

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Molecular Mechanisms of Resistance to Antifungals in *Candida albicans*

*Estela Ruiz-Baca, Rosa Isela Arredondo-Sánchez, Karina Corral-Pérez, Angélica López-Rodríguez, Iván Meneses-Morales, Víctor M. Ayala-García and Ana Lilia Martínez-Rocha*

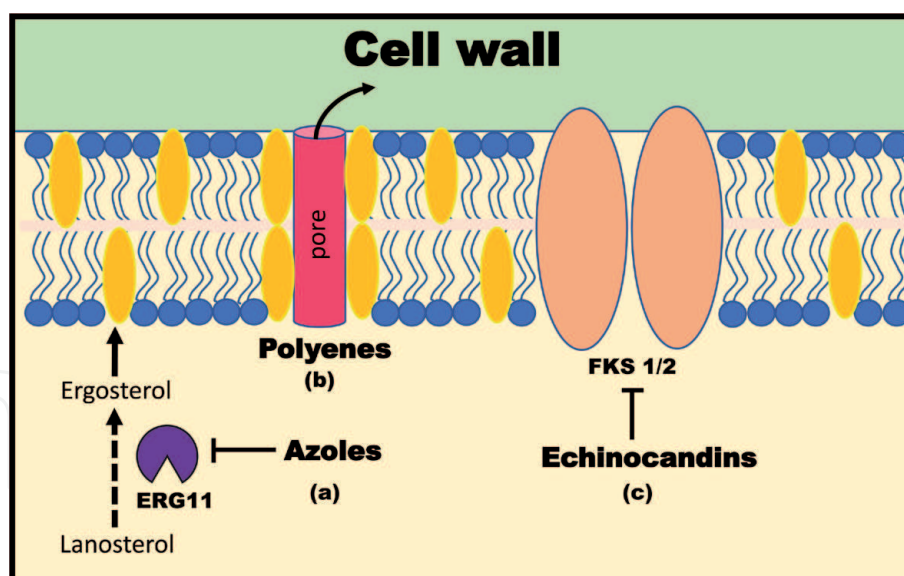
## Abstract

Invasive Candidiasis (IC) presents a global mortality rate greater than 40%, occupying the fourth place worldwide as the most frequent opportunistic nosocomial disease. Although the genus *Candida* consists of around 200 species, only 20 are reported as etiological agents of IC, being *Candida albicans* the most frequent causal agent. Even when there is a broad range of antifungals drugs for *Candida* infections, azoles, polyenes, and echinocandins are considered among the most effective treatment. However, there is some incidence for antifungal resistance among some *Candida* strains, limiting treatment options. Several molecular mechanisms with antifungal agents have been reported for *C. albicans* where insertions, deletions, and point mutations in genes codifying target proteins are frequently related to the antifungal drug resistance. Furthermore, gene overexpression is also frequently associated to antifungal resistance as well as an increase in the activity of proteins that reduce oxidative damage. This chapter summarizes the main molecular mechanisms to *C. albicans* antifungal drug resistance, besides offering an overview of new antifungal agents and new antifungal targets to combat fungal infections.

**Keywords:** resistance mechanism, antifungal, azoles, polyenes, echinocandins

## 1. Introduction

*Candida albicans* is the most important opportunistic commensal yeast that asymptotically colonizes the skin, oral cavity, gastrointestinal and genitourinary tracts in healthy people. However, it can cause superficial and invasive infections, especially in immunocompromised individuals [1–3]. Actually, invasive infections due to *Candida* species are considered among the main causes of morbidity and mortality in hospitalized patients. Although there are at least 15 *Candida* species related to human disease, more than 90% of the invasive diseases are related to *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* [4–6]. *C. albicans* infections is considered the fourth most common



**Figure 1.**

*Mechanisms of action of main antifungals families in the fungal cell. (a) Azoles disrupt the ergosterol synthesis by inhibiting the enzyme 14- $\alpha$ -lanosterol demethylase (ERG11) involved in the transformation of lanosterol into ergosterol in the endoplasmic reticulum. (b) Polyenes disrupt the cell membrane by binding to ergosterol resulting in pore formation. (c) Echinocandins inhibit 1,3- $\beta$ -d-glucan synthase (FKS 1/2) which causes disruption of the cell wall.*

opportunistic infection in hospitals. Invasive candidiasis (IC) is fatal in about 42% of the reported cases, despite the use of antifungal therapies [7, 8].

Nowadays, the most widely used antifungal drugs for IC include: A) azoles, for instance fluconazole (FLZ), itraconazole (ITC), voriconazole, posaconazole, isavuconazole; B) polyenes such as amphotericin B (AMB); C) echinocandins like caspofungin, micafungin, and anidulafungin [9–11].

These antifungal compounds act on different parts of the fungal cell (**Figure 1**). Azoles interrupt the ergosterol biosynthesis, the main component of the fungal membranes [10, 12, 13]. Polyenes such as AMB interact with ergosterol making pores in the cell membrane [10, 12–14]; while echinocandins act blocking the synthesis of  $\beta$ -d-glucan located in the fungal cell wall [13, 15]. The gradual risk increment for *Candida* infection and the greater use of antifungal agents has increased resistance towards *Candida* spp. Pharmacological failures in *Candida* spp. treatments have drawn attention to the problem of resistance to antifungals and their molecular mechanisms. *C. albicans* inherently is susceptible to azoles, polyenes, and echinocandins. Mono-resistance to azoles or echinocandins has been reported, as well as combined resistance to azoles and amphotericin, but resistance to multiple compounds that covers all three drug classes is a rare phenomenon and few cases have been reported in *C. albicans* [10, 12, 16].

The following chapter offers an overview of the main genetic mechanisms contributing to the antifungal resistance in *C. albicans*, besides giving an approach for alternative-compounds proposed against their infection.

## 2. Molecular mechanisms of antifungal resistance

### 2.1 Azoles

Fungi cell membrane is mainly integrated by ergosterol, a sterol contributing to several cellular functions, besides modulating membrane fluidity and the structure and function of membrane proteins. The azoles mechanism of action is to inhibit

