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Perspective Chapter: Antifungal Drug Resistance in *Candida* Species

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Abstract

*Candida* species, members of the normal body flora, are opportunistic mycosis agents that can cause infections associated with high morbidity and mortality rates in the presence of underlying predisposing factors. In recent studies, it has been reported that the incidence of invasive *Candida* infections caused by *Candida* species, such as non-albicans *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, and *Candida auris*, in which antifungal drug resistance is more common, has increased, in addition to *Candida albicans*, the most frequently detected *Candida* species. In this context, the objective of this review article is to discuss the molecular mechanisms and biofilm-related factors responsible for the antifungal drug resistance developed in *Candida* species.

**Keywords:** *Candida* spp., antifungal drug resistance, azoles, echinocandins, amphotericin B, biofilm

1. Introduction

*Candida* species found in the normal flora of the skin, mouth, and gastrointestinal tract are opportunistic mycosis agents that can cause life-threatening deep invasive infections in addition to superficial mucosal infections, especially in patients in the risk group, in the presence of facilitating factors, such as any condition where skin integrity is impaired, prolonged hospitalization, immunosuppression, surgeries, and widespread use of antimicrobial drugs and corticosteroids [1, 2].

The epidemiology of invasive *Candida* infections associated with high morbidity and mortality rates vary depending on the development of a resistance caused by the selective pressure resulting from the widespread use of antifungal drugs for prophylaxis and empirical treatment in patient groups at-risk [3]. The current understanding of the varying epidemiology and the related resistance profiles has been significantly enhanced by published data from various sentinel and population-based surveillance studies [4]. Although *C. albicans* continued to be the most frequently isolated *Candida* species in these studies, *Candida* species other than *albicans* resistant to antifungal therapy were also identified with increasing frequency, and the incidence of other *Candida* species varied from one center to another or according to the geographical region [5, 6].
Candida and Candidiasis

The results of the SENTRY study where antifungal resistance was investigated in Candida species isolated from various geographical regions indicated that the Candida parapsilosis, isolated from European and Latin American countries, had higher fluconazole resistance compared to the C. parapsilosis, isolated from Asia-Pacific countries (4.6, 4.3, and 0.6%, respectively). Another common Candida species, C. tropicalis, isolated from Asia-Pacific countries, was reported to have higher resistance to fluconazole compared to C. tropicalis isolated from other countries (9.2 and 1.1–2.9%, respectively [7–9]. Among other Candida species, C. glabrata reportedly had 10.2% resistance to fluconazole and 0–10% resistance against echinocandins in the United States. Varying rates have been reported for C. glabrata’s resistance against echinocandins in other parts of the world. In addition, it was reported that C. glabrata's resistance to echinocandin was frequently accompanied by its resistance to azoles and that this might result in an increase in the number of multi-drug resistant isolates [10, 11].

Candida auris, which is increasingly becoming a concern on a global scale, has become a pathogen of emphasis because of its multiple resistance to antifungal drugs, its long-term survival in the hospital environment, and its potential to cause epidemics [5]. The most important feature of Corynebacterium auris, in addition to its resistance to fluconazole (70%) and echinocandin (5%), is its resistance to amphotericin B (23%), which is interestingly not observed in other Candida species. Studies have reported resistance to two antifungal drug classes in 20% of C. auris isolates, as well as pan-resistant isolates with high minimum inhibitory concentration (MIC) values for all existing antifungal drug classes [10].

The progressive increase of Candida species other than albicans resistant to antifungals at the global level has increased the importance of identification of Candida species at the level of subspecies. Although fluconazole is still a widely preferred choice of treatment all over the world, both intrinsic and acquired resistance against fluconazole is increasing. The resistance to echinocandins currently remains low but may increase with their increased use. Therefore, improvement of diagnostic methods, development of international surveillance networks, and implementation of antifungal management programs are required for better epidemiological control of invasive Candida infections [6].

