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Review Article

Microbiology Section

Molecular Mechanisms of Antifungal Drug Resistance in *Candida* Species

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ABSTRACT

Invasive Candidal infections have emerged as one of the major threats to the world. Although, many new antifungal drugs have been developed in the recent years, the emergence of drug resistance has become a major deterrent in the antifungal therapy. *Candida* develops several molecular mechanisms to resist the exposure to antifungal drugs. Prolonged treatment in patients may trigger development of resistance to the prescribed drugs. Most of the antifungal therapeutic agents being fungistatic rather than fungicidal are the key reason for selection of resistant *Candida* strains. Overexpression or mutation of the target enzymes as well as transcriptional activation of genes encoding the drug efflux pumps of ATP Binding Cassette (ABC) and Major Facilitator Superfamilies (MFS) are some of the factors implicated in the development of drug resistance. Basic understanding of the underlying molecular mechanisms of antifungal drug resistance and their clinical impact is vital in planning of the effective management of Candidal infections. There is an interesting possibility of antifungal resistance in *Candida* becoming a marker in the assessment of the outcome of antifungal therapy in the future. This review describe and summarises the molecular mechanisms of drug resistance in *candida* species.

Keywords: Antifungal drugs, Invasive candidal infections, Resistance mechanisms

INTRODUCTION

Microorganisms adapt to the environmental changes by modulating the molecular intra-cellular mechanisms and their gene(s) expression(s) parsimoniously. Further, when they are in persistent contact with the environment for longer duration, they can generate molecular genetic alterations which could enable them to adapt to the adverse environment. Under selective pressure, these variants can evolve, develop and dominate within a short duration. A good example for this molecular evolution is the emergence of drug resistance in the microorganisms during the prolonged antimicrobial therapy. The increasing pattern of multidrug resistance is a major morbidity and mortality threat in the management of both bacterial and/or fungal infectious diseases. High mortality and morbidity rates due to fungal pathogens are of great concern today, due to the limited therapeutic options. In the present review, we shall deal with fungal drug resistance, particularly *Candida*, as it is one of the most common fungus causing infections in humans with widespread prevalence and morbidity.

Candida species are the members of microbiota of human gastrointestinal and urinary tracts which could lead to diseases when the host immune mechanisms are compromised. Since, human's immune system is able to counteract the candidal infections, the infections are usually asymptomatic. *Candida* albicans is a normal commensal of humans that resides in the oral cavity, gastrointestinal, vaginal and urinary tracts [1]. It acts as an opportunistic pathogen causing infections such as stomatitis, thrush, urinary tract-infections and can also cause severe systemic infections [1]. It causes infection when the host becomes debilitated or immunocompromised, the numbers of which are constantly increasing due to organ transplant, chemotherapy or due to the increase in prevalence of AIDS and hepatitis C [1,2]. *Candida* has various molecular mechanisms by which it maintains its character and pathogenicity. *C. albicans* has been found to be the predominant cause of invasive candidiasis [3]. However, in the recent years, many longitudinal studies have proved that Non Albicans species (NAC) have also been associated with clinical infections [4,5]. *Candida* is also isolated from nosocomial infections. Not only *C.albicans*, the other species of *Candida* like *C. glabrata, C. tropicalis, C. krusei, C. parapsilosis* are also found to cause hospital acquired infections [6,7]. As mentioned earlier, this could also be probably due to the rise in the number of immunocompromised patients, especially HIV cases, patients on immunosuppressive drugs and patients undergoing transplantation [3]. During the life long association with the human host, *Candida* generates genetically altered variants which could adapt to their new environment; the emergence of drug resistance is one of the major public health issues of global concern.

