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Candida and Candidiasis

Edited by Tulin Askun

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Meet the editor

Prof. Dr. Tulin Askun is head of the Molecular Biology Department (within the main Biology Department) in the Faculty of Sciences and Arts, Balikesir University, Turkey. She received both her master's degree and her Ph.D. in fungi from Balikesir University. In 2012, she received a Project Incentive Award in Basic Sciences from the same institution. She is responsible for implementing educational programs and scientific research, providing projects, and establishing and maintaining relationships with group members and project partners. She has published many articles, projects, papers, two edited volumes, and four book chapters.

Contents

Preface

Candidiasis is an infection caused by *Candida* spp., mostly *Candida albicans*. Although *Candida* species, a yeast, normally exists in the human body on the skin and in the mouth, throat, gut, and vagina, it does not cause infection; however, sometimes yeast proliferation occurs, and *Candida* spp. can cause several infections, such as an oral infection affecting the mouth and throat, called oropharyngeal candidiasis (thrush), as well as vulvovaginal, oesophageal, intestinal, and invasive candidiasis. Oesophageal candidiasis is a common infection in HIV/AIDS patients. The pathogenesis of candidiasis in humans is not completely understood. Infection caused by *Candida* spp. creates a serious problem in humans because of drug resistance. Investigation of new and effective active substances against pathogenic *Candida* spp. and a better understanding of the molecular mechanisms involved in the formation of antifungal resistance will help prevent *Candida* infection among individuals with immunological deficiency and will make antifungal therapy much more effective. This book provides a comprehensive overview of the latest information on *Candida* spp. and candidiasis, and will be useful to biologists, mycologists, chemists, molecular biologists, geneticists and agriculturists.

In the first section of the book, the history and taxonomic hierarchy, diversity, sexual reproduction, invasion biology, antifungal resistance, identification and genetic structure of *Candida* spp., novel and natural compounds for candidiasis treatment, and interactions between *Candida* spp. and cancer development are discussed. Molecular mechanisms and biofilm-related factors responsible for development of antifungal drug resistance in *Candida* species, together with antifungal drug resistance, multi-drug resistance, and related resistance mechanisms are reviewed. The second section examines the molecular identification of targeted DNA regions, and the advantages and disadvantages of each approach, as well as prospective new advances brought about by modern technology. Traditional and molecular laboratory methods for diagnosing candidiasis are reviewed, with a discussion of PCR-based and non-PCR-based methods. The third section reviews pathogenicity, signalling pathways, and drug delivery systems that have the ability to accelerate the accumulation of drugs in different cutaneous layers. The final section considers antibiofilm activity against *Candida* spp., and the new molecules capable of preventing the formation of biofilms or eradicating them. This section also discusses changes in fungal physiology, host-pathogen interactions between cells and antibiotics during the treatment of COVID-19 infection using proteomic studies to identify proteins associated with fungi, and antifungal activity of propolis as an alternative natural treatment method for *Candida* species.

I would like to thank IntechOpen for offering me the opportunity to participate in this project, which could not have been successfully concluded without a strong support group. First of all, my husband and my daughter supported me with love and understanding. Thanks for your unwavering support. Second, I am grateful to all the authors and my colleagues around the world who have collaborated with me on this

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Section 1

Candida spp. Growth Development, Invasion Biology Drug Resistance Genes and Molecular Mechanisms

Chapter 1

Perspective Chapter: *Candida* and Candidiasis – Recent Taxonomic Developments, Invasion Biology, and Novel Active Compounds

Tulin Askun

Abstract

Candida spp. infections are most predominantly caused by *Candida albicans*, followed by *C. glabrata*, *C. parapsilosis* and *C. tropicalis*. *Candida* spp. can cause a wide range of serious infections. Recent studies indicate that this genus has approximately 200 species. Candidiasis is a fungal infection caused by *Candida* spp. Sexual reproduction gives eukaryotic organisms some advantages, such as producing adaptable fertility to changing environments and eliminating harmful mutations. Relationships between epithelial cells and *Candida* spp. include responses to medically important fungal pathogens. Infection by *C. albicans*, which has significantly high virulence due to its biofilm formation feature, is rather difficult to manage. Invasive candidiasis is a serious infection that can affect the blood, brain, eyes, bones, heart or other parts of the body. Understanding *C. albicans* invasion kinetics is crucial to controlling the pathogen's intrusion into the cells. New and effective antifungal compounds are needed due to the limited number and competence of antifungal agents. The search for natural compounds with anti-candidiasis effects continues increasingly.

Keywords: *Candida*, candidiasis, invasion, diversity, antifungal resistance, sexual reproduction

1. Introduction

1.1 *Candida* and Candidiasis

Candida spp. (a kind of fungus) are ubiquitous. Candidiasis is a fungal infection caused by *Candida* species, such as oropharyngeal candidiasis, oesophageal candidiasis, vulvovaginal candidiasis, candidal gingivostomatitis, intestinal candidiasis, invasive candidiasis, disseminated candidiasis and bloodstream infections. The rate of death in hospitalised patient with bloodstream infections are one in four. *Candida* can live naturally on the surface of the skin, in the oral cavity, vagina and intestine without causing any trouble. *C. albicans* lives commensally on mucous membranes in healthy people and causes widespread candidiasis in the digestive system in vulnerable people [1, 2].

Candidemia is a *Candida* bloodstream infection, a familiar form of invasive candidiasis which is a severe infection that can affect many parts of the body such as the eyes, bones, blood and brain [3]. *Candida* infections also occur in immunocompetent patients, primarily affecting their nails, scalp and skin (onychomycosis) [4, 5].

Most *Candida* infections (around 80%) in people are caused by *C. albicans*, though infections caused by other *Candida* spp. are becoming more and more frequent [6]. It has been shown that *C. subhashii* isolated from peritonitis is genetically closely related to *C. tropicalis*, *C. albicans* and *C. dubliniensis* [7]. Although *C. albicans* infections can be easily treated, their nosocomial nature translates to a high mortality rate [8]. Pappas et al. reported that higher than 250,000 people are affected by invasive candidiasis each year [9, 10]. According to the studies conducted by Ferrer [11] and Farr et al. [12], vulvovaginal candidiasis caused by *C. albicans* is seen in 75% of women of reproductive age, 5% of which experience recurrent infections.

In a study conducted on different genotypic distributions of *C. albicans* strains in oral cancers, it was shown that *C. albicans* genotype-A had a significantly higher frequency of occurrence in patients with oral cancer. *C. albicans* was classified into four different genotypes in this study based on their PCR amplification products. *C. albicans* genotype-A contained 450 bp. Similarly, other types comprised genotype-B (840 bp), genotype-C (450 and 840 bp) and genotype-D (1080 bp). The results of this study showed that *Candida* spp. that settles in the mouth may play a role in the formation of oral cancer [13]. *Candida* infections still have high mortality rates [14]. The reports showed that multidrug-resistant *C. auris* causes healthcare-associated outbreaks with high mortality rate [15–17].

1.2 History and taxonomic hierarchy

The genus *Candida* was created by Dutch mycologist Christine Berkhout (according to her thesis published in 1923) with nine species, and then, the genus *Monilia* was added [18]. However, Berkhout's definition of the genus was not found to be sufficiently distinctive and decisive. Thus, it was later edited by Lodder and Kreger-van Rij. The generic name *Candida* is based on *Pseudomonilia albomarginata*, published by Arthur Geiger which is presently called *Candida mesenterica* [19]. Subcultures of the strain type were kept as CBS-602 as per the Dutch Centraalbureau Voor Schimmelcultures.

According to Barnet [18], presumably, Pseudomonilia (named in a publication in 1910) was the first genus used for budding, filamentous and asexual yeast. Then, Langeron and Talice [20] classified the yeast into eight genera. Subsequently, Kurtzman and Robnett divided the family Saccharomycetaceae into 11 well-structured clades [21]. Diezmann et al. [22] separated three major clades within Saccharomycetales in a well-structured manner. According to this, Clade 1 arises with node 29, clade 2 arises at node 16 and clade 3 arises at node 4. Clade 1 comprised six *Candida* spp. (*C. albicans*, *C. dubliniensis*, *C. maltosa*, *C. tropicalis*, *C. viswanathii* and *C. parapsilosis*) and Lodderomyces elongisporus. The current taxonomic hierarchy is as shown below:

Fungi, Dikarya, Ascomycota, Saccharomycotina, Saccharomycetes, Saccharomycetidae, Saccharomycetales, Debaryomycetaceae, *Candida*/Lodderomyces clade, *Candida*, *Candida albicans* [23].

1.3 Diversity

Approximately, a quarter of all yeast species belong to the genus *Candida* [24]. Recent studies indicate that this genus has approximately 200 species [25]. *Candida*

spp. is very common in different environments as its vegetative cells proliferate by budding or forming pseudo or septate hyphae [26]. Krohn et al. [27] detected large numbers of *C. albicans* and *C. glabrata* and lesser numbers of *C. guilliermondii*, *C. lusitaniae*, *C. tropicalis*, *C. kefyr*, *C. krusei* and *C. rugosa* in the duodenal fluid of patients with liver cirrhosis.

Barnett et al. [28] identified 12 new species of *Candida* (They were *C. aaseri*, *C. albicans*, *C. atlantica*, *C. haemulonii*, *C. intermedia*, *C. maris*, *C. zeylanoides*, *C. maritima*, *C. norvegica*, *C. sake*, *C. torresii* and *C. tropicalis*) from the marine environment. Li-J et al. [29] isolated and identified a new yeast species, *C. pseudorugosa* sp. nov., from the sputum of an acute pneumonia patient. According to the sequence analysis of the 26S rRNA gene D1/D2 domain and the internal transcribed spacer (ITS) region, the new species was closely like *C. rugosa*. Therefore, they proposed the name *C. pseudorugosa* sp. nov. for the new species. Wang et al. [26] added six more species (*C. intermedia*, *C. parapsilosis*, *C. quercitrusa*, *C. rugosa*, *C. zeylanoides* and *C. membranifaciens*) as marine yeast species.

C. auris was isolated and first identified from the external ear canal of an inpatient in a Japanese hospital by Satoh et al. [30]. Afterwards, Oh et al. [31] investigated 27 isolates including *C. haemulonii* group I and *C. pseudohaemulonii* by sequencing their ITS region and D1/D2 regions of the 26S ribosomal DNA from blood samples and ear canal swabs were taken from 23 patients. As a result of the study, they identified 15 of 27 isolates as *C. auris* obtained from ear specimens.

Infection of some *Candida* species such as *C. famata*, *C. kefyr*, *C. lusitaniae* and *C. zeylanoides* is sporadic in the bloodstream and other systemic infections. These species are considerable because they might have antifungal-resistant isolates that are occasionally discovered among them and it is possible that these isolates might be misidentified by commercial yeast identification systems. DNA sequencing or MALDI methods are reliable in identifying these potential antifungal-resistant isolates. Some of these species are frequently reported using teleomorph genus names such as *Wickerhamomyces canadensis* (*C. melinii*) and *Debaryomyces hansenii* (*C. famata*) (**Table 1**). Yeast or fungi may exist in both teleomorph (sexual stage) or anamorph (asexual stage) stages [32]**.** This confusing situation caused the species to be classified into different genera (**Table 1**). Good example is *Kluyveromyces lactis* (sexual state) and *Candida sphaerica* (asexual state) [33, 34].

1.4 Sexual reproduction

Sexual reproduction in eukaryotes can take many different forms. It has been reported that sexual reproduction may be essential for pathogenic fungi to create genetically diverse populations under extremely different environmental conditions [50]. Sexual reproduction gives eukaryotic organisms some advantages such as producing adaptable fertility to changing environments, eliminating harmful mutations, providing favourable genetic change and increasing genetic diversity [51, 52].

Tao et al. [53] reported that during mating, white cells (WHCs) and opaque cells (OPCs), separated from each other by their function and appearance, show organised function. Researchers reported that *C. albicans* may contain these cell types, WHCs and OPCs which are functionally and morphologically different. They studied three configurations of the mating-type locus in *C. albicans*. These are locus MTLa/α, locus a/and locus α/α. Most natural isolates have heterozygosis at the mating-type locus [54]. *C. albicans* may often change between two distinct cell types, WHC and OPC, [55]. Before mating, *C. albicans* must first be homozygous at the mating-type locus and then

Anamorph	Teleomorph	References
Candida vini	*Kregervanrija fluxuum*	[35]
Candida melinii	*Wickerhamomyces canadensis*	[24]
Debaryomyces hansenii var. hansenii	*Pichia kudriavzevii*	[36]
Candida famata var. famata	*Debaryomyces hansenii var. hansenii*	[37]
Candida famata var. flareri	*Debaryomyces hansenii var. flareri*	[38]
Candida globosa	*Citeromyces matritensis*	[39]
Candida guilliermondii	*Pichia guilliermondii*	[40]
Candida krusei	*Issatchenkia orientalis*	[41]
Candida lambica	*Pichia fermentans*	[42]
Candida lipolytica	*Yarrowia lipolytica*	[42]
Candida lusitaniae	*Clavispora lusitaniae*	[39]
Candida nitrativorans	*Pichia sydowiorum*	[42]
Candida opuntiae	*Clavispora opuntiae*	[42]
Candida pulcherrima	*Metschnikowia pulcherrima*	[22]
Candida sphaerica	*Kluyveromyces lactis* var. *lactis*	[42]
Candida valida	*Pichia membranifaciens*	[35]
Candida globosa	*Citeromyces matritensis*	[39]
Clavispora lusitaniae	*Clavispora lusitaniae*	[43]
Candida famata	*Debaryomyces hansenii*	[39, 44]
Candida. homilentoma	*Hyphopichia homilentoma*	[45]
Candida utilis	*Cyberlindnera jadinii*	[45]
Candida kunwiensis	*Metschnikowia kunwiensis*	[45]
Candida deformans	*Yarrowia yakushimensis*	[45, 46]
Candida lipophila	*Wickerhamiella lipophila*	[44]
Candida molischiana	*Kuraishia molischiana*	[44]
Candida pignaliae	*Ogataea pignaliae*	[47]
Candida Molischian	*Kuraishia Molischiana*	[48]
Candida bornbicola	*Starmerella bombicola*	[49]
Candida sphaerica	*Kluyveromyces lactis*	[43]

Table 1.
Some of Candida anamorph and teleomorph names according to the recent taxonomic developments.

switch from WHC to OPC type because only OPC can mate efficiently [56]. WHC represents the majority cell population in nature. However, minority OPCs are capable of matching. In sexual reproduction, WHC secretes sexual pheromones to stimulate both cell types (OPC and WHC). To initiate mating, the presence of opaque cells, WHCs release sexual pheromones and consequently creates favourable conditions for OPCs to mate with both sexes. These OPC and WHC connect through a pheromone signalling system. This coupling of WHC and OPC is thought to be the key to the fungus being an evolutionarily compatible and successful pathogen in the host [53].

1.5 Invasion biology

C. albicans normally exists as a commensal microorganism in human gastrointestinal and genital tracts. Fungi use the advantages of anisotropic growth, thus offering it advantages in terms of nutrient acquisition, movement capability and niche colonisation and mating [57, 58].

1.5.1 Candida albicans and epithelial cell interaction

Moyes et al. [59] reveal that the relation between the fungal pathogen (*Candida* spp.) and epithelial cells (EPCs) contains the key to host responses to fungi. According to previous general views, epithelial cells were thought of as a static barrier against invading fungi. There was a widespread belief that epithelial cells provided both an attachment for colonisation and a food source for invading fungi. However, in the light of recent studies, this view has changed significantly. It is now known that epithelial cells play a more active role in the differentiation of commensal and pathogen, immunity and damage mending. The interaction of *C. albicans* with epithelial cells proceeds as follows. (i) It attaches to epithelial cells of *C. albicans*, (ii) the fungus is recognised by EPCs, (iii) induction of endocytosis is initiated by the fungus, and (iv) *C. albicans* was taken into the cell and then initiates early apoptotic events. These events damage the epithelial cell. Thus, it is protected from phagocytosis. Endocytosis is induced by the interaction of *C. albicans* Als3 adhesive with E-cadherin in epithelial cells and with N-cadherin in endothelial cells [59].

In the light of recent research, the stages of *C. albicans* invasion are as follows: (1) A series of signalling circuits are initiated when *C. albicans* adheres to the EPCs, (2) certain morphological *Candida* species are recognised by the EPCs and endocytosis is initiated by the host cell, and 3) to escape from phagocytosis, early apoptotic events are initiated by *Candida* and this damages the EPCs. Early recognition events are important to reduce some of the damage at this early stage (**Figure 1**) [60, 61].

C. albicans is a dimorphic fungus that can be in yeast form or in the form of hyphae (germ tube, we mentioned as hyphae here). The first adhesion takes place through the yeast form. However, *C. albicans* can also be in the form of hyphae [62, 63]. It provides enhanced bonding, especially using surface portions expressed in the hyphal form. Thus, *C. albicans* hyphae adhere more strongly to ECs than to yeast cells [64, 65].

Early recognition events are important to alleviate some of the damage in this early step. The host-fungus interactions in EPCs have increased over time, and more research and information are needed in this regard. It is now known that EPCs are an important part of the host reaction mechanisms against fungal infections.

When *C. albicans* infected the host epithelial cells, the initial contact of the adherence including colonisation and invasion of fungal cells start. Many factors play a role in this process. In this step, cell-to-cell adherence occurs *via* epithelial receptors and *Candida* adhesins [66]. E-cadherin coexists with clathrin around hyphae endocytosed by epithelial cells [67]. Therefore, the fungus hyphae enter the epithelial cell.

The Als family, an adhesin family, has an important role in epithelial attachment. Als3 is a key hypha-specific protein [61, 66]. Furthermore, Als3 is one of the *C. albicans* invasins, which can induce endocytosis. It attaches to host cell receptors such as E-cadherin and N-cadherin and stimulates the host cells to endocytose the organism [68]. In the first step, adhesins such as Als3p bind to their target cellular receptors

or covalently bind to the host cell surface. In the second step, *C. albicans* invasins interact with target host receptors and initiate the activation of these receptors [59]. E-cadherin and actin microfilaments are proteins belonging to the septin family.

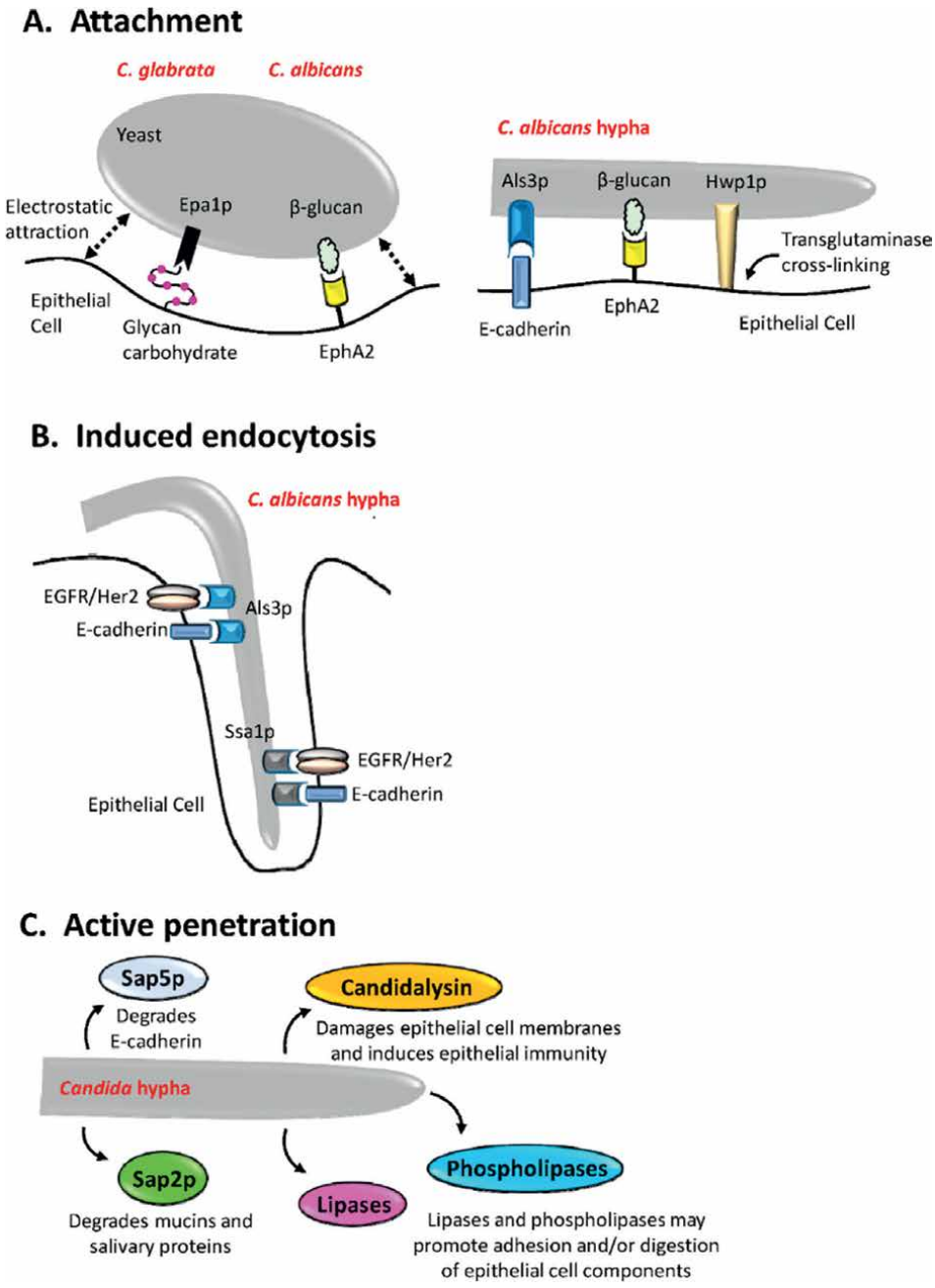

Figure 1.
Interactions of C. albicans *with host epithelial cells. (A)* C. albicans *binds to host epithelial cells in various ways via EphA2 and E-cadherin receptors or directly via transglutaminases. (B)* C. albicans *invasins interact with E-cadherin receptors to induce endocytosis. (C)* C. albicans *and some* Candida *species can reach mucosal tissues by secreting lipase, phospholipase, proteinases and secreted aspartic peptidases such as Sap2p and Sap5p. This figüre reproduced with permission of the authors [65].*

The septin family proteins are important as cytoskeletal elements for cell division in budding yeast. Septins play a key role in anchoring cell surface proteins of the specific regions of the cell membrane [69].

1.5.2 Candida albicans *and endothelial cell interaction*

C. albicans enter and invade endothelial cells by binding to N-(neural) cadherin (a transmembrane protein) and other cell surface receptors, acting as a mediator of cell–cell adhesion and affecting a range of biological activities (**Figure 2**) [70, 71]. Septin-7 is a filament-forming cytoskeletal GTPase and the septin family of proteins includes N-cadherin and actin microfilaments. Septins bind cell surface proteins to specific regions of the cell membrane in a particular way [70, 72].

Phan et al. [63, 73] investigated the accumulation of N-cadherin, SEPT7, and both N-cadherin and SEPT7 when yeast was added to endothelial cells. They showed that cells in the yeast phase germinated 30 minutes after adhering to the endothelium and were enveloped by SEPT7 after 60 minutes and they were surrounded by both SEPT7 and N-cadherin after 90 minutes. After this stage, endocytosis occurs in EPCs.

EPCs produce many cytokines such as GM-CSF, G-CSF, IL-1a, IL-1b and IL-6 in response to the presence of the fungus along with RANTES and IL-8. Today, the function of EPCs in terms of *C. albicans* is better understood. When *C. albicans* interact with EPCs, it turns into a dynamically reactive protector [59, 72]. It also produces antimicrobial peptides such as cathelicidin and b-defensins [74]. Adherence and recognition of *C. albicans* by EPCs result in cytoskeletal reorganisation. Neutrophils have great importance in epithelial anti-*Candida* defence. They release the secreting factors that stimulate EPCs preservation towards damage in a TLR4-dependent manner [75]. *Candida* can inactivate the antiapoptotic proteins in macrophages and neutrophils. Thus, it can cause apoptosis in EPCs. Furthermore, infection of EPCs by *C. albicans* causes premature initiation of apoptosis and then necrotic death [76, 77].

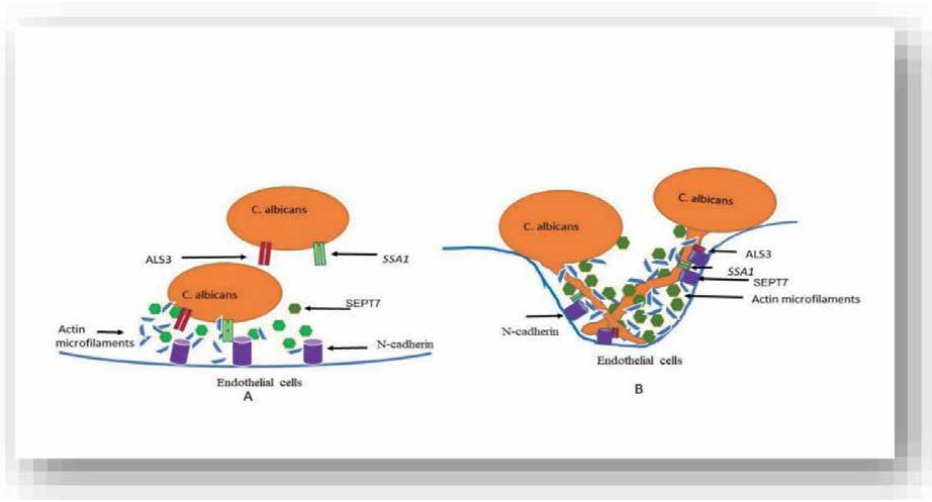

Figure 2.
Localization on the endothelial cell surface and endocytosis of C. albicans*. (A) Adhesion: At this stage, Als3 and Ssa1* C. albicans *proteins bind to endothelial cell-mediated N-cadherin. (B) Endothelial cell invasion: At this stage, SEPT7 and actin filaments gather around the hyphae.*

1.6 Antifungal resistance

C. albicans has very low levels of drug resistance; however, other types of *Candida*, such as *C. glabrata*, might be mostly resistant and more deadly. The most clinically important *Candida* spp. are *C. albicans*, *Candida parapsilosis* and *C. glabrata* [78].