In this context, molecular mechanisms and biofilm-related factors responsible for resistance to antifungal drugs in Candida species are discussed in this review article.

2. Antifungal drugs

The emergence of acquired drug resistance in common Candida species limits the treatment options for these species. Despite the ongoing need for more antifungal treatment options, the number of antifungals used in treatment remains limited [3, 12]. There are three main antifungal drug categories: azoles (fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole, etc.), echinocandins (caspofungin, micafungin and anidulafungin), and the amphotericin B (AMB), which is included in the polyene group [1].

Azoles bind to 14-α-demethylase, which is one of the critical enzymes (Erg11p) during ergosterol biosynthesis, leading to the disruption of fungal ergosterol synthesis and accumulation of toxic sterols. Echinocandins act by blocking the catalytic subunit of the glucan synthase enzyme encoded by the FKS gene, thereby inhibiting the biosynthesis of β-1,3-D-glucan, the primary cell wall polymer. Polyenes, on the
Other hand, bind to ergosterol, leading to the formation of pores in the cell membrane, disrupting the osmotic balance and ultimately the death of the fungal cell [1]. Fluconazole, which is included in the azole group, is often the drug of choice for the treatment of most Candida infections since it is inexpensive, has limited toxicity, and can be easily administered orally. However, in addition to the increase in the patient population at risk, the increase in Candida species with intrinsic or acquired resistance to antifungal drugs such as azoles caused by the selective pressure due to the use of antifungal drugs for prophylaxis or empirical treatment is increasingly becoming a concern [3, 13].

Given the limited number of antifungals used today, several clinical studies are underway for the development of antifungals. Some of these studies feature promising drugs, such as fosmanogepix (a novel Gwt1 enzyme inhibitor), ibrexafungerp (a first-in-class triterpenoid), olorofime (a novel dihydroorotate dehydrogenase enzyme inhibitor), opelconazole (a novel triazole optimized for inhalation), and rezafungin (an echinocandin designed to be dosed once weekly) are currently in the final phase [3].

3. Antifungal drug resistance

Antifungal drug resistance refers to stable genetic changes that increase the probability of failure in a treatment applied against a fungal pathogen included in a particular class of antifungal drugs [2]. In addition to several clinical factors pertaining to the host, the mechanisms of action of antifungals, the acquired resistance related to the mutations observed in Candida, and features such as biofilm structure are among the reasons for the failure of antifungal treatment [2, 14].

Generally speaking, resistance mechanisms cannot be transferred between Candida species. Thus, acquired resistance arises either in response to antifungal selection pressure in the individual patient or, rarely, due to horizontal transmission of resistant strains among patients [15]. The recent increase in the acquired resistance to the echinocandin group of antifungal drugs has been observed primarily in C. glabrata. Most patients who developed resistance to C. glabrata received 3–4 weeks of treatment containing the echinocandin group of antifungal drugs. However, the fact that there were also cases where resistant mutants have been reported in patients who received short-term treatments and even in patients who stayed in clinical services featuring resistant isolates even though they did not receive echinocandin suggests the potential for transfer among hospitalized patients also in this drug class [15]. Resistance to more than one antifungal drug is still not common; however, C. auris cases with multi-drug resistance have been increasingly reported [1, 15].

4. Detection of antifungal drug resistance

4.1 Phenotypic methods

In vitro antifungal susceptibility testing (AFST) is a tool commonly used to detect antifungal drug resistance or the possibility of failure of antifungal therapy. AFST measures the ability of a particular organism to grow in vitro in the presence of a particular drug. This measured growth indicates the minimal inhibitory
Concentration (MIC), that is, the lowest drug concentration that completely stops or significantly reduces fungal growth. Antifungal drug resistance is quantitatively determined phenotypically by determining the MIC value \[14, 16\]. To this end, broth microdilution (BMD) based reference methods that have been standardized for AFST by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST), are used \[14\].