There are relatively few classes of antifungal drugs. This restricts clinician's therapeutic usage and is further obliterated by the emergence of drug resistance [8]. The emergence of drug resistance in all pathogenic microorganisms, including fungi, is a process of evolution initiated by antimicrobial agents on prolonged exposure [9]. Whenever the pathogen population remains large even after drug treatment, the evolution of resistance becomes inevitable. The evolution of drug resistance depends on genetic variability, mostly mutation [9]. Resistance due to mutation arises in the pathogen by selection, genetic drift, recombination, and migration (including transmission between hosts). The emergence and spread of drug resistance depends on different possible mutations that enable the pathogen to avoid, remove or inactivate a drug [9]. The drug resistance can occur due to mutations in the drug targets, alteration in the sterol biosynthesis pathways, functional mutations in the transcription factors resulting in upregulation of ergosterol biosynthesis genes and multidrug efflux pumps. Genomic rearrangements resulting in gene amplification and loss of heterogenicity can also cause drug resistance.

Epidemiology of *Candida* infections: Centers for Diseases Control and Prevention (CDC) and the National Healthcare Safety Network has ranked *Candida* as fifth in causing hospital-acquired infections and fourth among Blood Stream Infection (BSI) pathogens [10]. Another international study by the SENTRY Antimicrobial Surveillance Program reported a total of 1239 *candida* BSI isolates from 79 medical centers in 2008-2009 [11]. This study reported

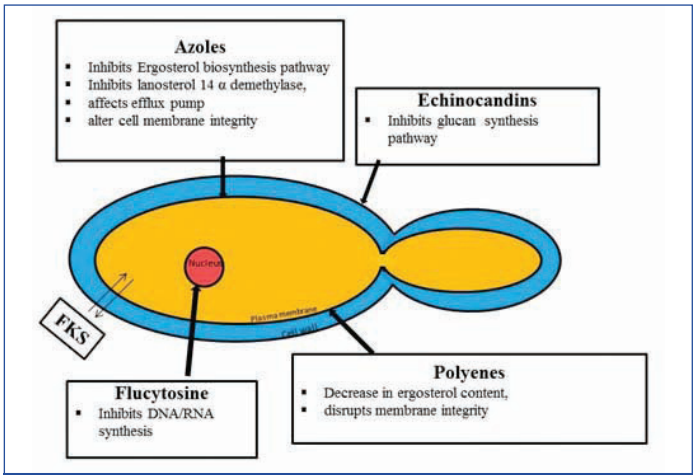
high antifungal resistance among the *C.glabrata* isolates with the resistance rates to echinocandins (16.7%), fluconazole (16.7%), posaconazole (5.0%) and voriconazole (11.0%) in patients of 20-39 years age [11].

Candida species are approximately the fourth most common cause of nosocomial infections in ICUs, according to data from the National Nosocomial Infections Surveillance System and the European Prevalence of Infection in Intensive Care [12]. *Candida* may cause hospital acquired infection when its incidence is increased in hospitals or due to involvement of invasive interventions and long-term antibiotic usage [10]. The incidence of fungal infections varies widely with solid organ–transplant recipients; it ranges 5% in renal transplantation, 35% in lung and heart transplant recipients and up to 40% in liver transplantation [13]. Serious fungal infections affect nearly more than 1 billion people per year [14]. Cancer chemotherapy and allogeneic bone marrow transplantation may also be related to fungal disease, and 30% of acute leukaemia patients have invasive fungal infections [13].

However, community-acquired cases cannot be ignored. The SENTRY antimicrobial surveillance program detected 1,354 infection episodes related to *Candida* species between 2008 and 2009 among which community-acquired cases were 36.5% and they also reported community-acquired candidemia is higher in North America (63.5%) than in Europe (22.4%) [10]. The Asian picture, regarding incidence of candidemia is not very clear due to lack of multicentric studies [15]. A Southern India based study reported an incidence rate of 5.7% for candidemia among children with onco-haematological malignancies [16].