There are various reports on fungal resistance mechanisms of several *Candida* spp. against fluconazole, itraconazole, voriconazole and several other azole drugs [79–81]. In a study, 27 mutations in the ERG11 gene were identified in azole-resistant *C. albicans* isolates. It is thought that these mutations may increase resistance to azole drugs and may be associated with the recurrence of vulvovaginal candidiasis [82].

Centers for Disease Control and Prevention reported that *Corynebacterium auris* is resistant to multiple antifungal drugs typically used to treat *Candida* infections, and identification of this species needs specific technology; therefore, standard laboratory methods are insufficient, and early identification and transmission prevention of *C. auris* for patients staying in the hospital is of great importance. Thus, it is possible to take precautions. Considering the above, it presents a dangerous global health threat. As a result of recent research, three classes of antifungals (azoles, echinocandins and amphotericin B) are found efficient against *Candida* spp. [17, 83].

Dagi et al. [84] tested 200 *Candida* strains isolated from bloodstream infections for drug resistance tests. The strains were *C. albicans*, *C. parapsilosis* complex and *C. glabrata* (47.5%, 14.0% and 18.0%, respectively). Except for *C. kefyr* strains, 11 *Candida* spp. were susceptible to amphotericin-B at an MIC value of 2 μg/mL. *C. glabrata* strains was resistant to fluconazole at MIC value ≥64 μg/mL. Others showed concentration-dependent susceptibility. The low MIC value of *C. pseudoaaseri* (0.016–1 μg/mL) against all types of antifungal drugs except flucytosine distinguished this species from *C. aaseri* with generally susceptible MICs between ≥0.008 and 0.5 μg/mL [7, 85]. *Candida auris* had a low MIC to echinocandin drugs (≤0.5 mg/L) and showed a close phylogenetic relationship to *C. haemulonii*; furthermore, resistance to azole, amphotericin B (AmB) and echinocandin had been reported in the species as well [7, 8].

In a recent study, Soliman et al. [86] investigated a green approach to control the proliferation of the 60-*Candida* species obtained from clinical samples. Tested *Candida* isolates were identified as *C. tropicalis*, *C. albicans*, *C. parapsilosis*, *C. krusei* and *C. glabrata*. *P. chrysogenum* was used as a biocatalyst for synthesising silver (Ag) nanoparticles. To do this, the metabolites of *P. chrysogenum* were used to reduce $AgNO_3$ to Ag-nanoparticles. The susceptibility test of *Candida* isolates to synthesise Ag-nanoparticles, fluconazole and amphotericin B was assessed. The 60 *Candida* isolates were found highly susceptible to Ag nanoparticles. Sensitivity to fluconazole and amphotericin B was 41.6% and 50.0%. This study shows very promise in eradicating *Candida* resistance.

Tan et al. [87] investigated how b-lactam antibiotics induce the spread of *C. albicans* in the gut. In their research, *C. albicans*, kept under pressure in the gastrointestinal tract of a healthy person, causes intensely released peptidoglycan production by autolysis of Gram (−) cells in the patients after treatment with b-lactam antibiotic and induces *C. albicans* hyphae production. They reported that the proliferation in hyphal cells causes penetration into mucosal barriers.

1.7 Identification and genetic structure of Candida

There are several methods using molecular technology for *Candida* spp. typing. These include the following methods: restriction fragment length polymorphism

analysis (RFLP), multilocus sequence typing (MLST), random amplified polymorphic DNA analysis (RAPD) and electrophoretic karyotyping [88, 89].

Studies for the correct identification of *Candida* spp., are ongoing. Arastehfar et al. [90] reported that they developed a precise, distinctive, saving cost and time, integrated and reliable test that can be incorporated into clinical laboratories without laborious DNA extraction steps. Using this method, they succeeded in distinguishing nine medically important complex species using a one-step multiplex PCR technique. The three cryptic *Candida* complex species found in samples were obtained from Iran (n = 135) and China (n = 145), which were *C. albicans* complex (*C. dubliniensis,* C. Africana*,* and *C. albicans*), *C. glabrata* complex (*C. bracarensis*, *C. nivariensis,* and *C. glabrata*) and C. parapsilosis complex (*C. metapsilosis*, *C. parapsilosis*, and *C. orthopsilosis*).

On the other hand, Al-Obaid et al. [91] examined 63 *C. tropicalis* strains identified by Vitek-2 and PCR isolated from different samples such as blood, respiratory tract, digestive tract and wound. They recorded 59 diploid-sequence-types (DST) with MLST. The study showed that most *C. tropicalis* isolates originated from diverse and unique strains. That is because they reported that 56 of the isolates from 48 patients were unique.

In MLST sequencing, various housekeeping genes (HKGs) of species responsible for infectious diseases are used to define DNA sequence polymorphisms between isolates. This technique has the advantage of providing information about the species' geographical origins and anatomical sources [92–93]. *Candida* spp. infections are most predominantly caused by *C. albicans*, followed by *C. glabrata*, *C. parapsilosis* and *C. tropicalis* [94].

Muñoz et al. [95] described the epidemiological profiles and the population structure of *C. albicans* by analysing the *C. albicans* MLST database. Therefore, they verified the general nature of *C. albicans* based on approximately 4300 database isolates with the inclusion of a group of DSTs from people. Some of them were exclusively healthy. The DST counts obtained from blood, oral and vaginal swabs were 32.4, 20.5 and 13.8, respectively. MLST was designed to allow the identification of unique DSTs based on nucleotide-polymorphisms in multiple HGKs. Selected HGK were those that encode ATP-dependent permease, aspartate aminotransferase, mannose phosphate isomerase, acetyl-CoA carboxylase and alanyl RNA synthetase [96]. They reported isolating the highest number of DSTs from blood (32.4%), oral swabs (20.5%) and vaginal swabs (13.8%). They described seven HGKs involved in the MSLT scheme with the highest genetic diversity.

1.8 Novel active compounds for candidiasis treatment

Its biofilm-forming ability makes it difficult to struggle and manage the highly virulent *C. albicans* infection. Therefore, studies for identifying effective novel compounds are ongoing. Some of the promising novel compounds reported during 2010–2020 are as follows. Nieminen et al. [97] reported the potent effects of D,L-2-hydroxyisocaproic acid on biofilm formation. They used XTT ((2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) to measure biofilm metabolic activity and assessed the results using a biomass marker. Biofilms were monitored using scanning electron microscopy (SEM), and they recorded the formation of abnormal, collapsed hyphal structures on incubation with D,L-2-hydroxyisocaproic acid at acidic pH.

Wong et al. [98] reported a new antifungal molecule called SM21 with a very potent MIC (0.2–1.6 μg/mL) against *Candida* infections. This molecule was toxic to fungi and was effective against biofilms of *Candida* species. They also stated that SM21 was effective in reducing tongue lesions in rats in oral candidiasis and suggested that it may have potential as a new antifungal agent. Oh et al. [31] observed the isolates of *C. auris*, *C. haemulonii* and *C. pseudohaemulonii* in terms of creating plaques *in vitro* in glucose-containing Sabouraud dextrose broth and deduced that all *C. auris* isolates were biofilm-negative while all isolates of *C. haemulonii* and *C. pseudohaemulonii* formed biofilms. They also arrive at a judgement that *C. pseudohaemulonii* formed a biofilm to induce central venous catheter-related fungemia in patients. Alam et al. [99] developed a novel noisome-based diallyl disulphide, an effective nanocarrier system, to treat disseminated *Candida* infection in murine. They showed that diallyl disulphide-loaded niosomes complex decreased the fungal cells and increased the life of cell tissue in contrast to the free formulation. Pałkowski et al. [100] investigated the structure–activity relationship of gemini imidazolium compounds using the chemical structure and surface-active properties and antifungal activity against *C. albicans* ATCC 90028 strain. They remarked that antifungal activity depends not only on the surface-active properties of the compound but also on the substituent type and the position at the chloride moiety of substituents. Szafrański et al. [101] synthesised novel 4-substituted N- pyridine-3-sulfonamides and converted them to triazole derivatives; then, they tested them against *Candida* spp. isolated from patients and found *C. albicans* strains, to be highly sensitive to the tested compounds. According to the docking study, based on inhibition of the cytochrome P-450-dependent lanosterol 14α-demethylase, the most active three compounds binding to *C. albicans* were determined as N-phenylpiperazine, pyrazole, and an alkylthio moiety of the compounds. Another study on novel compounds was done by Lino et al. [102]. They synthesised a novel series of 15 hydrazine-thiazole derivatives and tested the efficacy on six *Candida* spp. They reported that while some of the derivatives exhibited activity at the minimum inhibitory concentration MIC of 0.45–31.2 mM, some of them showed comparable or higher activity than standard drugs.

1.9 Natural compounds

Natural compounds effective on fungi are generally phenolic and are obtained from edible plants. Kim et al. [103] asserted that natural products increased the *in vitro* activity of fluconazole against strains of resistant filamentous fungi. They showed that cinnamic acid, benzoic acid, salicylic acid, thymol and 2,5- and 2,3-dihydroxybenzaldehyde exerted mainly additive or synergistic effects against fungal growth [104]. Conversely, the search for natural products with antifungal activities against *Candida* spp. and the search for natural molecules that can treat candidiasis are ongoing. Zida et al. [105] classified the compounds according to MICs and MFCs as given by the other authors. They classified 40 of 142 phytochemicals as significant according to their MIC values <100 μg/mL, and 24 of 142 showed moderate activity with MIC values between 100 and 625 μg/mL. In this group, ascosterosides from *Ascotricha amphitricha* and papulacandin-A from *Papularia sphaerosperma* exhibited the strongest activity with a MIC value of 0.1 μg/mL. Minooeianhaghighi et al. [106] investigated the efficacy of some essential oils (*Lavandula binaludensis* and *Cuminum cyminum*) against pathogenic *Candida* spp., and treatments for recurrent vulvovaginal candidiasis. They identified the oil components by comparing their mass spectra against the GC–MS library as well as using the existing literature. They determined

13 components using GC–MS analyses. The main components of *C. cyminum* and *L. binaludensis* essential oils were g-terpinene and 1,8-cineole (21.07%, 71.56%, respectively). They reported that *C. cyminum* (MIC 8.00 mg/ML) and *L. binaludensis* oils MIC 7.91 mg/mL) showed inhibitory activity. *L. binaludensis* inhibited 80% of *C. albicans* vaginal strains at a concentration of 7.81 mg/mL ($P < 0.05$). They reported that essential oils could be used as natural therapeutic inhibitors to prevent or limit the growth of the most significant pathogenic *Candida* species and against recurrent vulvovaginal candidiasis. In another study, Marangoni et al. [107] studied a blue-green alga, *Spirulina platensis* against 22 strains of *Candida* spp. (*C. albicans*, *C. glabrata*, *C. lusitaniae, C. tropicalis*, *C. krusei*, *C. Parapsilosis*, *C. Guillermondii*, among others). Faria et al. [108] examined the fungicidal activity of 12 natural phenolics against nine reference strains of *Candida* (*C. albicans* (3 strains), *C. parapsilosis* (2 strains), *C. glabrata, C. tropicalis, C. krusei* and *C. lusitaniae*). They showed that cinnamic acid, benzoic acid, salicylic acid, thymol and 2,5- and 2,3- dihydroxybenzaldehyde had mainly additive or synergistic efficacy against *C. albicans*.

1.10 Interactions between *Candida* spp. and cancer development

Some findings suggest a relationship between candidiasis and cancer. Ramirez-Garcia et al. [109] investigated the role of candidiasis in oral and oesophageal cancers. For this purpose, they studied oral *Candida* carriage by working with 52 oral cancer patients and 104 non-oral cancer subjects. The data obtained from the study showed that there is a significant relationship between the *Candida* species colonising the mouth in the formation of oral cancer. Li-D et al. [110] examined 207 invasive cancer patients. Patients with recurrent invasive candidiasis and patients with multiple infections were not included in this group. In their study, they detected 28% of deaths in 30 days. Invasive candidiasis was diagnosed based on the isolation of *Candida* species from the bloodstream. The rate of *Candida* species they obtained was as follows: *C. albicans* (48.3%), *C. glabrata* complex (24.2%) and *C. tropicalis* (10.1%), respectively. The results indicate that there may be a relationship between death cases and invasive *Candida* species. In another study performed by Choi et al. [111], they investigated the incidence of *Candida* infection in cancer patients (n = 17.797) and the risk of mortality in patients with *Candida*-infected cancer. Identified *Candida* species were 634, of which 75 had concerned bloodstream infection. The striking results were the high rate of *C. albicans* infections (85.8%) in the patients hospitalised in the intensive care unit.

2. Concluding remarks

Candida spp. contain the most common human fungal pathogens and *C. albicans* is a commensal inhabitant of the human mouth, gastrointestinal and genital region. *Candida* can weaken the mucosa and cause fatal conditions in situations such as the inadequacy of the host immunity or the presence of implanted medical devices. Immunocompromised patients can easily be exposed to *Candida*-related diseases. Therefore, the research and development of new anti-*Candida* drug active molecules are of great importance. There are various reports on fungal resistance mechanisms of several *Candida* spp. against fluconazole, itraconazole, voriconazole and several azole compounds. Natural compounds such as flavonoids contain a wide variety of biologically active compounds. Due to reasons such as low toxicity and rare side effects, they

have the advantage of potential usage. The synthesis of new compounds effective in *Candida* biofilm formation continues increasingly all over the world. On the other hand, the relationship between *Candida* and cancer is still somewhat blurred. More research is needed on whether *Candida* genus members cause cancer.

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References

[1] CDC, Centers for Disease Control and Prevention. 2019. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DFWED). Available from: https://www.cdc.gov/fungal/diseases/candidiasis/index.html. [Accessed: July 15, 2020]

[2] Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, et al. Antibiotic exposure as a risk factor for fluconazole-resistant Candida bloodstream infection. Antimicrobial Agents and Chemotherapy. 2012;**56**(5):2518-2523. DOI: 10.1128/AAC.05947-11

[3] Kullberg BJ, Arendrup MC. Invasive Candidiasis. The New England Journal of Medicine. 2015;**373**(15):1445-1456. DOI: 10.1056/NEJMra1315399

[4] Fortuna MC, Garelli V, Pranteda G, Carlesimo M, D'Arino A, Rossi A. Scalp infection by Candida Albicans in an immunocompetent patient: A description of a rare case. Journal of Chemotherapy (Florence, Italy). 2018;**30**(5):316-317

[5] Ge G, Li D, Mei H, Lu G, Zheng H, Liu W, et al. Different toenail onychomycosis due to *Rhodotorula mucilaginosa* and *Candida parapsilosis* in an immunocompetent young adult. Medical Mycology Case Reports. 2019;**24**:69-71. DOI: 10.1016/j.mmcr.2019.04.012

[6] Mark HB, Roberts SP. The Merck Manual of Diagnosis and Therapy. 18th ed. Merck Sharp & Dohme Corp: Kenilworth, NJ, USA; 2016

[7] Brandt ME, Lockhart SR. Recent taxonomic developments with *Candida* and Other opportunistic yeasts. Current Fungal Infection Reports. 2012;**6**(3):170-177. DOI: 10.1007/s12281-012-0094-x

[8] Ruiz-Gaitán AC, Cantón E, Fernández-Rivero ME, Ramírez P, Pemán J. Outbreak of Candida auris in Spain: A comparison of antifungal activity by three methods with published data. International Journal of Antimicrobial Agents. 2019;**53**(5):541-546. DOI: 10.1016/j.ijantimicag.2019.02.005

[9] Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nature Reviews Disease Primers. 2018;**4**(1):18026. DOI: 10.1038/nrdp.2018.26

[10] Ciurea CN, Kosovski IB, Mare AD, Toma F, Pintea-Simon IA, Man A. *Candida* and Candidiasis-opportunism versus pathogenicity: A review of the virulence traits. Microorganisms. 2020;**8**(6):857. DOI: 10.3390/microorganisms8060857

[11] Ferrer J. Vaginal candidosis: epidemiological and etiological factors. International Journal of Gynecology & Obstetrics. 2000;**71**(SUPPL. 1):21-27. DOI: 10.1016/S0020-7292(00)00350-7

[12] Farr A, Effendy I, Frey Tirri B, Hof H, Mayser P, Petricevic L, et al. Guideline: Vulvovaginal candidosis (AWMF 015/072, level S2k). Mycoses. 2021;**64**(6):583-602. DOI: 10.1111/myc.13248

[13] Alnuaimi AD, Wiesenfeld D, O'Brien-Simpson NM, Reynolds EC, McCullough MJ. Oral Candida colonization in oral cancer patients and its relationship with traditional

risk factors of oral cancer: A matched case-control study. Oral Oncology. 2015;**51**(2):139-145. DOI: 10.1016/j.oraloncology.2014.11.008

[14] Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infection Control and Hospital Epidemiology. 2013;**34**(1):1-14. DOI: 10.1086/668770

[15] Caceres DH, Rivera SM, Armstrong PA, Escandon P, Chow NA, Ovalle MV, et al. Case-case comparison of *Candida* auris versus other *Candida* species bloodstream infections: Results of an outbreak investigation in Colombia. Mycopathologia. 2020;**185**(5):917-923. DOI: 10.1007/s11046-020-00478-1

[16] Chowdhary A, Sharma C, Meis JF. *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathogens. 2017;**13**(5):e1006290. DOI: 10.1371/journal.ppat.1006290

[17] Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, GovenderNP,etal.Simultaneousemergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clinical Infectious Diseases. 2017;**64**(2):134-140. DOI: 10.1093/cid/ciw691

[18] Barnett JA. A history of research on yeasts 8: Taxonomy. Yeast (Chichester, England). 2004;**21**(14):1141-1193. DOI: 10.1002/yea.1154

[19] Geiger A. Beiträge zur Kenntnis der Sprosspilze ohne Sporenbildung. *Centralblatt f¨ur Bakteriologie*. *Parasitenkunde und Infektionskrankheiten*, Zweite Abteilung. 2010;**27**:97-149

[20] Langeron M, Talice R-V. Nouvelles m'ethodes d'´etude et essai de classification des champignons levuriformes. Annales de Parasitologie Humaine et Comparee. 1932;**10**:1-80

[21] Kurtzman CP. Phylogenetic circumscription of saccharomyces, Kluyveromyces and other members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma and Zygotorulaspora. FEMS Yeast Research. 2003;**4**(3):233-245. DOI: 10.1016/S1567-1356(03)00175-2

[22] Diezmann S, Cox CJ, Schönian G, Vilgalys RJ, Mitchell TG. Phylogeny and evolution of medical species of *Candida* and related taxa: A multigenic analysis. Journal of Clinical Microbiology. 2004;**42**(12):5624-5635. DOI: 10.1128/JCM.42.12.5624-5635.2004

[23] NCBI. Database resources of the National Center for Taxonomy Browser. 2022. Available from: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=1094981&lvl=3&lin=f&keep=1&srchmode=1&unlock

[24] Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for isolation, phenotypic characterization and maintenance of yeasts. In: InKurtzman CP, Fell JW, Boekhout T, editors. The Yeasts, a Taxonomic Study. 5th ed. Amsterdam, the Netherlands: Elsevier; 2011. pp. 87-110. DOI: 10.1016/B978-0-444-52149-1.00002-1

[25] Hizlisoy H, Onmaz NE, Al S, Yildirim Y, Gonulalan Z, Gumussoy KS, et al. Clonal diversity and antifungal susceptibility of *Candida* spp. recovered

from cow milk. Mljekarstvo. 2020;**70**:40-50. DOI: 10.1002/0470015330.ch7

[26] Wang L, Chi Z, Yue L, Chi Z, Zhang D. Occurrence and diversity of *Candida* genus in marine environments. Journal of Ocean University of China. 2008;**7**(4):416-420. DOI: 10.1007/s11802-008-0416-3

[27] Krohn S, Zeller K, Böhm S, Chatzinotas A, Harms H, Hartmann J, et al. Molecular quantification and differentiation of *Candida* species in biological specimens of patients with liver cirrhosis. PLoS One. 2018;**13**(6):e0197319. DOI: 10.1371/journal.pone.0197319

[28] Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and Identification. 3rd ed. Cambridge, England: Cambridge University Press; 2000. pp. 128-283

[29] Li J, Xu YC, Bai FY. *Candida pseudorugosa* sp. nov., a novel yeast species from sputum. Journal of Clinical Microbiology. 2006;**44**(12):4486-4490. DOI: 10.1128/JCM.01391-06

[30] Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiology and Immunology. 2009;**53**(1):41-44. DOI: 10.1111/j.1348-0421.2008.00083.x

[31] Oh BJ, Shin JH, Kim MN, Sung H, Lee K, Joo MY, et al. Biofilm formation and genotyping of *Candida* haemulonii, *Candida pseudohaemulonii*, and a proposed new species (*Candida auris*) isolates from Korea. Medical Mycology. 2011;**49**(1):98-102. DOI: 10.3109/13693786.2010.493563

[32] Hawksworth DL, Kirk PM, Sutton BC, Pegler DN. Ainsworth & Bisby's Dictionary of the Fungi. ed. 8 ed. Wallingford, UK: CAB International; 1995. p. 616

[33] Martini AV, Martini A. Taxonomic revision of the yeast genus Kluyveromyces by nuclear deoxyribonucleic acid Reassociation. International Journal of Systematic Bacteriology. 1987;**37**(4):380-385. DOI: 10.1099/00207713-37-4-380

[34] Hennebert GL, Weresub LK. Terms for states and forms of fungi, their names and types. Mycotaxon. 1977;**6**(1):207-211

[35] Kačániová M, Kunová S, Sabo J, Ivanišová E, Žiarovská J, Felsöciová S, et al. Identification of yeasts with mass spectrometry during wine production. Fermentation. 2020;**6**(1):5. DOI: 10.3390/fermentation6010005

[36] Nagarathnamma T, Chunchanur SK, Rudramurthy SM, Vineetha KR, Ramamurthy K, Joseph J, et al. Outbreak of Pichia kudriavzevii fungaemia in a neonatal intensive care unit. Journal of Medical Microbiology. 2017;**66**(12):1759-1764. DOI: 10.1099/jmm.0.000645

[37] Lodder J, Kreger-van Rij NJW. The Yeasts. A Taxonomic Study. Amsterdam: North-Holland Publishing Co; 1952. pp. 365-366

[38] Nguyen HV, Gaillardin C, Neuvéglise C. Differentiation of *Debaryomyces hansenii* and *Candida famata* by rRNA gene intergenic spacer fingerprinting and reassessment of phylogenetic relationships among *D. hansenii, C. famata, D. fabryi, C. flareri* (=*D. subglobosus*) and *D. prosopidis*: Description of D. vietnamensis sp. nov. closely related to D. nepalensis. FEMS Yeast Research. 2009;**9**(4):641-662. DOI: 10.1111/j.1567-1364.2009.00510.x

[39] Suzuki M, Nakase T. A phylogenetic study of ubiquinone-7 species of the genus *Candida* based on 18S ribosomal DNA sequence divergence. The Journal of General and Applied Microbiology. 2002;**48**(1):55-65. DOI: 10.2323/jgam.48.55

[40] Sullivan DJ, Henman MC, Moran GP, O'Neill LC, Bennett DE, Shanley DB, et al. Molecular genetic approaches to identification, epidemiology and taxonomy of non-albicans *Candida* species. Journal of Medical Microbiology. 1996;**44**(6):399-408. DOI: 10.1099/00222615-44-6-399

[41] Mohammadi K, Safari R, Khalesi M, Sajadi M, Bahri AH. Effects of Issatchenkia orientalis (*Candida krusei*) on aflatoxins in culture media and kilka fish meal. Toxin Reviews. 2018;**37**(1):35-38. DOI: 10.1080/15569543.2017.1313275

[42] Latouche GN, Daniel HM, Lee OC, Mitchell TG, Sorrell TC, Meyer W. Comparison of use of phenotypic and genotypic characteristics for identification of species of the anamorph genus *Candida* and related teleomorph yeast species. Journal of Clinical Microbiology. 1997;**35**(12):3171-3180. DOI: 10.1128/jcm.35.12.3171-3180.1997

[43] Desnos-Ollivier M, Moquet O, Chouaki T, Guérin A-M, Dromer F. Development of Echinocandin resistance in Clavispora lusitaniae during Caspofungin treatment. Journal of Clinical Microbiology. 2011;**49**(6):2304-2306. DOI: 10.1128/JCM.00325-11

[44] Wagner D, Sander A, Bertz H, Finke J, Kern WV. Breakthrough invasive infection due to Debaryomyces hansenii (teleomorph *Candida famata*) and Scopulariopsis brevicaulis in a stem cell transplant patient receiving liposomal amphotericin B and caspofungin for suspected aspergillosis. Infection. 2005;**33**(5-6):397-400. DOI: 10.1007/s15010-005-5082-4