Standardization of the tests enabled the determination of clinical breakpoints (CBP) and epidemiological cut-off values (ECV) for azoles, echinocandin, and AMB against Candida spp. CBPs are based on a combination of pharmacokinetic/pharmacodynamic (PK/PD) parameters and clinical outcome data, that is, the MIC value that predicts an organism's in vivo response. On the other hand, ECVs are based on the MIC value that distinguishes resistant and non-resilient wild strains. However, the clinical response cannot be reliably detected using the ECV \[14, 17\].

Today, in addition to the reference methods, commercial tests (E-test (Biomerieux)), automated test platforms (Vitek-2 [Biomerieux] bioMerieux, Inc., Marcy l’ Etoile, France), and YeastOne Sensititre (TREK Diagnostic Systems, Inc., Cleveland, OH) are used for AFST. On the other hand, the studies for the standardization of these tests are in progress \[4, 5, 14, 18\].

### 4.2 Molecular methods

Phenotypic AFST has some major limitations. Therefore, other methods have been developed for the molecular detection of resistance-related genetic mutations independent of culture for the isolation of Candida \[5\].

Molecular detection of resistance relies on DNA technologies used in the detection of relevant mutations in genes associated with drug resistance, including methods, such as Sanger sequencing, pyrosequencing, real-time PCR, Luminex technology, and next-generation sequencing (NGS). Among these methods, NGS has the ability to detect novel mutations that play a role in the phenotypic resistance of clinical isolates. However, these methods have limited use in the direct detection of resistance. Although there are specific tests for the direct detection of FKS and ERG11 mutations, the interpretation of the test results is challenging as the effect on susceptibility depends on codon, amino acid change, and species. For instance,azole resistance rarely develops due to ERG11 mutations alone. In fact, these mutations further complicate the molecular detection of resistance due to the multiple underlying mechanisms. Nevertheless, molecular tests are more successful in detecting the FKS mutants. It is envisaged that the aforementioned problems can be overcome with the development of new techniques in the near future \[5, 11, 14, 19\].

### 5. Antifungal resistance mechanisms

Candida species generally develop antifungal resistance by changing the density and structure of antifungal target proteins and the sterol composition in the cell membrane or releasing efflux pumps that reduce the accumulation of drugs into the cell. Another mechanism that contributes to the development of resistance is biofilm formation \[12, 16\].
5.1 Resistance mechanisms to azole group of antifungal drugs

Ergosterol, which makes up most of the sterols in the fungal cell membrane, is formed by the conversion of lanosterol to ergosterol by the enzyme lanosterol 14-alpha-demethylase, which is encoded by the \textit{ERG11} gene in \textit{Candida} species [9]. Determination of the molecular resistance mechanisms of \textit{Candida} species to azoles poses a challenge, given the fact that the resistance mechanisms, especially in the case of resistance to azoles such as fluconazole, vary according to the species, and depend on a combination of several factors [10].

Among the molecular resistance mechanisms developed against the azole group of antifungal drugs are alteration or overproduction of lanosterol 14-alpha-demethylase, which is involved in the synthesis of ergosterol and is the target of the antifungal drug and mechanisms that ensure the excretion of the antifungal drug out of the cell [3].

5.1.1 Point mutations in the \textit{ERG11} gene

Mutations leading to amino acid changes in the hotspot (HS) region of the \textit{ERG11} gene can cause azole resistance by causing changes in the structure of the target protein and a decrease in the binding affinity of the drug [20].

Most of the amino acid changes that occur in the \textit{ERG11} gene occur in the three HS regions of the protein, especially between amino acid sequences 105–165, 266–287, and 405–488, rather than being distributed throughout the coding region [19].