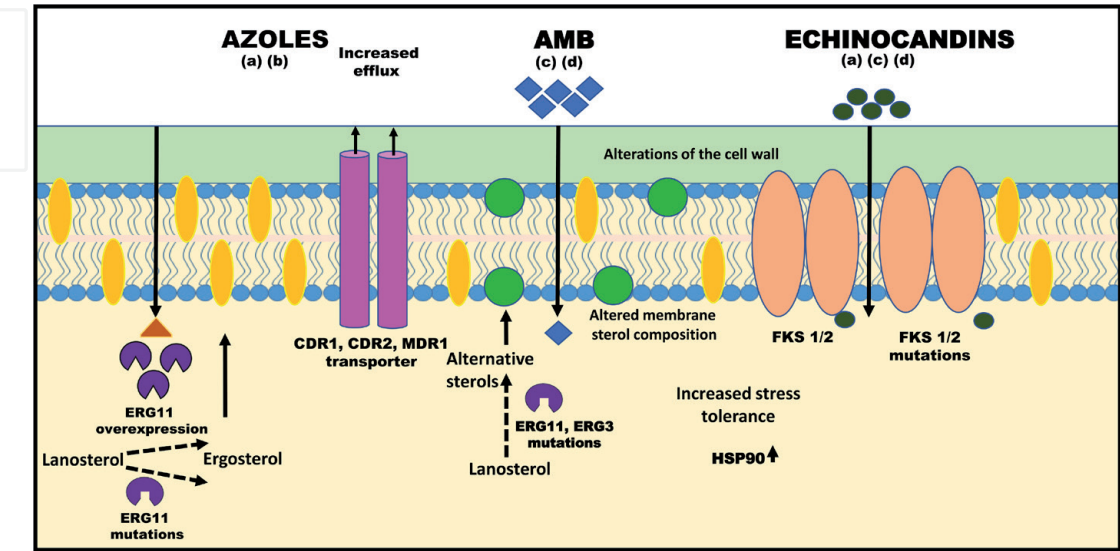
14 $\alpha$ -lanosterol demethylase, encoded by the *ERG11* gene, which converts lanosterol to ergosterol in the cell membrane (**Figure 1**). This enzyme contains an iron protoporphyrin unit in its active site. Azoles bind to iron causing the blockage of the ergosterol biosynthetic pathway [17–19]. The interruption of ergosterol synthesis allows the accumulation of 14 $\alpha$ -methyl sterols, which alters the membrane's stability, permeability, and the action of the enzymes bound to it [20].

The evolution of antimicrobial agent's resistance is common, as there are many microbes able to develop strategies against drugs action. The incremented azoles resistance is mainly a result of their fungistatic rather than fungicidal nature [17–19]. The mechanisms of resistance to azole antifungal agents have been elucidated in *Candida* spp. species and can be classified mainly as: 1) changes in cell wall or in plasma membrane, leading to poor drug absorption; 2) alterations in the affinity of the target drug (i.e. *ERG11* gene), due to a site mutation or its overexpression; 3) drug efflux mediated by membrane transporter proteins belonging to the transporters of the ATP-binding cassette (ABC), namely CDR1 and CDR2 or the transporter of the major facilitator superfamily (MFS), CaMDR1; 4) biofilm formation [18–21]. Although the resistance described in *C. albicans* strains is usually a combination of the mechanisms mentioned above (**Figure 2**) [16].

2.1.1 Mutations of the *ERG11* target enzyme

Mutations in the *C. albicans* *ERG11* gene reduce the affinity for fluconazole and have a moderate effect on posaconazole [17–19]. Several point mutations have been identified in the *ERG11* gene. In resistant strains, there are more than 140 substitutions reported, most of them have a functional additive effect. Two of the most common alterations in *C. albicans* (R467K and G464S), are located near the heme-binding site [20]. Other substitutions related to resistance are A114S, Y132H, Y132F, K143R, Y257H, and K143Q, which contribute to a significant increased resistance (more than four times) to fluconazole and voriconazole [22].

Some clinical isolates share common mutations with environmental azole-resistant strains, suggesting that some azole-resistant clinical isolates could have their origin in the environment [23]. This resistance appears to be driven by the



**Figure 2.** Schematic overview of the main mechanisms of drug resistance against azoles, AMB, and echinocandins adopted by *Candida albicans*. (a) Alteration of the enzyme target (azoles and echinocandins), (b) overexpression of drug efflux proteins (azoles), (c) Reduction of sterols in the plasma membrane (AMB), (d) increased stress tolerance and altered the fungal cell wall (echinocandins and AMB).



agricultural use of azoles. In patients without azoles treatment, resistance has been identified derived from the environment. These cases involved a Cyp51A substitution at position 98 (from leucine to histidine), and a 34 base tandem repeat (TR) in the *cyp51A* promoter, leading to overexpression. Both changes are necessary to confer resistance. In particular, these resistant isolates can be crossed with susceptible strains, suggesting that resistance could be transferred through the sexual cycle. Strains with these alterations have emerged throughout Europe and beyond. Additionally, a new environmentally selected resistance mutation (TR46, Y121F, T289A) was reported among patients in the Netherlands [20].

### 2.1.2 Dysregulation of the target enzyme *ERG11*

One way to decrease the drug effective concentration is the overexpression of *ERG11* [17]. This overexpression is common among azole-resistant *C. albicans* clinical isolates. This contributes directly to resistance, since an increase in the target requires more drug for inhibition, reducing susceptibility [19]. *ERG11* overexpression arises either from genetic dosing through gene duplication or from positive regulation of the gene by trans-acting factors [23]. Multiple mechanisms explain the constitutive overexpression of *ERG11* in azole-resistant clinical strains. First, amplification of the *ERG11* gene can occur by the formation of an isochromosome with two copies of the left arm of chromosome 5 [i (5 L)], in which *ERG11* resides, or by duplication of the entire chromosome. Second, the activation of mutations in the gene encoding the transcription factor Upc2 positively regulates most of the ergosterol biosynthesis genes [18, 20].

### 2.1.3 Alteration of the ergosterol biosynthesis pathway (point mutations in *ERG* genes)

Brief exposures of two to three hours to azoles cause transient upregulation of the *ERG* gene family in *C. albicans*. These data suggest a common regulation of ergosterol biosynthetic pathway in the presence of inhibitors. Longer *in vitro* exposures to azoles (minimum 24 h) leads to constitutive up-regulation of the *ERG* genes decreasing drug susceptibility [23].

Modification of the metabolic pathway can be effective at different points, as example, alteration of the last steps of biosynthesis through the inactivation of the *ERG3* gene results in no toxic methylated sterols production, leading to azole cross-resistance. Furthermore, mutations in non-essential genes of this pathway (*ERG3*, *ERG6*, *ERG24*, and *ERG2*) also lead to a decrease, or even a total absence, of ergosterol in the plasma membrane [17]. Lanosterol demethylase inactivity or defectiveness due to azoles induce ergosterol depletion and toxic 14 $\alpha$ -methyl-3,6-diol sterols accumulation. The presence of 14 $\alpha$ -methyl sterols can modify the function and fluidity of the plasma membrane [21]. The additive mutation in the *ERG3* gene prevents the formation of this toxic product from 14 $\alpha$ -methylfecosterol and leads to the accumulation of non-toxic sterols (Mishra, 2007; Shukla, 2016). Although this mechanism is not the most frequent one, it has been identified in several clinical isolates of *C. albicans* [23]. Mutations in *ERG3* are sufficient to induce azole resistance in *Candida* spp., but they are rarely associated with high resistance [20].

Four clinically isolated *C. albicans* *erg3* mutants (CA12, CA488, CA490, and CA108) were reported as resistant to fluconazole, voriconazole, itraconazole, ketoconazole, and clotrimazole under CLSI test conditions. Importantly, CA12 and CA108 retained an azole-resistant phenotype even when tested in the presence of FK506, a multi-drug flux inhibitor. In contrast, CA488, CA490, along with three isolates (CA6, CA14, and CA177, in which ergosterol comprised more than 80% of the total sterol

fraction and ergosta 7,22-dienol was undetectable) exhibited azole sensitive phenotypes in the inhibitor FK50 presence. CA108 mutant strain contains multiple amino acid substitutions in ERG3, but only a single conserved polymorphism (E266D) in sterol 14 $\alpha$ -demethylase (ERG11). CA12 contains a substitution (W332R) in ERG3 and no residue changes in ERG11. Furthermore, CA488 and CA490 were found to harbour multiple residue changes in both ERG3 and ERG11 [24]. Furthermore, the residue 193 in ERG3 was found to play an important role in azole resistance [25].