There is a lot of variation among the incidence and prevalence reports quoted from different parts of India. An incidence rate of 6.9% for *Candida* species in blood stream infections was reported by Sahni V et al., from Maulana Azad Medical College, New Delhi [17]. A study done in New Delhi gave a prevalence rate of 18% for *Candida* species among isolates of blood culture [18]. A similar 5-year study (2001-2005) by Xess I et al., from AIIMS, New Delhi, reported a prevalence rate of 6% for *Candida* species [19]. Another study from Rohtak, Northern India, reported an isolation rate of 8.1% for *Candida* species among neonatal septicaemia cases [20]. A 13-year study from a tertiary care hospital in Thailand showed a prevalence of 6.14% for *Candida* species among blood culture isolates [21]. Based on a study from SGPGI Lucknow, *Candida* species is stated as eighth among all isolates from BSI and reported an incidence rate of 1.61 per 1000 hospital admissions for candidemia [22].

Antifungal drugs in clinical treatment: Though, there are various classes of antifungal drugs, the classes of drug in current therapeutic use in *Candida* infections are relatively few [23,24]. Azoles, echinocandins, polyenes, nucleoside analogues and few other antifungal agents like allylamines, thiocarbamates are the various antifungal drugs in use. Azoles are the inhibitors of Lanosterol 14-α-Demethylase enzyme. The azole drugs include imidazoles (miconazole, econazole, clotrimazole, and ketoconazole) and triazoles (fluconazole, itraconazole, and voriconazole (second-generation, synthetic triazole derivative of fluconazole) and posaconazole (hydroxylated analogue of traconazole) [25,26]. Azoles are widely used for topical usage as well as in invasive Candidal infections [25]. Echinocandins are inhibitors of glycan synthesis. Caspofungin, micafungin, and anidulafungin are few echinocandins used in oesophageal and invasive candidiasis [27,28]. Polyenes act by binding to ergosterol. Polyenes like nystatin and amphotericin B have a broad spectrum antifungal action. Nucleoside analogues like flucytosine are inhibitors of DNA/RNA synthesis. Allylamines and thiocarbamates inhibit squaline epoxide enzyme used in the biosynthesis of ergosterol [29]. Griseofulvin is a tricyclic spirodiketone, acting by inhibiting fungal mitosis [Table/Fig-1].



[Table/Fig-1]: Mechanism of action of antifungal drugs.

Antifungal Drug Resistance in *Candida* Species

Phenotype, genotype, serotype of the yeast, initial MICs, biofilm formation, fungistatic nature of the drug, drug dosage and pharmacokinetics, drug interactions, site and severity of the infections, immune status of the patient, noncompliance of the patient, etc., are amongst the various factors which may precipitate the emergence of drug resistance in the *Candida* species.

Resistance to azoles: Azoles are the largest family of antifungal drugs. Fluconazole, clotrimazole, ketoconazole, itraconazole, miconazole are some of the common antifungal drugs in azole group. Fluconazole is currently the widely used drug in treating the candidal infections. Resistance to these drugs is one of the major challenge in antifungal therapy and public health. Widespread, irrational and chronic usage of these drugs is one of the reasons for development of resistance to azole drugs.

Azole group of drugs generally act by disrupting the fungal cell membrane by inhibiting an enzyme namely lanosterol 14-α-sterol Demethylase (DM) [26]. This enzyme is basically a cytochrome P 450 enzyme having heme cofactor azole binding site and is involved in the biosynthesis of ergosterol by mediating the conversion of lanosterol to ergosterol. Inhibition of this enzyme by azole leads to accumulation of the toxic product 14-α-methyl-3,6-diol and reduction in ergosterol content of the fungal plasma membrane which ultimately leads to disruption of the integrity of the fungal cell membrane resulting in reduced fungal growth. Various mechanisms of resistance to azoles have been reported in *Candida* species [30]. More than one type of resistance may be present in any strain which may even lead to cross resistance [31]. Any change in the target enzyme namely lanosterol 14-α-sterol demethylase may lead to development of drug resistance. Alterations in the affinity of azoles to the lanosterol 14-α-sterol demethylase enzyme may result in reduced binding of azoles to that enzyme [32,33]. Also, target site mutation or over expression of *ERG11* genes (Ergosterol Biosynthesis enzyme-Lanosterol (C-14) demethylase) may lead to increased cellular content of 14 DM resulting in increased ergosterol synthesis. In addition, any alteration in the cell wall or the cell membrane may cause variation in the uptake of azoles. Any change in the sterol or phospholipid content of the plasma membrane may cause poor penetration of the drug into the membrane. Also, pumping out through over expressed efflux systems may cause decrease in the intracellular concentration of the azoles [32,33]. This induction of multidrug pumps resulting in decrease in concentration of drugs in the fungal cell membrane at the target