[45] Ribeiro LR, Santos A, Groenewald M, Smith M, Lara CA, Góes-Neto A, et al. Description of Hyphopichia buzzinii f.a., sp. nov. and Hyphopichia homilentoma comb. nov., the teleomorph of *Candida homilentoma*. Antonie Van Leeuwenhoek. 2017;**110**(7):985-994. DOI: 10.1007/s10482-017-0870-2

[46] Groenewald M, Smith MT. The teleomorph state of *Candida deformans* Langeron & Guerra and description of Yarrowia yakushimensis comb. nov. Antonie Van Leeuwenhoek. 2013;**103**(5):1023-1028. DOI: 10.1007/s10482-013-9882-8

[47] Péter G, Tornai-Lehoczki J, Dlauchy D. *Ogataea pignaliae* sp. nov., the teleomorph of *Candida pignaliae*. International Journal of Systematic and Evolutionary Microbiology. 2010;**60**(Pt 10):2496-2500. DOI: 10.1099/ijs.0.019174-0

[48] Péter G, Dlauchy D, Tornai-Lehoczki J, Kurtzman CP. *Kuraishia molischiana* sp. nov., the teleomorph of *Candida molischiana*. Antonie Van Leeuwenhoek. 2005;**88**(3-4):241-247. DOI: 10.1007/s10482-005-7267-3

[49] Rosa CA, Lachance MA. The yeast genus Starmerella gen. Nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. International Journal of Systematic Bacteriology. 1998;**48**(Pt 4):1413-1417. DOI: 10.1099/00207713-48-4-1413

[50] Heitman J. Sexual reproduction and the evolution of microbial pathogens. Current Biology: CB. 2006;**16**(17):R711-R725. DOI: 10.1016/j.cub.2006.07.064

[51] Barton NH, Charlesworth B. Why sex and recombination? Science (New York, N.Y.). 1998;**281**(5385):1986-1990

[52] Otto SP, Lenormand T. Resolving the paradox of sex and recombination. Nature Reviews. Genetics. 2002;**3**:252-261

[53] Tao L, Cao C, Liang W, Guan G, Zhang Q, Nobile CJ, et al. White cells facilitate opposite- and same-sex mating of opaque cells in *Candida albicans*. PLoS Genetics. 2014;**10**(10):e1004737. DOI: 10.1371/journal.pgen.1004737

[54] Lockhart SR, Pujol C, Daniels KJ, Miller MG, Johnson AD, Pfaller MA, et al. In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics. 2002;**162**(2):737-745. DOI: 10.1093/genetics/162.2.737

[55] Slutsky B, Staebell M, Anderson J, Risen L, Pfaller M, Soll DR. "white-opaque transition": A second high-frequency switching system in *Candida albicans*. Journal of Bacteriology. 1987;**169**(1):189-197. DOI: 10.1128/jb.169.1.189-197.1987

[56] Miller MG, Johnson AD. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell. 2002;**110**(3):293-302. DOI: 10.1016/s0092-8674(02)00837-1

[57] Desai JV. *Candida albicans* hyphae: From growth initiation to invasion. Journal of fungi (Basel, Switzerland). 2018;**4**(1):10. DOI: 10.3390/jof4010010

[58] Harris SD. Hyphal morphogenesis: An evolutionary perspective. Fungal Biology. 2011;**115**:475-484

[59] Moyes DL, Richardson JP, Naglik JR. *Candida albicans*-epithelial interactions and pathogenicity mechanisms: Scratching the surface. Virulence. 2015;**6**(4):338-346. DOI: 10.1080/21505594.2015.1012981

[60] Naglik JR, Moyes DL, Wächtler B, Hube B. *Candida albicans* interactions with epithelial cells and mucosal immunity. Microbes and Infection. 2011;**13**(12-13):963-976. DOI: 10.1016/j.micinf.2011.06.009

[61] Zhu W, Filler SG. Interactions of *Candida albicans* with epithelial cells. Cellular Microbiology. 2010;**12**(3):273-282. DOI: 10.1111/j.1462-5822.2009.01412.x

[62] Korting HC, Hube B, Oberbauer S, Januschke E, Hamm G, Albrecht A, et al. Reduced expression of the hyphal-independent *Candida albicans* proteinase genes SAP1 and SAP3 in the efg1 mutant is associated with attenuated virulence during infection of oral epithelium. Journal of Medical Microbiology. 2003;**52**(Pt 8):623-632. DOI: 10.1099/jmm.0.05125-0

[63] Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, et al. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biology. 2007;**5**(3):e64. DOI: 10.1371/journal.pbio.0050064

[64] Sandin RL, Rogers AL, Patterson RJ, Beneke ES. Evidence for mannose-mediated adherence of *Candida albicans* to human buccal cells in vitro. Infection and Immunity. 1982;**35**(1):79-85. DOI: 10.1128/iai.35.1.79-85.1982

[65] Richardson JP, Ho J, Naglik JR. *Candida*-epithelial interactions. Journal of Fungi (Basel, Switzerland). 2018;**4**(1):22. DOI: 10.3390/jof4010022

[66] Chaffin WL. *Candida albicans* cell wall proteins. Microbiology and

Molecular Biology Reviews: MMBR. 2008;**72**(3):495-544. DOI: 10.1128/MMBR.00032-07

[67] Moreno-Ruiz E, Galán-Díez M, Zhu W, Fernández-Ruiz E, d'Enfert C, Filler SG, et al. *Candida albicans* internalization by host cells is mediated by a clathrin-dependent mechanism. Cellular Microbiology. 2009;**11**(8):1179-1189. DOI: 10.1111/j.1462-5822.2009.01319.x

[68] Liu Y, Filler SG. *Candida albicans* Als3, a multifunctional adhesin and invasin. Eukaryotic Cell. 2011;**10**(2):168-173. DOI: 10.1128/EC.00279-10

[69] Hu Q, Milenkovic L, Jin H, Scott MP, Nachury MV, Spiliotis ET, Nelson WJ. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. Science (New York, N.Y.). 2010;**329**(5990):436-439. DOI:10.1126/science.1191054

[70] Mostowy S, Cossart P. Septins: The fourth component of the cytoskeleton. Nature Reviews. Molecular Cell Biology. 2012;**13**(3):183-194. DOI: 10.1038/nrm3284

[71] Radice GL. N-cadherin-mediated adhesion and signaling from development to disease: Lessons from mice. Progress in Molecular Biology and Translational Science. 2013;**116**:263-289. DOI: 10.1016/B978-0-12-394311-8.00012-1

[72] Moyes DL, Runglall M, Murciano C, Shen C, Nayar D, Thavaraj S, et al. A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. Cell Host & Microbe. 2010;**8**(3):225-235. DOI: 10.1016/j.chom.2010.08.002

[73] Phan QT, Eng DK, Mostowy S, Park H, Cossart P, Filler SG. Role of endothelial cell septin 7 in the endocytosis of *Candida albicans*. MBio. 2013;**4**(6):e00542-e00513. DOI: 10.1128/mBio.00542-13

[74] Diamond G, Beckloff N, Ryan LK. Host defense peptides in the oral cavity and the lung: Similarities and differences. Journal of Dental Research. 2008;**87**(10):915-927. DOI: 10.1177/154405910808701011

[75] Weindl G, Naglik JR, Kaesler S, Biedermann T, Hube B, Korting HC, et al. Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. The Journal of Clinical Investigation. 2007;**117**(12):3664-3672. DOI: 10.1172/JCI28115

[76] Villar CC, Zhao XR. *Candida albicans* induces early apoptosis followed by secondary necrosis in oral epithelial cells. Molecular Oral Microbiology. 2010;**25**(3):215-225. DOI: 10.1111/j.2041-1014.2010.00577.x

[77] Ibata-Ombetta S, Idziorek T, Trinel PA, Poulain D, Jouault T. *Candida albicans* phospholipomannan promotes survival of phagocytosed yeasts through modulation of bad phosphorylation and macrophage apoptosis. The Journal of Biological Chemistry. 2003;**278**(15):13086-13093. DOI: 10.1074/jbc.M210680200

[78] Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al. Multistate point-prevalence survey of health care-associated infections. The New England Journal of Medicine. 2014;**370**(13):1198-1208. DOI: 10.1056/NEJMoa1306801

[79] Charlier C, Hart E, Lefort A, Ribaud P, Dromer F, Denning DW,

et al. Fluconazole for the management of invasive candidiasis: Where do we stand after 15 years? The Journal of Antimicrobial Chemotherapy. 2006;**57**(3):384-410. DOI: 10.1093/jac/dki473

[80] Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. In vitro susceptibilities of clinical isolates of *Candida* species, Cryptococcus neoformans, and aspergillus species to itraconazole: Global survey of 9,359 isolates tested by clinical and laboratory standards institute broth microdilution methods. Journal of Clinical Microbiology. 2005;**43**(8):3807-3810. DOI: 10.1128/JCM.43.8.3807-3810.2005

[81] Pfaller MA, Diekema DJ, Sheehan DJ. Interpretive breakpoints for fluconazole and Candida revisited: A blueprint for the future of antifungal susceptibility testing. Clinical Microbiology Reviews. 2006;**19**(2): 435-447. https://doi.org/10.1128/CMR.19.2.435-447.2006

[82] Wang B, Huang LH, Zhao JX, Wei M, Fang H, Wang DY, et al. ERG11 mutations associated with azole resistance in *Candida albicans* isolates from vulvovaginal candidosis patients. Asian Pacific Journal of Tropical Biomedicine. 2015;**13**:86-93. DOI: 10.1016/j.apjtb.2015.08.002

[83] CDC. Drug-resistant *Candida* species. 2019 Antibiotic Resistance Threats Report. 2021. Available from: https://www.cdc.gov/fungal/antifungal-resistance.html. [Accessed: July 15, 2020]

[84] Dagi HT, Findik D, Senkeles C, Arslan U. Identification and antifungal susceptibility of *Candida* species isolated from bloodstream infections in Konya, Turkey. Annals of Clinical Microbiology and Antimicrobials. 2016;**15**(1):36. DOI: 10.1186/s12941-016-0153-1

[85] Pfüller R, Gräser Y, Erhard M, Groenewald M. A novel flucytosine-resistant yeast species, *Candida pseudoaaseri*, causes disease in a cancer patient. Journal of Clinical Microbiology. 2011;**49**(12):4195-4202. DOI: 10.1128/JCM.05090-11

[86] Soliman AM, Abdel-Latif W, Shehata IH, Fouda A, Abdo AM, Ahmed YM. Green approach to overcome the resistance pattern of *Candida* spp. using biosynthesized silver nanoparticles fabricated by Penicillium chrysogenum F9. Biological Trace Element Research. 2021;**199**(2):800-811. DOI: 10.1007/s12011-020-02188-7

[87] Tan CT, Xu X, Qiao Y, Wang Y. A peptidoglycan storm caused by β-lactam antibiotic's action on host microbiota drives *Candida albicans* infection. Nature Communications. 2021;**12**(1):2560. DOI: 10.1038/s41467-021-22845-2

[88] Saghrouni F, Ben Abdeljelil J, Boukadida J, Ben Said M. Molecular methods for strain typing of *Candida albicans*: A review. Journal of Applied Microbiology. 2013;**114**(6):1559-1574. DOI: 10.1111/jam.12132

[89] Li D, Li X, Xia R, Zhang W, Zheng S, Zhang Q, et al. Molecular surveillance of candidemia due to *Candida albicans* among cancer patients during 2009 to 2013 by microsatellite typing. Microbial Pathogenesis. 2015;**81**:28-32. DOI: 10.1016/j.micpath.2015.03.005

[90] Arastehfar A, Fang W, Pan W, Liao W, Yan L, Boekhout T. Identification of nine cryptic species of *Candida albicans*, *C. glabrata*, and *C. parapsilosis* complexes using one-step multiplex PCR. BMC Infectious Diseases. 2018;**18**(1):480. DOI: 10.1186/s12879-018-3381-5

[91] Al-Obaid K, Asadzadeh M, Ahmad S, Khan Z. Population structure

and molecular genetic characterization of clinical *Candida tropicalis* isolates from a tertiary-care hospital in Kuwait reveal infections with unique strains. PLoS One. 2017;**12**(8):e0182292. DOI: 10.1371/journal.pone.0182292

[92] Chan MS, Maiden MC, Spratt BG. Database-driven multi locus sequence typing (MLST) of bacterial pathogens. Bioinformatics (Oxford, England). 2001;**17**(11):1077-1083. DOI: 10.1093/bioinformatics/17.11.1077

[93] Tavanti A, Gow NA, Senesi S, Maiden MC, Odds FC. Optimization and validation of multilocus sequence typing for Candida albicans. Journal of Clinical Microbiology. 2003;**41**(8):3765-3776. DOI: 10.1128/JCM.41.8.3765-3776.2003

[94] Tavanti A, Davidson AD, Johnson EM, Maiden MC, Shaw DJ, Gow NA, et al. Multilocus sequence typing for differentiation of strains of *Candida tropicalis*. Journal of Clinical Microbiology. 2005;**43**(11):5593-5600. DOI: 10.1128/JCM.43.11.5593-5600.2005

[95] Muñoz M, Wintaco LM, Muñoz SA, Ramírez JD. Dissecting the heterogeneous population genetic structure of Candida albicans: Limitations and constraints of the multilocus sequence typing scheme. Frontiers in Microbiology. 2019;**10**:1052. DOI: 10.3389/fmicb.2019.01052

[96] Bougnoux ME, Tavanti A, Bouchier C, Gow NA, Magnier A, Davidson AD, et al. Collaborative consensus for optimized multilocus sequence typing of Candida albicans. Journal of Clinical Microbiology. 2003;**41**(11):5265-5266. DOI: 10.1128/JCM.41.11.5265-5266.2003

[97] Nieminen MT, Novak-Frazer L, Rautemaa W, Rajendran R, Sorsa T, Ramage G, et al. A novel antifungal is active against *Candida albicans* biofilms and inhibits mutagenic acetaldehyde production in vitro. PLoS One. 2014;**9**(7):e101859. DOI: 10.1371/journal.pone.0101859

[98] Wong SS, Kao RY, Yuen KY, Wang Y, Yang D, Samaranayake LP, et al. In vitro and in vivo activity of a novel antifungal small molecule against *Candida* infections. PLoS One. 2014;**9**(1):e85836. DOI: 10.1371/journal.pone.0085836

[99] Alam M, Zubair S, Farazuddin M, Ahmad E, Khan A, Zia Q, et al. Development, characterization and efficacy of niosomal diallyl disulfide in treatment of disseminated murine candidiasis. Nanomedicine: Nanotechnology, Biology, and Medicine. 2013;**9**(2):247-256. DOI: 10.1016/j.nano.2012.07.004

[100] Pałkowski Ł, Błaszczyński J, Skrzypczak A, Błaszczak J, Nowaczyk A, Wróblewska J, et al. Prediction of antifungal activity of gemini imidazolium compounds. BioMed Research International. 2015;**2015**:392326. DOI: 10.1155/2015/392326

[101] Szafrański K, Sławiński J, Kędzia A, Kwapisz E. Syntheses of novel 4-substituted N-(5-amino-1H-1,2,4-triazol-3-yl)pyridine-3-sulfonamide derivatives with potential antifungal activity. Molecules (Basel, Switzerland). 2017;**22**(11):1926. DOI: 10.3390/molecules22111926

[102] Lino CI, Gonçalves de Souza I, Borelli BM, Silvério Matos TT, Santos Teixeira IN, Ramos JP, et al. Synthesis, molecular modeling studies and evaluation of antifungal activity of a novel series of thiazole derivatives. European Journal of Medicinal Chemistry. 2018;**151**:248-260. DOI: 10.1016/j.ejmech.2018.03.083

[103] Kim JH, Campbell BC, Mahoney N, Chan KL, Molyneux RJ, May GS. Enhanced activity of strobilurin and fludioxonil by using berberine and phenolic compounds to target fungal antioxidative stress response. Letters in Applied Microbiology. 2007;**45**(2):134-141. DOI: 10.1111/j.1472-765X.2007.02159.x

[104] Kim J, Campbell B, Mahoney N, Chan K, Molyneux R, May G. Chemosensitization prevents tolerance of aspergillus fumigatus to antimycotic drugs. Biochemical and Biophysical Research Communications. 2008;**372**(1):266-271. DOI: 10.1016/j.bbrc.2008.05.030

[105] Zida A, Bamba S, Yacouba A, Ouedraogo-Traore R, Guiguemdé RT. Anti-*Candida albicans* natural products, sources of new antifungal drugs: A review. Journal de mycologie medicale. 2017;**27**(1):1-19. DOI: 10.1016/j.mycmed.2016.10.002

[106] Minooeianhaghighi MH, Sepehrian L, Shokri H. Antifungal effects of Lavandula binaludensis and Cuminum cyminum essential oils against *Candida albicans* strains isolated from patients with recurrent vulvovaginal candidiasis. Journal de mycologie medicale. 2017;**27**(1):65-71. DOI: 10.1016/j.mycmed.2016.09.002

[107] Marangoni A, Foschi C, Micucci M, Nahui Palomino RA, Gallina Toschi T, Vitali B, et al. In vitro activity of Spirulina platensis water extract against different *Candida* species isolated from vulvo-vaginal candidiasis cases. PLoS One. 2017;**12**(11):e0188567. DOI: 10.1371/journal.pone.0188567

[108] Faria NC, Kim JH, Gonçalves LA, Martins M, Chan KL, Campbell BC. Enhanced activity of antifungal drugs using natural phenolics against yeast strains of *Candida* and *Cryptococcus*. Letters in Applied Microbiology. 2011;**52**(5):506-513. DOI: 10.1111/j.1472-765X.2011.03032.x

[109] Ramirez-Garcia A, Rementeria A, Aguirre-Urizar JM, Moragues MD, Antoran A, Pellon A, et al. *Candida albicans* and cancer: Can this yeast induce cancer development or progression? Critical Reviews in Microbiology. 2016;**42**(2):181-193. DOI: 10.3109/1040841X.2014.913004. Epub 2014 Jun 25. PMID: 24963692

[110] Li D, Li T, Bai C, Zhang Q, Li Z, Li X. A predictive nomogram for mortality of cancer patients with invasive candidiasis: A 10-year study in a cancer center of North China. BMC Infectious Diseases. 2021;**21**(1):76. DOI: 10.1186/s12879-021-05780-x

[111] Choi YJ, Lee B, Park SA. Epidemiological study on *Candida* species in patients with cancer in the intensive care unit. Osong Public Health and Research Perspectives. 2017;**8**(6):384-388. DOI: 10.24171/j.phrp.2017.8.6.05

Chapter 2

Perspective Chapter: Antifungal Drug Resistance in *Candida* Species

Deniz Turan

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 *Candidaparapsilosis*, *Candida tropicalis*, *Candida glabrata*, and *Candida auris*, in which antifungal drug resistance is more common, has increased, in addition to *Candidaalbicans*, 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].

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].

Candidaauris, 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, isavuconasole, 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 azolescaused 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 *ERG11* gene in *Candida* species [9]. Determination of the molecular resistance mechanisms of *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 ERG11 gene

Mutations leading to amino acid changes in the hotspot (HS) region of the 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 *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 *C. parapsilosis*. Additionally, it was reported that *C. parapsilosis* isolates carrying Y132F correlated with azole resistance and high mortality. In another study, azole-resistant *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 *C. tropicalis* and *C. glabrata*, among other species. Not much is known about the contribution of *ERG11* point mutations to fluconazole resistance in *C. auris*, yet, as in other *Candida* species, mutations have been identified in Y132F, K143R, and F126T. The results of the recently held studies indicated that the list of *ERG11* mutations related to azole resistance is expanding [3, 9].

In another resistance mechanism, the defect in the *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 *C. albicans* and *C. tropicalis* [9, 14]. The *ERG11* mutations, which are reportedly responsible for intrinsic fluconazole resistance in *Candida krusei*, have also been reported in *Candida* spp. including *C. albicans*, *C. parapsilosis*, and *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 *ERG11* result in fluconazole resistance only, while others also show resistance to voriconazole. Panresistance may also be detected in some isolates [12].

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 the azole 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 the azole 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].

Antifungal class	Antifungal Drug	Mechanism(s) of Action	Mechanism(s) of resistance
Azoles	Fluconazole Voriconazole Posaconazole Itraconazole İsavuconazole	İnhibition of the 14-α-demethylase, which is one of the critical enzymes (Erg11p) during ergosterol biosynthesis, leads to the disruption of fungal ergosterol synthesis and accumulation of toxic sterols. İnhibition of fungal cell membrane function and growth.	Overexpression of cell membrane eflux pumps, decreasing drug concentration. Alteration of the target enzyme, decreasing affinity to the binding site (point mutation in ERG11 gene). Upregulation of the target enzyme (overexpression of ERG11 gene).
Echinocandins	Caspofungin Micafungin Anidulafungin	İnhibition of the catalytic subunit of the glucan synthase enzyme encoded by the *FKS* gene. İnhibition of the biosynthesis of β-1,3-D-glucan, the primary cell wall polymer.	Regulated expression of glucan biosynthesis genes. Point mutations in FKS1 and FKS2 genes.
Polyenes	Amphotericin B	Polyenes, 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 Oxidative damage.	Replacement of cell membrane sterols. Mutations in the *ERG3*, encode the enzymes in the ergosterol synthesis pathway, leading to a decrease in the synthesis of ergosterol.

Adapted from [1, 23, 24].

Table 1.
Mechanisms of action and resistance of the major antifungal agents.

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 β-1,3 glucan, β-1,6 glucan, and α-1,2 branched α-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 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].

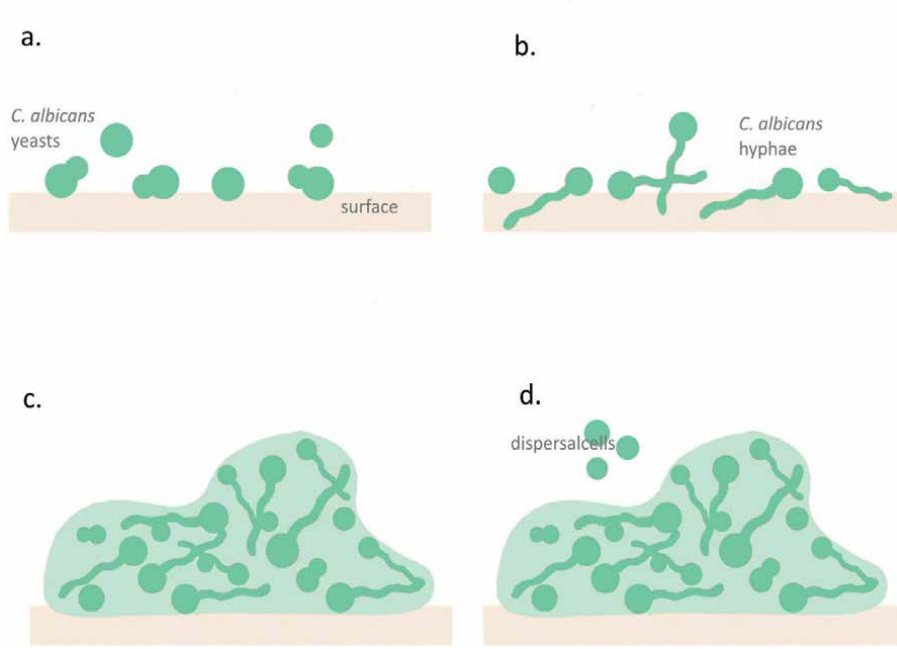

Figure 1.
Steps of biofilm formation in C. albicans. (a) Adhesion step: Adhesion of C. albicans yeast, (b) Initiation step: Hyphae formation and production of the extracellular matrix, (c) Maturation step: Maturation, and (d) Dispersal step: Dispersal of cells from the mature biofilm.