#### 2.1.4 Efflux pumps

A mechanism to decrease the azoles intracellular concentration is increasing their output. This class of resistance is mediated by the activity of transport systems such as the pleiotropic drug resistance (PDR) class of ATP-binding cassette transporters (ABC) and major facilitators superfamily (MFS) transporters [17]. These membrane proteins translocate compounds across cell membranes actively using different energy sources. ABC proteins are primary transporters that use ATP hydrolysis. MFS pumps are secondary transporters that use the motive force of the proton across the plasma membrane. Both types of transporters contain distinctive protein domains that confer substrate specificity: nucleotide-binding domains (NBD) in ABC pumps and transmembrane domains (TMD) in ABC and MFS pumps. Fungal PDR proteins appear to share common features on both sides of the two TMDs that separate the cytosolic from the outer cytosolic space [18, 26]. This probably reflects the fact that the cytosolic part is the motor that drives the transport of a variety of substrates through the lipid bilayer through the core of the protein into the outer cytosolic space or the outer layer of the lipid bilayer [26].

*C. albicans* contains 28 ABC proteins and 96 potential MFS transporters [18]. In this species, the main transporters, related to resistance, of the ABC proteins are CDR1 and CDR2 (resistance drugs to *Candida* 1 and 2) [21], while for MFS it is MDR1 (Multidrug Resistance 1). CDR1 and CDR2 overexpression improves drug output and reduces its accumulation in cells [23]. Positive regulation of MDR1 results in increased azole output [17]. Several cis-acting regulatory elements responsible for the regulation of the *CDR1* and *CDR2* genes have been identified. Promoter deletion studies have revealed five different regulatory elements in the *CDR1* promoter, including one BEE (basal expression element), one DRE (drug-sensitive element), two SRE (steroid sensitive element), and one NRE (negative regulatory element). Internal deletions of the BEE and DRE motifs in the *CDR1* promoter affect baseline *CDR1* expression and drug-induced expression, respectively. SRE1 and SRE2 are involved in steroid hormone responses: SRE1 responds only to progesterone and SRE2 to progesterone and  $\beta$ -estradiol. Finally, the deletion of the NRE motif leads to an increase in the baseline expression of *CDR1*. In contrast to *CDR1*, the *CDR2* promoter contains only one DRE motif. Among these diverse cis-acting elements, DRE is the only element involved in constitutive high expression and transient up-regulation of *CDR1* and *CDR2*. In *C. albicans*, *CDR1* is the main contributor to azole resistance among ABC transporters [23, 26].

In *C. albicans* a gene encoding a CaNdt80p protein similar to the *Saccharomyces cerevisiae* meiosis-specific transcription factor Ndt80p has been identified. Alteration of CaNdt80 affects the basal expression of *CDR1* and reduces its ability for up-regulation in the presence of miconazole. More recently, Ndt80p was involved in the global effect of azole resistance through its regulon, including several genes implicated in ergosterol metabolism [23]. Additionally, MDR1 is the only MFS transporter involved in the azole resistance of clinical isolates. MDR1 usually does not express detectable levels in fluconazole-susceptible isolates but is constitutively up-regulated in some fluconazole-resistant strains. A region called BRE (benomyl response

element) or MDRE (*MDR1* drug resistance element), respectively, was identified. This region is responsible for the constitutively high expression of *MDR1* in fluconazole-resistant isolates. Hyperactive alleles confer a constitutive overexpression of *MDR1* and therefore, resistance to fluconazole [23]. *MDR1* expression in *C. albicans* cells is enhanced by benomyl, methotrexate, and several other unrelated drugs, and found to be more pronounced in some of the azole-resistant clinical isolates [21].

The up-regulation of ABC and MFS transporters is mediated by specific regulations in resistant fungal pathogens. Point mutations defined as gain-of-function (GOF) mutations in these regulators confer an inherently high level of expression of the transporters in drug-resistant strains. GOF mutations in the transcription factor Upc2p led to increased resistance to fluconazole in *C. albicans* [17]. GOF mutations in the transcription factors TAC1 and MRR1 lead to upregulation of the CDR1/CDR2 and *MDR1* drug efflux pumps, respectively [16, 18]. An important question related to strategies to overcome efflux-mediated antifungal resistance is the relative contribution of each efflux pump protein to clinically significant antifungal resistance in *C. albicans*. It is now clear that the CDR1, CDR2, and *MDR1* transporters are the main efflux pumps that mediate resistance of *C. albicans* to azole drugs. However, *MDR1* is relatively specific for fluconazole, while many azole drugs can act as substrates for CDR1 and CDR2. Interestingly, several fluconazole-resistant *C. albicans* isolates overexpress only CDR1 and CDR2, but not *MDR1*, while other strains overexpress only *MDR1*, reflecting the existence of at least two different transcriptional pathways that are responsible for the upregulation of these genes in azoles [26].

## 2.2 Polyenes

The potent fungicidal activity of polyenes derives from their ability to selectively bind sterol at the fungal cell membrane (**Figure 1**). Four models have been proposed as the mode of action for polyenes: 1) the pore formation model, 2) the surface adsorption model, 3) the sterol sponge model, and 4) the oxidative damage model [14]. The pore formation model is the most studied mechanism, where polyenes are directly intercalated with the ergosterol membrane forming ion channels that permeabilize and kill yeast cells [14, 27]. Additionally, indirect mechanisms of fungal cells damage have been identified due to the effect of polyene compounds, such as those mediated by reactive oxygen species (ROS) and by the secretion of interleukin-1 $\beta$  (IL-1 $\beta$ ) by host cells [28, 29].

The polyene AMB is a broad-spectrum drug and is one of the main antifungals used for ICs [10, 14]. AMB is heptane isolated from *Streptomyces nodosus* producing high toxicity. Hence, a liposomal AMB (Ambisome R) has been developed to minimize side effects and increase treatment efficacy [10, 14, 30, 31]; however, the high costs of this drug limited its use. Resistance to AMB is rare, despite 50 years of clinical use as monotherapy, although resistant *C. albicans* strains have been found in different studies [32–35]. The alterations in the composition of the sterols and phospholipids of the membrane, the regulation of oxidative stress, and alterations of the fungal cell are the more frequent resistance mechanisms described for AMB in fungi [10, 12, 14]. In *C. albicans*, resistance to AMB is associated with ergosterol replacement by a precursor molecule or by sterols reduction at the plasma membrane (**Figure 2**) [10, 12, 14].