Mutation of Y132F either alone or in combination with R398I has been reported in fluconazole-resistant \textit{C. parapsilosis}. Additionally, it was reported that \textit{C. parapsilosis} isolates carrying Y132F correlated with azole resistance and high mortality. In another study, azole-resistant \textit{C. parapsilosis} isolates carrying Y132F were detected in environmental sources, including the hands of healthcare workers or the devices used by the healthcare workers in the clinic, even though none of the healthcare workers in question had received azole treatment [3]. Fluconazole resistance with Y132F mutation was also detected in a small number of \textit{C. tropicalis} and \textit{C. glabrata}, among other species. Not much is known about the contribution of \textit{ERG11} point mutations to fluconazole resistance in \textit{C. auris}, yet, as in other \textit{Candida} species, mutations have been identified in Y132F, K143R, and F126T. The results of the recently held studies indicated that the list of \textit{ERG11} mutations related to azole resistance is expanding [3, 9].

In another resistance mechanism, the defect in the \textit{ERG3} gene, which is responsible for the production of the other enzyme D5,6-sterol desaturase involved in the ergosterol synthesis pathway, leads to the synthesis of alternative toxic sterols with low affinity for azole group of antifungal drugs instead of ergosterol, which, in turn, gives rise to azole resistance [9]. This resistance mechanism has been detected in \textit{C. albicans} and \textit{C. tropicalis} [9, 14]. The \textit{ERG11} mutations, which are reportedly responsible for intrinsic fluconazole resistance in \textit{Candida krusei}, have also been reported in \textit{Candida} spp. including \textit{C. albicans}, \textit{C. parapsilosis}, and \textit{C. tropicalis} [14].

On the other hand, chemical diversity in a core unit structure within the azole family facilitates the development of cross-resistance. For example, some mutations in \textit{ERG11} result in fluconazole resistance only, while others also show resistance to voriconazole. Panresistance may also be detected in some isolates [12].

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5.1.2 Overexpression of the ERG11 gene

Often the level of overexpression is minimal or can be observed with other resistance mutations. Studies have shown that overexpression often involves Upc2p, a zinc cluster transcription factor induced upon depletion of ergosterol. Mutations in Upc2p result in gain-of-function (GOF) for this regulator, resulting in constitutive transcriptional activity and increased Erg11p production [3]. In addition to managing the regulation of many other genes (not only ERG11) involved in the ergosterol biosynthesis pathway, Upc2p appears to also play an essential role in the response given to azoles [19].

However, overexpression of ERG11 is not always observed together with UPC2 mutation. This resistance mechanism has been described especially in C. albicans and less frequently in C. parapsilosis and C. tropicalis. The role of ERG11 overexpression in fluconazole resistance in C. glabrata, C. krusei, and C. auris has yet to be elucidated [2, 9].

5.1.3 Overexpression of membrane transportes

The efflux pumps are the proteins responsible for the excretion of exogenous or endogenous substances out of the cell by transporting them across the cell membrane. Accordingly, the efflux pumps throw drugs out of the cell, reducing their intracellular concentrations and thus their effects on the cell. There are two types of efflux pumps associated with drug resistance: ATP binding cassette (ABC) transporters (CDR1 and CDR2) and major facilitator superfamily (MFS) transporters (MDR1 and MDR2). Efflux pumps are responsible for the active excretion of toxic molecules such as theazole group of antifungal drugs. Overexpression of genes encoding efflux pumps (CDR and MDR) is one of the most important resistance mechanisms against the azole group of antifungal drugs in Candida species [2, 21].

The ABC and MFS transporters in pathogenic yeasts are mainly overexpressed by GOF mutations in TAC1 and MRR1, respectively. It is noteworthy that these transcription factors play a role in virulence as well as drug resistance [3]. The increased expression of the CDR and MDR genes encoding ABC transporters in C. albicans (MDR1, CDR1, CDR2), C. glabrata (CgCDR1, CgCDR2), and C. krusei is associated with a broad spectrum of antifungal resistance, whereas the increased expression of MDR genes encoding MFS has been described in C. albicans and C. parapsilosis [4, 9, 14]. Stimulation of the efflux pumps encoded by the CDR gene generally tends to affect all drugs in theazole group and is sufficient for the development of resistance in certain strains. On the other hand, the efflux pumps encoded by MDR genes are generally selective for fluconazole [4].