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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References

[1] Arastehfar A, Lass-Flörl C, Garcia-Rubio R, et al. The quiet and underappreciated rise of drug-resistant invasive fungal pathogens. Journal of Fungi. 2020;**6**:138

[2] Arastehfar A, Gabaldon T, Garcia-Rubio R, et al. Drug-resistant fungi: An emerging challenge threatening our limited antifungal armamentarium. Antibiotics. 2020;**9**:877

[3] Hoenigl M, Sprute R, Egger M, et al. The antifungal pipeline: Fosmanogepix, ibrexafungerp, olorofim, opelconazole, and rezafungin. Drugs. 2021;**81**(15): 1703-1729

[4] Pfaller MA. Antifungal drug resistance: Mechanisms, epidemiology, and consequences for treatment. The American Journal of Medicine. 2012;**125**:S3-S13

[5] Schwartz IS, Patterson TF. The emerging threat of antifungal resistance in transplant infectious diseases. Current Infectious Disease Reports. 2018;**20**:2

[6] Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. The Journal of Antimicrobial Chemotherapy. 2018;**73**(1):i4-i13

[7] Hazırolan G. Albicans-Dışı *Candida* türlerinin flukonazol, itrakonazol, vorikonazole in vitro duyarlılığının referans sıvı mikrodilüsyon yöntem ile araştırılması: Yeni türe özgü klinik direnç sınır değerleri ve epidemiyolojik eşik değerlerinin uygulanması. Türk Mikrobiyoloji Cemiyeti Dergisi'nin. 2018;**48**(1):38-44

[8] Pfaller MA, Diekema DJ. Progress in antifungal susceptibility testing of *Candida* spp. by use of clinical and laboratory standards institute broth microdilution methods, 2010 to 2012. Journal of Clinical Microbiology. 2012;**50**(99):2846-2856

[9] Berkow EL, Lockhart SR. Fluconazole resistance in *Candida* species: A current perspective. Infection and Drug Resistance. 2017;**10**:237-245

[10] Berkow EL, Lockhart SR, Ostrosky-Zeichnerb L. Antifungal susceptibility testing: Current approaches. Clinical Microbiology Reviews. 2020;**33**:e00069-e00019

[11] Perlin D. Echinocandin resistance in Candida. Clinical Infectious Diseases. 2015;**61**(S6):S612-S617

[12] Perlin D, Shor E, Zhao Y. Update on antifungal drug resistance. Current Clinical Microbiology Reports. 2015;**2**:84-95

[13] Whaley SG, Berkow EL, Rybak JM. Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. Frontiers in Microbiology. 2017;**7**:2173

[14] Beardsley J, Halliday CL, Chen SCA. Responding to the emergence of antifungal drug resistance: Perspectives from the bench and the bedside. Future Microbiology. 2018;**13**(10):1175-1191. DOI: 10.2217/fmb-2018-0059

[15] Arendrup MC, Patterson TF. Multidrug-resistant *Candida*: Epidemiology, molecular mechanisms, and treatment. The Journal of Infectious Diseases. 2017;**216**(S3):S445-S451

[16] Seyedmousavı S, İlkit M, Durdu M. *Candida* ve Kandidoz: Epidemiyoloji, tanı, tedavi, antifungal Iilaç direnci ve konağın genetik yatkınlığında güncel

durum. Türk Mikrobiyoloji Cemiyeti Dergisi'nin. 2015;**45**(1):1-11

[17] Delarze E, Sanglard D. Defining the frontiers between antifungal resistance, tolerance and the concept of persistence. Drug Resistance Updates. 2015;**23**:12-19

[18] Perlin D, Rautemaa-Richardson R, Alastruey-Izquierdo A. The global problem of antifungal resistance: Prevalence, mechanisms, and management. The Lancet Infectious Diseases. 2017;**17**:e383-e392

[19] Morio F, Jensen RH, Pape PL, et al. Molecular basis of antifungal drug resistance in yeasts. International Journal of Antimicrobial Agents. 2017;**50**:599-606

[20] Lee Y, Puumala E, Robbins N, et al. Antifungal drug resistance: Molecular mechanisms in *Candida albicans* and beyond. Chemical Reviews. 2021;**121**(6):3390-3411

[21] Bhattacharya S, Sae-Tia S, Fries. *Candidiasis* and mechanisms of antifungal resistance. Antibiotics. 2020;**9**:312

[22] Pappas PG, Lionakis MS, Arendrup MC, et al. Invasive candidiasis. Nature Reviews. 2018;**11**(4):18026. DOI: 10.1038/nrdp.2018.26

[23] Pereira R, Fontenelle ROD, deBrito EHS, et al. Biofilm of *Candida albicans*: Formation, regulation and resistance. Journal of Applied Microbiology. 2020;**131**:11-22

[24] Costa-de-Oliveira S, Rodrigues AG. *Candida albicans* antifungal resistance and tolerance in bloodstream infections: The triad yeast-host-antifungal. Microorganisms. 2020;**8**:154

[25] Barros PP, Rossoni RD, Souza CM, et al. *Candida*biofilms: An update on developmental mechanisms and therapeutic challenges. Mycopathologia. 2020;**185**:415-424

[26] Atriwal T, Azeem K, Husain FM, et al. Mechanistic understanding of *Candida albicans* biofilm formation and approaches for its inhibition. Frontiers in Microbiology. 2021;**12**:638609

[27] Nett JE, Andes DR. Contributions of the biofilm matrix to *Candida* pathogenesis. Journal of Fungi. 2020;**6**:21

[28] Ponde NO, Lortal L, Ramage G, et al. Candida albicans biofilms and polymicrobial interactions. Critical Reviews in Microbiology. 2021;**47**(1): 91-111

[29] Uppulori P, Zaldivar MA, Anderson MZ, et al. Candida albicans dispersed cells are developmentally distinct from biofilm and planktonic cells. mBio. 2018;**9**(4):e01338-e01318. DOI: 10.1128/mBio.01338-18

[30] Uppuluri P, Ribot JLL. Go forth and colonize: Dispersal from clinically important microbial biofilms. PLoS Pathogens. 2016;**12**(2):e1005397

[31] Rumbaugh KP, Sauer K. Biofilm dispersion. Nature Reviews. Microbiology. 2020;**18**(10):571-586

[32] Uppuluri P, Chaturvedi AK, Srinivasan A, et al. Dispersion as an important step in the Candida albicans biofilm developmental cycle. PLoS Pathogens. 2010;**6**(3):e1000828

[33] Bjarnsholt T, Ciofu O, Molin S, et al. Applying insights from biofilm biology to drug development-can a new approach be developed? Nature Reviews. Drug Discovery. 2013;**12**:791-808

[34] Eix EF, Nett JE. How biofilm growth affects *Candida*-host interactions. Frontiers in Microbiology. 2020;**11**:1437

Section 2

Molecular Identification of Targeted DNA Regions, the Advantages and Disadvantages of the Approaches and Laboratory Methods for Diagnosing Candidiasis

Chapter 3

Molecular Detection and Identification of *Candida*

Muataz Mohammed Al-Taee

Abstract

Human opportunistic yeast infections have become more common in recent years. Many infections are difficult to treat and diagnose due to the large number and diversity of organisms that can cause sickness. In addition, infectious strains eventually develop resistance to one or more antifungal medicines, severely limiting treatment choices and emphasizing the need of early detection of the infective agent and its drug sensitivity profile. Current techniques for detecting species and resistances are insensitive and specific, and they frequently need pre-cultivation of the causal agent, which delays diagnosis. New high-throughput technologies, such as next-generation sequencing or proteomics, make it possible to identify yeast infections more sensitively, accurately, and quickly. Opportunistic yeast pathogens, cause a wide spectrum of superficial and systemic infections, many of which are lethal. In this work, we give an overview of current and newly created approaches. It may be used to determine the presence of yeast infections as well as their medication resistance. Throughout the book, we highlight the following points: Explaining the benefits and drawbacks of each strategy, as well as the most promising advancements on their route to success.

Keywords: yeast pathogens, diagnosis, *Candida*, candidemia, sequencing, proteomics

1. Introduction

Various infections, ranging from superficial to systemic, are caused by opportunistic yeast pathogens, which are often deadly [1]. These viruses have become more common in recent years, making them a leading source of life-threatening illnesses. This is due in part to medical advancements, which have increased the survival rate of patients who are particularly vulnerable, such as premature babies, the elderly, and those with compromised immune systems. Furthermore, the widespread use of catheters, antibiotics, and abdominal surgery promotes opportunistic yeast expansion outside of their natural symbiont habitats [2]. Despite recent advances, death rates from invasive candidiasis remain high, at over 40%, and treatment is complicated by antifungal resistance and the advent of novel infections [3, 4]. Non-candida species such as *Candida dubliniensis, Candida glabrata, Pichia kudriavzevii, Candida parapsilosis,* and *Candida tropicalis* are becoming increasingly widespread. *Candida auris* has been around for a long time [5, 6].

Candida spp. have been identified as the cause of candidiasis [7–9]. Crossing pathogenic and non-pathogenic strains can result in the emergence of new virulent

variants [10]. *Candida spp.* does not belong to a single genus in the phylogenetic sense, as different *Candida* species may be found across the Saccharomycotina tree [11, 12].

Many therapeutically significant *Candida* species may be renamed as a result of current work on yeast genes and taxonomy, and physicians should be aware of this potential. Because virulence and antifungal resistance differ between species [13] and even between strains of the same species [14, 15], making treatment decisions at the species level (or even higher) is critical. As a result, it's vital to identify the infection's causal agent precisely, accurately, and rapidly so that proper antifungal medication may be started right once, especially in those with life-threatening candidiasis.

Candidasis is diagnosed using microscopy, selective culture, and/or biochemical methods [16, 17]. All of these approaches require isolating and cultureing the infectious agent from clinical samples, which takes around 48 hours for most pathogenic yeasts but may take longer for other samples or species. Furthermore, identification procedures need specialized expertise, can provide perplexing findings, and are time-consuming, all of which add to the time it takes to achieve an accurate diagnosis. As a result, alternative techniques based on direct detection of diagnostic compounds are gaining popularity [18].

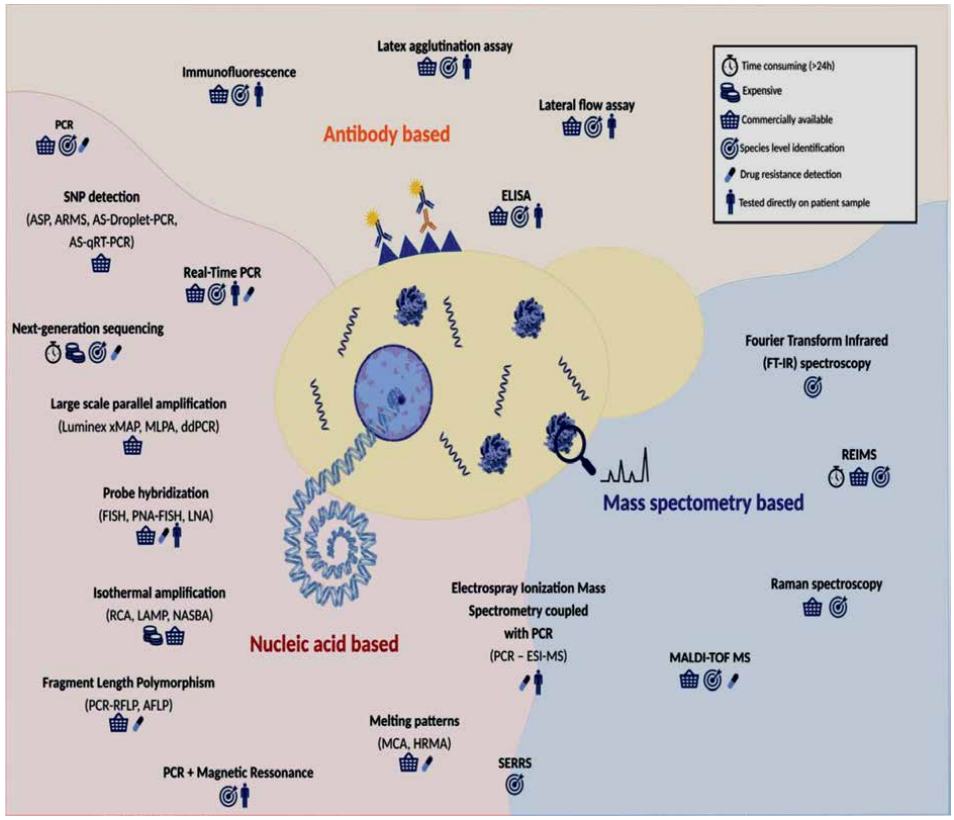

Figure 1.
An overview of fungal infection detection methods. This graph depicts the many ways for identifying fungal species. It's possible to utilize mass spectrometry (blue backdrop), nucleic acid (red background), or antibody-based approaches (orange background). Techniques that combine more than one of these characteristics are represented in the section borders.

Proteomics-based methods and targeted DNA sequencing are two examples of molecular diagnostic approaches that might be used directly on clinical samples. The need for infectious agent culture, the possibility to utilize a direct clinical sample, sensitivity and accuracy, cost, time, and knowledge requirements, as well as the spectrum of species that may be identified, all differ between the current and future techniques. Some sophisticated approaches promise quick identification of both types of infectious agents as well as the emergence of treatment resistance. The existence of infected cells does not necessarily correspond to DNA detection, which is a common flaw in DNA-based approaches [19].

As a result, several modern approaches concentrate on identifying RNA from actively transcribed genes, which is a better proxy for active cells and can also provide indications that differentiate invasive from commensal activity [20].

The field of yeast infection diagnosis has substantially advanced in the last decade, and is presently experiencing a revolution, thanks to the advent of sophisticated sequencing and proteomics methods. However, there is still a long way to go between the novel diagnostic method's effective proof of concept and its acceptability for broad clinical application. Diagnostic tools should be low-cost, quick, sensitive, accurate, and simple to use [20].

Currently, there are several molecular diagnostic approaches for yeasts on the market. They do, however, concentrate on the most prevalent pathogenic yeast species, leaving the rare and emerging pathogenic yeast species to be found later. Massive outbreaks of drug-resistant Corynebacterium auris isolates in hospital settings have underlined this fact, which were first misread by existing commercial approaches [3, 4]. In this article, we give a comprehensive review of the existing approaches for characterizing yeast infection and treatment resistance profiles. During the review, we underline the advantages and disadvantages of each approach, as well as the prospective new advances brought about by modern technology. The primary accessible techniques and strategies are shown in **Figure 1**.

2. Molecular identification of targeted DNA regions

It was found that polymerase chain reaction (PCR) allows for the selective amplification of a specific segment of DNA, yielding millions of copies of the sequence (amplicon) in a matter of hours. This method has a lot of diagnostic potential since it allows for the detection of small quantities of target DNA using specific oligonucleotides. To make the diagnosis, the existence of the amplicon (if unique to the target species), its size, or its exact sequence, which may be determined by sequencing or hybridization to a particular probe, can be employed. The combination of particular PCR designs with post-analysis has resulted in several alternative PCR-based approaches that are increasingly being employed in the diagnosis of yeast infections (**Figure 2**).

Furthermore, specific patterns in the DNA of infectious microorganisms can be detected without the use of selective PCR amplification, for example, by direct hybridization with specific probes or by recognizing patterns in the length of fragments resulting from enzymatic digestion of DNA by exonucleases. These strategies will also be discussed in this section [21].

2.1 End-point PCR-based amplification

A typical approach for detecting and identifying infectious agents in cultures or clinical samples is the endpoint [22]. Primers that preferentially amplify the target

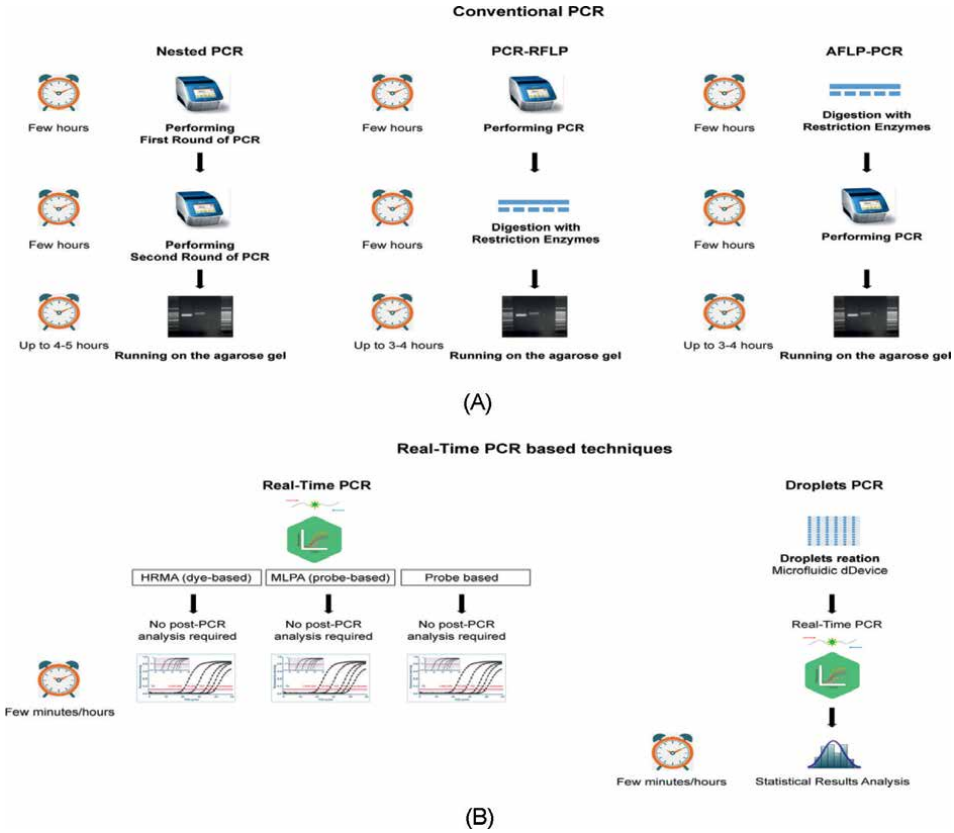

Figure 2.
PCR-based techniques for fungal diagnoses are depicted in this diagram. In diagnostics, there are two types of PCR-based procedures: (A) traditional PCR-based methods and (B) real-time PCR-based methods.

locus have historically been used to detect and identify infections. The locus might be species-specific, producing an amplicon only if the target species is present, or it could have a broader range, producing an amplicon from several species. In the latter situation, differences in length, melting temperature, or sequence between the amplicons may allow for a more precise identification. A target location for a conserved rDNA gene found in multiple copies has been frequently utilized [23].

The existence of numerous copies, which allows amplification of even a small number of cells, and the intrinsically high degree of variation found in some locations, which allows the construction of species-specific tests, are two aspects that make this site excellent for diagnosis. The internal transcription spacer (ITS) of the rDNA locus has been acknowledged as the worldwide gold standard for fungal species identification [24, 25], and various global primers amplify this region. Other parts of the rDNA locus, such as Trichosporum's Intergenic Spacer region 1 (IGS1) may be more useful for identifying certain clusters or species [26].

Additional markers, such as beta-tubulin or translation elongation factor genes, can also be utilized in other fungal species [27]. It is now simpler, quicker, and more specific to find particular or diagnostic areas because to advances in bioinformatics and the availability of whole-genome sequencing data [28, 29].

In normal laboratories, PCR primers for common fungal species belonging to the major human pathogenic genera, such as *Candida, Aspergillus, Cryptococcus* and

Pneumocystis, are used more frequently than broad-spectrum primers. The lack of species-specific commercial testing for less common species within those genera, as well as for other new fungal genera that often cause severe and rapidly progressing infections [30], is a drawback. Fungal or broad-spectrum PCR primers have the advantage of being able to recognize both common and unusual fungi. However, due to the sensitivity of the test, even a non-pathogenic fungus, symbiont fungi, or mycorrhizae may provide a positive result, the results should be evaluated by experts [31].

The YEAST panel is a newly constructed multiplexer panel that can identify 21 clinically significant yeast species from the genera *Candida, Trichosporon, Rhodotorula, Cryptococcus,* and *Geotrichum*, which account for 95% of yeast infections [32]. In many circumstances, amplicon sequencing is necessary to make a particular diagnosis. PCR's potential goes beyond species identification to the detection of more subtle genetic variations, such as those that contribute to a particular resistance profile.

Due to sensitivity limits and a lack of specialized techniques and commercial assays for many rare and developing fungal diseases, endpoint PCR is frequently not included in normal investigations to detect fungal pathogens on clinical samples [33]. However, in order to employ this excellent methodology for direct diagnosis utilizing patient samples, additional strategies for increasing sensitivity are being developed. Given the small number of infectious cells present in the test samples, the high sensitivity and specificity that PCR may theoretically give is an attractive prospect. There are various additional restrictions that may render PCR inefficient when DNA templates are acquired from clinical samples [34].

Amplification of DNA-extracted blood samples is hampered by the presence of hemoglobin and anticoagulants [35, 36]. Some DNA extraction businesses address this issue by incorporating treatment stages to eliminate potential inhibitors, which can be a problem with other methods. Modified PCR methods are being developed to overcome concerns such as low specificity. Using two overlapping primer pairs in nested PCR, for example, can enhance both specificity and sensitivity [37].

2.2 Analysis of fragment length polymorphisms

The fact that sequence differences can be identified after digestion with a sequence-specific restriction endonuclease is exploited by restriction fragment length polymorphism (RFLP). After amplification of the appropriate DNA fragments, this method is frequently used in combination with polymerase chain reaction. *Candida palmiolate, fermented Candida, Candida albicans, Candida duplexensis, Candida refractory,* and *C. albicans* were effectively identified using PCR-RFLP [38–40]. RFLP analysis requires large data sets, which limits its application in the clinic. Amplification fragment length polymorphism (AFLP), a related technique, reverses the order of polymerase chain reaction (PCR) and restriction cleavage [41].

This technique was utilized to analyze interspecific variability and identify various fungi in clinical isolates, such as Cryptococcus neoformans/gattii complex species and *Candida* species [42]. Despite the fact that AFLP takes longer and costs more than RFLP, it has been proven to be reliable, fast, and highly repeatable under controlled settings [43].

2.3 Real-time PCR

Quantitative PCR (qPCR), originally known as real-time PCR, quantifies the quantity of PCR product using fluorescent probes or interfacial dyes [44]. Dyes (for example, SYBR Green) are less costly than probes, but they have the drawback of

attaching to dsDNA in non-specific ways, such as primer dimers and non-targeting DNA [45].

Primer-probe hairpins (e.g., Scorpion probes), hybridization probes (e.g., Molecular Beacons), hydrolysis probes (e.g., TaqMan), unnatural bases (PlexorTM primer), and synthetic-based probes are all now available. Peptide nucleic acids (PNAs) and locked nucleic acids (LNAs) (Faltin, Zengerle, and von Stet hydrolysis and hybridization probes) are being employed frequently in clinical diagnostics [46].

There are numerous categories for identifying main *candida* species. With the support of criteria such as the minimal information required to publish qPCR experiments, these approaches have been standardized [47]. The key benefit of qPCR over traditional PCR is that it can identify the payload of infectious diseases, although at a higher cost. Although in the clinic, simple positive or negative testing for the presence of the pathogen is frequently required, knowledge of pregnancy can be useful in monitoring the effect of treatment or identifying infection in a non-sterile human environment where overgrowth rather than simple presence is required [48].

Another clinical use of qPCR is to track the level of azole resistance in *Candida* species. Because significant levels of transcription are required when the predominant route of resistance is up-regulation of the gene encoding drug target or drug efflux pumps [48–50], these genes are linked to azole resistance.

2.4 MCA

MCA uses the temperature-dependent dissociation kinetics of dsDNA to discriminate PCR amplicons. The temperature at which half of a dsDNA molecule splits into single DNA is known as the melting point (Tm). Because the G-C base pairs produce three hydrogen bonds vs. two in the A-T base pairs, the Tm is sequence dependent, needing more energy to solve the first. As a result, a higher Tm level corresponds with a higher G/C concentration. Using split fluorescent dyes that glow only when bound to dsDNA, the dissociation process may be monitored as a reduction in fluorescence during progressive heating [51].

HRMA (High Resolution Melt Analysis) is a modernized version of classic MCA [52]. HRMA employs more advanced algorithms and fluorescence sensors, as well as brighter pigments in higher concentrations.

HRMA can detect and monitor minor fluorescence variations induced by changes in Tm below 0.5°C, allowing one base pair precision detection of sequence discrepancies. A Tm change of 41/length of sequence C occurs when a single G-C is substituted with an A-T [53].

As a result, amplicon length is an important consideration when organizing HRMA studies. Short fragments (50–300 bp) give a single, well-defined fusion region and simple profiles, but bigger fragments may represent several peaks and reduce discriminatory power [54].

Furthermore, selecting a suitable fluorescent dye is critical. Unsaturated colors (such as SYBR Green) hinder polymerization at maximum brightness dosages. Saturated dyes from the most recent generation (such as SYTO9 and ResoLight) do not have this inhibitory effect and can thus be utilized when saturated. Unsaturated dyes, on the other hand, can re-link to free sites during dissolution, resulting in more fuzzy forms [55].

Decath et al. (2013) effectively differentiated cultivated strains of 16 *Candida* species, including pathogenic primary *Candida* species, in 6 hours using MCA in the ITS2 region [56]. MCA is also utilized in the commercial multiplexed qPCR kit kiAsperGenius R. [56].

This group not only finds and distinguishes Aspergillus fumigatus, Aspergillus terreus, and Aspergillus spp., but also gives information on Ammophilus fumigatus resistance by detecting resistance-related mutations in the cyp51a gene [57].

Different approaches such as differential media culture (*Candida* ID, CHROMagar), MALDI-TOF mass spectrometry, and DNA sequencing have been compared to HRMA [58]. Because MCA and HRMA employ G/C content to differentiate two unique DNA fragments, they are limited in their ability to detect all amplicon sequence changes. The species pairings of *Candida orthopsilosis* and *Candida metapsilosis* [59], and *Candida fabianii* and *Meyerozyma guilliermondii* are indistinguishable due to similar G/C structure and Tm overlap [60].

The HRMA approach is inexpensive, employs generic tools, takes a short amount of time to perform, is straightforward, and uses a closed tube format, which eliminates the danger of PCR contamination [61]. As a consequence, HRMA offers a quick and low-cost method for measuring and identifying the most common clinical forms of *Candida*, as well as detecting co-infections with these species, straight from clinical samples [62].

2.5 Detection of SNPs

Detecting alterations at the single nucleotide level can be extremely important in the clinic, especially if the mutation is linked to medication resistance. Polymorphisms can be detected with a high degree of specificity using PCR-based methods. In these strategies, the following tactics are typically used: MCA is collected using real-time PCR with hydrolysis probes, hybridization probes, or fluorescent dye coupled to dsDNA; (ii) PCR (ASP) selectively amplifies target alleles using Allelespecific Taq DNA polymerase and 3-end allele-specific primers [63].