### 2.2.1 Alteration in the composition of sterols in the cell membrane (mutations in *ERG* genes)

The most common mechanism for acquired resistance to AMB in *C. albicans* is attributed to alterations in the composition of sterols of the fungal cell membrane [10, 12, 14, 36]. Different mutations in *ERG* genes (*ERG11*, *ERG3*, *ERG2*, and



*ERG6*) have been associated with this mechanism in *Candida* spp. [14, 37, 38]. Loss of function of the *ERG11* and *ERG3* genes (lanosterol 14 $\alpha$ -demethylase and C-5 sterol desaturase, respectively), leads to the exchange of ergosterol for alternative sterols such as lanosterol, eburicol, and 4,14-dimethyl-zymosterol in the membrane of *C. albicans*, [14, 36, 39]. Resistance to AMB in *C. albicans* is also associated with an aminoacidic substitution in *ERG11* and with *ERG5* (sterol desaturase C-22) disfunction, again associated with an alternative membrane sterol composition [14, 39, 40]. In other *Candida* spp., the inactivation of *ERG6* [11, 14, 37] and *ERG2* had a similar effect [11, 14]. Resistance to AMB is rarely found in combination with resistance to other antifungal drugs, although certain mutations that induce resistance to polyenes can lead to cross-resistance to azoles [14, 36, 41].

### 2.2.2 Response to oxidative stress and alterations in the cell wall

Fungal resistance mechanisms are also related to oxidative stress regulation, allowing the cell to tolerate exposure to AMB [14, 30]. In *C. albicans*, one of the described mechanisms of stress tolerance to AMB includes the heat shock protein 90 (Hsp90) molecular chaperone, which regulates a large number of proteins involved in several fungal cellular processes [42, 43]. In addition to alterations in the composition of sterols in the plasma membrane and the regulation of oxidative stress, studies in fungi have correlated resistance to AMB with fungal cell wall alterations [14, 44, 45]. In AMB resistant *C. tropicalis* strains, an enlargement of the cell wall has been observed with increased levels of 1,3- $\beta$ -glucans [14, 44], suggesting an affectation in the penetration of AMB through the cell wall [14, 45].

## 2.3 Echinocandins

Echinocandins are lipo-peptides that inhibit 1,3- $\beta$ -d-glucan synthetase, which is responsible for the biosynthesis of 1,3- $\beta$ -d-glucan, one of the main components of the fungal cell wall, causing osmotic instability and therefore the death of fungal cells (**Figure 1**) [10, 13]. This class of drugs has certain advantages attributable to its effects on the fungal cell wall, including a lower risk of side effects since animal cells do not have this structure [10]. Echinocandins have a limited spectrum, but for *Candida* species, they have broad fungicidal activity. The 1,3- $\beta$ -d-glucan synthetase target comprises a GTP binding protein Rho, which helps regulate the biosynthetic capacity of glucan synthetase, and a catalytic subunit, FKS, which encodes three related genes, *FKS1*, *FKS2*, and *FKS3*. *FKS1* is essential in *C. albicans* and other *Candida* spp. Whereas *FKS1* and *FKS2* are functionally redundant in *C. glabrata*, *FKS3* is very low expressed compared to other genes [46], not being a significant contributor to biosynthetic capacity in general.

Echinocandins are the first major new class of antifungal drugs on the market in decades. Consequently, it is of vital importance to assess the nature of the resistance mechanism to this class of drugs. Mutations that affect the target site are the most likely resistance mechanism that exists (**Figure 2**), since unlike azoles, echinocandins are poor substrates for drug exit through efflux transporters, ruling out this mechanism of resistance [10, 13]. Specific mutations have already been reported in two highly conserved regions of the Fks1 subunit of glucan synthetase, a membrane protein, which can confer resistance *in vitro* in *Candida* isolates to caspofungin, the first echinocandin approved for the treatment of yeast infections [10, 13, 47, 48]. Other ways in which there may be the acquisition of resistance to echinocandins in *C. albicans* is through different response pathways to cellular stress, as well as some clinical factors such as empirical therapy, prophylaxis, gastrointestinal reservoirs, or intra-abdominal infections.



### 2.3.1 Acquired FKS mutations

Resistance-associated amino acid substitutions occur in two highly conserved hot-spot (HS) regions of the FKS genes. The residues they encompass are Phe641–Pro649 and Arg1361 in *C. albicans* and other *Candida* spp. Substitutions of amino acids Ser645 and Phe641 cause 75% resistance in *C. albicans* [10, 13]. Pharmacodynamic studies conducted in murine models infected with *C. albicans* demonstrated that mutations in the *FKS1* gene confer resistance to echinocandins [48, 49]. Mutations in *FKS1* lead to a decrease in the virulence of *C. albicans* in murine models of IC. Furthermore, high doses of caspofungin are effective against *C. albicans*, including resistant isolates that presented point mutations in *FKS1* [50, 51]. Several studies have reported that mutations in the *FKS1* gene produce changes in the morphology of the cell wall of *C. albicans*, observing a decrease in 1,3- $\beta$ -d-glucan levels in contrast to the increased amount of chitin in response to echinocandin exposure [51]. Data suggest that increased chitin in the *C. albicans* cell wall could provide a window of opportunity to acquire mutations in *FKS1*, even without exposure to caspofungin [52].

### 2.3.2 Adaptive stress responses

The fungal cell wall is a dynamic structure that changes during growth and development, requires 1,3- $\beta$ -d-glucan crosslinking, an essential polymer for the survival of the fungal cell. Echinocandins alter the integrity of the cell wall and induce stress in the cell. In response to this, the fungal cell possesses a repertoire of mechanisms to protect the cell against such destabilization. Protection against cell wall weakening is induced through a variety of stress adaptation mechanisms, which involve protein kinase C (PKC), calcineurin, and Hsp90 [10, 13]. Stress signals in the cell wall are transmitted through the Rho GTPase, which mobilizes various effectors. Its activation alters several carbohydrate polymers along with the structure and remodelling of the cell wall. The Hsp90 heat shock protein organizes a cellular stress response circuit that has a major impact on resistance to echinocandins. Also, the genetic or chemical modulation of the Hsp90 protein reduces tolerance to echinocandins [52]. In response to the inhibition of FKS by the action of echinocandins, a greater amount of chitin is produced helping to maintain the integrity of the cell wall as chitin replaces 1,3- $\beta$ -d-glucan, thus reducing sensitivity to drugs [10, 13, 48].

## 3. New antifungals

The resistance of *C. albicans* and other pathogenic fungi to current antifungal agents has established the need to find new antifungal targets with a novel mechanism of action. Resistant strains are increasing in number for some classes of antifungal agents, particularly for azoles and echinocandins [53]. Consequently, it is necessary to face the challenge of successfully managing fungal infections. To achieve this, one of the main points is the continuation of the development of new antifungal drugs [54]. The main issues faced by the development of new drugs are: 1) they must have a broad spectrum against emerging filamentous yeasts and fungi and 2) they must have a more efficient fungicidal activity to eliminate pathogens quickly and totally [55–59]. Besides, invasive candidiasis occurs in very frail patients who do not tolerate much organ toxicity, since such patients are often taking many other therapeutic agents, so drug–drug interactions must be carefully considered [60].