5.2 Resistance mechanisms to echinocandin group of antifungal drugs

Echinocandins act by inhibiting the two catalytic subunits of the BDG synthase enzyme complex encoded by the FKS1 and FKS2 genes [14]. Although in vitro fungicidal is still effective against most Candida species, the prevalence of intrinsic or acquired resistance to echinocandins is increasing [19].

Studies have identified several mutations associated with echinocandin resistance in the HS1 and HS2 regions of FKS1 and FKS2 in C. albicans and other species [11]. In particular, amino acid changes at positions Phe641 and Ser645 in FKS1 are the most prominent mutations associated with clinical failure in C. albicans. The most
common cause of echinocandin resistance in *C. glabrata* is the mutations in FKS1’s HSP1 (Phe625, Ser629) and FKS2 (Phe659, Ser663). *C. parapsilosis* and *Candida guillermondii* naturally contain mutations in FKS1 that are responsible for their reduced susceptibility to echinocandins; nevertheless, the clinical impact of this mutation has yet to be determined [5, 14].

In recent studies, in addition to stating that the most appropriate way to determine the echinocandin resistance mechanisms is the sequence analysis of the HS region, the importance of whole-genome analysis of the *FKS* gene has also been emphasized, given that mutations have been identified in regions other than HS [1]. Additionally, it has been stated that the change in the lipid content of the microenvironment surrounding the *FKS* gene may play a role in echinocandin resistance [3].

The fact that echinocandin-resistant isolates, especially *C. glabrata*, are also often resistant to fluconazole presents a severe clinical picture [22]. Acquired mutations in *FKS1* occur in *C.albicans*, *C.tropicalis*, *C.krusei*, and *C.glabrata* after long-term drug exposure. However, acquired resistance mutations in *FKS2* have only been observed in *C. glabrata* so far [14]. Echinocandin resistance can be altered by the expression of the *FKS* genes. FKS2 expression in *C. glabrata* is calcineurin-dependent; hence, FKS2-dependent resistance may be abolished after treatment with the calcineurin inhibitor FK506 (tacrolimus) [12].

### 5.3 Resistance mechanisms to polyene group of antifungal drugs

Polyenes are a group of antifungal drugs that target ergosterol-containing membranes and bind to sterols in the cell membrane, forming channels, and thereby disrupting the integrity of the membrane [19].

AMB is fungicidal, and resistance to AMB is usually observed intrinsically. Acquired resistance in susceptible species is rare [18]. The mechanism deemed to be responsible for AMB resistance in *Candida* species involves the mutations in the ERG2, ERG3, ERG5, ERG6, and ERG11 genes that encode the enzymes in the ergosterol synthesis pathway, leading to a decrease in the synthesis of ergosterol, the target of the drug [15].

Some strains of the *Candida lusitaniae* and *Candida haemulonii* complex show intrinsic resistance to AMB. Decreased polyene susceptibility has been reported in *C. albicans* isolates, in which ERG3, ERG11, and ERG5 mutations were detected along with changes in the ergosterol pathway involving ERG2. Additionally, in *C. glabrata*, mutations in ERG2 and ERG6 have been implicated in reduced susceptibility. Furthermore, it has been reported that all these changes, except for ERG6 mutation, may also cause the development of cross-resistance to azoles [19].