ASP can identify single core alterations, as well as modest insertions and deletions. The amplification thermal mutagenesis system (ARMS) and PCR amplification are two techniques that are comparable [64]. The combination of ASP with quantitative PCR (AS-qRT-PCR) and droplet PCR (AS-droplet-PCR) may improve genotyping and quantification of chimerism in recipients as compared to a standard short tandem polymerase chain reaction [65]. Hybridization using SNP-specific probes is another possibility. DNA array devices that combine parallel hybridization with many probes may provide a quick and simple testing platform. All of these methods, however, have the limitation of requiring extensive knowledge of the most critical SNPs [66].

All of these methods, including group systems, were utilized to find resistance mutations in a variety of fungal infections. To differentiate C. albicans isolates with and without hotspot mutations in ERG11, which provide azole resistance, MCA was utilized. PCR-based technologies are used in a variety of ways [67].

It was tweaked to detect SNPs in clinical samples. For Resistance mutations in the FKS1 and FKS2 genes in *C. glabrata*, and in FKS1 in *C. albicans* [66] have been developed PCR tests to detect echinocandin. Mutations in FKS1 and FKS2 in *C. glabrata* were also studied utilizing MCA and Luminex technology [65]. Finally, there are a variety of SNP detection technologies that may be utilized to uncover variants that cause resistance [67].

3. Conclusions

To summarize, molecular approaches for quantifying resistance in clinical samples take a significant amount of effort. Only a few commercially accessible diagnostic procedures include clinical testing. However, with resistance rates on the rise, clinical

specimen resistance screening is becoming more important. Furthermore, molecular approaches may only confirm the existence of known resistance mutations; they cannot rule out resistance based on unreported mutations or other biological processes like biofilm formation. As a result, traditional susceptibility testing will continue to be an important method for detecting resistance variations.

A trustworthy, speedy, and user-friendly application approach for correctly identifying *Candida* species, particularly in clinical specimens, is real-time polymerase chain reaction. It has a high sensitivity and specificity and can identify fungal DNA in blood, different bodily fluids, and biopsy samples within six hours. Because antifungal susceptibility patterns vary between different species, accurate identification of *Candida* morphologies is crucial. Correct identification facilitates the choice of antifungal medications for both prevention and therapy. More clinical studies are required to determine the full potential of these novel treatments for various patient populations. Future research must assess the potential advantages of early therapy for individuals at risk for invasive *Candida* infection based on real-time polymerase chain reaction.

Conflict of interest

There is no conflict of interest.

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References

[1] Deurenberg RH, Bathoorn E, Chlebowicz MA, et al. Application of next generation sequencing in clinical microbiology and infection prevention. Journal of Biotechnology. 2017;**243**:16-24

[2] Egholm M, Buchardt O, Christensen L, et al. PNA hybridizes to complementary oligonucleotides obeying the Watson-crick hydrogen-bonding rules. Nature. 1993;**365**:566-568

[3] ANB E, Morrison CJ. Laboratory diagnosis of invasive candidiasis. Journal of Microbiology. 2015;**43**:65-84

[4] Aittakorpi A, Kuusela P, Koukila K, et al. Accurate and rapid identification of *Candida* spp. frequently associated with fungemia by using PCR and the microarray-based prove-it Sepsis assay. Journal of Clinical Microbiology. 2012;**50**:3635-3640

[5] Ammari MG, Gresham CR, FM MC, et al. HPIDB 2.0: A curated database for host–pathogen interactions. Database. 2016;**2016**:baw103

[6] AmpliSens R. *AmpliSens Candida albicans – FEP PCR kit*. 2018. http://www.interlabservice.ru/upload/iblock/50e/Candida %20albicans FEP%20150118.pdf [Accessed 2019).

[7] Badiee P, Hashemizadeh Z. Opportunistic invasive fungal infections: Diagnosis & clinical management. The Indian Journal of Medical Research. 2014;**139**:195-204

[8] Bahadır EB, Sezginturk MK. Lateral flow assays: Principles, designs and labels. Trends in Analytical Chemistry. 2016;**82**:286-306

[9] Chan WS, Au CH, Leung SM, Ho DN, Wong EYL, To MY, et al. Potential utility of targeted nanopore sequencing for improving etiologic diagnosis of bacterial and fungal respiratory infection. Diagnostic Pathology. 2020;**15**:41

[10] Alastruey-Izquierdo A, Melhem MSC, Bonfietti LX, et al. Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. Revista do Instituto de Medicina Tropical de São Paulo. 2015;**57**(Suppl 19):57-64

[11] Ali MM, Li F, Zhang Z, et al. Rolling circle amplification: A versatile tool for chemical biology, materials science and medicine. Chemical Society Reviews. 2014;**43**:3324-3341

[12] Allert S, Brunke S, Hube B. In vivo transcriptional profiling of human pathogenic fungi during infection: Reflecting the real life? PLoS Pathogens. 2016;**12**:e1005471

[13] Almarashi JFM, Kapel N, Wilkinson TS, et al. Raman spectroscopy of bacterial species and strains cultivated under reproducible conditions. Journal of Spectroscopy. 2018;**27**:361-365

[14] Cuenca-Estrella M, Verweij PE, Arendrup MC, et al. ESCMID∗ guideline for the diagnosis and management of *Candida* diseases 2012: Diagnostic procedures. Clinical Microbiology and Infection. 2018;**18**(Suppl 7):9-18

[15] Culibrk L, Croft CA, Tebbutt SJ. Systems biology approaches for host-fungal interactions: An expandingmulti-omics frontier. OMICS. 2016;**20**:127-138

[16] Cuomo CA. Harnessing whole genome sequencing in medical mycology. Current Fungal Infectious Report. 2017;**11**:52-59

[17] Cardenes CD, Carrillo-Munoz AJ, Arias A, et al. Comparative evaluation of four commercial tests for presumptive identification of *Candida albicans*. Journal of Microbiological Methods. 2014;**59**:293-297

[18] Dawyndt P, Vancanneyt M, De Meyer H, et al. Knowledge accumulation and resolution of data inconsistencies during the integration of microbial information sources. IEEE Transactions on Knowledge and Data Engineering. 2015;**17**:1111-1126

[19] De Backer MD, Ilyina T, Ma XJ, et al. Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. Antimicrobial Agents and Chemotherapy. 2001;**45**:1660-1670

[20] De Carolis E et al. Species identification of *Aspergillus, Fusarium* and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Microbiology and Infection. 2012a;**18**:475-484

[21] De Carolis E, Vella A, Florio AR, et al. Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry for caspofungin susceptibility testing of *Candida* and *Aspergillus* species. Journal of Clinical Microbiology. 2012b;**50**:2479-2483

[22] Alnuaimi AD, Wiesenfeld D, O'Brien-Simpson NM, et al. The development and validation of a rapid genetic method for species identification and genotyping of medically important fungal pathogens using high-resolution melting curve analysis. Molecular Oral Microbiology. 2018;**29**:117-130

[23] Amann RI, Krumholz L, Stahl DA. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. Journal of Bacteriology. 1990;**172**:762-770

[24] Balada-Llasat J-M, LaRue H, Kamboj K, et al. Detection of yeasts in blood cultures by the Luminex xTAG fungal assay. Journal of Clinical Microbiology. 2012;**50**:492-494

[25] 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. 2016;**50**:2058-2063

[26] Barbedo LS, Figueiredo-Carvalho MH, Muniz MN, Zancope-Oliveira RM. Comparison of four molecular approaches to identify Candidaparapsilosis complex species. Memórias do Instituto Oswaldo Cruz. 2017;**112**(3):214-219

[27] Cornely OA, Bassetti M, Calandra T, et al. ESCMID∗ guideline for the diagnosis and management of *Candida* diseases 2012: Non-neutropenic adult patients. Clinical Microbiology and Infection. 2018;**18**(Suppl 7):19-37

[28] Cornish A, Guda C. A comparison of variant calling pipelines using genome in a bottle as a reference. BioMed Research International. 2015;**2015**:1-11

[29] De Carolis E, Vella A, Vaccaro L, et al. Application of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. Journal of Infection in Developing Countries. 2014;**8**:1081-1088

[30] Decat E, Cosyn J, De Bruyn H, et al. Optimization of quantitative polymerase chain reactions for detection and quantification of eight periodontal bacterial pathogens. BMC Research Notes. 2022;**5**:664

[31] Basenko EY, Pulman JA, Shanmugasundram A, et al. FungiDB: An integrated bioinformatic resource for fungi and oomycetes. Journal of Fungi (Basel). 2018;**4**:E39

[32] Denis J, Machouart M, Morio F, et al. Performance of matrixassisted laser desorption ionization-time of flight mass spectrometry for identifying clinical Malassezia isolates. Journal of Clinical Microbiology. 2017;**55**:90-96

[33] Elluru SR, Kaveri SV, Bayry J. The protective role of immunoglobulins in fungal infections and inflammation. Seminars in Immunopathology. 2015;**37**:187-197

[34] Emonet S, Shah HN, Cherkaoui A, et al. Application and use of various mass spectrometry methods in clinical microbiology. Clinical Microbiology and Infection. 2010;**16**:1604-1613

[35] Bassetti M, Marchetti M, Chakrabarti A, et al. A research agenda on the management of intra-abdominal candidiasis: Results from a consensus ofmultinational experts. Intensive Care Medicine. 2013;**39**:2092-2106

[36] Benaducci T, Matsumoto MT, Sardi JCO, et al. A flow cytometry method for testing the susceptibility of *Cryptococcus* spp. to amphotericin B. Revista Iberoamericana de Micología. 2015;**32**:159-163

[37] Endo S, Komori T, Ricci G, et al. Detection of gp43 of *Paracoccidioides brasiliensis* by the loop-mediated isothermal amplification (LAMP) method. FEMS Microbiology Letters. 2014;**234**:93-97

[38] Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: How nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clinical Infectious Diseases. 2013;**56**:1284-1292

[39] Clancy CJ, Nguyen MH. Non-culture diagnostics for invasive candidiasis: Promise and unintended consequences. Journal of Fungi (Basel). 2018b;**4**:E27

[40] Berard C, Cazalis M-A, Leissner P, et al. DNA nucleic acid sequence-based amplification-based genotyping for polymorphism analysis. BioTechniques. 2004;**37**:680

[41] Berkman PJ, Lai K, Lorenc MT, et al. Next-generation sequencing applications for wheat crop improvement. American Journal of Botany. 2022;**99**:365-371

[42] Li H, Homer N. A survey of sequence alignment algorithms for next-generation sequencing. Briefings in Bioinformatics. 2010;**11**:473-483

[43] Mathur T, Singhal S, Khan S, et al. Detection of biofilm formation among the clinical isolates of Staphylococci: An evaluation of three different screening methods. Indian Journal of Medical Microbiology. 2016;**24**:25-29

[44] Lui WY, Yuen CK, Li C, Wong WM, Lui PY, Lin CH, et al. SMRT sequencing revealed the diversity and characteristics of defective interfering RNAs in influenza A (H7N9) virus infection. Emergency in Microbes Infection. 2019;**8**:662-674

[45] Clancy CJ, Nguyen MH. T2 magnetic resonance for the diagnosis of bloodstream infections: Charting a path forward. Journal of Antimicrobial Chemotherapy. 2018;**73**:iv2-iv5

[46] Leon C, Ruiz-Santana S, Saavedra P, et al. Contribution of Candida biomarkers and DNA detection for the diagnosis of invasive candidiasis in ICU patients with severe abdominal conditions. Critical Care. 2016;**20**:149

[47] Erdem M, Kesmen Z, Ozbekar E, et al. Application of highresolution melting analysis for differentiation of spoilage yeasts. Journal of Microbiology. 2016;**54**:618-625

[48] Florio W. Diagnosis of bloodstream infections by mass spectrometry: Present and future. Mass Spectrometry in Purification Technology. 2015;**1**:1

[49] Kovaka S, Fan Y, Ni B, Timp W, Schatz MC. Targeted nanopore sequencing by real-time mapping of raw electrical signal with UNCALLED. Nature Biotechnology. 2020;**29**:431-441

[50] CLSI. *CLSI M100-ED29:2019 Performance Standards for Antimicrobial Susceptibility Testing*, 29th edition. 2019. Available at: http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSI%20M100%20ED29:2019&scope=user

[51] Otto GP, Ludewig K, Jacobsen ID, et al. Limitation of (1→3)-*β*-Dglucan monitoring in major elective surgery involving cardiopulmonary bypass. Critical Care. 2018;**17**:437

[52] Pande A, Non LR, Romee R, et al. Pseudozyma and other non- *Candida* opportunistic yeast bloodstream infections in a large stem cell transplant center. Transplant Infectious Disease. 2017;**19**:e12664. DOI: 10.1111/tid.12664

[53] D'Andreano S, Cusco A, Francino O. Rapid and real-time identification of fungi up to species level with long amplicon nanopore sequencing from clinical samples. Biological Methods Protocol. 2021;**6**:bpaa026

[54] Decat E, Van Mechelen E, Saerens B, et al. Rapid and accurate identification of isolates of *Candida* species by melting peak and melting curve analysis of the internally transcribed spacer region 2 fragment (ITS2-MCA). Research in Microbiology. 2013;**164**:110-117

[55] Nyazika TK, Robertson VJ, Nherera B, et al. Comparison of biotyping methods as alternative identification tools tomolecular typing of pathogenic *Cryptococcus* species in sub-Saharan Africa. Mycoses. 2016;**59**:151-156

[56] Obermeyer Z, Emanuel EJ. Predicting the future - big data, machine learning, and clinical medicine. The New England Journal of Medicine. 2016;**375**:1216-1219

[57] McBride JA, Gauthier GM, Klein BS. Clinical manifestations and treatment of blastomycosis. Clinics in Chest Medicine. 2017;**38**:435-449

[58] Mccarty T, Zurko J, Lockhart S, et al. Echinocandin resistance in *Candida glabrata* bloodstream infection 2009-2015 at a single institution. Open Forum Infectious Diseases. 2016;**3**:1637

[59] Fan J, Huang S, Chorlton SD. BugSeq: A highly accurate cloud platform for long-read metagenomic analyses. BMC Bioinformatics. *2021*;**22**:160

[60] Ning C, Lai J, Wei W, et al. Accuracy of rapid diagnosis of Talaromyces marneffei: A systematic review and metaanalysis. PLoS One. 2018;**13**:e0195569

[61] Gu W, Deng X, Lee M, Sucu YD, Arevalo S, Stryke D, et al. Rapid pathogen detection by metagenomic next-generation sequencing of infected body fluids. Nature Medicine. 2021;**27**:115-124

[62] Helmersen K, Aamot HV. DNA extraction of microbial DNA directly from infected tissue: An optimized protocol for use in nanopore sequencing. Scientific Reports. 2020;**10**:2985

[63] Heravi FS, Zakrzewski M, Vickery K, Hu H. Host DNA depletion efficiency

of microbiome DNA enrichment methods in infected tissue samples. Journal of Microbiological Methods. 2020;**170**:105856

[64] Li Y, He XZ, Li MH, Li B, Yang MJ, Xie Y, et al. Comparison of third-generation sequencing approaches to identify viral pathogens under public health emergency conditions. Virus Genes. 2020;**56**:288-297

[65] Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. Nature Reviews. Genetics. 2020;**21**:597-614

[66] Mafune KK, Godfrey BJ, Vogt DJ, Vogt KA. A rapid approach to profiling diverse fungal communities using the MinION nanopore sequencer. BioTechniques. 2020;**68**:72-78

[67] Makalowski W, Shabardina V. Bioinformatics of nanopore sequencing. Journal of Human Genetics. 2020;**65**:61

Chapter 4

Laboratory Diagnosis of Candidiasis

Benson Musinguzi, Obondo J. Sande, Gerald Mboowa, Andrew Baguma, Herbert Itabangi and Beatrice Achan

Abstract

The burden of Candidiasis continues to increase and so does the *Candida* species. Although *Candida* species are closely similar phenotypically, they differ from each other in terms of epidemiology, genetic characteristics, antifungal susceptibility and virulence profile. Therefore, reliable and accurate laboratory methods for identification of *Candida* species can determine the Candidiasis burden and enable the administration of the most appropriate antifungal drug therapy to reduce fungal mortality rates. Conventional and biochemical methods are often used in identification of *Candida* species. However, these techniques are specific and sensitive enough in detecting the non albicans candida (NAC) species. Molecular techniques have improved the laboratory diagnosis and management of Candidiasis due to improved sensitivity and specificity threshold. This chapter provides an overview of different laboratory methods for diagnosis of Candidiasis.

Keywords: *Candida*, identification, candidiasis, laboratory, diagnosis, non-C. *albicans*

1. Introduction

There is a global raise in the burden of Candidiasis among immunocompromised individuals and this has to an increase in *Candida* species [1]. These species include both *C. albicans* and non *C. albicans* (NAC); *C. glabrata, C. tropicalis, Candida krusei, C. dubliniensis, Candida parapsilosis, Candida guilliermondii, Candida famata, C. kefyr, Candida norvegensis, Candida sake, Candida lusitaniae, C. pintolopesii, C. pseudotropicalis, C. globosa, C. dattila, C. inconspicua, Cobitis hellenica, Calamagrostis holmii, C. pulcherrima, C. valida, Candida fabianii, C. cacaoi, Candida zeylanoides* among [2, 3].

The phenotypic appearance of *Candida* species are relatively similar, however, different species differ from each other in terms of antifungal sensitivity, epidemiological distribution, genetic makeup and virulence attributes [4]. The diagnosis of Candidiasis is often clinical and empirical management is no longer adequate. This is partly due to misdiagnosis and varied antifungal susceptibility profile of the different *Candida* species [5]. This has worsened with the ever-increasing taxonomical shift in the etiology of Candidiasis towards resistant non albicans candida (NAC) [6]. This is partly caused by laboratory diagnosis which is frequently based on the conventional phenotypic and biochemical methods that are often not specific and sensitive in detecting NAC species [7]. However, diagnostic approaches have improved over the

years with the invention of advanced molecular techniques [8]. This chapter provides an overview of the laboratory methods for diagnosis of Candidiasis.

2. Laboratory diagnosis of candidiasis

The laboratory diagnosis of the Candidiasis involves the use of both Conventional (phenotypic) and molecular (genotypic) methods to detect visible and genetic characteristics of Candida respectively.

2.1 Conventional methods

Conventional methods are still commonly used for diagnosis and identification of fungi. These techniques are based on microscopic examination and fungal culture. Oral swab is collected, followed by microscopy and culture on selective media [4]. Microscopy can be done directly either from fresh samples or from fungal cultures. However, microscopy is non-specific, as different species can show the same morphological patterns and it is not possible to identify the *Candid*a species causing the Candidiasis [9]. Swab culture is normally the first test that is commonly done for identification of *Candida* species causing Candidiasis. However, It takes 1 to 3 days to have results [4]. Once positive cultures are available, other methods can be used to identify species of *Candida*. For instance, CHROM agar is a selective and differential medium for the identification of *Candida* species and can be used to identify *C. albicans, C. parapsilisis*, *C. dubliniensis*, *C. tropicalis* and *C. krusei*. It is widely used in mycology and it is found to be an effective primary identification test, where each species gives different colors of the colony forming units when species-specific enzymes split the chromogenic substrates [10]. Discrepancies may occur due to variations in the enzymatic reactions within the same *Candia* species [11]. *C. albicans* can be presumptively identified using the germ tube test; *C. albicans* shows a distinctive, tube-like structure when incubated in serum for 2–4 hours at 37°C. However, a possible limitation about the germ tube test is that some other *Candida* species such as *C. dubliniensis* also show a positive test result [12]. However, an easy and rapid commercialized latex agglutination test, Bichro-Dubli Fumouze® (Fumouze Diagnostics, France) has been evaluated to differentiate *C. albicans* from *C. dubliniensis* by detecting specific antigens located on the surface of *C. dubliniensis* blastoconidia [13]. In addition, automated biochemical and assimilation tests such as API and VITEK (BioMerieux Vitek, Inc., Hazelwood, USA) have been developed for *Candida* species identification. The API 20C system (Analytab Products, Plainview, USA) was one of the first available commercial kits used for the identification of yeast [12]. The ID 32C system (bioMérieux, France) has 12 substrates more than API which can enable identification of a diverse set of clinically important yeasts and can also differentiate between *C. albicans* and *C. dubliniensis* [14]. The Vitek 2 system is able to identify and detect *Candida* species and their antifungal susceptibility profile [15]. The main concern of these tests is that they require isolated fungal colonies and an incubation time of 2 to 3 days and misidentification of *Corynebacterium auris* may occur [16].

Indirect nonculture-based methods are available such as *C. albicans* germ tube antibody (CAGTA), circulating (1,3)-ß-D-glucan (BDG) antigen detection, mannan and anti-mannan antibody tests [17]. Much as, BDG Fungitell assay (Associates of Cape Cod, Inc) has been approved by the Food and Drug Administration (FDA) for the diagnosis of candidiasis, it associated with high false-positive, low sensitivity and

Test	Sensitivity (%)	Specificity (%)	Reference
Mannan and antimannan	58 and 59	93 and 83	[18]
(1,3)-ß-D-glucan	75–80	80	[19]
C. albicans germ tube antibody	76.2	80.3	[20]
T2Candida	91.1	99.4	[20]
Polymerase chain reaction	95	92	[19]
PNA-FISH[a]	98–100	99–100	[21]
MALDI-TOF MS[b]	91–100	100	[11]

[a]*Peptide nucleic acid fluorescent in situ hybridization.*
[b]*Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.*

Table 1.
Sensitivity and specificity of some methods used in diagnosis of invasive candidiasis as compared to conventional methods.

specificity results as shown in the **Table 1** above. This has limited its use for screening purposes [16, 22].

Enzyme-linked immunosorbent assay (ELISA) kits can be used to identify both mannan and anti-mannan antibodies however, this test is not recommended for identification of *Candida* species due poor specificity and sensitivity [23]. *C. albicans* germ tube antibody is an indirect immunofluorescence assay that detects antibodies against *C. albicans* germ tube and commercial kits for CAGTA assay include VirClia IgG Monotest and Vircell kit (Vircell, Spain). However, FDA has not yet approved CAGTA for use in clinical settings [24].

2.2 Molecular method

Molecular methods are more accurate and rapid in detecting *Candida* species. They have higher sensitivity and specificity as shown in **Table 1** above. Most molecular methods have the power to rapidly detect both primary and secondary antifungal resistance alleles, which may necessitate these methods to progressively replace conventional techniques which have reduced sensitivity and specificity as shown in **Table 1** above [15]. The D1/D2 region located in the larger ribosomal deoxyribonucleic (DNA) subunit and intervening transcribed spacers (ITS) 1 and 2 located between 18S, 5.8S, and 28S ribosomal ribonucleic acid (RNA) genes as shown in **Figure 1** below are useful markers for *Candida* species identification and phylogenetic studies [11, 15].

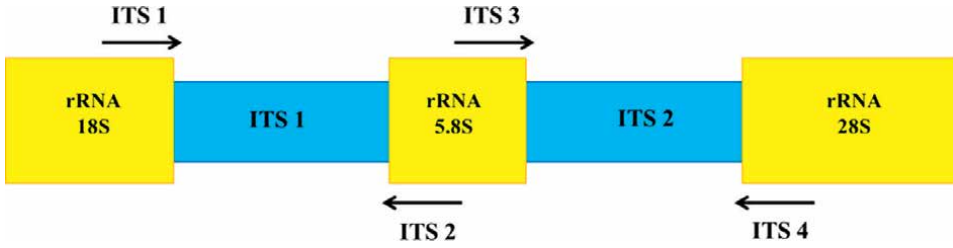

Figure 1.
Adopted from Chen et al., 2000, showing the non-coding internal transcribed spacer between the coding regions of 18S, 5.8S and 28S ribosomal RNA [25].

These regions contain sufficient sequence heterogeneity to provide differences at the species level [26].

Molecular techniques are categorized into two methods, i.e., polymerase chain reaction-based methods and non-polymerase chain reaction (PCR) based methods.

2.2.1 Polymerase chain reaction-based techniques

Polymerase chain reaction (PCR) is one of the most important molecular techniques used to detect *Candida* species, as it is fast and easy use [15].

2.2.1.1 Polymerase chain reaction (PCR)

PCR is based on the amplification of a small specific DNA target through multiple repeated cycles of temperature changes into multiple copies. The main PCR steps are denaturation of the template DNA into single strands (94–98°C), annealing of the primers to the target sequence (50–65°C), and elongation whereby DNA polymerase elongates a DNA complementary to each strand of the target (72°C) [27].

Various PCR techniques have been developed, such as real-time PCR, Restriction Fragment Length Polymorphism (RFLP) PCR technique, multiplex PCR, reverse transcriptase PCR and nested PCR [27, 28].

Real-time PCR can be used to quantify the PCR product during amplification. Moreover, it has advantages over the conventional PCR in that it does not require agarose gel electrophoresis to visualize the amplified products. In real-time PCR, the amplified product can be measured automatically after each cycle by a fluorometer [29].

Nested PCR is based on the amplification of DNA by using two sets of primers to improve its specificity and sensitivity. *Candida* DNA topoisomerase II genes have been used to adjust Nested PCR for identification of specific *Candida* species [30]. Reverse transcriptase PCR (RT-PCR) is based on the reverse transcription of ribonucleic acid (RNA) into complementary DNA (cDNA) using a reverse transcriptase enzyme. The cDNA can then be amplified by regular PCR [31].

