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Review Article

ANTIFUNGAL RESISTANCE IN CANDIDA SPECIES: AN OVERVIEW

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ABSTRACT

Over the past few decades, the prevalence of fungal infections has dramatically increased due to the growing number of populations at high risk. Although *Candida albicans* is considered as the most prevalent species, in recent years non *albicans Candida* (NAC) spp. are increasingly reported from various clinical types of *Candida* infections. NAC spp. are either innately resistant to antifungal drugs or may acquire resistance during course of therapy. Antifungal resistance once rarely documented in *Candida* spp. is now being increasingly reported from various parts of world. The development of antifungal drug resistance in *Candida* spp. with different mechanisms has potential clinical impact. In this review, various aspects of antifungal resistance in *Candida* spp. are discussed.

Keywords: Antifungal resistance, azoles, echinocandins, *Candida* species, Non-*albicans Candida* spp., Polyenes.

INTRODUCTION

Over the past few decades, the number and variety of fungi causing infections has dramatically increased (Deorukhkar *et al.*, 2014). Several factors like advent of HIV/AIDS, advances in therapeutic and diagnostic technology, the use of increasingly aggressive chemotherapeutic regimens and indwelling medical devices are known to boost fungal infections (Silva *et al.*, 2012).

Among various fungal pathogens, the majority of infections are caused by *Candida* spp. (Deorukhkar *et al.*, 2014). Currently, *Candida* spp. is reported to be either third or fourth among the microorganisms and first among the fungal pathogens isolated from blood stream infections (Deorukhkar and Saini, 2016).

Although *Candida albicans* is considered as the most prevalent species, in recent years non *albicans Candida* (NAC) spp. are increasingly reported from various clinical types of *Candida* infections (Lokhart, 2014; Deorukhkar *et al.*, 2014). The shift towards NAC spp. is of concern because these emerging pathogens often demonstrate reduced susceptibility to commonly used antifungal drugs (Deorukhkar *et al.*, 2014).

NAC spp. are either innately resistant to antifungal drugs or may acquire resistance during course of therapy (Deorukhkar and Saini 2015). The clinical consequences of antifungal resistance lead to treatment failure and emergence of resistant *Candida* spp. Therefore it is very important to understand the mechanisms involved in resistance of *Candida* spp. In this review article the overview of various aspects of antifungal resistance in *Candida* spp. is presented.

ANTIFUNGAL AGENTS: TYPES AND MECHANISM OF ACTION

Compared to bacterial counterparts, the armamentarium against fungi is limited. This can be attributed to various factors including identification of distinct targets on eukaryotic fungal cell type without being toxic to host cells (Srinivasan *et al.*, 2014). The antifungal arsenal against *Candida* is currently classified on the basis of their target of activity.

Azoles interact with cytochrome P-450 and inhibit the ergosterol biosynthesis by interfering with C-14 demethylation of lanosterol (Odds *et al.*, 2003). Ergosterol is the major component of fungal cell membrane and acts as a bioregulator of membrane fluidity, asymmetry and integrity (Kathiravan *et al.*, 2012). It has a hormone-like role in fungi and stimulates the growth. Fluconazole and itraconazole are the most commonly used azoles for treatment of *Candida* infections (Silva *et al.*, 2012). They demonstrate either fungistatic or fungicidal activity against *Candida* spp.

Owing its convenient administration, high bioavailability, cost effective and extended activity, fluconazole is most attractive azole used in prevention and treatment of *Candida* infections (White *et al.*, 1998). Voriconazole and posaconazole are 2nd generation triazole having broad spectrum of activity against *Candida* spp. However, high price limits their use (Deorukhkar and Saini, 2015).

Polyenes interact with the ergosterol component within the cell membrane and generate pores leading to leakage of cytoplasmic contents and death of the fungal cell (Andriole 2000). Nystatin and amphotericin B are examples of polyenes used for treatment of *Candida* infections (White *et al.*, 1998). Amphotericin B is generally regarded to have the broadest spectrum of antifungal activity and used in life-threatening disseminated infections whereas, the use of nystatin is limited to superficial and mucocutaneous infections (Silva *et al.*, 2012; Kathiravan *et al.*, 2012).