### 5.4 Multi-drug resistance and related resistance mechanisms

Although intrinsic multi-drug resistance (MDR) is rare among *Candida* species, *C. auris* isolates, which are resistant to all three antifungal drug classes consisting of fluconazole, AMB, and echinocandins, have begun to be detected. Heteroresistance may also develop against fluconazole, along with increased resistance to echinocandin, especially in *C. glabrata*. In addition, echinocandin resistance, albeit rarely, may occur in *C. krusei*. The resistance may occur sporadically in some species. On the other hand, cross-resistance to azoles and AMB may develop in relation to the specific mutations in the ergosterol biosynthetic pathway [19].
The resistance to azole usually develops over time depending on more than one mechanism, including the ERG11 or MDR1 upregulation with combinations of CDR1/CDR2 upregulation and ERG11 modifications as the most common mechanisms. Although CDR1/CDR2 and MDR1 upregulation can be explained by TAC1 and MRR1 GOF mutations, ERG11 upregulation is not always associated with UPC2 GOF mutations and requires additional regulatory factors. The gradual introduction of point mutations in ERG11, MRR1, TAC1, and UPC2 has been shown in vitro to induce the development of resistance in drug-susceptible C. albicans isolates. In contrast to the case in C. albicans, where the resistance develops based on the gradual increases in the mutations caused by multiple mechanisms, the resistance in C. glabrata may usually develops via GOF mutations in CgPDR1 in a single step [9, 12, 15].

The mechanisms of action of antifungal drugs used in the treatment of Candida species and the mechanisms of antifungal resistance are given in Table 1 [1, 23, 24].

### 5.5 Biofilm and antifungal resistance

The most fundamental features of Candida are their ability to form biofilms, which develop a high tolerance to antifungal drugs [25]. Biofilms are complex three-dimensional structures consisting of a central cluster of microbial cells attached to host tissue or abiotic surfaces and embedded in an extracellular polysaccharide substance (EPS) that protects microorganisms [23]. Biofilm structures are a dynamic cluster of multiple cell types, the formation of which is regulated by a transcriptional regulatory network [25].

Biofilm development progresses through four main phases over a 24- to 48-hour period: adherence, initiation, maturation, and dispersal [25, 26]. Accordingly, the adherence of the yeast cell to the surface (adherence phase) is followed by the cell proliferation phase (initiation phase), which is accompanied by hyphal growth. Subsequently, the maturation of the biofilm structure (maturation phase) begins with the assembly of hyphae and the aggregation of the extracellular matrix (ECM). Finally, yeast cells detached from the upper parts of the biofilm layer are dispersed to the environment in order to initiate the same process in other foci (dispersal phase) [23, 26].

#### 5.5.1 Adhesion phase

During the adhesion phase, yeast cells adhere to a surface and form a basal layer that will anchor the biofilm to the surface. Adhesins specific to C. albicans’ hyphae structure, such as ALS3 and hyphal wall protein (HWP1), play a role during this adhesion process. The presence of genes responsible for adhesion, regulated by the transcription factor BCR1, is essential for adhesion during biofilm formation [23, 25].

#### 5.5.2 Initiation phase

The adhesion phase is followed by the initiation phase, which is characterized by the onset of hyphae formation and leads to the formation of a hyphae network that will contribute to the overall strength of the biofilm. This phase is critical for the healthy development of the biofilm. At this phase, virulence factors specific to the cell type and transcriptional regulators play a role [23, 25].
5.5.3 Maturation phase

The next phase is maturation. Hyphal yeast cells produce exo-polymeric substances (EPS), which virtually act as adhesives. A mature *C. albicans* biofilm is preserved within an ECM structure composed of glycoproteins (55%), carbohydrates (25%), lipids (15%), and nucleic acids (5%). Although the macromolecule structures of the polysaccharide components of *Candida* biofilms are similar to those of cell walls, there are significant differences between the cell wall and the ECM. It has been reported that the presence of \( \beta-1,3 \) glucan, \( \beta-1,6 \) glucan, and \( \alpha-1,2 \) branched \( \alpha-1,6 \) mannan in the ECM structure contributes to the antifungal resistance of *Candida* biofilms, particularly against fluconazole. In addition, excess polysaccharide content in ECM was found to protect *Candida* biofilms against disinfectants and oxidative stressors [25, 27].

