



# Antifungal Drug Resistance in *Candida* Species

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## ABSTRACT

There has been a significant increase in the number of reports of mucosal and systemic infections caused by *Candida* spp. in recent years. Despite the increase in the infection rates by *Candida* spp., therapeutic options for their treatment are relatively limited. In the recent years, there has been a marked increase in the incidence of treatment failures in candidosis patients receiving long term therapy, which poses a serious problem in the treatment of infections caused by *Candida* spp.

**Key words:** Candidosis, *Candida*, species, antifungal agents, drug resistance

## Kandida Türlerinde Antifungal İlaç Direnci

### ÖZET

Son yıllarda kandida türlerinin neden olduğu mukozal ve sistemik enfeksiyonların sayısında önemli bir artış olmuştur. *Candida* türlerine bağlı olarak enfeksiyon oranlarındaki artışa rağmen tedavi seçenekleri nispeten sınırlıdır. Son yıllarda uzun süre tedavi alan alan kandidiyazis hastalarında tedavi başarısızlığı insidansında belirgin bir artış olmuştur.

**Anahtar kelimeler:** Kandidiyazis, kandida, tür, antifungal ajanlar, ilaç direnci

## INTRODUCTION

There has been a significant increase in the number of reports of mucosal and systemic infections caused by *Candida* spp. in recent past. This is mainly attributed to a dramatic rise in the number of immunocompromized individuals, especially those infected with the human immunodeficiency virus (HIV), and patients receiving immunosuppressive therapy for malignancy and those undergoing transplantedation. *Candida albicans* and non-*albicans* species have acquired considerable significance in the recent past due to the enhanced susceptibility of immunocompromized patients. *Candida* spp. are now recognized as important causative agents of hospital acquired infections. Although, *Candida albicans* is a potential pathogen most commonly isolated from clinical specimens, many recent reports have documented emergence of non-*albicans* species of *Candida*, as nosocomial pathogens. *C.*

*tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, etc. have been reported to cause nosocomial infections (1,2).

Despite the increase in the infection rates by *Candida* spp., therapeutic options for their treatment are relatively limited. In the recent years, there has been a marked increase in the incidence of treatment failures in candidosis patients receiving long term therapy, which poses a serious problem in the treatment of infections caused by *Candida* spp. The wide spread use of antifungal agents because of the limited availability and because of increase in the incidence of opportunistic infections by *Candida* spp. result into evolution of drug resistance. The emergence of drug resistance poses a serious public health concern. The emergence of antifungal drug resistance is an evolutionary process that proceeds on temporal, spatial and genomic scales (3).

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### **Resistance to Polyenes**

The most important polyenes commonly used in the treatment of candidosis are amphotericin B, nystatin, natamycin and others. The most important agent, as far as development of resistance is concerned, is amphotericin B. Polyenes act by causing disruption of fungal cytoplasmic membrane, i.e. by interacting with ergosterol - an important component of fungal cell membrane, essential for maintaining fluidity and integrity of the membrane as well as for proper functioning of the membrane - bound enzymes. Amphotericin B intercalates into the membrane and generates channels and pores, through which many cellular components, particularly potassium and magnesium ions, come out and destroy the proton gradient within the membrane and cause death of the fungal cell (4).

### **Development of Resistance to Polyenes**

Polyenes act by interacting with ergosterol. The affinity of polyenes is high for ergosterol and less for 3-hydroxy or oxosterols. This low affinity for sterols such as fecosterol and episterol plays significant role in emergence of resistance to polyenes (5). Studies using polyene resistant strains revealed a marked decrease in membrane ergosterol content; the ergosterol, which is the favoured sterol target of polyenes, is replaced by biosynthetic precursors such as lanosterol, fecosterol, lichesterol and episterol. The change in sterol composition is frequently associated with an overall increase in the membrane sterol content and some changes in phospholipids and thus results in either quantitative or qualitative changes in sterol content of the cell influencing the amount or the availability of ergosterol for the action of polyenes. These changes in ergosterol content may contribute to the development of resistance to polyenes, especially the amphotericin B.

The quantitative changes in ergosterol content that contribute to development of resistance include:

- Decrease in the content of ergosterol because of inhibition of its synthesis
- Alteration of sterol content, i.e. replacement of ergosterol with sterols with reduced affinity and
- Alterations in the ratio of sterol to phospholipids (5,6).

