Drug Resistance in Candida krusei: A Literature Review on Mechanisms and Pathways

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Candida krusei has been identified as a potential multi-drug resistant (MDR) pathogen. In vitro antifungal tests have shown a significant decrease in the susceptibility of *C. krusei* to fluconazole. *Candida krusei* commonly causes systemic infections. Effective systemic antifungal agents for invasive candidiasis are divided into 4 groups: the first group, Polyenes, includes amphotericin B (AmB) deoxycholate, liposomal AmB, AmB lipid complex (ABLC), and AmB colloidal dispersion (ABCD). The second group, Triazoles, includes fluconazole, itraconazole, voriconazole, and posaconazol. This group is the most commonly used for the treatment of candidiasis worldwide. Due to its widespread use, it has led to antifungal resistance, especially in certain types of Candida, such as *C. krusei*. The third group, Echinocandins, includes caspofungin, anidulafungin, and micafungin. Currently, this classification of antifungals is widely used for the treatment of Candida and is effective for certain types of Candida. The fourth group used is Flucytosine.[1]

Polyenes and azoles (imidazoles and triazoles) are the most commonly used antifungal agents. Fluconazole, one of the most widely used triazoles for antifungals, is a low molecular weight triazole that is highly active against several pathogens that cause systemic mycosis. Fluconazole has unique pharmacokinetics, high water solubility, weak protein binding, high cerebrospinal fluid penetration, and is well absorbed after oral administration. Fluconazole belongs to the azole drug class, where its target is the cytochrome P450-dependent lanosterol 14α demethylase Erg11p enzyme, which is encoded by the ERG11 gene. This enzyme catalyzes the sterol 14α demethylase reaction in ergosterol biosynthesis. [2]

Ergosterol is one of the main sterol compounds in yeasts and molds. This cellular membrane component is necessary for cell proliferation. Azole drugs bind to the active site of the protein via heme, specifically the iron atom of the heme group, which interferes with the activation of oxygen atoms, resulting in the demethylation of lanosterol. The fungistatic effect of azoles is not due to disruption of the ergosterol biosynthesis pathway, but rather due to the production and accumulation of toxic 14-methylated compounds in yeast cells, such as 14-methylergosta-8,24(28)-dien-3,6-diol, eburicol, lanosterol, obtusifoliol, 14a-methyl-fecosterolobtusifoliol, or 14a-methyl fecosterol. The accumulation of toxic compounds, together with the deficiency of ergosterol, alters the function and fluidity of the plasma membrane.[3]

Fluconazole is more fungistatic than fungicidal, so treatment with fluconazole provides more opportunities for the development of resistance. [2] Fluconazole is the first-line antifungal drug for the treatment of stable clinical patients and for empirical treatment of suspected disseminated candidiasis in non-neutropenic patients in countries where the resistance level of species such as *C. krusei* and *C. glabrata* is below 10-15%.[3]

The mechanisms of resistance to antifungal drugs vary among different fungal species. Some mechanisms of fungal resistance include: firstly, mutations or overexpression of the ERG11 gene encoding the drug target enzyme lanosterol 14α-demethylase- Erg11p. Erg11p is a

cytochrome P450 enzyme belonging to the CYP51 family. This enzyme converts lanosterol to ergosterol. Inhibition of Erg11p causes the accumulation of 14α-methyl sterol, which interferes with ergosterol biosynthesis and causes damage to the integrity of the cell membrane.[4]

In addition to mutations in the Erg11p gene, mutations in genes encoding other enzymes in the ergosterol biosynthesis pathway, such as the ERG3 gene, can also trigger resistance. Mutations in the FKS1/2 genes can also induce resistance. Other mechanisms include compensating for the accumulation of toxic ergosterol in fungal cells. Then, by overexpressing efflux pumps such as ATP Binding Cassette (ABC) and Major Facilitator Superfamily (MFS) in transporter proteins. In addition to mutations and molecular pumps, the emergence of complex multicellular structures such as biofilms can increase or induce resistance to antifungals.[3]

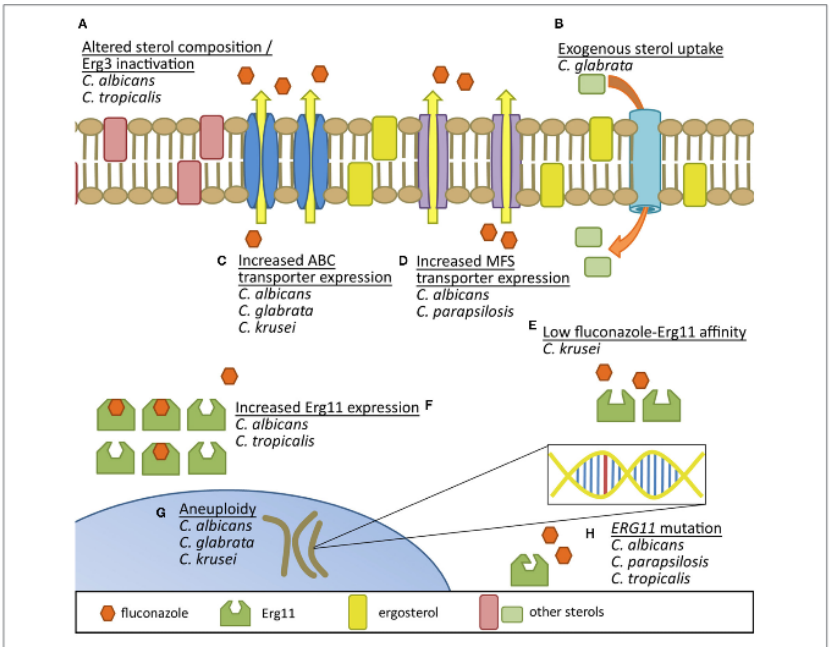

Figure 1. Drug resistant mecanism of Candida spp.[5]

Candida krusei has intrinsic resistance to fluconazole, which is related to efflux pumps mediated by ABC proteins and the low affinity of the target enzyme Erg11p for fluconazole.[6] This difference in resistance mechanisms distinguishes it from other Candida species, which are mostly caused by mutations in the ERG11 gene. This was confirmed by a study conducted by Alison et al., which found that in the *C. krusei* isolates studied, fluconazole resistance was largely the result of a decrease in the resistance of 14α-demethylase to the inhibitory effects of fluconazole.[7]

The mechanism of fluconazole resistance in *C. krusei* is due to the presence of efflux pumps mediated by ABC transporters. There are two types of these transporters, namely ABC proteins encoded by the Candida Drug Resistant (CDR) gene, which use ATP as an energy source to expel fluconazole from the cell, and MFS proteins encoded by the Multidrug Resistant (MDR1) gene, which use a proton gradient to cross the cell membrane. In *C. krusei*, ABC proteins are used. ABC proteins are composed of two Trans-Membrane Domains (TMD) and two Nucleotide Binding Domains (NBD). The TMD protein forms the substrate binding site but is unable to transport the

substrate across the phospholipid bilayer membrane. This transport requires energy from ATP hydrolysis, which is carried out in the NBD located on the cytoplasmic side. Therefore, both ABC proteins will work together to expel fluconazole from the *C. krusei* cell. The expulsion of fluconazole by this fungus is very rapid, within 90 minutes it can expel 60% of fluconazole that enters the fungal cell, so that fluconazole is unable to work.[3]

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