5.5.4 Dispersal phase

The final phase is characterized by the dispersal of the mature forms of yeast cells and/or biofilm fragments. In this way, biofilm formation occurs in different regions, and the infection becomes systemic [23, 25]. The ability of biofilm-associated yeast cells to evade the immune system contributes to the success of infection and dissemination of *Candida* biofilms. The ability of biofilm-associated yeast cells to evade the immune system contributes to the success of infection and dissemination of *Candida* biofilms.
Candida and Candidiasis

cells to disperse and thereby initiate new biofilm formation is of clinical significance in terms of giving rise to invasive diseases and candidemia [26]. Various components, such as transcriptional regulators, cell wall proteins, and chaperones play an important role in this phase [23, 25]. The steps of biofilm formation of C. albicans are shown in Figure 1 [28].

C. albicans dispersed cells manifest a different development process compared to biofilms and planktonic phases. The majority of the persistent cells are the lateral yeast cells originating from the hyphal layers of the biofilm. It has been reported that persistent yeast cells originating from biofilms adhere better to host cells, are more resistant to azoles, and have higher virulence characteristics compared to free-floating planktonic C. albicans yeast cells [29].

Despite their fundamental similarities, bacterial and fungal biofilms differ in structural and developmental aspects. Dispersal from the bacterial biofilms occurs predominantly at the end of the biofilm life cycle. On the other hand, dispersal from the fungal biofilms featuring C. albicans, subject to most biofilm studies, occurs not only at the final stage, but the cell release, which mostly involves unbudded yeast cells, also occurs throughout the growth cycle [30, 31].

Studies investigating environmental signals regulating the dispersion from fungal biofilms are still in their infancy and have particular aspects, which differ from the studies that investigate the dispersion from bacterial biofilms. Dispersal from bacterial biofilms is triggered by factors, such as nutrition, carbon limitation, hypoxia, low nitric oxide (NO) levels, and a decrease in cellular bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) levels [30, 31].

Figure 1.
Steps of biofilm formation in C. albicans. (a) Adhesion step: Adhesion of C. albicans yeast, (b) Initiation step: Hyphae formation and production of the extracellular matrix, (c) Maturation step: Maturation, and (d) Dispersal step: Dispersal of cells from the mature biofilm.
In comparison, dispersal from the fungal biofilms featuring *C. albicans* is triggered by a carbon source such as glucose but restricted by other sources, such as maltose, galactose, and phosphate-buffered saline (PBS). In addition, it has been demonstrated that the pH of the growing medium also affects the dispersion. Accordingly, dispersion is increased in acidic and decreased in alkaline conditions [30, 32].

Dispersion from fungal biofilms depends on the balance between yeast and the hyphae community. PES1 (Pescadillo ribosomal biogenesis factor-1), which controls the production of lateral yeast from hyphal filaments as key regulators of dispersion, and NRG1 (Neuregulin-1), a negative regulator of filamentation, have been reported to play a role in this balance [29]. In addition, it was stated in another study that the presence of histone deacetylase, which enables proper biofilm formation and multifactorial drug resistance development in *C. albicans*, also plays a regulatory role in dispersion [30].

Dispersin B, which is among the matrix-degrading enzymes in bacteria, and DNase I (Deoxyribonuclease I), another enzyme, have attracted attention as an antibiofilm and pro-dispersal agent. In fungal biofilms, a complex hyphae structure and the presence of abundant EPS (extracellular polymeric substances) prevent fragmentation. Although none of the well-known dispersins has been identified in fungal biofilms, DNase has been found to cause degradation by acting on the biofilm matrix in the treatment of *C. albicans* biofilms [30, 31].