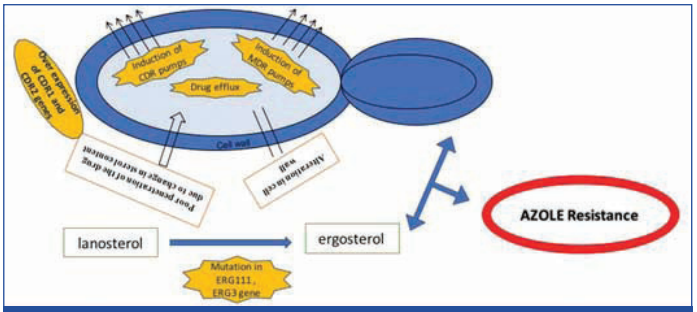
enzyme lanosterol 14-α-sterol demethylase is one of the vital mechanisms of azole resistance.

Over expression of plasma membrane efflux pump is one of the major mechanisms of azole resistance. The major families of efflux proteins namely ABC and MFS are of great clinical significance in the azole resistance mechanisms. These proteins actively transport the compounds across the fungal cell membrane. ABC proteins are primary transporters and MFS are secondary transporters, both containing distinctive protein domains namely Nucleotide Binding Domains (NBDs) in ABC transporters and Transmembrane Domains (TMDs) in ABC and MFS transporters. *Candida* Drug Resistant (CDR) genes of the ATP-binding cassette superfamily and MDR genes of major facilitator super-family, encode the efflux pumps of the *Candida* species. Induction of CDR encoded efflux pumps has been found to be the common resistance mechanism in *Candida* to almost all azole groups of drugs, while induction of MDR encoded efflux pumps play an important role in fluconazole resistance [30].

Molecular studies have shown that the other important mechanisms of azole resistance in *Candida* is mutation of the genes encoding target enzyme *ERG11* and over expression of the genes encoding for membrane transport proteins [32,34]. The *ERG11* gene encodes for enzyme target lanosterol 14-α-sterol demethylase. Studies have shown several genetic alterations in *ERG11* gene in *Candida* species. Any alteration or upregulation of this target enzyme may lead to azole resistance. Mutation in the *ERG11* gene prevents binding of the azole drugs to the enzyme target [35]. It is said that the reduced affinity of *ERG11* gene to fluconazole may be the cause for the intrinsic resistance exhibited by the *C. krusei* to the fluconazole [36]. However, it is reported that this mechanism plays a limited role in clinical resistance in *Candida* species to azoles [30]. Several point mutations have been identified in resistant strains of *Candida* species during the *ERG11* gene sequence analysis. In a study, when the clinical strains of *Candida* were tested, azole resistance was found to be associated with a point mutation at amino acid 467 where arginine is replaced with lysine [37]. *D116E* and *E266D* are few other observed mutations, not necessarily associated with resistance [34]. However, few studies reported that though point mutation causing replacement of arginine and overexpression of *ERG11* encoding the efflux pump systems are found in resistant strains, the overexpression of *ERG11* gene may not be associated with azole resistance [34].