### 3.1 Discovery and development of new antifungal drugs

This part of the chapter provides an overview of ongoing efforts to develop new classes of antifungal drugs (**Table 1**). Although there are several strategies for the development of these drugs, these include those obtained from new chemical agents, from reusing existing drugs, from peptides with antimicrobial properties, and finally from natural compounds extracted from plants [10, 55, 58].

Several new chemical-antifungals are designed specifically to target either 1,3- $\beta$ -D-glucan (such as Rezafungin and Ibrexafungin) or ergosterol (such as the compound VT-1161). These compounds are very specific for fungal infections or they have a longer half-life, offering better efficacy [58, 60–62]. At the same time, several of these antifungal agents have new targets and subsequently, new mechanisms of action. For instance, fosmanogepix, formerly APX001, and aureobasidin A, which act by inhibiting inositol acyltransferase, and inositol phosphorylceramide synthase, respectively [63, 64]. Efungumab (or Mycograb) and geldanamycin-like agents can inhibit the HSP90 chaperone, which has been also shown to confer resistance to antifungals [65, 66]. The AR-12 compound deregulates chaperone's activity by blocking fungal acetyl-CoA synthase [67]. The T-2307 compound is an aryl-amidine that inhibits the respiratory chain complex and is active against yeast and filamentous fungi [68]. Finally, the VL-2397 compound has a similar structure to the ferrichrome siderophore, and whose mechanism of action or its target is unknown, but it is known to be transported by the Sit1 protein [69]. Some compounds that have been already tested for other types of diseases are now receiving a new focus as antifungals. These include two compounds that enhance the antifungal activity, such as rifampin, which acts on RNA polymerase [70], and verapamil, which acts on a calcium channel [71]. We have also given importance to alternative compounds such as peptides and plant extracts; many molecules are actually studied with promising results, especially against *C. albicans*. Some peptides such as lysozyme, lactoferrin, defensins, Histatin-5, and cathelicidins are known to have antifungal properties. The main mechanism of action is due to the enhancement of substances traffic through the fungal membrane, which favours permeabilization [10, 72–76]. Plant extracts are another prominent source of new antifungals, they can act either alone or synergistically with existing antifungals to improve their function. The compounds extracted from plants are essential oils, terpenes, and flavonoids among many others. They have diverse mechanisms of action, such as alteration of the plasma membrane, binding to ergosterol, induction of apoptosis, inhibition of growth, filamentation, and biofilm formation in *C. albicans* [10, 77–81].

### 3.2 New targets and alternative approaches

Despite the efforts made to discover, repositioning, or create new antifungal drugs, it is imperative to find new targets to help eliminating *Candida* spp. infection. The new antifungal targets include biosynthetic and signal transduction pathways, which are key players for fungal survival processes. The sphingolipids biosynthesis is a biosynthetic pathway considered as a promising target. Sphingolipids are a part of cell membranes, that act as signalling molecules regulating processes such as apoptosis. As fungal sphingolipids are structurally different to mammalian sphingolipids, they are excellent candidates for antifungal design [82]. The heat-shock proteins (Hsps) represent another potential antifungal target as they control several basic physiological activities, and heat-shock protein disruption in *C. albicans* inhibits growth or reverses tolerance to antifungals [83]. A recently studied pathway as a potential target is the ionic homeostasis signalling pathway, which is central to the fungus survival by regulating gene expression,

Source	Compound	Target	Mechanism of action	Reference
Chemicals	Rezafungin (CD101)	$\beta$ -d-glucan	$\beta$ -d-glucan synthase inhibition	[60]
	Ibrexafungerp (SCY-078)	$\beta$ -d-glucan	$\beta$ -glucan synthase inhibition	[61]
	VT-1161	Ergosterol	Specific for fungal Cyp51	[62]
	Fosmanogepix (APX001]	Glycosyl phosphatidylinositol	GPI biosynthesis inhibition	[63]
	Aureobasidin A	Inositol phosphorylceramide synthase	Sphingolipids biosynthesis inhibition	[64]
	Efungumab (or Mycograb)	HSP90	Antibody binds to fungal HSP90	[65]
	Geldanamycin-like agents	HSP90	HSP90 inhibition	[66]
	AR-12	Probably blocks fungal acetyl-CoA synthetase 1	Downregulation of chaperone proteins	[67]
	T – 2307	Mitochondrial membrane potential	Respiratory chain complexes inhibition	[68]
Repurposed compounds	VL-2397 (ASP2397)	Unknown	Unknown, but taken up by Sit1	[69]
	Rifampin	RNA polymerase	Enhance the antifungal activity	[70]
Promising Peptides	Verapamil	Calcium channel	Enhance the antifungal activity	[71]
	Lysozyme	Secreted aspartic protease (SAP)	Reduces SAP activity and secretion	[72]
	Lactoferrin (hl.f)	Antimicrobial activity	Production of cationic antimicrobial peptide lactoferricin	[73]
	Human b-defensins (HBD)	Cell membrane	Increases membrane permeability	[74]
	Histatin-5	Non-lytic ATP efflux	Inhibition of adhesion	[75]
	Cathelicidins	Cell membrane	Increases membrane permeability	[76]
	<i>Scutellaria aicalensis</i> (flavonoid baicalein)	Unknown	Induces apoptosis in <i>C. albicans</i>	[77]
	<i>Cymbopogon nardus</i> (essential oils)	Unknown	Inhibits hyphal growth in <i>C. albicans</i>	[78]

Source	Compound	Target	Mechanism of action	Reference
Plant	<i>Artemisia judaica</i> (essential oil)	Germination	Inhibits the formation of germination tube and biofilms in <i>C. albicans</i>	[79]
Natural compounds	Thymol (terpene)	Ergosterol	Binds to ergosterol in the membrane resulting in cell death	[80]
	Carvacrol (terpene)	Cell membrane	Alters cellular cytoplasmic membrane and induces apoptosis	[81]

**Table 1.**  
*Antifungal compounds in development against C. albicans or Candida spp.*

morphological transition, response to stress, and resistance to antifungals [84]. The Ras-cAMP-PKA signal transduction pathway is essential for cellular metabolism and controls morphogenesis, adhesion, and biofilm formation, making the inactivation of this signalling cascade attractive as a target for new antifungals [85].

Finally, an alternative approach to conventional antifungal drugs is the use of nanotechnology, which produces the so-called “nanoantibiotics”. These nanoantibiotics are unique due to their improved physicochemical properties, such as reduced toxicity and biocompatibility as well as their size that must be less than 100 nm [86]. The antimicrobial properties of silver have been known for a long time, so silver nanoparticles were tested as antimicrobials and showed potent activity against drug-resistant fungal biofilms [87].

4. Conclusions

A better understanding of the resistance mechanisms of azoles, polyenes, and echinocandins, along with the discovery of new cellular and clinical factors promoting resistance, will facilitate the design of more effective strategies to overcome and prevent resistance to antifungal agents. Even though several biomedical research offer a window hoping to reduce the incidence of *C. albicans* and the complications those systemic infections by this fungus entail; the quest for new targets with novel mechanisms of action continues to be the priority.

Acknowledgements

ALMR thanks the National Council of Science and Technology of Mexico (CONACyT) for the postdoctoral fellowship granted. RIAS and KCP are thankful for the scholarship granted by the National Council of Science and Technology of Mexico (CONACyT).