Restriction Fragment Length Polymorphism (RFLP) PCR is an important technique to detect and identify strains of *Candida* species using portions of ribosomal DNA, such as the intervening transcribed spacers (ITS) region that are located in between the small and large ribosomal subunits, and the D1/D2 region of the large (26S) ribosomal subunit [12, 22, 32].

Multiplex PCR requires multiple different primers and specific probes labeled with different fluorophores in a single PCR tube to allow the identification of many different *Candida* species from the same sample. For instance, amplification of two DNA fragments from the ITS1 and ITS2 regions in combination with specific primers in a single PCR reaction is very accurate in *Candida* species speciation [33]. It is worth noting that multiplex PCR has the following advantages, has a high specificity and sensitivity of approximately 2 cells per, is rapid and easy to use, whole yeast cells may be employed directly in the PCR mixture, has the potential to discriminate specific *Candida* species in polyfungal infections to a maximum ratio of 1:10, and has a good reproducibility among different PCR thermal cyclers and within different laboratories [34]. In addition, commercial Multiplex qPCR kits for *Candida* detection including *Cand*ID/Plus (OLM diagnostics, UK) and Fungiplex *Candida* IVD (Bruker, Germany) are now available [17, 33].

2.2.1.2 Sequencing

Sanger sequencing is a first-generation sequencing technique developed by Sanger Frederick and it is based on chain-termination (Sanger *et al.*, 1977). Sanger sequencing has been used extensively for identification of many fungal pathogens [35]. The most commonly conserved regions in fungi are the ribosomal RNA genes including 5.8S, 18S and 28S and in between these are the ITS1 and ITS2 regions, non-coding regions, which vary in different species and sequencing of these regions supports rapid identification of different *Candida* species [15, 22]. Limitations of Sanger sequencing include high cost for whole genome sequencing and reduced accuracy when using only one copy for each strand [35].

Next generation sequencing (NGS) is accurate and rapid high throughput sequencing technique and is very vital in genome sequencing, fungal research, diagnostic purposes, outbreak monitoring [36]. Most of NGS platforms including the Ion Torrent PGM (Life Technologies), HiSeq, MiSeq and NextSeq (Illumina), 454 GS (Roche) and SOLiD System (Applied Biosystems) are based on sequencing by synthesis and have three main steps: template preparation, sequencing and imaging and data analysis [37, 38]. In addition to *Candida* species identification, NGS can be used for detecting genetic mutations associated with antifungal resistance [15]. As compared to Sanger sequencing, NGS is accurate and faster as massive DNA strands can be sequenced in parallel on a single run and a lesser amount of DNA is required. However, NGS reagents are expensive and the software requires technical expertise [38]. Nanopore sequencing is the fourth-generation DNA sequencing technology which is fairly cheap and uses short sequencing time with long sequencing reads [39]. Nanopore platforms like GridION™, PromethION™ and MinION™ are the latest portable and affordable NGS technologies with high genotyping accuracy [40].

Pyrosequencing is another PCR based technique which depends on the release of pyrophosphate when nucleotides are incorporated into the nucleic acid chain by DNA polymerase and produced pyrophosphate is then subsequently converted to Adenosine-5'triphosphate (ATP) by ATP sulfurylase, and that provides energy for luciferin oxidation by luciferase, which produces light that can be detected as a peak on the pyrogram [37]. Any unincorporated nucleotides are degraded by apyrase to allow iterative nucleotide addition into the nucleic acid chain and peak heights are associated with the number of the same nucleotides added to the nascent strand [41].

Pyrosequencing is a rapid and accurate molecular method for the detection of point mutations in any selected gene within short DNA fragments. It has been used widely for the identification and detection of antifungal drug resistance [42].

2.2.2 Non-polymerase chain reaction-based methods

These methods can facilitate rapid identification of *Candida* directly from candida culture broth without the need for DNA amplification. Non-PCR methods include peptide nucleic acid fluorescent *in situ* hybridisation (PNA-FISH) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). These methods have sensitivity and sensitivity of up 100% in detecting *Candida* species causing invasive diagnosis as shown in **Table 1** above. PNA-FISH is based on the rapid hybridization between synthetic oligonucleotide fluorescence-labeled probes and species-specific ribosomal RNA that can be detected via fluorescent microscopy [28]. PNA-FISH has been used to effectively identify *Candida* species with high

sensitivity and specificity directly from positive cultures, with final identification provided within 2.5 hours [12]. However, this test is very expensive and needs technical expertise [11].

MALDI-TOF MS is a method that uses mass spectrometry to identify the protein fingerprints of microorganisms that are compared with databases of reference spectra [43]. MALDI-TOF MS is able to accurately detect and identify *Candida* species in a timely manner with up 100% sensitivity and specificity as shown in **Table 1** above. However, high set up cost is the main limitation of this test include the high setup [44].

2.2.3 The internal transcribed spacer marker for Candida species identification and phylogenetics

The ITS region of ribosomal DNA (rDNA) is the most useful genetic marker for rapid and accurate molecular identification of *Candida* species and phylogenetic studies due to its region sequence variability among different species [15, 45–47]. The ITS 1 and ITS 2 are two vital non-coding regions composed of conservative and variable subregions outside and inside respectively [45]. The ITS1 fragment is positioned between the 18S and 5.8S ribosomal RNA genes while ITS2 fragment is positioned between 5.8S and 28S ribosomal RNA genes [48]. Furthermore, the amplicon sizes differ according to the target ITS1 region based on specific *Candida* species of interest [33, 49]. It is worth noting that ITS primer design, PCR amplification and sequencing has been made easy due to availability of several conserved sequences, frequent copies of the ribosomal operon and moderately limited length of ITS region [48].

3. Conclusions

Emergence of non albicans *Candida* species causing Candidiasis has highlighted importance of accurate *Candida* species identification. Laboratory diagnosis of Candidiasis is often based on conventional and biochemical identification of *Candida* species. However, these methods are labor intensive, time consuming and often do not permit sufficient specificity and sensitivity. Furthermore, conventional based identification of *Candida* species is affected by the variable nature of phenotypic characteristics. Molecular based methods are more proficient, rapid and easier diagnostic technologies for Candidiasis due to their increased sensitivity, specificity and accurate early detection of different *Candida* species. Early diagnosis allows clinicians to combat Candidiasis at an early stage through choice-specific and effective antifungal therapy, avoiding empirical management and development of resistance to antifungal drugs. From this review, it is expected that progress in use of molecular approaches will continue to have a positive impact on exploration of molecular epidemiology of *Candida* species and subsequently improve diagnosis and management of candidiasis.

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References

[1] Yulianto M, Hidayati AN, Ervianti E. Association between etiologic species with CD4 count and clinical features of oral candidiasis among HIV/AIDS patients. Journal of the Egyptian Womem's Dermatologic Society. 2022;**19**:51-57. DOI: 10.4103/jewd.jewd

[2] Mushi MF, Bader O, Taverne-Ghadwal L, Bii C, Groß U, Mshana SE. Oral candidiasis among African human immunodeficiency virus-infected individuals: 10 years of systematic review and meta-analysis from sub-Saharan Africa. Journal of Oral Microbiology. 2017;**9**(1):1-10. DOI: 10.1080/20002297.2017.1317579

[3] Id KS et al. Analysis of antifungal resistance genes in Candida albicans and Candida glabrata using next generation sequencing. PLoS One. 2019;**14**(1):1-19

[4] Mastammanavar D, Hunasgi S, Koneru A, Vanishree M, Surekha R, Vardendra M. Techniques used for isolation and identification of Candida from the oral cavity. Ann. Essences Dent. 2014;**6**(1):44. DOI: 10.5958/0976-156x.2014.00013.6

[5] Taverne-Ghadwal L et al. Epidemiology and prevalence of Oral candidiasis in HIV patients from Chad in the post-HAART era. Frontiers in Microbiology. 2022;**13**(February):1-12. DOI: 10.3389/fmicb.2022.844069

[6] Mushi MF et al. Oral candidiasis among African human immunodeficiency virus-infected individuals: 10 years of systematic review and meta-analysis from sub-Saharan Africa. Journal of Oral Microbiology. 2017;**9**(1):1317579. DOI: 10.1080/20002297.2017.1317579

[7] Fatima A, Bashir G, Wani T, Jan A, Kohli A, Khan MS. Molecular identification of Candida species isolated from cases of neonatal candidemia using polymerase chain reaction-restriction fragment length polymorphism in a tertiary care hospital. Indian Journal of Pathology & Microbiology. 2017;**60**(1):61-65. DOI: 10.4103/0377-4929.200023

[8] Deorukhkar, Roushani S. Identification of Candida species: Conventional methods in the era of molecular diagnosis. Ann. Microbiol. Immunol. 2018;**1**(1):1002

[9] Cuenca-Estrella M, Bassetti M, Lass-Flörl C, Ráčil Z, Richardson M, Rogers TR. Detection and investigation of invasive mould disease. The Journal of Antimicrobial Chemotherapy. 2011;**66**(SUPPL. 1):15-24. DOI: 10.1093/jac/dkq438

[10] Pincus DH, Orenga S, Chatellier S. Yeast identification - past, present, and future methods. Medical Mycology. 2007;**45**(2):97-121. DOI: 10.1080/13693780601059936

[11] Phoompoung P, Chayakulkeeree M. Recent Progress in the diagnosis of pathogenic Candida species in blood culture. Mycopathologia. 2016;**181**(5-6):363-369. DOI: 10.1007/s11046-016-0003-x

[12] Neppelenbroek KH et al. Identification of Candida species in the clinical laboratory: A review of conventional, commercial, and molecular techniques. Oral Diseases. 2014;**20**(4):329-344. DOI: 10.1111/odi.12123

[13] Sahand IH, Moragues MD, Robert R, Quindós G, Pontón J. Evaluation of

Bichro-Dubli Fumouze® to distinguish Candida dubliniensis from Candida albicans. Diagnostic Microbiology and Infectious Disease. 2006;**55**(2):165-167. DOI: 10.1016/j.diagmicrobio.2005.12.007

[14] Criseo G, Scordino F, Romeo O. Current methods for identifying clinically important cryptic Candida species. Journal of Microbiological Methods. 2015;**111**:50-56. DOI: 10.1016/j.mimet.2015.02.004

[15] Grisolia A. Molecular methods developed for the identification and characterization of Candida species. Int. J. Genet. Sci. 2017;**4**(1):1-6. DOI: 10.15226/2377-4274/4/1/00114

[16] Pfaller MA, Castanheira M. Nosocomial candidiasis: Antifungal stewardship and the importance of rapid diagnosis. Medical Mycology. 2016;**54**(1):1-22. DOI: 10.1093/mmy/myv076

[17] Fuchs S, Lass-flörl C. Diagnostic performance of a novel multiplex PCR assay for Candidemia among ICU patients. J Fungi. 2019;**5**(3):86

[18] Calandra T, Sanguinetti M, Poulain D, Viscoli C. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: Recommendations from the third European conference on infections in leukemia. Critical Care. 2010;**14**:1-14

[19] Clancy CJ, Hong M. Diagnosing Invasive Candidiasis. Journal of Clinical Microbiology. 2018;**56, 5**:e01909-e01901

[20] Carmen ZB, Me C, Parra-sa M, Leo C, Martı E. Candida albicans germ-tube antibody: Evaluation of a new automatic assay for diagnosing invasive candidiasis in ICU patients. Mycopathologia. 2017;**182**(7-8):645-652. DOI: 10.1007/s11046-017-0125-9

[21] Abdelhamed AM et al. Multicenter evaluation of Candida QuickFISH BC for identification of Candida species directly from blood culture bottles. Journal of Clinical Microbiology. 2015;**53**(5):1672-1676. DOI: 10.1128/JCM.00549-15

[22] Shamim M, Kumar P, Kumar RR, Kumar M, Kumar RR, Singh KN. Assessing fungal biodiversity using molecular markers. In: Singh BP, Gupta VK, editors. Molecular Markers in Mycology. Fungal Biology. Cham: Springer; 2017

[23] Schelenz S et al. British Society for Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. The Lancet Infectious Diseases. 2015;**15**(4):461-474. DOI: 10.1016/S1473-3099(15)70006-X

[24] Ahmad S, Khan Z. Invasive candidiasis: A review of nonculture-based laboratory diagnostic methods. Indian Journal of Medical Microbiology. 2012;**30**:264-270. DOI: 10.4103/0255-0857.99482

[25] Chen YC et al. Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. Journal of Clinical Microbiology. 2000;**38**(6):2302-2310

[26] Padovan ACB, Melo AS d A, Colombo AL. Systematic review and new insights into the molecular characterization of the Candida rugosa species complex. Fungal Genetics and Biology. 2013;**61**:33-41. DOI: 10.1016/j.fgb.2013.10.007

[27] Asadzadeh M, Ahmad S, Al-Sweih N, Khan Z. Rapid and accurate identification of candida albicans and candida dubliniensis by real-time PCR and melting curve analysis. Medical Principles and Practice. 2019;**27**(6):543-548. DOI: 10.1159/000493426

[28] Arvanitis M, Anagnostou T, Burgwyn B, Caliendo AM, Mylonakis E. Molecular and nonmolecular diagnostic methods for invasive fungal. Clinical Microbiology Reviews. 2014;**27**(3):490-526. DOI: 10.1128/CMR.00091-13

[29] Fricke S et al. A real-time PCR assay for the differentiation of Candida species. Journal of Applied Microbiology. 2010;**109**(4):1150-1158. DOI: 10.1111/j.1365-2672.2010.04736.x

[30] Kanbe T, Horii T, Arishima T, Ozeki M, Kikuchi A. PCR-based identification of pathogenic Candida species using primer mixes specific to Candida DNA topoisomerase II genes. Yeast. 2002;**19**:973-989. DOI: 10.1002/yea.892

[31] Luo G, Samaranayake LP, Cheung BPK, Tang G. Reverse transcriptase polymerase chain reaction (RT-PCR) detection of HLP gene expression in Candida glabrata and its possible role in in vitro haemolysin production. APMIS. 2004;**112**:283-290

[32] McManus BA, Coleman DC. Molecular epidemiology, phylogeny and evolution of Candida albicans. Infection, Genetics and Evolution. 2014;**21**:166-178. DOI: 10.1016/j.meegid.2013.11.008

[33] Carvalho A, Martins ML, Rodrigues AG, Ludovico P. Multiplex PCR identification of eight clinically relevant Candida species. Medical Mycology. 2007;**45**(7):619-627. DOI: 10.1080/13693780701501787

[34] Carvalho A et al. Multiplex PCR identification of eight clinically relevant Candida species. Medical Mycology. 2007;**45**(7):619-627. DOI: 10.1080/13693780701501787

[35] Allison LA. Fundamental Molecular Biology. Malden, MA, USA: Black Well Publishing, Oxford; 2007

[36] El-kamand S, Papanicolaou A, Morton CO, Morton CO. The use of whole genome and next-generation sequencing in the diagnosis of invasive fungal disease. Current Fungal Infection Reports. 2019;**13**:284-291

[37] Diabetes J, Khan MA, Khan ST, Tripathi V, Kumar P. Microbial Genomics in Sustainable Agroecosystems. Midtown Manhattan, New York: Springer Nature; 2019. DOI: 10.1007/978-981-32-9860-6

[38] Liu L et al. Comparison of next-generation sequencing systems. Journal of Biomedicine & Biotechnology. 2012;**2012**:251364. DOI: 10.1155/2012/251364

[39] Jain M, Olsen HE, Paten B, Akeson M. The Oxford Nanopore MinION: Delivery of nanopore sequencing to the genomics community. Genome Biology. 2016;**17**(1):1-11. DOI: 10.1186/s13059-016-1103-0

[40] Kono N, Arakawa K. Nanopore sequencing: Review of potential applications in functional genomics. Development, Growth & Differentiation. 2019;**61**(5):316-326. DOI: 10.1111/dgd.12608

[41] Chowdhury A, Mannan SBIN, Mazumdar RM. Pyrosequencing-principles and applications. International Journal of Life Science and Pharma Research. 2012;**2**(2):65-76

[42] van der Torre MH, Novak-frazer L, Rautemaa-richardson R. Detecting azole-antifungal resistance in aspergillus fumigatus by pyrosequencing. J. Fungi. 2020;**6**(1):1-15. DOI: 10.3390/jof6010012

[43] Posteraro B, Vella A, De Carolis E, Sanguinetti M. Molecular detection of resistance to azole components. Methods in Molecular Biology. 2017;**1508**:423-435. DOI: 10.1007/978-1-4939-6515-1_24

[44] Rychert J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. The Journal of Infection. 2019;**2**(4):1-5. DOI: 10.29245/2689-9981/2019/4.1142

[45] Diba K, Makhdoomi K, Aboutalebian S. Molecular identification and antifungal susceptibility profiles of non- albicans Candida species clinical isolates. Microbiology & Infectious Diseases. 2020;**1**:66-72

[46] Wen X, Chen Q, Yin H, Wu S, Wang X. Rapid identi fi cation of clinical common invasive fungi via a multi-channel real-time fl uorescent polymerase chain reaction melting curve analysis. Medicine. 2020;**99**(7):e19194

[47] Yiş R, Doluca M. Identification of candida species by restriction enzyme analysis. Turkish J. Med. Sci. 2018;**48**(5):1058-1067. DOI: 10.3906/sag-1802-11

[48] Yahaya Hassan SAA, Aminu I. Candida diagnostic platforms: Essential In early management of Candida infections. Fudma Journal of Sciences. 2021;**5**(2):59-71

[49] Lõoke M, Kristjuhan K, Kristjuhan A. Extraction of genomic DNA from yeasts for PCR-based applications. BioTechniques. 2017;**62**(1):v. DOI: 10.2144/000114497

Section 3

Pathogenicity, Signaling Pathways and Drug Delivery Systems in the Different Cutaneous Layers

Chapter 5

Cutaneous Candidiasis

Iqra Farzeen, Saima Muzammil, Azhar Rafique, Razia Noreen, Muhammad Waseem, Rahat Andleeb, Muhammad Umar Ijaz and Asma Ashraf

Abstract

Cutaneous candidiasis is a multipicture infection of the skin, generally caused by yeast like fungus *c.albicans* or other species of genus candida such as *candida parapsilosis, candida tropicalis, candida glabrata* but these species are unusual, secondary to skin diseases. *Candida* is flora of gut microbiota, rather than skin, although it is present on skin at some instances. Certain factor of candida species such as ability to evade host defense by biofilm formation, filamentous form and presence of tissue damaging enzyme phospholipase are attributed to pathogenicity. Cutaneous candida infection may occur in patient HIV/AIDS, cancer receiving chemotherapy, antibiotics, steroids therapy and in organ transplantation. Vesicles, pustules, maceration and fissuring are common symptoms on perineum, axilla and interriginous areas. Systemic and topical therapies are common treatment with different drugs. Single drug therapy as combination of anti-fungal, antibacterial and topical corticosteroid has marvelous results. Nystatin, Clotrimaziole and miconazole are efficiently reviewed topical drugs with 73–100% cure.

Keywords: *C.albicans*, pathogenicity, systemic therapies, topical therapies and drugs

1. Introduction

Fungi like to reside in different areas such as air, soil, waterbodies, nutriment, attire, human body, flora and fauna. Coccidioidomycetes, Histoplasmosis and blastomycosis are human mycoses, native to different geographical regions and may found in any organism [1]. However, few individuals, effected by inhalation of these dimorphic fungi, show symptoms. On the other hand, opportunistic fungal infection mostly caused by some fungal species such as Candida, Aspergillus and Zygomycetes species, it basically present in immunosuppressed patients [2]. Among opportunistic fungi Candida classified as particularly threatened species to diseased person with week defense system. Cutaneous candidiasis is a skin infection caused by *C. albicans*. From 20 species of candida, 30 are causing infection in human [3–5].

Medically most important species of genus candida are *C. albicans, C.krusei, C. parapsilosis, C. glbrata, C. guilermondii, C. tropicalis* and *C. kefyr*. Invasive candidiasis present in those who have immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS), hematological disorders and cancer and may involve any internal organ or site [6]. By ingesting and killing opsonized candida,

immune system is very useful in host body defense against invasive candida [7]. In immunodeificient patient such as innate anemia and impaired natural immunity by physiological abnormalities in phagocytic cells, candida is primary threat [8, 9]. Cutaneous candidiasis is well defined disease characterized by candida infection of skin [4]. So the present study was designed to review pathogenicity, causes, signaling pathways and drug delivery system having ability to accelerate accumulation of drug in different cutaneous layer.

2. Overview of common clinical feature of cutaneous candidiasis

A considerable ratio of healthy individuals carry detectable number colonizing candida on skin, oral, gastrointestinal tract. Oral candida account 25–75% in healthy population [10], colonize mostly on the surface of oral cavity and most frequently present in dorsum of tongue. Review of literature showed that oral candida was continuous and regular. Adherence on epithelium by *C. albicans* blastocconidia and hyphae followed integrin like molecule such as INT1p factor [11, 12]. Arginine-glycine-aspartic acid (RGD) sequence recognition by adhesion molecule on epithelial cells and themselves express RGD sequence identified by mammalian integrins enhance adhesion of yeast to epithelium. Carriages of Candida species rarely establish into mucocutaneous so cutaneous and mucosal candidiasis poorly progresses to invasive candida disease.

A well known cutaneous candidiasis fungal infection may categorized or may be limited to integument on limited body surface. Candida skin infection expresses by interdigital candidiasis between fingers and toe may develop after softening and torn away finger's skin. Like fungi, candida develops in hot, dark and humid area which answered why it occur between skin surface, close to each other. Fissuring, maceration, pimples and cyst are mostly present on interdiginious skin area. Candida also cause rashes affecting anus and buttock area usually known as diaper dermatitis. Rash is common causing agent of blanitis, spread to scrotum, thigh and gluteal area. Candida is major source of folliculitis in immunosuppressed and obese patients [13].

Approximately 1% outpatient [14] reported with common disease of skin, cutaneous candidiasis affect all life stages as compared to 7% [15] of all inpatient visits in skin medical centre. Candida is primary source of skin disease or may develop secondary to other skin infections such as allergic dermatitis, psoriasis or existing diaper dermatitis. Cutaneous candidiasis develop in all body regions but frequently manifestation include intertrigo, inflammation, diaper, rashes and candidiasis of finger web [14]. Diversification of albicans species present but *C. albicans* play major role in human skin candidiasis [14]. Synergetic *candida albicans* fungus origin of wide human pathologies ranging from persistent or mild mucocutaneous infection to acute, lethal and disseminated disorder. It colonize at mucosal surface or skin and progression into candidiasis in immunocompromising, barrier disruption or wide antibiotic use [16–18]. Fourth major reason of nosocomial infection is prevalence of cutaneous candidiasis by C. albicans, pledged with 20–80% fatality. CD4+ T cells play role in HIV+ /AIDS patients susceptible to oropharyngeal candidiasis paly role in defense of this candidiasis [19].

In finding related to human, Consequences of interleukins-23 and interleukins-17 pathway briefly evaluated to protect mice candidiasis. It is also showed that IL-23, IL-17RC and Act-1deficient mice are also vulnerable to oropharyngeal

candidiasis(OPC) [20–23]. Similar signaling pathway for cutaneous candidiasis is also described in mouse model [24–26]. Altogether, it is showed that mouse is faithful animal to study immune response in candidiasis.

The IL-17 cytokines family comprise 6 related members. Interleukins member-17A, 17B, 17C,17D,17E (Interleukins-25) and 17F [27]. Still, limited data available about antifungal function of IL-17 family cytokines aside from IL-17A and IL-17F. IL-17A and IL-17F signal by heterodimeric receptor composed of IL-17RA and IL-17RC [28, 29]. Additionally, IL17RA combine with other partner of interleukin-17 family of receptors to make a binding complex for other IL-17 family cytokines [30] and predicted as interleukin −17 family of receptors signaling subunit.

Specifically, receptor complex (Interleukins-17RA and interleukins-17RE has cytokines interleukins-17C signal [27]. In comparison to iterleukins-17F and 17A, a lymphocyte derived, interleukins-17C is mainly released by upper integumentary tissue layer and keratinocytes [27, 31]. IL-17C like IL-17A govern natural defense at mucosal surface and skin by invigorating chemokines, inflammatory cytokines and antimicrobial peptides production. Interleukins-17A and interleukin-17C translated genes overlapping [32–34]. It is reported that interleukins-17C magnify direct signaling response by T-helper17 on T-helper 17 cells by interleukins-17RE/17RA [35]. Many studies has demonstrated protective role of IL-17C in gut and skin, but it is still poorly understood [36–38].

Local skin condition like occlusion, incomplete skin barrier, humidity and altered microbial flora promote cutaneous candidiasis infection. Many risk factor are known such as medical or disease related immunosuppression, endocrine disorders, malnutrition, pregnancy, steroid therapy, malignant diseases and compromised blood flow [16, 39].

Human cutaneous candidiasis is noxious dermatotosis defined by visible and microscopic cyst that are basically polymorphonuclear leukocytes. Epicutaneous implementation of candida blastospore occlusion for 24 hours in rodents create intraepidermal microabscesses and subcorneal cyst at sites of hyphal invasion. Only two species *C. albicans and C. stellatoidea* produces lesion after penetration in skin, remaining species fail to elicit inflammatory response [40]. Chemotactic stimuli generation may be related to clinical mechanism accountable for neutrophil cellular migration. In vitro studied explained migration of polymorphonuclear leukocytes by *C. albicans.* This response dependent on heat labile serum factor and independent of organism's viability [41]. Stratum corneum act as barrier but only to few species of candida. After penetration barrier, complement system mediates an acute neutrophil pustular response by inhibiting candida proliferation and prevents deep invasion of tissue [40].