Another mechanism of azole resistance is development of bypass pathways. It is already mentioned that inhibition of lanosterol 14-α-sterol demethylase due to exposure to azoles may lead to the accumulation of the toxic product 14-α-methyl-3,6-diol. Mutations in the *ERG3* gene prevents the formation of the toxic product 14-α-methyl-3,6-diol from 14-α-methylfecosterol [38]. Hence, if the ergosterol is replaced with the latter sterol, the fungal cell membrane will become functional and this may negate the azole leading to membrane disruptive effects.

Molecular studies revealed two types of efflux pumps namely ABC transporters and MFS proteins, responsible for the development of resistance to azoles in *Candida* species. The ABC transporter gene CDR and the MSF gene (*CaMDR1* gene)-BEN-R are the genes for these transporters. In resistant strains, these genes are shown to be overexpressed. Few studies have reported that BEN-R gene is responsible for resistant to fluconazole in *C.albicans* [37,39]. *CDR1* and *CDR2* in *C. albicans* strains and *CgCDR1* has been found to be responsible for resistance to azoles in *C. glabrata* [40]. Overexpression of *CDR2* gene showing cross resistance to azoles in *C. albicans* have been reported [39,41] [Table/Fig-2].



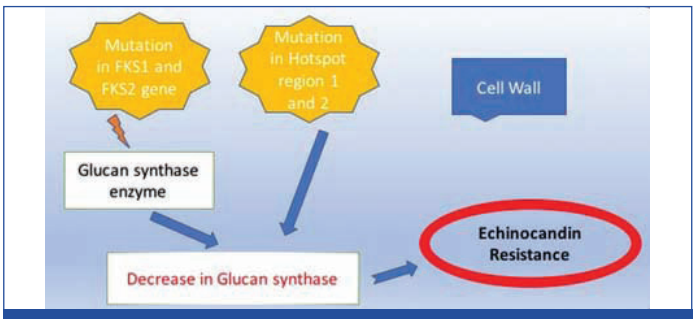
[Table/Fig-2]: Mechanism of resistance to azole group of drugs.

Resistance to echinocandins: β(1,3)D-glucan synthase is an enzyme which helps in the biosynthesis of β(1,3)D-glucan, an important component in the fungal cell wall [31]. Echinocandins act by inhibiting this β(1,3) D-glucan synthase enzyme causing defective fungal cell wall formation leading to cell death [31].

Point mutation and intrinsic mutation of the genes encoding FKS (Glucan synthase gene) subunits of the enzyme β(1,3) D-glucan synthase are responsible for the resistance or decrease in susceptibility to echinocandins [42]. There are some highly conserved regions in *Candida* species where the specific point mutation have been found to cluster around, known as "hot spot regions" [43]. Mutations in such hot spot regions may cause increase in Minimum inhibitory concentration (MIC), reduced β(1,3) D-glucan synthase sensitivity, and cross-resistance among the echinocandins [44,45].

In *C.albicans*, these hot spot regions namely HS1 is found in amino acid positions 641 to 649 and HS2 is found in amino acid positions 1345 to 1365 in FKS1 [44]. Such hot spot mutations FKS1 have also been reported in other species of *Candida* namely *C. glabrata, C. dubliniensis, C. krusei, C. tropicalis* [44,46,47]. Mutations in FKS2 (paralog of FKS1) can also lead to resistance to echinocandins in *C. glabrata* species [48]. However, *C. parapsilosis*, is less susceptible to echinocandins due to intrinsic mutation in FKS1. Studies have shown that *C. parapsilosis* exhibit a higher range of MIC values when compared with other *Candida* species [49].

Initiation of adaptive stress response is another mechanism of echinocandin resistance [50]. Studies report that when echinocandins inhibit β(1,3)D-glucan synthase enzyme, there is an increase in chitin synthesis in *Candida* species mediated by protein kinase C, high-osmolarity glycerol response and Ca^{2+} calcineurin signalling pathways [51,52]. Few studies have shown that few *C. albicans* strains can grow at supra-MIC concentrations of capsofungin by paradoxical effect [53-56]. These strains have high chitin levels at supra MIC concentration of capsofungin when compared to lower levels of capsofungin [54] [Table/Fig-3].