Conflict of interest

All authors declare no conflicting interests.



IntechOpen

IntechOpen

### **Author details**

Estela Ruiz-Baca\*, Rosa Isela Arredondo-Sánchez, Karina Corral-Pérez,  
Angélica López-Rodríguez, Iván Meneses-Morales, Víctor M. Ayala-García  
and Ana Lilia Martínez-Rocha\*  
Faculty of Chemistry Science, Juarez University of Durango State, Durango, México

\*Address all correspondence to: eruiz@ujed.mx and analilia.martinez@ujed.mx

### **IntechOpen**

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

## References

- [1] Rodriguez DL, Quail MM, Hernday AD, Nobile CJ. Transcriptional circuits regulating developmental processes in *Candida albicans*. *Frontiers in Cellular and Infection Microbiology*. 2020; **10**:605711.
- [2] Maheronnaghsh M, Fatahinia M, Dehghan P, Teimoori A. Identification of *Candida* species and antifungal susceptibility in cancer patients with oral lesions in Ahvaz, Southern West of Iran. *Advanced Biomedical Research*. 2020; **9**:50.
- [3] Kan S, Pang Q, Song N, Mei H, Zheng H, Li D, et al. Study on vulvovaginal candidiasis: clinical epidemiology and *in vitro* susceptibility of pathogenic yeasts in China. *Social Science Research Network*. 2020; **10**:2139.
- [4] Forastiero A, Garcia-Gil V, Rivero-Menendez O, Garcia-Rubio R, Monteiro MC, Alastruey A, Jordan R, et al. Rapid development of *Candida krusei* echinocandin resistance during caspofungin therapy. *American Society for Microbiology*. 2015; **59**:6975-6982.
- [5] Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*. 2016; **62**: e1–e50.
- [6] Espinel-Ingroff A, Cantón E, Pemán J. Antifungal Resistance among Less Prevalent *Candida* Non-*albicans* and Other Yeasts versus Established and under Development Agents: A Literature Review. *Journal of Fungi*. 2021; **7**(1):24.
- [7] Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen RA, Powderly W, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clinical Infectious Diseases*. 2003; **37**:634-643.
- [8] Ford CB, Funt JM, Abbey D, Issi L, Guiducci C, Martinez DA, et al. The evolution of drug resistance in clinical isolates of *Candida albicans*. *eLife*. 2015; **4**: e00662.
- [9] Ruiz CI, Cuenca EM. Antifungals for systemic use. *Enfermedades Infecciosas y Microbiología Clínica*. 2009; **27**(6):353-362.
- [10] de Oliveira Santos GC, Vasconcelos CC, Lopes AJO, Cartágenes MSS, Philo AKDB, Nascimento FRF, et al. *Candida* infections and therapeutic strategies: Mechanisms of action for traditional and alternative agents. *Frontiers in Microbiology*. 2018; **9**(1351):1-25.
- [11] Ahmad S, Joseph L, Parker JE, Asadzadeh M, Kelly SL, Meis JF et al. ERG6 and ERG2 are major targets conferring reduced susceptibility to amphotericin B in clinical *Candida glabrata* isolates in Kuwait. *Antimicrobial Agents and Chemotherapy*. 2019; **63**.
- [12] Cuenca EM. Antifungal drug resistance mechanisms in pathogenic fungi: from bench to bedside. *Clinical Microbiology and Infection*. 2014; **20**:54-59.
- [13] Houšť J, Spížek J, Havlíček V. Antifungal Drugs. *Metabolites*. 2020; **10**(106): 1-16.
- [14] Carolus H, Pierson S, Lagrou K, Van Dijck P. Amphotericin B and other polyenes-discovery, clinical use, mode of action and drug resistance. *Journal of Fungi (Basel)*. 2020; **6**(4):321.
- [15] Patil A, Majumdar S. Echinocandins in antifungal pharmacotherapy. *Journal*

of Pharmacy and Pharmacology. 2017; **69**:1635-1660.

[16] Jensen RH, Thyssen KM, Vale L, Sanglard D, Jorgensen R, Fog K, et al. Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance *in vivo* in *Candida albicans* orchestrated by multiple genetic alterations. *Journal of Antimicrobial Chemotherapy*. 2015; **70**:2551-2555.

[17] Campoy S, Adrio JL. Antifungals. *Biochemical Pharmacology*. 2016.

[18] Sanglard D, Coste AT. Activity of isavuconazole and other azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. *Antimicrob Agents Chemother*. 2016; **60**(1):229-238.

[19] Shukla PK, Singh P, Yadav RK, Pandey S, Bhunia SS. Past, present, and future of antifungal drug development. *Communicable Diseases of the Developing World*. 2016; 125-167.

[20] Cowen LE, Sanglard D, Howard SJ, Rogers PD, Perlin DS. Mechanisms of antifungal drug resistance. *Cold Spring Harbor Perspectives in Medicine*. 2015; **5**.

[21] Mishra NN, Prasad T, Sharma N, Payasi A, Prasad R, Gupta DK, et al. Pathogenicity and drug resistance in *Candida albicans* and other yeast species a review. *Acta Microbiologica et Immunologica Hungarica*. 2007; **54**(3):201-235.

[22] Xiang MJ, Liu JY, Ni PH, Wang S, Shi C, Wei B, et al. ERG11 mutations associated with azole resistance in clinical isolates of *Candida albicans*. *Federation of European Microbiological Societies*. 2013; **13**:386-393.

[23] Vandeputte P, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal

infections. *International Journal of Microbiology*. 2012.

[24] Martel CM, Parker JE, Bader O, Weig M, Gross U, Warrilow AG, et al. A clinical isolate of *Candida albicans* with mutations in ERG11 (encoding sterol 14 $\alpha$ -demethylase) and ERG5 (encoding C22 desaturase) is cross resistant to azoles and amphotericin B. *Antimicrobial Agents and Chemotherapy*. 2010; **54**:3578-3583.

[25] Morio F, Pagniez F, Lacroix C, Miegerville M, Pape PL. Amino acid substitutions in the *Candida albicans* sterol D5,6-desaturase (ERG3p) confer azole resistance: characterization of two novel mutants with impaired virulence. *Journal of Antimicrobial Chemotherapy*. 2012; **67**:2131-2138.

[26] Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya M, et al. Efflux-mediated antifungal drug resistance. *Clinical Microbiology Reviews*. 2009; **22**(2): 291-321.

[27] Kinsky SC. Polyene antibiotics. In *Antibiotics*, Springer: 1967; pp. 122-141

[28] Delattin N, Cammue BP, Thevissen K. Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms. *Future Medicinal Chemistry*. 2014; **6**(1):77-90.

[29] Darisipudi MN, Allam R, Rupanagudi KV. Polyene macrolide antifungal drugs trigger interleukin-1 $\beta$  secretion by activating the NLRP3 inflammasome. *PLOS one*. 2011; **6**(5):1-6.

[30] Mesa-Arango AC, Scorzoni, L, Zaragoza O. It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Frontiers in Microbiology*. 2012; **3**.