5-flucytosine (5FC) is an antifungal agent that works through conversion to 5-fluorouracil within target cells. This nucleoside analogue gets incorporated into RNA and affects the fungal protein synthesis (Silva *et al.*, 2012). It is fungus specific as human cells contain minimal or no cytosine deaminase. 5FC is used as an adjunctive rather than primary therapeutic agent due to development of resistance (Pfaller, 2007).

Echinocandins are most recent addition to antifungal armamentarium. This group of antifungal agents selectively targets the fungal cell wall and inhibits 1-3- and 1-6- β -D-glucan

synthesis (Deorukhkar and Saini, 2016). As the target for echinocandins is not present in mammalian cells, these drugs are non toxic to humans (Silva *et al.*, 2012).

This class of antifungal agents is now increasingly used as first line drugs for treatment and management of candidemia and other form of invasive candidiasis among patients having recent history of exposure to azole and are either colonized or infected with fluconazole resistant *Candida* spp (Deorukhkar and Saini, 2016).

As per current guidelines all three echinocandins (casprofungin, micafungin and anidulafungin) are equally effective for the treatment of disseminated candidiasis (Deorukhkar and Saini, 2016). In one of our study, casprofungin was found to be the most efficient echinocandin drug for treatment of candidemia due to fluconazole resistant species (Deorukhkar and Saini, 2016).

ANTIFUNGAL RESISTANCE: CONCEPT AND TYPES

The resistance to an antifungal agents exhibited by infecting *Candida* spp. can either be microbiological or clinical.

Microbiological resistance is defined as the non-susceptibility of an infecting fungal species to an antifungal drug by *in vitro* susceptibility testing, in which the minimum inhibitory concentration (MIC) of the drug exceeds the susceptibility breakpoint for that organism (Kanafani and Perfect, 2008). In some cases, patient responds clinically to treatment with the antifungal drug to which an infecting *Candida* spp. has shown resistance *in vitro*. This is either due to eradication of pathogen by host's immune mechanisms or due to unusually higher concentration of antifungal drug at the infected site or due to synergistic action of antifungal agent with other molecules at the site of infection (Sanglard and Odds, 2002).

Microbiological/microbial resistance may be classified as primary or intrinsic resistance and secondary or acquired resistance.

Primary microbiological resistance is naturally occurring resistance among certain fungal species without prior exposure to the antifungal drug. It emphasizes the importance of species identification of fungal isolate from clinical specimen. An example of primary resistance is fluconazole resistance in *C. krusei* isolates (Kanafani and Perfect, 2008).

Secondary resistance develops in intrinsically susceptible fungal species after exposure to the antifungal drug (Kanafani and Perfect, 2008). Development of fluconazole resistance in *C. albicans* is an example of secondary resistance. Secondary resistance emphasizes the importance of antifungal susceptibility testing of fungal isolate from clinical specimen.

In contrast to clinical resistance, microbiological resistance can be objectively defined, scientifically measured and investigated (Sanglard and Odds, 2002).

Clinical resistance is defined as a condition in which there is failure in eradication of fungal infection in spite of administration of antifungal agent with an *in vitro* activity against the

infecting fungus (Kanafani and Perfect, 2008). This type of resistance is due to variety factors related to the host, the antifungal drug or the infecting fungal species.

Susceptibility/resistance to an antifungal agent, cell type and size of fungal populations are fungal properties associated with treatment failure. Host factors responsible for clinical resistance include immune status of the patient, presence of foreign materials including medical devices, site of infection and undrained abscesses whereas inappropriate dosage, fungistatic nature of the antifungal agent, poor absorption, distribution, or metabolism and drug-drug interaction are properties of antifungal agents leading to treatment failure (Canuto and Rodero, 2002).

EPIDEMIOLOGY OF ANTIFUNGAL RESISTANCE: PREVALENCE AND RISK FACTORS

Antifungal resistance once rarely documented in *Candida* spp. is now being increasingly reported from various parts of world. The emergence of NAC spp. and utilization of simple and rapid methods of antifungal susceptibility testing like disc diffusion and agar based techniques are important among various reasons.

Azole Resistance

Among all classes of antifungal drugs, azole resistance in *Candida* spp. is extensively studied. Azole resistance particularly fluconazole resistance was increasingly reported from HIV infected patients with oropharyngeal candidiasis (OPC) (Kanafani and Perfect, 2008). The prevalence of azole resistance was reported to be 21 to 32% in symptomatic patients and upto 14% in asymptomatic patients (Kanafani and Perfect, 2008). Both intrinsic and acquired resistance has been reported in *Candida* spp. isolated from HIV infected patients with OPC.