Candida, human mucosal microbiota, not of skin carries by fingers. By microscopy and culture cutaneous candidiasis characterized and clinical diagnosis established. In these days different techniques namely polymerase chain reaction (PCR) and matrix-assisted-laser-desorption–ionization time-of-flight-mass-spectrometry (MALDI TOF-MS) manifested sensitive, rapid, well characterized method of candidiasis identification [42, 43].

The differentiation between living and dead fungi require cultures. National treatment guidelines exist for few invasive yeast infection [44] but lacking for skin yeast infection. Current treatment includes wide range of oral and topical therapies with anti-fungal and anti-inflammatory effects. Furthermore, topical treatment with corticosteroid have been established, but it is not clear whether these are preferable or not.

3. Pathogenesis stages

3.1 Biofilm formation

C. parapsilosis, C. albicans and other species are capable of biofilm formation, promoting incursion and inhibit killing by antifungal therapy (**Figure 1**). Yeast, pseudohyphae, hyphae and germ tubes, are multiple morphologies of *C. albicans* are whereas those formed by *Parapsilosis* morphologies consist of only two cells type: hyphae and yeast. All these biofilm attaches to medical instruments and limited penetration of antifungals. Biofilm start by attachment of yeast cell with surface layer [45]. This explain the need to central line tube elimination to clear candidiasis and improve outcome [46].

3.2 Evading host immune system

C. albicans has mechanism to dodge normal immune response, making it vulnerable to premature infants who are immunocompromised. *C. albicans* can conceal its surface structure and improper *Candida* phagocytosis because its hyphae surface protein mimic receptor of complement system. Fungus gas ability to eradicate cellular C3b complement, minimizing immune response. In case, candida recognition, its

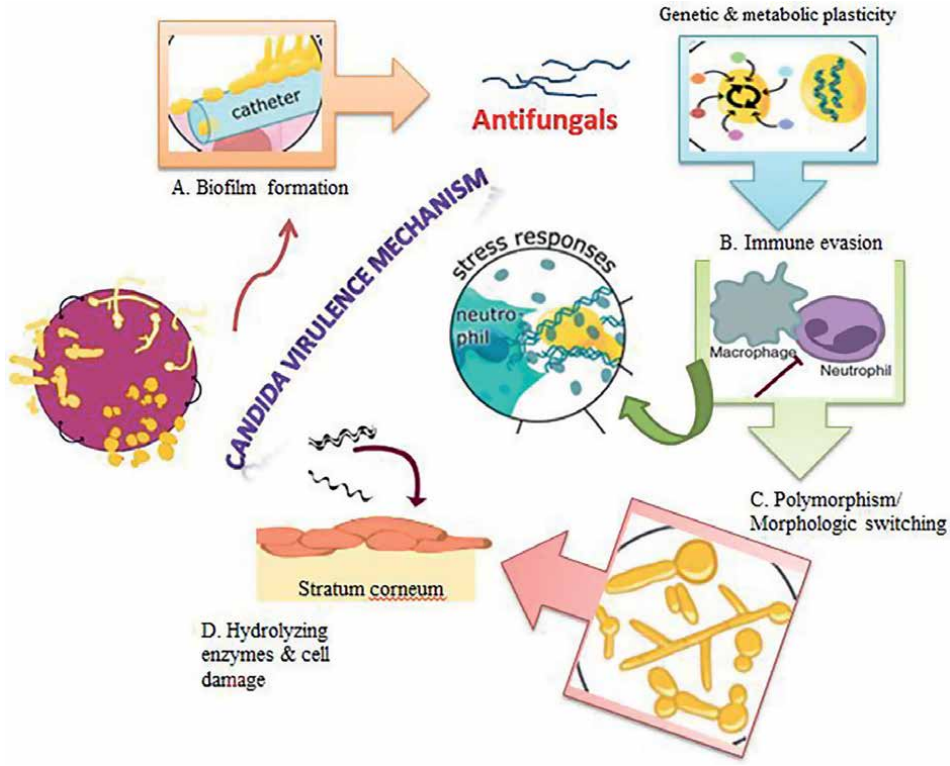

Figure 1.
Mechanism of C. albicans virulence. (A) Biofilm production, (B) evading host immune system, (C) morphologic switching, and (D) release of hydrolyzing enzymes.

hyphae involve in lysosomal fusion as well as elimination of macrophages to promote puncturing via filament elongation for destruction.

3.3 Morphologic switching

Morphologic switching shows capability of *C. albicans* to get both filamentous and yeast form. A filamentous hyphae exist in two forms either hyphae or pseudophyae. Changes in both form occur by ecological factor such as pH, temperature and amino acid presence. In vivo studies shows infection with wild type strain is more lethal and had greatest mortality rate than either filamentous only or yeast form [47]. The yeast form ease tissue and cell adhesion. In human cells, pseudohyphae enhance destruction and attenuated immune system. Another study indicated, both hyphal form of *C. albicans* are virulent, psudohyphal form has less virulency and can be more easily cleared from tissue as compared to hyphal form [48].

3.4 Hydrolyzing enzymes

C.albicans produced enzymes such as protease, phospholipase, lipase to accelerate digestion of epithelial cells and invasion in host tissue. A gene named as Int1 helped explain sticking mechanism, genes like *C. albicans* phospholipase B (caPLB 1) explain importance of phospholipase enzyme penetration in host cell [49]. Another group of enzymes, secreted aspartic acid (SAPs) help colonization of candida species. SAPs breaks different proteins including keratin and collagen, beneficial for host tissue [50]. Excluding *C. glabrtata,* almost all candida species produced SAPs*, C.albicans* produced highest amount of SAPs.

4. Virulence factors

There are many factors responsible for C. albicans virulence in colonization and intrusion of mucosal and cutaneous sites. The first indispensable step at epithelial cells, adhesion depend on upper surface elements of fungi affinity for epithelia. Candida adhesion molecule generally has three types.

One of these, a surface glycoprotein's protein constituent bind with arginine-glycine-aspartate (RGD), common to fibronectin, collagen, vitronectin and other extracellular matrix glycoprotein. In the second, binding of surface glycoprotein in lecithin like manner to sugar component of host glycoprotein. Surface mannoprotein a third one, least defined, polysaccharide constituent of candida attached with unknown host receptors. Different adhesion patterns have been reviewed in detail by calendar and Braun [51]. HIV/AIDS, mononucleosis, cancer and antibiotics can also increase risk of candidiasis [52]. Many studies suggested role of SAPs in colonization of oral epithelia [53]. Ex vivo analysis indicated, pepstatin inhibit a process, Pit formation by Candida albicans yeast in mouse corneocytes [54], which inhibit aspartyl proteinase enzymes, demonstrating Corneal layer invasion of proteinase. Pepstatin cannot hindered C. albicans attachment to corneocytes, only penetration, pointing out, colonization and adhesion of epidermal cells restricted by proteinase. Protein dependent penetration do not require morphologic alteration of yeast cell of candida to hyphae.

Hyphae formation is essential step for virulence and penetration of *C. albicans.* There is little evidence of hyphae role invasion, while yeast forms are associated with colonization. But hyphal form important colonizer in buccal epithelium So hyphae formation is not obligatory step for epidermal invasion. In vitro experiment shown hyphal property such as thigmotropism [55]. Histopathologic studies of *C.albicans* infected tissue, hyphae are unevenly distributed. Whereas keratinized cells have specific pattern along keratinocytes strata or perpendicular to it. Such pattern are not consistent as some plant fungal species exist perpendicular to cell boundaries.

It is suggested hyphae may prefer to grow appropriately towards all time available nutrients due to thigmotropism to micro surface in keratinized layer. A general factor, surface hydrophobicity, regulating cell adhesion by wander walls forces [56]. *C albicans* show wide hydrophobicity with respect to growth temperature [57], these variation associated with epithelial cell adhesion [58]. As a result hydrophobicity play role in pathogenesis of cutaneous candidiasis. Virulence factor by *C. albicans* rarely and discontinuously expressed in all possible microenvironment. There is no clear evidence to explain *C. albicans* high potential for rapid switching of expressed phenotypes [59] by transcriptional regulation expression [60]. This phenomena helpful to adapt different microenvironment to regulate gene expression of virulence during colonization and penetration [61].

5. Diagnosis

Adjunctive diagnosis is miracle, not any replaced culture but they useful only for high risk patients treated antifungal therapy and monitoring therapy response. Fungal outer membrane polysaccharide such as Beta-D-glucana de mannan and polymerase chain reaction assist detecting non-blood stream infection and treatment. Currently they do not give good result in identifying true infection and costly. BDG level is also helpful in detecting antifungal therapy. Serum BDG level in infants with invasion is 364 pg./ml vs.89 pg./ml in noninfected infants, decreases by antifungal therapy to 58 pg./ml [59].

Another assay to decide treatment therapy is direct buffy coated fluorescent assay [60]. In this analysis, fluorescent stain bind to cellulose and chitin containing structure. Fluorescent test helpful to recognize hyphae and spores following 2 hours duration. Bimolecular techniques such as PCR and DNA microarray technology, recognize fungi and its antifungal potential more quickly and with higher sensitivity than blood culture. PCR is helpful during higher fungal infection expression. PCR can detect candidiasis, nonblood stream infection, candida peritonitis and endotracheal colonization. Adjunctive test and PCR test are not critically studied.

6. Cutaneous candidiasis treatment

Immunocompromised patients are susceptible to fungal infection. The incidence of superficial and deep seated infection [62]. Cutaneous candidiasis is most common problem in human beings [63]. Various treatment strategies such as systemic therapy, topical drug treatment are used by different drug delivery system. Many treatment strategies are challenging and restricted to small group of compound, azole.

6.1 Systemic therapy

Systemic antifungal therapy is miracle for cutaneous candidiasis treatment with until complete remission occur with multiple week requirement. Various other infection such as catheter site and fungal digital escher are cured by continuous systemic therapy. Epithelization of skin lesion is helpful. Untreated primary skin infection extends far from visible range of lesion so systemic antifungal therapy applied before debridement (2–3 weeks). However, debridement role in neutropenic patient is unclear.

6.2 Treatment by fluconazole

A synthetic Trazole, fluconazole drug of 3rd generation is most preferable in clinical patients [64, 65]. FLZ is used as tablet and (i.v) injection, but restricted due to side effects such as skin and gastrointestinal irritation and taste disturbance. Application of solid lipid nanoparticles (SLN) and nanostructured lipid carrier (NLCs) for drug application in topical therapy of cutaneous candidiasis is another approach of encapsulation. The SLNS has great ability to direct active molecule and enhance therapeutic effectiveness with significant practicality of induction of lipophilic and hydrophilic drug. It has more efficacy, physical maintaince and low cost as compared to liposomes. Gold nanoparticles are also widely used in drug application [66].

Moreover, Physiological lipid core with epidermal attacking, follicular transfer and controlled emission of active molecule with increased skin hydration caused by occlusivity demonstrated high biocompatibility and biodegradability. Administration of FLZ require prolonged therapy, however systemic therapy application leads to disastrous by products [67]. It urges to develop fruitful FLZ preparation to avoid problem.

6.3 Treatment by Sertaconazole

Sertaconazole nitrate, a broad range topical antifungal factor belonging to azole class of drugs consisting of benzothiophene radical and excess lipophilic content [68, 69]. Synthesis reaction with lipophilic benzithiophene ether cause penetration of sertaconazole into keratinized layer of skin [70]. Similarly, these method recognize molecule without systemic absorption [70]. Chemical alteration to imidazole loop of sertaconazole nitrate boost up its in vitro antifungal activity against candida than other molecule of same antifungal class [68]. Sertaconazole nitrate shown high stability under different physiochemical condition [69].

6.4 Mechanisms of action of sertaconazole

Candida species show 3 main antifungal drug targets cell membrane, cell wall and nucleic acid [71]. It interacts with biosynthetic pathway of ergosterol by suppressing cytochrome P450-dependent 14 alpha-lanosteroldemethylase or Erg11p, regulated by ERG11 gene. This inhibition decreases addition of methyl to lanosterol to from ergosterol, hence ergosterol amount in fungal cell wall decreases resulting of toxic sterol mechanism intermediate humus to inhibit cell growth (**Figure 2**). Consequently, high lanosterol leads to absence of main fluidity, symmetry, permeability and integrity controlling factors [72–76]. Azole basically are fungistatic. Sertaconazole has an

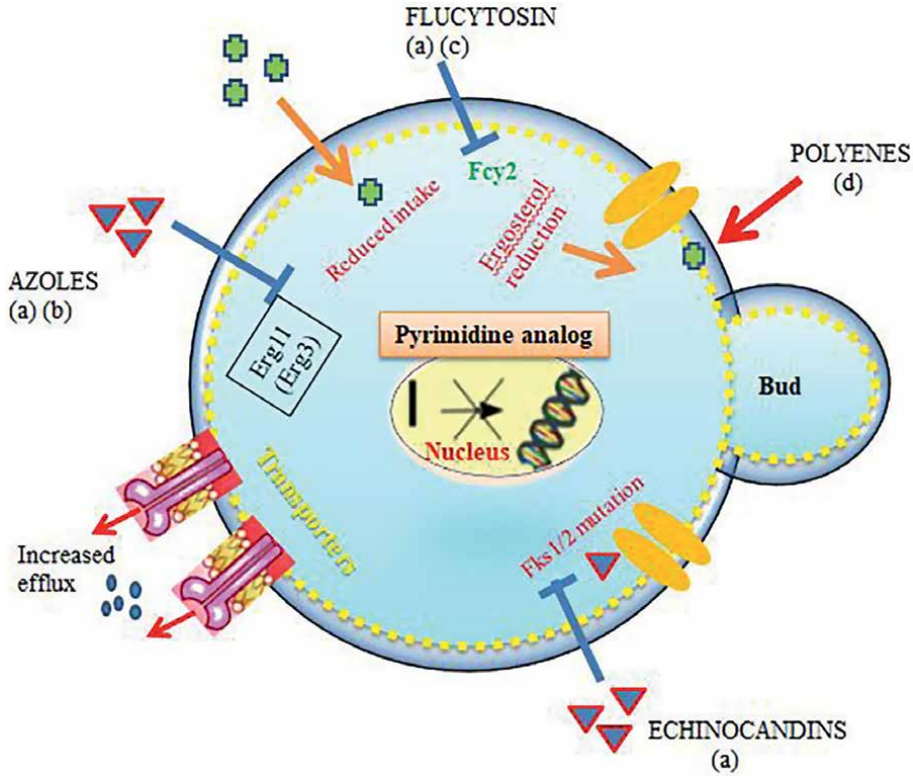

Figure 2.
Antifungal drug site of action in yeast-like cell.

additional effect, bind to nonsterol lipid of plasma membrane like other azoles, to change cell viability [77].

Azole and iron atom group of heme interaction leads to inactivation of 14 alpha-lanosterol demethylase blockage of yeast mycelium transformation. This step inhibit attachment of fungus to human skin surface, initial step of treatment [78]. Ketoconazole possess anti-fungal activity against Candida and dermatophyte infections, leading to KTZ development with hope that drug is not absorbed hence reduced drug related side effect. Although some studies have shown to moderate skin and eye irritation by using topical KTZ application, but systemic toxicity in anima is reported in literature [79]. Sertaconazole is also capable of inhibiting hyphae growth and conglomeration of shorter, poorly developed and cluttered blastocconidia [80]. Sertoconazole also bind directly with nonsterol lipid in the cell membrane in the absence of ergosterol synthesis, interacting permeability regulation, and cause leakage of intracellular components ATP, similar to miconazole and ketoconazole [81].

6.5 Topical therapy

It helpful for local treatment, noninvasive and applicable directly to site of action [82]. Topical formulation creams, lotion, gels, spray resulted in skin absorption, itching, thus failed to proper removal of yeast [83, 84]. In Switzerland, liposomal gel, 1st topical gel econazole established in 1994 [85, 86]. FLZ topical drug delivery

developed by gel micellar emulsion [87], gel designing [67], Organogel with lecithin content [88] and FLZ containing hydrogel [67] extensively studied.

For sustained drug release, skin aggregation for localized effect in skin and less permeation of drug required. So vesicular carrier is best and effective way of topical drug delivery [85, 86]. Vesicular carrier helped in best drug transfer, increase concentration and improved potency [89, 90]. Localization of drug at application site is characterized by vesicular carrier for reservoir, reducing dose, dosing frequency and systemic side effects [90].

6.6 Liposomal treatment

Over last few decades, fungal infection susceptibility and vulnerability upgraded in terms of frequency and major causative agent of death [91, 92]. Immunosuppressed patients are more prone than impaired immunity system [62]. Outer layer and deep seated infection rate rises gradually [93, 94] skin fungal infection is common problem, treatment strategy is challenging and limited to small number of compounds such as azole [63]. A wide range of azole antifungal agent are exploited, however, due to toxicity clinical use is restricted.

Phospholipid and nonionic surfactants containing liposomes and niosomes respectively more advantageous over conventional method. Niosomes and liposomes are analogous, best chemical structure, moderate cost and diversification of surfactant as compared to liposomal based vesicles. Most important characteristics are amphipathic nature allowing carring both hydrophilic and hydrophobic drugs [95, 96]. Liposome and niosomes act as dissolvable matrix and no specific point of drug delivery [97] Clotrimaziole loaded liposomes might be effective for skin candidiasis, as localized effect [83]. Literature reviewed that liposomes and niosomes have wide ability for TRA (Tretinoin) and niosomes carrying Ketoconazole are dominant over plain drug solution [98] showed that ketoconazole-containing niosomes offer a considerable advantage over plain drug solution. Literature reviewed that liposomes and niosomes have wide ability for Tretinoin and enhanced cutaneous drug accretion [96, 99–100]. Additionally, delivery of drug deeper layer of skin with highest accumulation of finasteride in follicular region is reported [97].

Similarly, toxicity of drug is lessened by drug pharmacology and bioavailability modification [101]. All these carrier system met desired sustained drug release characteristics versus local delivery. On the other hand, they show drawbacks such as liquid type and wash out problem. Furthermore, they can also be applied to commonly used dermal vehicle such as hydrogel, proper semisolid stability with appropriate dermal use [83, 102].

7. Conclusion

Genetic basis of immunodeficiency disease lead to best diagnosis and treatment and increased knowledge to pathophysiology of immune system. Clotrimaziole, nystatin and miconazole most important drugs with high clinical and mycological cure. Moving towards oral therapy, fluconazole only available evidence based option for systemic treatment. FLA loaded CA lipid based SLNs explained better activity against fungus by localized drug depot formation. This delivery system has great ability for dermal delivery of drug to treat cutaneous candidiasis. In perspective, resistance to candida species increases, these is need of further investigation and treatment option to reduce use of drugs.

Authors contributions

Iqra Farzeen, Rahat Andleeb, Asma Ashraf & Muhammad Waseem has substantially contributed to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work.

Saima Muzammil, Azhar Rafique & Razia Noreen participated in drafting or revising the work.

Rahat Andleeb, Asma Ashraf & Muhammad Umar Ijaz approved the final version of the work to be published.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Pfaller MA. Epidemiology and control of fungal infections. Clinical Infectious Diseases. 1994;**19**(Suppl. 1):8-13

[2] Koundal S, Cojandaraj L. Candida species–morphology, medical aspects and pathogenic spectrum. European Journal of Molecular & Clinical Medicine. 2020;**7**(07):4015-4021

[3] Pfaller MA, Diekema DJ. Epidemiology of invasive mycoses in North America. Critical Reviews in Microbiology. 2010;**36**:1-53

[4] Espinosa-Hernández VM, Morales-Pineda V, Martínez-Herrera E. Skin infections caused by emerging Candida species. Current Fungal Infection Reports. 2020;**14**:99-105

[5] Nurdin RSC et al. Cutaneous candidiasis caused by Candida kefyr. Pan African Medical Journal. 2021;**38**(178):1-8

[6] Neofytos D, Fishman JA, Horn D, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transplant Infectious Disease. 2010;**12**:220-229

[7] Maródi L, Johnston RB Jr. Host-fungal infections relevant to the newborn infant. In: Polin RA, Fox WW, Abman SH, editors. Fetal and Neonatal Physiology. 4th ed. Philadelphia, PA: Elsevier Saunders; 2011. pp. 1566-1569

[8] Rezaei N, Moin M, Pourpak Z, et al. The clinical, immunohematological, and molecular study of Iranian patients with severe congenital neutropenia. Journal of Clinical Immunology. 2007;**27**:525-533

[9] Segal BH, Herbrecht R, Stevens DA, et al. Defining responses to therapy and study outcomes in clinical trials of invasive fungal diseases: Mycoses study group and European Organization for Research and Treatment of Cancer consensus criteria. Clinical Infectious Diseases. 2008;**47**:674-683

[10] Cannon RD, Holmes AR, Mason AB, Monk BC. Oral Candida: clearance, colonization, or candidiasis? Journal of Dental Research. 1995;**74**:1152-1161

[11] Hostetter MK. RGD-mediated adhesion in fungal pathogens of humans, plants and insects. Current Opinion in Microbiology. 2000;**3**:344-348

[12] Hostetter MK. The iC3b receptor of Candida albicans and its roles in pathogenesis. Vaccine. 2008;**26**(Suppl. 8):108-112

[13] Maródi L. Mucocutaneous candidiasis. In: Stiehm's Immune deficiencies. Vol. 1. Tokyo: Academic Press; 2014. pp. 775-802

[14] Sei Y. 2011 epidemiological survey of dermatomycoses in Japan. Journal of Medical Mycology. 2015;**56**:129-135

[15] Peñate Y, Guillermo N, Melwani P, et al. Dermatologists in hospital wards: An 8 -year study of dermatology consultations. Dermatology. 2009;**219**:225-231

[16] Yost CC, Cody MJ, Harris ES, et al. Impaired neutrophil extracellular trap (NET) formation: A novel innate immune deficiency of human neonates. Blood. 2009;**113**:6419-6427

[17] Huppler AR, Bishu S, Gaffen SL. Mucocutaneous candidiasis: The IL-17 pathway and implications for targeted immunotherapy. Arthritis Research & Therapy. 2012;**14**(4):2

[18] Glocker E, Grimbacher B. Chronic mucocutaneous candidiasis and congenital susceptibility to Candida. Current Opinion in Allergy and Clinical Immunology. 2010;**10**:542-550

[19] Fidel PL. Candida-host interactions in HIV disease: Implications for oropharyngeal candidiasis. Advances in Dental Research. 2011;**23**(1):45-49

[20] Conti H, Peterson A, Huppler A, Brane L, Hernández-Santos N, Whibley N, et al. Oral-resident 'natural' Th17 cells and γδ-T cells control opportunistic Candida albicans infections. The Journal of Experimental Medicine. 2014;**211**(10):2075-2084

[21] Conti H, Shen F, Nayyar N, Stocum E, Sun JN, Lindemann M, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. The Journal of Experimental Medicine. 2009;**206**(2):299-311

[22] Ferreira MC, Whibley N, Mamo AJ, Siebenlist U, Chan YR, Gaffen SL. Interleukin-17-induced protein lipocalin 2 is dispensable for immunity to oral candidiasis. Infection and Immunity. 2014;**82**(3):1030-1035

[23] Ho A, Shen F, Conti H, Patel N, Childs E, Peterson A, et al. IL-17RC is required for immune signaling via an extended SEFIR domain in the cytoplasmic tail. Journal of Immunology. 2010;**185**:1063-1070

[24] van de Veerdonk FL, Kullberg BJ, Verschueren IC, Hendriks T, van der Meer JW, Joosten LA, et al. Differential effects of IL-17 pathway in disseminated candidiasis and zymosan-induced multiple organ failure. Shock. 2010;**34**(4):407-411

[25] Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic antiCandida albicans host defense in mice. The Journal of Infectious Diseases. 2004;**190**(3):624-631

[26] Kagami S, Rizzo HL, Kurtz SE, Miller LS, Blauvelt A. IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal skin host defense against Candida albicans. Journal of Immunology. 2010;**185**(9):5453-5462

[27] Kashem SW, Kaplan DH. Skin immunity to Candida albicans. Trends in Immunology. 2016;**37**(7):440-450

[28] Toy D, Kugler D, Wolfson M, Van den Bos T, Gurgel J, Derry J, et al. Cutting edge: Interleukin-17 signals through a heteromeric receptor complex. Journal of Immunology. 2006;**177**(1):36-39

[29] Kuestner R, Taft D, Haran A, Brandt C, Brender T, Lum K, et al. Identification of the IL-17 receptor related molecule, IL-17RC, as the receptor for IL-17F. Journal of Immunology. 2007;**179**:5462-5467

[30] Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. Immunity. 2011;**34**:149-162

[31] Ramirez-Carrozzi V, Sambandam A, Luis E, Lin Z, Jeet S, Lesch J, et al. IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. Nature Immunology. 2011;**12**(12):1159-1166

[32] Golden JB, McCormick TS, Ward NL. IL-17 in psoriasis: Implications for therapy and cardiovascular comorbidities. Cytokine. 2013;**62**(2):195-201

[33] Yamaguchi Y, Fujio K, Shoda H, Okamoto A, Tsuno NH, Takahashi K, et al. IL-17B and IL-17C are associated

with TNF-alpha production and contribute to the exacerbation of inflammatory arthritis. Journal of Immunology. 2007;**179**(10):7128-7136

[34] Li H, Chen J, Huang A, Stinson J, Heldens S, Foster J, et al. Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(2):773-778