The qualitative changes in ergosterol that may lead to development of resistance include reorientation or masking of ergosterol in the cell membrane because of which there is no binding with polyenes (7). In some polyene resistant strains no apparent alternation in their mem-

brane sterol content was seen. In such strains, possibility of changes in cell wall permeability to polyenes is the mechanism proposed. Another mechanism that thought to mediate resistance to amphotericin B is the increased catalase activity, which diminishes oxidative damage caused by this agent. In recent times, another mechanism proposed regarding the development of resistance to amphotericin B is related to the growth phase of the fungal cell. According to this, during the log phase of growth, break-down and resynthesis of the cell wall occurs at a higher rate that provides enhanced access of amphotericin B to the cell membrane. However, during the stationary phase of growth, break-down and synthesis of the cell wall occurs at a much lower rate that leads to the development of relative resistance to amphotericin B (5).

It has been also observed that most of the clinically isolated polyene-resistant *Candida* are the species other than *C. albicans* notably *C. tropicalis* and *C. lusitanae*. The potential for polyene resistance is reported to be high in *C. glabrata* and *C. parasilopsis*. In view of its haploid nature, *C. glabrata* can mutate frequently, and develop resistance faster than *C. albicans* and in *C. parasilopsis*, which is inhibited readily like other *Candida* species by polyenes but is less readily killed by them (8).

### **Molecular Aspects of Amphotericin B Resistance**

Amphotericin B acts by interacting with ergosterol. Several enzymes take part in the synthesis of ergosterol. The two important enzymes that participate in ergosterol synthesis are :

- C-8 sterol isomerase that catalyzes the production of episterol from fecosterol.

Activity of this enzyme is regulated by ERG 2 gene and

- C-5 sterol desaturase responsible for conversion of episterol into ergosterol. This enzyme is encoded by ERG 3 gene. Mutations in ERG 2 and ERG 3 genes encoding two important enzymes participating in ergosterol synthesis are responsible for amphotericin B resistance. Clinical strains of *C. albicans* showing resistance to amphotericin B with defective ERG2 and ERG 3 genes, and reduced ergosterol content have been reported (5).

### **Resistance to Azoles**

The azole group includes fluconazole, clotrimazole, itraconazole, ketoconazole and miconazole. Currently, flu-

conazole is the most widely used drug for treating candidiasis. Wide spread and prolonged use of azoles promote rapid development of the phenomenon of multidrug resistance, which poses a major problem in antifungal therapy. The azoles like polyenes, act by targeting ergosterol in the fungal plasma membrane. Azoles inhibit a key enzyme in the biosynthetic pathway for ergosterol. The target enzyme for azoles is a lanosterol 14  $\alpha$  demethylase (14 DM). It is a cytochrome P-450 enzyme containing a heme cofactor in the catalytic site to which the azoles bind. This binding inhibits cytochrome P-450 dependent 14-  $\alpha$ - demethylation of lanosterol. The inhibition of demethylation results into depletion of ergosterol and accumulation of sterol precursors into the plasma membrane, thereby disrupting the integrity of the membrane, its functions such as nutrient transport, chitin synthesis and reduce the effectiveness of several membrane associated enzymes. This finally leads to inhibition of fungal growth

The polyenes preferentially bind to membranes containing ergosterol. They form pores in the plasma membrane and cause leaking of essential cytosolic components from the cells (5,9,10.)

#### **Possible Mechanisms of Azole Resistance**

The mechanism of resistance to azole antifungal agents in *Candida* species may originate because of

- Qualitative or quantitative changes in the target enzyme lanosterol 14  $\alpha$  - demethylase. The qualitative change leads into the alternations in the affinity of the drug target, i.e. enzyme 14 DM to azoles, that ultimately results in reduced binding affinity of the enzymes to azoles (11,12). The quantitative change leads to increase cellular content of 14 DM due to target site mutation or overexpression of ERG 11 gene that finally results into increased ergosterol synthesis.

- Changes in the cell wall or plasma membrane, which lead to impaired azole uptake. This poor penetration of azoles across the membrane may be due to the alternations in sterol and/or phospholipid composition of the membrane and related reduced permeability. Alternatively, reduction in the intracellular concentration of readily accessed azole to its target may be due to pumping out by overexpressed efflux systems (5, 7, 11,12).

#### **Molecular Aspects of Azole Resistance**

Molecular studies on azole resistance have revealed dif-

ferent molecular mechanisms of resistance. Mechanisms that have been identified include:

- Alterations in the gene encoding the target enzyme ERG 11.
- Overexpression of genes coding for membrane transport proteins of the ATP binding cassette (ABC) transporter (CDR1/ CDR2) or the major facilitator (MDR1) superfamilies of transporters (12-14) .