Acquired resistance is reported in *C. dubliniensis* after exposure to azoles (Deorukhkar and Saini 2016). Many researchers have documented the role of empirical treatment or prophylaxis with azole in selection of azole resistant *Candida* spp. However, with introduction of highly active antiretroviral therapy (HAART) in 1996 as standard care for the treatment of HIV reduced incidence of OPC and eventually the azole resistance in *Candida* isolates (Kanafani and Perfect, 2008).

Infections due to azole resistant *Candida* spp. have been also reported in HIV non infected patients. Several researchers have documented emergence of azole resistant *Candida* spp. in malignancy and bone marrow transplant patients (Kanafani and Perfect, 2008). Azole resistance has been reported from cases of recurrent vulvovaginal candidiasis (RVVC).

As happened with OPC in 1990s, there was also an increase in the incidence of candidemia due to NAC spp. like *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. *C. krusei* and many strains of *C. glabrata* are innately resistant to fluconazole (Krcmery and Barnes 2002). *C. tropicalis* was initially regarded as fluconazole susceptible species, however recent studies have documented emergence of fluconazole resistance in this NAC spp (Deorukhkar *et al.*,

2014). The emergence of fluconazole resistance in *C. tropicalis* isolates is of concern because it is one of the most common NAC spp. isolated from various clinical types of *Candida* infections. Resistance to the azole group of antifungal is disquiet as these antifungals (especially fluconazole) are commonly used for prophylaxis and treatment of candidiasis. In one of our study, fluconazole resistance was more common in *C. tropicalis* isolated from blood cultures (Deorukhkar *et al.*, 2014).

Polyene Resistance

As compared to azole resistance to amphotericin B is rarely encountered (Akins, 2005). For many years amphotericin B has been the only antifungal polyene that can be administrated systemically for treatment of visceral candidiasis.

Although amphotericin B resistance is rare during treatment, there are recent reports of isolates demonstrating elevated minimum inhibitory concentrations (MICs). *C. glabrata* and *C. krusei* tend to have higher MICs to amphotericin B compared to *C. albicans* (Canuto and Rodero, 2002). NAC spp. like *C. lusitanae* and *C. guilliermondii* are reported to be innately resistant to amphotericin B (Kanafani and Perfect, 2008). Acquisition of these NAC spp. have been documented in patients receiving amphotericin B therapy. Acquired resistance is also reported in strains of *C. albicans* during treatment with amphotericin B (Sanglard and Odds, 2002).

Echinocandin Resistance

MICs of all three echinocandins are comparatively much lower than that for amphotericin B and fluconazole against most of commonly isolated *Candida* spp. However, the MIC values for echinocandins tend to be high for *C. parapsilosis* and *C. guilliermondii*. In one of our study, MIC for all three echinocandins was higher in *C. parapsilosis* compared to other *Candida* spp (Krcmery and Barnes 2002; Silva *et al.*, 2012). Matsumoto *et al.* (2014) reported *C. parapsilosis* to be the only *Candida* spp. resistant to micafungin.

Chamilos *et al.* (2007) reported that at concentration above MIC for echinocandins, these drugs paradoxically promote the in vitro growth of *C. parapsilosis*. Therefore, echinocandins should be used with caution for treating *C. parapsilosis* infections. Echinocandins appears to be promising antifungals as resistant mutants don't exhibit cross-resistance against other classes of antifungal drugs, and conversely, *Candida* isolates resistant to other antifungal drugs are not cross-resistant to echioncandins. As echinocandins are new addition to antifungal arsenal, the burden of resistance to this antifungal class is still not completely appreciated.

MECHANISMS FOR ANTIFUNGAL RESISTANCE: CELLULAR AND MOLECULAR

Azole Resistance

Mechanisms of azole resistance in particular and fluconazole in specific have been most extensively studied. Following mechanisms are usually described for azole resistance in *Candida* spp. (Sanglard and Odds, 2002):

- (i) Efflux of drug by multi-drug transporters such as ATP-binding cassette (ABC) gene family.
- (ii) Amino acid substitution to *ERG11* gene affecting drug-target binding.
- (iii) Overexpression of *ERG11* minimizing effect of azoles.
- (iv) Mutation in *ERG3* alleles leading to change in toxic sterol concentration.