The aim of the biofilm dispersion is to prevent biofilm-induced infections and to develop new treatment approaches [31]. The key step in the fight against microorganisms in the biofilm is the disintegration and dissolution of the biofilm structure or its conversion into a planktonic cell form with no antibiotic resistance properties [33].

Biofilm formation is a complex and multi-phase process controlled by a wide variety of transcriptional regulators (TR). TRs play a key role in the microbial response given to environmental stimuli and regulate the cellular development and routine biological functions of the cells. Studies have shown that a network of nine basic TRs (BCR1, EFG1, NDT80, ROB1, TEC1, BRG1, FLO8, GAL4, and RFX2) is required for biofilm formation both in vitro and in vivo [23, 25].

Cells aggregated within biofilm clusters induce the host’s immune response and the development of resistance to antifungals. Candidiasis, which features a versatile interaction with the host, often involves the formation of surface-associated biofilms. Compared to planktonic cells, *Candida* biofilms resist phagocytosis by neutrophils, monocytes, and macrophages. In addition, biofilm formation also alters mononuclear cell cytokine profiles, which affects immunity. Biofilms modulate immunity throughout various developmental stages. During the formation of mature biofilm, the ECM contributes to the resistance against the host defense. It has been reported that yeast-like cells dispersed from mature biofilms, compared to standard planktonic cells, are more virulent and adhere better to surfaces, forming new biofilms [23, 27, 34].

The effect of biofilm formation on the development of antifungal resistance is multifactorial. Factors, such as the increase in the density of cells and cell membrane sterols, the presence of a complicated extracellular matrix, and the expression of antifungal resistance genes may lead to the development of antifungal resistance [26].

*C. albicans*, most commonly isolated from clinical specimens, has been used as a model to study fungal biofilms. In addition to *C. albicans*, the biofilm-forming properties of other species, including *C. tropicalis*, *Candida parapsilosis*, and *C. glabrata*, have gained prominence. Accordingly, it has been reported that
Corynebacterium auris, which has emerged recently and attracted attention with its multi-drug resistance, easily forms biofilms on artificial materials and its frequency of isolation as an infection factor increases in patients using medical devices [27]. Candida species differ significantly in terms of biofilm formation and structure, ECM variations, and antifungal resistance [26].

Candida spp., along with other Candida species and bacteria, can form polymicrobial biofilms at many sites of infection. In such environments, ECM produced by one of the organisms may contribute to the collective preservation of other organisms within the biofilm. For example, polymicrobial biofilms formed by C. Albicans and Staphylococcus aureus exhibit increased antibiotic resistance compared to monomicrobial Staphylococcus biofilms. The coexistence of C. albicans and other Candida species can be observed in oropharyngeal candidiasis in particular [27, 34].

Biofilms formed by Candida species cause chronic or recurrent infections. These biofilms are also tolerant and/or resistant to different antifungal compounds in addition to the innate immune system. This can be explained by several factors, such as increased metabolic activity in the early stages of biofilm development, the presence of ECM, activation of efflux pumps responsible for azole resistance, and changes in gene expression, including overexpression of drug targets [23, 25, 27]. Further clarification of these processes can be beneficial in the development of new strategies to combat biofilm-derived infections [26, 27].

6. Conclusion

In conclusion, the incidence of invasive Candida infections that are resistant to antifungal drugs, which are associated with high mortality and morbidity, is increasing. Resistance to antifungal drugs may develop due to widespread antifungal use and other factors as well as predisposing factors pertaining to the patients. Therefore, it is crucial to identify the causative Candida species at an early stage and to analyze the antifungal susceptibility profiles to determine the epidemiology of resistance. In this way, the management of appropriate antifungal use would be possible, and only then the unnecessary use of antifungals can be reduced, and the drug-related undesirable side effects and the development of multi-drug resistance can be prevented. In addition, understanding the mechanisms of antifungal resistance will contribute to the development of molecular methods for rapid detection of antifungal resistance and the development of new fungi-specific antifungal drugs.
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