[Table/Fig-3]: Mechanism of resistance to Echinocandins.

Resistance to polyenes: The commonly used polyenes in treating Candida infections are amphotericin B, nystatin, etc. Though there has been only a minimal resistance to amphotericin B documented in the literature over the past few decades; however, it is a matter of great concern. The major problem with the usage of this drug

is the side effects and toxicity [57,58]. Resistance pattern differs with different species. Though, *C. krusei* and *C. glabrata* show a higher MIC to the polyenes than *C. albicans*, these species are often considered to be susceptible to amphotericin B.

Polyenes generally act by disrupting the fungal cytoplasmic membrane by interacting with ergosterol. Ergosterol is essential for maintaining the integrity of the fungal cell membrane and functioning of the membrane bound enzymes. The polyenes generate pores in the cell membrane through which potassium and magnesium ions of the cellular components escape and cause destruction of the proton gradient of the cell membrane finally leading to fungal cell death [57].

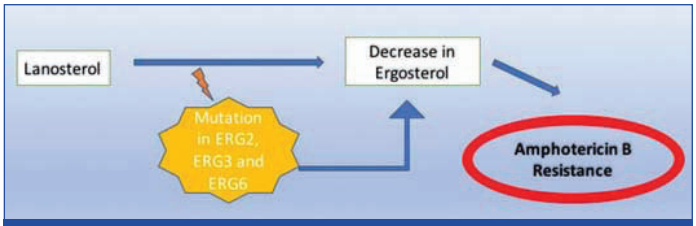
Polyenes have high affinity for ergosterol and low affinity for 3 hydroxy or oxosterols like fecosterol and episterol which is one of the important reasons in the emergence of resistance to polyenes drugs [59]. Studies reveal that polyenes favoured sterol namely ergosterol has been replaced with other biosynthetic precursors like lanosterol, episterol, fecosterol, lichesterol, etc. Though, this change may lead to overall increase in membrane sterol content but the availability of ergosterol to the polyenes may be affected significantly. This in turn may be one of the causes of development of resistance to the polyenes like amphotericin B.

Thus, the major causes of development of resistance to polyenes include inhibition of ergosterol synthesis leading to decrease in ergosterol levels, replacement of ergosterols with other biosynthetic precursors causing alteration in sterol content and change in the sterols phospholipids ratio [59,60]. Masking of the ergosterol in the cell membrane causing non-availability for binding with the polyenes is another mechanism of development of drug resistance [61]. In few resistant strains with no alteration in the membrane sterol content, the development of resistance is attributed to the change in the cell wall permeability to the polyenes. Increase in catalase activity of amphotericin B leading to decrease in the oxidative damage by the drug is also proposed to be another mechanism of drug resistance. Growth phase of the cell is also found to play a role in the development of resistance to polyenes. It is found that higher rate of breakdown and synthesis of cell wall occurs in the log phase leading to higher rate of access of amphotericin B to the cell membrane. However, there is lower rate of breakdown and synthesis of cell wall during the stationary phase of growth leading to the development of resistance to the polyenes.

It has been reported frequently that the polyene resistance is found to be higher in *Candida* non albicans species like *C. tropicalis, C. glabrata, C. lusitaniae, C. parapsilosis*, etc. *C. glabrata* is found to mutate frequently making it more likely to develop resistance to polyenes than the *C. albicans* [62].