These mechanisms usually function separately, but in many situations they may get combined to contribute to a step-by-step acquisition of resistance to azoles.

As compared to *C. albicans* and other commonly isolated NAC spp., relatively little is known about the mechanism of azole resistance in *C. tropicalis*. Overexpression of *C. tropicalis* (*ctERG11*) associated with missense mutations have been described as the mechanism for azole resistance in *C. tropicalis* (Forastiero *et al.*, 2013). Additionally, upregulation of two multidrug efflux transporter genes, *ctMDR1* and *ctCDR1* are linked to development of fluconazole resistance in *C. tropicalis* (Forastiero *et al.*, 2013).

Polyene Resistance

Although isolation of polyene resistant strains has been reported, polyene resistance is not a significant clinical problem till date. Polyene resistant *Candida* isolates have significant low ergosterol content in their cell membrane. Qualitative and quantitative changes in the sterol content of the cell membrane have been reported in polyene resistant *Candida* spp (Canuto and Rodero, 2002). Mutations in the *ERG3* gene involved in ergosterol biosynthesis results in accumulation of other sterols in the fungal cell membrane (Kanafani and Perfect, 2008). Alternation in *POL* gene family is another mechanism known for polyene resistance (Canuto and Rodero, 2002).

Molecular mechanisms involved in polyene resistance include the decrease in net ergosterol content of cell, replacement of few or total polyene-binding sterols, and change or masking of the polyene binding sterols (Canuto and Rodero, 2002). Amphotericin B resistance may be also related to elevated catalase activity and decreased susceptibility to oxidative damage (Kanafani and Perfect, 2008).

Echinocandin Resistance

The mechanisms of echinocandin resistance in *Candida* spp. is yet to be completely elucidated. Generation of insufficient target enzyme β -1-3-D-glucan synthase and production of an alternative form of the enzyme with decreased echinocandin binding are possible mechanisms proposed for innate resistance (Kanafani and Perfect, 2008).

Acquired echinocandin resistance is linked with mutations in *FKSI* gene of β -1-3-D-glucan synthase complex (Kanafani and Perfect, 2008). Products of *FKSI* gene are alternate subunits of β -1-3-D-glucan synthase enzyme complex (Denning 2003).

BIOFILM FORMATION AND ITS ROLE IN ANTIFUNGAL RESISTANCE

Candida spp. is unique mycotic pathogen that has established itself both as commensals and pathogen in humans (Deorukhkar, 2017). The transition from a commensal to a potent pathogen is mediated by various virulence factors like adhesion to host tissue and medical devices, biofilm formation and secretion of extracellular hydrolytic enzymes (Deorukhkar and Saini, 2014).

Among various virulence factors attributing to pathogenicity of *Candida* spp. biofilm formation appears to be most important. Biofilm are specific and well organised surface associated communities of microorganisms embedded within an extracellular matrix. *Candida* spp. is capable of producing biofilm on most, if not all, medical devices (Deorukhkar and Saini, 2016). Biofilm production not only increases the ability of *Candida* spp. to withstand host defense mechanisms but also confers significant resistance to antifungal therapy.

The exact mechanism of biofilm resistance to antifungal agents is not known. It is postulated that the presence of the matrix limits the penetration of antifungal drugs by producing a diffusion barrier (Sardi *et al.*, 2013).

Several researchers have reported total resistance to antifungal drugs in biofilm forming isolates. In one of our study, azole and polyene resistance was more common in biofilm forming isolates compared to non biofilm producers (Deorukhkar *et al.*, 2014). Triazoles like voriconazole and posaconazole and all three echinocandins are approved for treatment and prevention of infections due to biofilm forming *Candida* isolates (Sardi *et al.*, 2013).

CONCLUSION

Candida infections are one of the major causes of morbidity and mortality in immunocompromised patients. Antifungal resistance once rarely documented in *Candida* spp. has emerged as important health-care problem worldwide. Various clinical, cellular, and molecular factors contribute to antifungal drug resistance in *Candida* spp. These mechanisms usually function separately, but in many situations they may get combined to contribute to a step-by-step acquisition of resistance. Understanding resistance mechanisms employed by *Candida* spp. is very essential to circumvent the problem of emergence of drug resistance and treatment failure.

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