulika Prasad, Neeraj Sharma, Anurag Payasi, Rajendra Prasad, Dwijendra K. Gupta and Randhir Singh. Pathogenicity and Drug Resistance In *Candida Albicans* And Other Yeast Species A Review. Acta Microbiologica et Immunologica Hungarica, 2007; 54(3): 201–235.
3. Arunaloke Chakrabarti Drug resistance in fungi – an emerging problem. Regional Health Forum, 2011; 15(1): 97-103.
4. Patrick Vandeputte, Selene Ferrari, and Alix T. Coste. Antifungal Resistance and New Strategies to Control Fungal Infections. Hindawi Publishing Corporation, International Journal of Microbiology, 2012; Article ID 713687, 26.
5. Derek Law, Caroline B. Moore and David W. Denning. Amphotericin B resistance testing of *Candida* spp.: a comparison of methods. *Journal of Antimicrobial Chemotherapy*, 1997; 40: 109–112.
6. Luis Ostrosky-Zeichner, Kieren A. Marr, John H. Rex and Stuart H. Cohen. Amphotericin B: Time for a New "Gold Standard". Clinical Infectious Diseases, 2003; 37: 415–25.
7. I.A. Casalinuovo, P. Di Francesco, E. Garaci. Fluconazole resistance in *Candida albicans:* a review of mechanisms. European Review for Medical and Pharmacological Sciences, 2004; 8: 69-77.
8. Richard D. Cannon, Erwin Lamping, Ann R. Holmes, Kyoko Niimi, Koichi Tanabe, Masakazu Niimi and Brian C. Monk. *Candida albicans* drug resistance – another way to cope with stress. Microbiology, 2007; 153: 3211–3217.
9. Nagendra Nath Mishra, Tulika Prasad, Neeraj Sharma, Anurag Payasi, Rajendra Prasad, Dwijendra K. Gupta and Randhir Singh. Pathogenicity and Drug Resistance In *Candida Albicans* And Other Yeast Species A Review. Acta Microbiologica et Immunologica Hungarica, 2007; 54(3): 201–235.

10. Michael A. Pfaller. Antifungal Drug Resistance: Mechanisms, Epidemiology, and Consequences for Treatment. The American Journal of Medicine, 2012; 125: S3–S13.
11. Paul L. Fidel, Jr., Jose A. Vazquez and Jack D. Sobel. *Candida glabrata*: Review of Epidemiology, Pathogenesis, and Clinical Disease with Comparison to *C. albicans* Clinical Microbiology Reviews, Jan, 1999; 12(1): 80–96.
12. Christopher B Ford, Jason M Funt, Darren Abbey, Luca Issi, Candace Guiducci, Diego A Martinez, Toni Delorey, Bi yu Li, Theodore C White, Christina Cuomo, Reeta P Rao, Judith Berman, Dawn A Thompson, Aviv Regev. The evolution of drug resistance in clinical isolates of *Candida albicans.* eLife, 2015; 4: e00662.
13. Rajendra J. Kothavade, M. M. Kura, Arvind G. Valand and M. H. Panthaki. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. Journal of Medical Microbiology, 2010; 59: 873–880.