Mutations in the genes encoding the enzymes involved in the synthesis of ergosterol may also lead to the development of resistance to the polyenes. C8 sterol isomerase and C5 sterol desaturase are the two important enzymes in the biosynthesis of ergosterol. C8 sterol isomerise is involved in the conversion of fecosterol into episterol. *ERG2* gene regulate the activity of this enzyme. C5 sterol desaturase catalyses the episterol conversion into ergosterol. *ERG3* genes encode this C5 sterol desaturase enzyme. Hence, any mutation or defect in the *ERG2* and *ERG3* gene may cause amphotericin B resistance. Clinical studies reported *C.albicans* resistant strains with *ERG2* and *ERG3* gene defects and reduced ergosterol content [59] [Table/Fig-4].

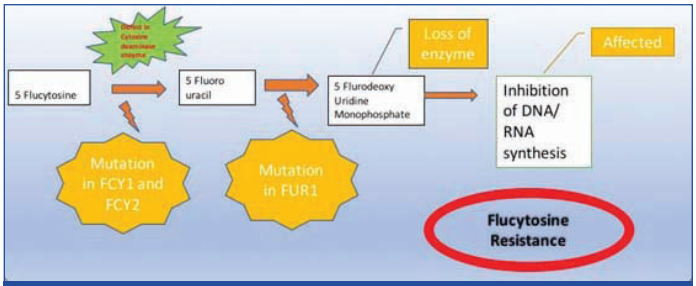
Resistance to flucytosine (5-flurocytosine): Resistance to 5-flurocytosine has been reported in around 10% of *C.albicans* cases. The drug flurocytosine acts by inhibition of fungal protein synthesis and nucleic acid synthesis. Fungal cytosine permease



[Table/Fig-4]: Showing mechanism of antifungal drug resistance to polyenes.

takes the drug inside the cell. Flurocytosine is first deaminated to 5 fluro uracil and then split into 5 flurodeoxyuradine monophosphate and 5 flurouridylic acid and then phosphorylates into 5 flurouracil triphosphate, which is catalysed by uracil phosphoribosyltransferase. DNA synthesis is inhibited by 5 flurodeoxyuridine monophosphate by inhibiting thymidine synthetase. Protein synthesis is inhibited by 5 flurouracil triphosphate by incorporating into RNA [59].

The loss of the enzyme uridine monophosphate pyrophosphorylase is found to be one of the important causes of resistance to 5 Fluorouracil [63]. It has also been suggested that defect in cytosine deaminase activity may lead to primary resistance and decrease in uracil phosphoribosyltransferase activity which may lead to secondary resistance [64]. In addition, any loss of permease activity may also cause resistance to 5 Fluorouracil [59] [Table/Fig-5].



[Table/Fig-5]: Mechanism of drug resistance to flucytosine.

Monotherapy may lead to development of resistance to 5 Fluorouracil. Interestingly, it has been found that when 5 Fluorouracil is given in combination with amphotericin B, the occurrence of drug resistance in *C.albicans* strains has been reduced. Acquired resistance may be due to failure in conversion of 5-fluorocytosine into 5-fluorouracil triphosphate and 5-flurodeoxyuridinemonophosphate or due to loss of feedback control of pyrimidine biosynthesis. Intrinsic resistance to 5 Fluorouracil may be due to deficiency of enzymes involved in the metabolism of the 5 Fluorouracil pathway.

An overview of various antifungal drugs along with their mechanism of action and drug resistance mechanisms are shown in [Table/Fig-6].

Antifungal resistance and its clinical impact: The resistance exhibited by the *Candida* species is found to be associated with rise in MICs leading to poor prognosis of the patients, high management costs, etc. It has been reported that the clinical outcome of patients infected with *Candida* species is significantly poorer in cases of resistant MICs for fluconazole and voriconazole when compared with the susceptible MICs [31]. Few case reports also describe the resistance to echinocandins is associated with high MICs and poor clinical outcomes [65-67]. Various studies have reported breakthrough infections in bone marrow and solid organ transplant patients on fluconazole prophylaxis and epifunginprophylaxis [68,69]. Invasive Candidiasis can also lead to prolonged hospital stay and high management costs due to difficulty in diagnosis and identification of resistant strains [70,71]. However, sparse data is available on the financial impact of resistant Candidal infections.