#### **Alterations in ERG11**

Various genetic alterations in ERG11 of *C. albicans* have been observed (15). Analysis of the ERG11 gene sequence identified several point mutations in resistant strains of *C. albicans*. Seven different point mutations with azole resistance have been defined so far. A point mutation leading to replacement of arginine with lysine at amino acid 467 has been found to be associated with azole resistance in a clinical strain of *C. albicans* when matured strains were tested (16). Two of the most common point mutations in ERG11 of *C. albicans*, associated with resistance, D 116E and E 266 D are the most frequently observed mutations, which are not necessarily associated with resistance (14)

In Addition to target site mutations, overexpression of the ERG 11 gene has been observed in azole resistant clinical isolates of *C. albicans*, but the role of this phenomenon to the development of resistance is not exactly known. Although, alterations such as the point mutation leading to replacement of arginine and the overexpression of the genes encoding efflux pump systems are seen in the resistant isolates, the recent data suggests that overexpression of ERG 11 in *C. albicans* is not associated with azole resistance (14). Molecular studies have revealed that there are two types of efflux pumps, which are responsible for the development of azole resistance in *Candida* spp. These include ATP- binding cassette (ABC) transporters and major facilitators superfamily (MFS) proteins, which are responsible for the low level of accumulation of azole antifungal agents. Two genes for these transporters, the ABC transporter gene CDR and the MSF gene (Also known as CaMDR1 gene)- BEN-R were shown to be overexpressed in resistant isolates. Most recent studies suggest that the overexpression of BEN-R is responsible for the specific resistance of clinical isolates of *C. albicans* to fluconazole (14,16,18).

CDR1 and CDR2 have been found to be responsible for development of resistance to azole in *Candida albicans* strains, however CgCDR1 was found to be responsible for

azole resistance in *Candida glabrata* (14,19,20). Some of other multidrug efflux transporter genes of both classes existing in *C. albicans* have been cloned. These are ABC-transporter genes : CDR2, CDR3, CDR4, CDR5 and the MFS gene FLU1. Over expression of CDR2 gene in *C. albicans* isolates showing cross resistance to azole derivatives have been reported (17,18).

### Cross Resistance in Azole

Cross - resistance in azole has also been reported (21). Fluconazole resistance has been rarely reported but *C. albicans* resistant to ketoconazole are cross resistant to fluconazole. Ketoconazole resistant *C. albicans* have also been found to be cross resistant to itraconazole and miconazole (9). White et al reported extensive cross-resistance for fluconazole, clotrimazole, itraconazole and ketoconazole (14). CDR overexpression and R467 K point mutation in ERG 11 appear to be responsible for azole cross resistance. However, MDR1 overexpression does not lead to cross resistance to other azole because of its specificity for fluconazole (22,23).

### Resistance to Flucytosine(5-Fluorocytosine)

Fluorocytosine acts by inhibiting nucleic acid and protein synthesis in fungi. It is taken inside the cell by fungal cytosine permease and then it is deaminated to 5 - fluorouracil (5- FU), which is initially converted to 5 - fluorodeoxyuridine monophosphate and 5 - fluorouridylic acid. Further phosphorylation results into production of 5 - fluorouracil triphosphate. This reaction is catalysed by uracil phosphoribosyl transferase. 5 - fluorodeoxyuridine monophosphate inhibits DNA synthesis via inhibition of thymidine synthetase. However, 5 - fluorouracil triphosphate gets incorporated into RNA and inhibits protein synthesis (5).

### Mechanism of Resistance

Resistance to 5 - fluorocytosine may be encountered in about 10% of primary isolates of *C. albicans*. Resistance arises during treatment by selection of resistant mutants. The most common cause of resistance appears to be loss of the enzyme uridine monophosphate pyrophosphorylase (9). It has been observed that a defect in cytosine deaminase activity usually results in primary resistance. However, a decrease in the activity of uracil phosphoribosyl transferase leads to secondary resistance. In addition to this, loss of permease activity is also responsible for development of resistance in *Candida* species (5).

Resistance of *Candida* species to 5 - fluorocytosine is acquired during monotherapy. Combination of 5 - fluorocytosine and amphotericin - B reduces the occurrence of resistance in *C. albicans* isolates. It has been observed that this acquired resistance results on account of failure to metabolise 5 -fluorocytosine into 5 - fluorouracil triphosphate and 5 - flurodeoxyuridine monophosphate or from the loss of feedback control of pyrimidine biosynthesis. Deficiency of enzymes involved in the uptake or metabolism of 5 - fluorocytosine or deregulation of pyrimidine synthesis pathway are the factors leading to development of intrinsic resistance to 5 - flurocytosine (17, 24).

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