[31] Nett JE, Andes DR. Antifungal agents: spectrum of activity, pharmacology, and clinical indications.

Infectious Disease Clinics of North America. 2016; **30**:51-83.

[32] Rambach G, Oberhauser H, Speth C, Lass CF. Susceptibility of *Candida* species and various moulds to antimycotic drugs: use of epidemiological cutoff values according to EUCAST and CLSI in an 8-year survey. Medical Mycology. 2011; **49**:856-863.

[33] Ostrosky LZ, Rex JH, Pappas PG, Hamill RJ, Larsen RA, Horowitz HW, et al. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrobial Agents and Chemotherapy. 2003; **47**:3149-3154.

[34] Maraki S, Mavromanolaki VE, Stafylaki D, Nioti E, Hamilos G, Kasimati A. Epidemiology and antifungal susceptibility patterns of *Candida* isolates from Greek women with vulvovaginal candidiasis. Mycoses. 2019; **62**:692-697.

[35] Badiie P, Alborzi A. Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five-year study. Iranian Journal of Microbiology. 2011; **3**:183-188.

[36] Sanglard D, Ischer F, Parkinson T, Falconer D, Bille J. *Candida albicans* mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents. Antimicrobial Agents and Chemotherapy. 2003; **47**:2404-2412.

[37] Young LY, Hull CM, Heitman J. Disruption of ergosterol biosynthesis confers resistance to amphotericin B in *Candida lusitanae*. Antimicrobial Agents and Chemotherapy. 2003; **47**:2717-2724.

[38] Silva LN, Oliveira SS, Magalhães LB, Andrade VV, Torres-Santos EC, Carvalho MD, Pereira MD, Branquinho MH, Santos AL. Unmasking the amphotericin B resistance mechanisms in *Candida haemulonii*

species complex. ACS Infectious Diseases. 2020; **6**:1273-1282.

[39] Vincent BM, Lancaster AK, Scherz RS, Whitesell L, Lindquist S. Fitness trade-offs restrict the evolution of resistance to amphotericin B. PLOS Biology. 2013; **11**: e1001692.

[40] Martel CM, Parker JE, Bader O, Weig M, Gross U, Warrilow AG, et al. Identification and characterization of four azole-resistant ERG3 mutants of *Candida albicans*. Antimicrobial Agents and Chemotherapy. 2010; **54**(11):4527-4533.

[41] Kelly S, Lamb D, Kelly D, Manning N, Loeffler J, Hebart H, et al. Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol  $\Delta 5, 6$ -desaturation. FEBS letters. 1997; **400**:80-82.

[42] LaFayette SL, Collins C, Zaas AK, Schell WA, Betancourt-Quiroz M, Gunatilaka AL, et al. PKC signaling regulates drug resistance of the fungal pathogen *Candida albicans* via circuitry comprised of Mkc1, calcineurin, and Hsp90. PLOS Pathog. 2010; **6**:e1001069.

[43] Cowen LE, Lindquist S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. Science. 2005; **309**:2185-2189.

[44] Mesa-Arango AC, Rueda C, Román E, Quintin J, Terrón MC, Luque D, et al. Cell wall changes in amphotericin B-resistant strains from *Candida tropicalis* and relationship with the immune responses elicited by the host. Antimicrobial Agents and Chemotherapy. 2016; **60**:2326-2335.

[45] Seo K, Akiyoshi H, Ohnishi Y. Alteration of cell wall composition leads to amphotericin B resistance in *Aspergillus flavus*. Microbiology and Immunology. 1999; **43**:1017-1025.



- [46] Katiyar SK, Alastruey-izquierdo A, Healey KR, Jhonson ME, Perlin DS, Edlind TD. Fks1 and Fks2 are functionally redundant but differentially regulated in *Candida glabrata*: implications for echinocandin resistance. *Antimicrobial Agents and Chemotherapy*. 2012; **56**(12):6304-6309.
- [47] Balashov SV, Park S, Perlin DS. Assessing Resistance to the echinocandin antifungal drug caspofungin in *Candida albicans* by profiling mutations in FKS1. *Antimicrobial Agents and Chemotherapy*. 2006; **50**(6):2058-2063.
- [48] Perlin DS. Mechanisms of echinocandin antifungal drug resistance. *Annals of the New York Academy of Science*. 2015; **1354** (Pt 1): 1-11.
- [49] Garcia-Effron G, Lee S, Park S, Cleary JD, Perlin DS. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: implication for the existing susceptibility breakpoint. *Antimicrobial Agents and Chemotherapy*. 2009; **53** (Pt 9):3690-3699.
- [50] Ben-Ami R, García-Effron G, Lewis R, Gamarra S, Leventakos K, Perlin D, et al. Fitness and virulence costs of *Candida albicans* FKS1 hot spot mutations associated with echinocandin resistance. *The Journal of Infectious Diseases*. 2011; **204**:626-635.
- [51] Wiederhold NP, Najvar LK, Bocanegra RA, Kirkpatrick WR, Patterson TF. Caspofungin dose escalation for invasive candidiasis due to resistant *Candida albicans*. *Antimicrobial Agents and Chemotherapy*. 2011; **55**(7):3254-3260.
- [52] Imtiaz T, Lee KK, Munro CA, Maccallum DM, Shankland GS, Johnson EM, et al. Echinocandin resistance due to simultaneous FKS mutation and increased cell wall chitin in a *Candida albicans* bloodstream isolate following brief exposure to caspofungin. *Journal of Medical Microbiology*. 2012; **61**(Pt 9):1330-1334.
- [53] Singh SD, Robbins N, Zaas AK, Schell WA, Perfect JR, Cowen LE. Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. *PLOS Pathogens*. 2009; **5**(7): e1000532.
- [54] Gray KC, Palacios DS, Dailey I, Endo MM, Uno BE, Wilcock BC, et al. Amphotericin primarily kills yeast by simply binding ergosterol. *Proceedings of the National Academy of Sciences*. 2012; **109**(7):2234-2239.
- [55] Perfect JR. The antifungal pipeline: a reality check. *Nature Reviews Drug Discovery*. 2017;**16**(9):603.
- [56] Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. *Clinical Infectious Diseases*. 2017; **64**(2):134-140.
- [57] Parente-Rocha JA, Bailão AM, Amaral AC, Taborda CP, Paccez JD, Borges CL, et al. Antifungal resistance, metabolic routes as drug targets, and new antifungal agents: an overview about endemic dimorphic fungi. *Mediators of Inflammation*. 2017.
- [58] Wall G, Lopez-Ribot JL. Current antimycotics, new prospects, and future approaches to antifungal therapy. *Antibiotics*. 2020; **9**(8):445.
- [59] Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. *Nature Reviews Disease Primers*. 2018; **4**(1):1-20.