Antifungal class of drugs	Examples	Mode of action	Mechanism of drug resistance	Molecular basis of Resistance	Final change that leads to resistance	References
Azoles	Miconazole Clotrimazole Ketoconazole Fluconazole Itraconazole Voriconazole	Inhibitors of lanosterol 14-α-demethylase	Decreased binding affinity by the drug to lanosterol 14-α-demethylase Increase in the concentration levels of lanosterol 14-α-demethylase, Upregulation of drug transporters and alterations in ergosterol biosynthetic pathway	1.Modifications in the ERG11 gene by: a) Point mutations b) Up regulation	1.a) Reduced drug affinity for the target enzyme b) Overexpression Increased ergosterol synthesis	[35] [32,34]
				2. Alterations in ergosterol biosynthetic pathway	2. Production of various sterols supporting growth; cross-resistance to other azoles and AmB	[32,33]
				3. verexpresssion of CDRs and MDR genes encoding efflux pumps	3. Reduced drug accumulation in the cell	[30,39,41]
Echino-candins	Caspofungin Micafungin Anidulafungin	Inhibitors of (1,3)-α-D-glucan synthase	Decrease in the glycan synthesis processing	1. Point mutation in FKS subunits of enzyme β(1,3) D-glucan synthase	1. Decrease in susceptibility to echinocandins	[42]
				2. Mutation in hotspot region	2. Increase in Minimum inhibitory concentration (MIC), reduced β(1,3) D-glucan synthase sensitivity, and cross-resistance among the echinocandins.	[43-45]
Polyenes	Nystatin Amphotericin B	Bind to ergosterol	Decrease in the ergosterol content in the cell	1.inhibition of ergosterol synthesis	1. Decrease in ergosterol levels, replacement of ergosterols with other biosynthetic precursors	[59,60]
				2. Masking of the ergosterol in the cell membrane	2. Non availability for binding with the polyenes	[61]
				3.Point mutations in ERG3 and ERG6	3. Defect in enzymes involved in the synthesis of ergosterol	[59]
Nucleoside analogues	Flucytosine	Inhibitor of DNA/ RNA synthesis	loss of the enzyme uridine monophosphate pyropho--sphorylase, defect in cytosine deaminase activity, decrease in uracil phosphoribo-syltran-sferase activity, loss of permease activity	1.Point mutations in FCY1, FCY2, FUR1	1. Deficiency in the enzymes necessary for cellular transport and uptake of 5-FC or for its metabolism	[72]
				2. Increased synthesis of pyrimidines	2. It competes with the fluorinated antimetabolites of 5-FC and thus diminish its antimycotic activity.	[72]

[Table/Fig-6]: Mechanisms of antifungal drug resistance.

CONCLUSION

Infections caused by *Candida* species is one of the major clinical threats in immunocompromised patients. Although, many antifungal drugs are currently in clinical practice, further novel antifungal drugs and new drug targets are in need due to the fear of antifungal drug resistance and re-emergence of infections. It has become imperative to understand the molecular mechanisms of drug resistance to combat the multidrug resistant fungal infections. Recently, many strategies are being implemented for the prevention of emergence of drug resistance in Candidal infections. Aggressive surveillance of resistance and development of appropriate protocol and guidelines for antifungal drug therapy is the major need today. Antifungal susceptibility testing in all cases is recommended for effective management of Candidal infections. Furthermore molecular, genetic and biological research is necessary to understand the underlying molecular drug resistance mechanisms in antifungal therapy and to discover newer antifungal drugs with high efficacy. Also, there is a need for integration of different disciplines in establishing the management protocol for Candidal infections. Furthermore, exploiting the diagnostic methods including the molecular techniques facilitates effective clinical management to reduce the morbidity and mortality due to candidal infections.

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