- [60] Garcia-Effron G. Rezafungin—mechanisms of action, susceptibility and resistance: similarities and differences with the other echinocandins. *Journal of Fungi*. 2020; **6**(4):262.
- [61] Schell WA, Jones AM, Borroto-Esoda K, Alexander BD. Antifungal activity of SCY-078 and standard antifungal agents against 178 clinical isolates of resistant and susceptible *Candida* species. *Antimicrobial Agents and Chemotherapy*. 2017; **61**(11).
- [62] Warrilow AGS, Hull CM, Parker JE, Garvey EP, Hoekstra WJ, Moore WR, et al. The clinical candidate VT-1161 is a highly potent inhibitor of *Candida albicans* CYP51 but fails to bind the human enzyme. *Antimicrobial Agents and Chemotherapy*. 2014; **58**(12):7121-7127.
- [63] Berkow EL, Lockhart SR. Activity of novel antifungal compound APX001A against a large collection of *Candida auris*. *Journal of Antimicrobial Chemotherapy*. 2018; **73**(11):3060-3062.
- [64] Takesako k, Kuroda H, Inoue T, Haruna F, Yoshikawa Y, Kato I, et al. Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. *The Journal of Antibiotics*. 1993; **46**(9):1414-1420.
- [65] Louie A, Stein DS, Zack JZ, Liu W, Conde H, Fregeau C, et al. Dose range evaluation of Mycograb C28Y variant, a human recombinant antibody fragment to heat shock protein 90, in combination with amphotericin B-desoxycholate for treatment of murine systemic candidiasis. *Antimicrobial Agents and Chemotherapy*. 2011; **55**(7):3295-3304.
- [66] Lamothe F, Alexander BD, Juvvadi PR, Steinbach WJ. Antifungal activity of compounds targeting the Hsp90-calcineurin pathway against various mould species. *Journal of Antimicrobial Chemotherapy*. 2015; **70**:1408-1411.
- [67] Koselny K, Green J, Favazzo L, Glazier VE, DiDone L, Ransford S, Krysan DJ. Antitumor/antifungal celecoxib derivative AR-12 is a non-nucleoside inhibitor of the ANL-family adenylylating enzyme acetyl CoA synthetase. *ACS Infectious Diseases*. 2016; **2**(4):268-280.
- [68] Yamashita K, Miyazaki T, Fukuda Y, Mitsuyama J, Saijo T, Shimamura S, et al. The novel arylamidine T-2307 selectively disrupts yeast mitochondrial function by inhibiting respiratory chain complexes. *Antimicrobial Agents and Chemotherapy*. 2019; **63**(8).
- [69] Dietl AM, Misslinger M, Aguiar MM, Ivashov V, Teis D, Pfister J, et al. The siderophore transporter Sit1 determines susceptibility to the antifungal VL-2397. *Antimicrobial Agents and Chemotherapy*. 2019; **63**(10).
- [70] Christenson JC, Shalit I, Welch DF, Guruswamy A, Marks MI. Synergistic action of amphotericin B and rifampin against *Rhizopus* species. *Antimicrobial Agents and Chemotherapy*. 1987; **31**:1775-1778.
- [71] Liu S, Yue L, Gu W, Li X, Zhang L, Sun S. Synergistic effect of fluconazole and calcium channel blockers against resistant *Candida albicans*. *PLoS One*. 2016; **11**(3): e0150859.
- [72] Wu T, Samaranayake LP, Leung WK, Sullivan PA. Inhibition of growth and secreted aspartyl proteinase production in *Candida albicans* by lysozyme. *Journal Medical Microbiology* 1999; **48**:721-730.
- [73] Samaranayake YH, Samaranayake LP, Wu PC, So M. The antifungal effect of lactoferrin and lysozyme on *Candida krusei* and *Candida albicans*. *Journal of Antimicrobial Chemotherapy*. 1999; **43**:103-110.

of Pathology, Microbiology and Immunology. 1997; **105**:875-883.

[74] Krishnakumari V, Rangaraj N, Nagaraj R. Antifungal activities of human beta-defensins HBD-1 to HBD-3 and their C-terminal analogs Phd1 to Phd3. *Antimicrobial Agents and Chemotherapy*. 2009; **53**:256-260.

[75] Edgerton M, Koshlukova SE. Salivary histatin 5 and its similarities to the other antimicrobial proteins in human saliva. *Advances in Dental Research*. 2000; **14**:16-21.

[76] Tsai PW, Yang CY, Chang HT, Lan CY. (2011). Human antimicrobial peptide LL-37 inhibits adhesion of *Candida albicans* by interacting with yeast cell-wall carbohydrates. *PLoS One*. 2011; **6**: e17755.

[77] Serpa R, França EJ, Furlaneto-Maia L, Andrade CG, Diniz A, Furlaneto MC. *In vitro* antifungal activity of the flavonoid baicalein against *Candida* species. *Journal Medical Microbiology* 2012; **61**:1704-1708.

[78] De Toledo LG, Ramos MADS, Spósito L, Castilho EM, Pavan FR, Lopes ÉDO, et al. Essential oil of *Cymbopogon nardus* (L.) Rendle: a strategy to combat fungal infections caused by *Candida* species. *International Journal of Molecular Sciences*. 2016; **17**:E1252.

[79] Köse YB, İşcan G, Göger F, Akalın G, Demirci B, Baser KHC. Chemical composition and biological activity of *Centaurea baseri*: new species from Turkey. *Chemistry and Biodiversity*. 2016; **13**:1369-1379.

[80] de Castro RD, de Souza TMP, Bezerra LM, Ferreira GL, Costa EM, Cavalcanti A L. Antifungal activity and mode of action of thymol and its synergism with nystatin against

*Candida* species involved with infections in the oral cavity: an *in vitro* study. 2015; *BMC Complementary Medicine and Therapies*. **15**:417.

[81] Dalleau S, Cateau E, Bergès T, Berjeaud JM, Imbert C. *In vitro* activity of terpenes against *Candida* biofilms. *International Journal Antimicrobial Agents*. 2008; **31**:572-576.

[82] Mota FC, Del Poeta M (2020). Fungal sphingolipids: role in the regulation of virulence and potential as targets for future antifungal therapies. *Expert Review of Anti-infective Therapy*. 2020.

[83] Gong Y, Li T, Yu C, Sun S. *Candida albicans* Heat shock proteins and Hsps-associated signaling pathways as potential antifungal targets. *Frontiers in Cellular and Infection Microbiology*. 2017; **7**:520.

[84] Li Y, Sun L, Lu C, Gong Y, Li M, Sun S. Promising antifungal targets against *Candida albicans* based on ion homeostasis. *Frontiers in Cellular and Infection Microbiology*. 2018; **8**:286.

[85] Rajasekharan SK, Kamalanathan C, Ravichandran V, Ray AK, Satish AS, Mohanvel SK. Mannich base limits *Candida albicans* virulence by inactivating Ras-cAMP-PKA pathway. *Scientific Reports*. 2018; **8**(1):1-9.

[86] Beyth N, Houri-Haddad Y, Domb A, Khan W, Hazan R. Alternative antimicrobial approach: nano-antimicrobial materials. *Evidence-based Complementary and Alternative Medicine*, 2015.

[87] Lara HH, Romero-Urbina DG, Pierce C, Lopez-Ribot JL, Arellano-Jiménez MJ, Jose-Yacamán M. Effect of silver nanoparticles on *Candida albicans* biofilms: an ultrastructural study. *Journal of Nanobiotechnology*. 2015; **13**(1):1-12.