[35] Chang SH, Reynolds JM, Pappu BP, Chen G, Martinez GJ, Dong C. Interleukin-17C promotes Th17 cell responses and autoimmune disease via interleukin-17 receptor E. Immunity. 2011;**35**(4):611-621

[36] Friedrich M, Tillack C, Wollenberg A, Schauber J, Brand S. IL-36γ sustains a Proinflammatory Selfamplifying loop with IL-17C in anti-TNF-induced Psoriasiform skin lesions of patients with Crohn's disease. Inflammatory Bowel Diseases. 2014;**20**(11):1891-1901

[37] Martin DA, Towne JE, Kricorian G, Klekotka P, Gudjonsson JE, Krueger JG, et al. The emerging role of IL-17 in the pathogenesis of psoriasis: Preclinical and clinical findings. Journal of Investigative Dermatology. 2013;**133**(1):17-26

[38] Johnston A, Fritz Y, Dawes SM, Diaconu D, Al-Attar PM, Guzman AM, et al. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. Journal of Immunology. 2013;**190**(5):2252-2262

[39] Del Rosso JQ, Kircik LH. Optimizing topical antifungal therapy for superficial cutaneous fungal infections: Focus on topical naftifine for cutaneous dermatophytosis. Journal of Drugs in Dermatology. 2013;**12**:165-171

[40] Ray TL, Wuepper KD. Experimental cutaneous candidiasis in rodents. The Journal of Investigative Dermatology. 1976;**66**:29-33

[41] Denning TJV, Davies RR. Candida albicans and the chemotaxis of polymorphonuclear neutrophils. Sabouraudia. 1973;**11**:210-221

[42] Maibach HI, Kligman AM. The biology of experimental human cutaneous moniliasis (Candida albicans). Archives of Dermatology. 1962;**85**:233-254

[43] Rebora A, Marples RR, Kligman AM. Experimental infection with Candida albicans. Archives of Dermatology. 1973;**108**:69-73

[44] Arendrup MC, Boekhout T, Akova M, Meis JF. ESCMID † and ECMM ‡ joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. Clinical Microbiology and Infection. 2014;**20**:76-98

[45] Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, et al. Candida albicans. The virulence factors and clinical manifestations of infection. Journal of Fungi. 2021;**7**:79

[46] Benjamin DK, Stoll BJ, Fanaroff AA, et al. Neonatal candidiasis among extremely low birth weight infants: Risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006;**117**:84-92

[47] Bendel CM, Hess DJ, Garni RM, et al. Comparative virulence of Candida albicans yeast and filamentous forms in orally and intravenously inoculated mice. Critical Care Medicine. 2003;**31**:501-507

[48] Cleary IA, Reinhard SM, Lazzell AL, et al. Examination of the pathogenic potential of Candida albicans

filamentous cells in an animal model of haematogenously disseminated candidiasis. FEMS Yeast Research. 2016;**16**:11

[49] Leidich SD, Ibrahim AS, Fu Y, et al. Cloning and disruption of caPLB1, a phospholipase B gene involved in the pathogenicity of Candida albicans. The Journal of Biological Chemistry. 1998;**273**:26078-26086

[50] Naglik JR, Challacombe SJ, Hube B. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. Microbiology and Molecular Biology Reviews. 2003;**67**:400-428

[51] Calderone RA, Braun PC. Adherence and receptor rclationships of Candida albicans. Microbiological Reviews. 1991;**55**:1-20

[52] Udemezue O. Therapeutic Potential of Natural Compounds for Cutaneous VandidiasisTreatment using Murine Models. by Udemezue, O. I. May 2021. pp. 1-220

[53] Borg M, Ruchel R. Expression of extracellular acid proteinase by proteolytic Candida spp during experimental infection of the oral mucosa. Infection and Immunity. 1988;**56**:626-631

[54] Ray TL, Payne CD. Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: A role of Candida acid proteinase. Infection and Immunity. 1988;**56**:1942-1949

[55] Sherwood J, Gow NAR, Oooday OW, Gregory OW, Marshall D. Contact sensing in Candida albicans: A possible aid to epithelial penetration. Journal of Medical and Veterinary Mycology. 1992;**30**:461-470

[56] Klotz SA, Drutz DJ, Zajic E. Factors governing adherence of Candida species to plastic surfaces. Infection and Immunity. 1985;**50**:97-101

[57] Antley PP, Hazen KC. Role of yeast cell growth temperatureon Calldida albicans virulence in mice. Infection and Immunity. 1988;**56**:2884-2890

[58] Hazen KC, Brawner DL, Riesselman MH, Jutila MA, Cutler JE. Differential adherence of hydrophobic and hydrophilic Candida albicans yeast cells to mouse tissues. Infection and Immunity. 1991;**59**:907-912

[59] Soli DR. High-frequency switching in Candida albicans. Clinical Microbiology Reviews. 1992;**5**:183-203

[60] Soli DR, Morrow B, Srikantha T. High-frequency phenotypic switching in Candida albicans. Trends in Genetics. 1993;**9**:61-65

[61] Soil DR, Galsask R, Schmid J, Hanna C, Mac K, Morrow B. Genetic dissimilarity of commensal strains of Candida spp carried in different anatomical locations of the same healthy women. Journal of Clinical Microbiology. 1991;**29**:1702-1710

[62] Soni N, Wagstaff A. Fungal infection: Focus on critical care: Antibiotics and ICU. Current Anaesthesia & Critical Care. 2005;**16**:231-241

[63] Calzavara-Pinton PG, Venturini M, Sala RA. Comprehensive overview of photodynamic therapy in the treatment of superficial fungal infections of the skin. Journal of Photochemistry and Photobiology B: Biology. 2005;**78**:1-6

[64] Ruhnke M, Hartwig K, Kofla G. New options for treatment of candidaemia in critically ill patients.

Clinical Microbiology and Infection. 2008;**14**:46-54

[65] Alekha KD, William FE. Fluconazole. In: Florey K, editor. Analytical Profiles of Drug Substances and Excipients. London, UK: Academic Press; 2006. pp. 57-113

[66] Kareem HA, Samaka HM, Abdulridha WM. Evaluation of the effect of the gold nanoparticles prepared by green chemistry on the treatment of cutaneous candidiasis. Current Medical Mycology. 2021;**7**(1):1-5

[67] Abdel-Mottaleb MMA, Mortada ND, El-Shamy AA, Awad GAS. Physically cross-linked polyvinyl alcohol for the topical delivery of fluconazole. Drug Development and Industrial Pharmacy. 2009;**35**:311-320

[68] Raga MM, Moreno-Manas M, Cuberes MR, et al. Synthesis and antimycotic activity of (benzo[b]thienyl) methyl ethers of 1-(2,4- dichlorophenyl)-2-(1H-imidazol1-yl)- ethanol and of (Z)-1 (2,4-dichlorophenyl)-2-(1H-imidazol-1-yl) ethanone oxime. Arzneimittel Forschung. 1992;**42**:691-694

[69] Albet C, Fernández JM, Sacristán A, Ortíz JA. Physicochemical properties, analytical determinations and stability of sertaconazole nitrate. Arzneimittel Forschung. 1992;**42**:695-698

[70] Farré M, Ugena B, Badenas JM, Márquez M, Roset P, Ortiz JA. Pharmacokinetics and tolerance of sertaconazole in man after repeated percutaneous administration. Arzneimittel-Forschung. 1992;**42**(5A):752-754

[71] Cannon RD, Lamping E, Holmes AR, et al. Candida albicans drug resistance—Another way to cope with stress. Microbiology. 2007;**153**:3211-3217

[72] Odds FC, Brown AJ, Gow NA. Antifungal agents: Mechanisms of action. Trends in Microbiology. 2003;**11**:272-279

[73] Ghannoum MA, Rice LB. Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clinical Microbiology Reviews. 1999;**12**(4):501-517

[74] Akins RA. An update on antifungal targets and mechanisms of resistance in Candida albicans. Medical Mycology. 2005;**43**(4):285-318

[75] Hargrove TY, Friggeri L, Wawrzak Z, Qi A, Hoekstra WJ, Schotzinger RJ, et al. Structural analyses of Candida albicans sterol 14α-demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol biosynthesis. Journal of Biological Chemistry. 2017;**292**(16):6728-6743

[76] Palacín C, Tarragó C, Ortiz JA. Sertaconazole: Pharmacology of a gynecological antifungal agent. International Journal of Gynaecology and Obstetrics. 2000;**71**(Suppl. 1): 37-46

[77] Carrillo-Muñoz AJ, Giusiano G, Ezkurra PA, Quindós G. Antifungal agents: Mode of action in yeast cells. Revista Española de Quimioterapia. 2006;**19**(2):130-139

[78] Agut J, Palacín C, Sacristán A, Ortiz JA. Inhibition of ergosterol synthesis by sertaconazole in Candida albicans. Arzneimittel-Forschung. 1992;**42**(5A):718-720

[79] Choi FD, Juhasz MLW, Natasha AM. Topical ketoconazole: A systematic review of current dermatological applications and future developments. Journal of Dermatological Treatment. 2019;**30**(8):760-771

[80] Figueras MJ, Cano JF, Guarro J. Ultrastructural alterations produced by sertaconazole on several opportunistic pathogenic fungi. Journal of Medical and Veterinary Mycology. 1995;**33**(6):395-401

[81] Agut J, Palacín C, Salgado J, Casas E, Sacristán A, Ortíz JA. Direct membrane damaging effect of sertaconazole on Candida albicans as a mechanism of its fungicidal activity. Arzneimittel Forschung. 1992;**42**:721-724

[82] Murdan S. Drug delivery to the nail following topical application. International Journal of Pharmaceutics. 2002;**236**:1-26

[83] Ning M, Guo Y, Pan H, Chen X, Zhongwei G. Preparation, in vitro and in vivo evaluation of liposomal/niosomal gel delivery systems for clotrimazole. Drug Development and Industrial Pharmacy. 2005;**31**:375-383

[84] Bidkar S, Jain D, Padsalg A, Patel K, Mokale V. Formulation development and evaluation offluconazole gel in various polymer bases. Asian Journal of Pharm. 2007;**1**:63-68

[85] Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. Journal of Controlled Release. 1994;**30**:l-15

[86] Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Ponec M. Structure of the skin barrier and its modulation by vesicular formulations. Progress in Lipid Research. 2003;**42**:1-36

[87] Laithy HMEI, EI-Shaboury KMF. The development of cutina lipogels and gel microemulsion for topical administration of fluconazole. AAPS PharmSci. 2002;**3**:1-9

[88] Jadhav KR, Kadam VJ, Pisal SS. Formulation and evaluation of lecithin organogel for topical delivery of fluconazole. Current Drug Delivery. 2009;**6**:174-183

[89] Yimei J, Jolya L, Omria A. Liposomes as a carrier for gentamicin delivery: Development and evaluation of the physicochemical properties. International Journal of Pharmaceutics. 2008;**59**:254-263

[90] Manosroi A, Wongtrakul P, Manosroi J, Sakai H, Sugawara F, Yuasa M, et al. Characterization of vesicles prepared with various nonionic surfactants mixed with cholesterol. Colloids and Surfaces B: Biointerfaces. 2009;**30**:129-138

[91] Shao P, Huanga L, Hsueh P. Recent advances and challenges in the treatment of invasive fungal infections. International Journal of Antimicrobial Agents. 2007;**30**:487-495

[92] Ramsdale M. Programmed cell death in pathogenic fungi. Biochimica et Biophysica Acta. 2008;**1783**:1369-1380

[93] Donnelly R, McCarron P, Tunney M. Antifungal photodynamic therapy. Microbiological Research. 2008;**163**:1-12

[94] Segal E, Elad D. Fungal vaccines and immunotherapy. Journal of Medical Mycology;**16**:134-151

[95] Uchegbu IF, Vyas SP. Nonionic surfactant-based vesicles (niosomes) in drug delivery. International Journal of Pharmaceutics. 1998;**2006**(172):33-70

[96] Araujo VHS, Duarte JL, Carvalho GC, Silvestre ALP, Fonseca-Santos B, Marena GD, et al. Nanosystems against candidiasis: A review of studies performed over the last two decades. Critical Reviews in Microbiology. 2020;**46**(5):508-547

[97] Tabbakhian M, Tavakoli N, Jaafari MR, Daneshamouza S. Enhancement of follicular delivery of finasteride by liposomes and niosomes I. In vitro permeation and in vivo deposition studies using hamster flank and ear models. International Journal of Pharmaceutics. 2006;**323**:1-10

[98] Satturwar PM, Khandare JN, Nande VS. Niosomal delivery of ketoconazole. Indian Drugs. 2001;**38**:620-624

[99] Foong WC, Harsanyi BB, Mezei M. Biodisposition and histological evaluation of topically applied retinoic acid in liposomal, cream, and gel dosage forms. In: Hanin I, Pepeu G, editors. Phospholipids. 1990. pp. 139-154

[100] Sinico C, Manconi M, Peppi M, Lai F, Valenti D, Fadda AM. Liposomes as carriers for dermal delivery of tretinoin: in vitro evaluation of drug permeation and vesicle-skin interaction. Journal of Controlled Release. 2005;**103**:123-136

[101] Junginger HE, Hofland HEJ, Bouwstra JA. Liposomes and niosomes interactions with human skin. Cosmetics and Toiletries. 1991;**105**:45-50

[102] Pavelic Z, Skalko-Basnet N, Schubert R. Liposomal gels for vaginal drug delivery. International Journal of Pharmaceutics. 2001;**219**:139-149

Section 4

Candida Treatments, Cytotoxicity, New Molecules Capable of Preventing Biofilms, Proteome Identification, Diagnosis, Proteomic Approach Advancements, New Effective Compounds and Natural Compounds

Chapter 6

Novel Treatment Approach against *Candida* spp.: Evaluation of Antifungal and Antibiofilm *In Vitro* Activity of Dendritic Molecules

Natalia Gómez-Casanova, José Luis Copa-Patiño and Irene Heredero-Bermejo

Abstract

Infections caused by the genus *Candida* are a serious threat, especially in the sanitary field. These pathogens are able to generate biofilms, which is one of the main problems because they are difficult to eradicate and are associated with a high mortality rate. These biofilms provide *Candida* species with increased resistance to health care drugs and disinfectants. Currently, the resistance to antifungals is increasing gradually and there are few drugs accepted for clinical use capable of combating them, and, unfortunately, these substances are sometimes toxic at the effective doses required. Therefore, finding new molecules capable of preventing the formation of biofilms or eradicating them once generated is of vital importance. In addition, it is essential to know the appropriate techniques to evaluate a new compound, guaranteeing reliable and precise data. Studies with dendritic systems of cationic nature are recently being carried out, presenting interesting and encouraging results as antimicrobials, against cells cancer cells, surface activating agents, and encapsulation of antibiotic, among others. In this chapter, we will focus on its antifungal capacity, especially its antibiofilm activity against *Candida* spp.

Keywords: *Candida*, treatments, cytotoxicity, biofilms, microscopy, dendrimers, dendrons, dendritic compounds, resazurin

1. Introduction

In recent years, the cases of fungal infections are gradually increasing. These infections range from skin lesions to systemic infections that can lead to the death of the patient. Unfortunately, patients who are immunosuppressed or that suffered from invasive procedures are particularly affected and at risk of taking these kind of infections [1]. One pathogen of particular interest in the clinical field are the species belonging to *Candida* genus. *Candida* spp. are a group of opportunistic pathogens that are usually part of the human microbiota. Under favorable conditions, such as those mentioned above, they can invade tissues and lead to a significant infection

that can result in the death of the patients. There are two common types of *Candida* species: *Candida albicans* and non-albicans *Candida* (NAC). *C. albicans* has been the most frequent species related to fungal infections over the years and, therefore, the most studied. However, studies and infections by other *Candida* species are becoming more frequent, and even also are being isolated more frequently in the clinical environment. Among these NAC species, the most frequent are *C. glabrata, C. tropicalis, or C. parapsilosis* [2]. These pathogens are responsible for the majority of nosocomial fungal infections [3]. The mortality rate associated is around 40%, therefore, these pathogens are highly relevant to hospital environment. In addition, it is important to mention the appearance of new *Candida* species. For example, *C. auris* is associated with mortal candidemia and exhibits multidrug resistance [4].

A relevant virulence factor that some species of the genus *Candida* present is their ability to develop biofilms on diverse surfaces. These biofilms are highly organized communities of cells that are attached to biotic or abiotic surfaces and are surrounded by a self-produced extracellular matrix [5]. This condition is of special clinical relevance because these pathogens can grow and form biofilms in surgical materials, such as catheters or prosthesis. The main problem associated with these biofilms is that in this state, *Candida* cells are much more protected both from environmental conditions, antifungal exposure, and from the immune system. Consequently, treatment failure may occur and, unfortunately, on some occasions, it can cause the death of hospitalized or immunocompromised patients.

The increase in the resistance to current antifungals, their low effect against biofilms, and that they are associated with greater cytotoxicity, make it strictly necessary to find new compounds that may offer new alternatives against these pathogenic yeasts. A new alternative in the search for effective molecules for the treatment of these human pathogens is the use of dendritic systems. Among these systems, there are dendrimers and dendrons, with monodisperse branched structures with a high capacity for multifunctionalization [6, 7]. These structures serve a large number of applications, such as diagnostic agents, drug vectors, drug encapsulation, cancer treatment, antimicrobial treatment, or nucleic acid delivery, among others [8, 9]. Therefore, these molecules can be highly versatile and cost-effective. In addition, they are very interesting not only because they have antimicrobial activity by themselves, but also because they could serve as nanocarriers to increase the bioavailability of antifungal drugs with poor solubility [10, 11].

This chapter will focus on the use of different dendritic compounds as a new antifungal strategy against *Candida s*pp. biofilms. Besides, it will discuss different colorimetric methods for evaluating the biocide activity of these new compounds.

2. *Candida*: Biofilm and stages of biofilm development

Biofilms are extraordinarily complex microbial communities that are adhered to surfaces (biotic or abiotic) and surrounded by a self-produced extracellular matrix, which, among other things, provides them with protection. Biofilm formation of *C. albicans* begins with the adherence of cells to a solid surface, creating a basel layer. This is followed by a phase of cell proliferation and early-stage of filamentation of attached cells. Then, *Candida* cells can invade the surface they have been attached to. The invasion is caused by the secretion of some hydrolytic enzymes, such as hemolysins, proteinases, and phospholipases, being the most studied secreted aspartyl

proteases (SAP) [12]. Afterward, the biofilm matures, and during this phase, we can find both hyphal cells, pseudohyphae cells, and round yeast cells. During the maturation, different extracellular polymeric substances (EPS) are secreted, such as β-1,6-glucan, α-mannan, and β-1,3-glucan, among others. All these substances are part of the extracellular matrix that surrounds the set of cells that constitute the biofilm. Finally, there is the dispersion stage. This stage is particularly important and problematic because it can cause new foci of infection in a patient, worsening the prognosis and can be lethal. The release and dispersal of yeast cells from the biofilm aim to grow in a different location and generate a new biofilm [5, 13].

3. Determination of the viability of a *Candida* biofilm *in vitro*

As it is known, there are different colorimetric methods using redox indicators that allow us to determine the viability of biofilms. These redox indicators are 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl) commonly known as XTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazol named as MTT, or 7-Hydroxy-3H-phenoxazin-3-one 10-oxide commonly known as resazurin, among others. One of the problems associated with the first two viability assays is that they required other substances, such as menadione or phenazine methosulfate [14–17], to evaluate a *Candida* biofilm. On the other hand, another drawback is the use of dimethyl sulfoxide (DMSO) to dissolve the salts in the MTT assay, making it an end-point assay that does not allow to measure viability at different times from the same plate. Performing experiments with dyes, such as MTT, XTT, or similar, would force the researcher to duplicate the number of plates, reagents, and experiments (one plate to achieve the value of absorbance and the other to determine the value of plating on agar plates. These aspects will be carefully explained below). In addition, if there are different time points, more plates will be required. Therefore, the use of these end-point assays leads to a large expenditure of money, time, and resources, tremendously valuable factors in science.

On the other hand, biofilms present among their structure some persister cells, a type of cells that are resistant and do not grow or die in the presence of antifungals, presenting low or no metabolic activity that cannot be fully detected by some colorimetric methods that measure cell viability [18]. For this reason, assays using MTT or XTT could only give us the values obtained after cell metabolic reduction and the absorbance measurement. Therefore, we would be missing tremendously relevant information and we would be giving inaccurate information. However, studies carried out with the resazurin method have shown good efficacy and reliable and complete results [19–22]. Resazurin does not require additives or dissolve salts with DMSO, as is the case with the dyes mentioned above (**Figure 1**).

On the other hand, it is common to find articles that evaluate the efficacy of a compound against biofilms (eukaryotes and prokaryotes) exclusively using crystal violet. This dye stains the biofilm surface that has been generated, however, it lacks value to quantify the viability of the cells that remains viable after *in vitro* treatment. For that reason, the use of crystal violet with the aim of quantifying the biofilm biomass is interesting and advisable. This assay is adequate to determine easily and effectively the amount of biofilm, an essential approach. However, in order to assess the activity of antimicrobials, the ideal is to determine the viability percentage quantitatively using colorimetric assays (resazurin) and qualitatively growing on the agar plates using the "drop plate method" (explained below).

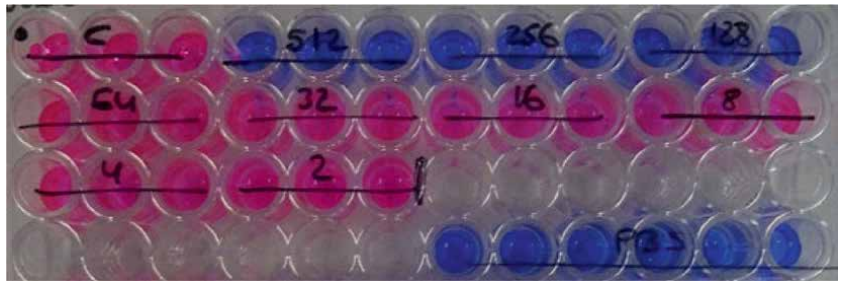

Figure 1.
C. albicans *biofilm treated* in vitro *(c: control, compound concentrations: ranging from 512 to 2 mg/L). Viability determination using resazurin after treatment with an antifungal. Viable cells (active metabolism): pink wells and non-viable cells (low or non-active metabolism): blue wells.*

4. General concepts of biofilm *in vitro* treatment and nomenclature used

The *in vitro* treatment of biofilms can be approached at two stages of their development: 1) treating and inoculating the microorganism at the same time, that is, before biofilm generation, and 2) treating the biofilm after it adheres to a surface and form a mature biofilm. Both procedures give us different results. In the first case, it would indicate the capacity of the compound to prevent the generation of a *Candida* biofilm, while in the second case; it would indicate the ability of the treatment to eliminate a mature and highly organized biofilm with complex functions. In the literature, different types of acronyms can be found to indicate the values obtained in biofilm studies. Usually, it is common to mention MBIC (minimum biofilm inhibitory concentration) and MBEC (minimum biofilm eradication concentration). However, other acronyms that provide more information on the damage caused by the compounds have recently emerged and that will be explained in detail next.

Referring to the different treatments we can differentiate studies [19–22]:

1. *Treatment to evaluate the prevention of biofilm formation*: In these studies, the microplates are washed after treatment, and resazurin is added to each well. After 24 hours, the absorbance is quantified. Subsequently, a homogeneous suspension from each well is grown on an agar medium for 24–48 hours. This will reach to obtain different data:

   - In the first step, we would obtain the value of the minimum biofilm inhibition concentration (MBIC). The minimum concentration inhibits the formation of biofilm structure, although the cells are not dead.

   - In the second step, we would obtain the value of the minimum fungicidal concentration in biofilm (MFCB). The minimum concentration completely avoids the formation of biofilm structure, because the cells are dead.

2. *Treatments to evaluate the eradication of established biofilms:* In this case, the microplates with previously established biofilms are washed after treatment, and resazurin is added to each well. After 24 hours, the absorbance is quantified. Subsequently, a homogeneous suspension from each well is cultured on an agar medium for 24–48 hours. This will reach to obtain different data:

- In the first step, we would obtain the value of the minimum biofilm damaging concentrations (MBDC). The minimum concentration damages cells by disrupting an established biofilm, though they may not all be dead.

- In the second step, we would obtain the value of the minimum biofilm eradicating concentration (MBEC). The minimum concentration completely kills the microbial cells of an established biofilm.

The data obtained by this method and in this way, allow us to consider even the persister cells that are not assessed with other methods due to the inefficiency of the technique. The MFCB and MBEC values allow us to determine 100% cell death if no colonies grow on the agar plates. Therefore, the results obtained with the colorimetric assay, such as resazurin or other dyes, should never be independent of the culture on agar plates to determine these values.

5. Dendritic cationic compounds

The problem associated with the high rate of resistance against commercial antifungals is a real fact and threat in the hospital setting. These resistances are mainly due to the inadequate and massive use of these antimicrobials [23, 24]. Besides and unfortunately, the number of antifungals approved for clinical use is relatively limited, and the required doses are frequently associated with high toxicity [25]. Therefore, it is crucial to find compounds capable of eradicating *Candida* cells, not generating resistances, and not being cytotoxic.

In this regard, cationic dendritic systems are of special interest and are actively studied due to their attractive characteristics and properties. The hydrophobic/hydrophilic balance of these systems is determined by increasing dendritic generation and, in consequence, it determines how they interact with the cell membranes [21, 26]. Among these kinds of molecules, two main types can be differentiated: *dendrimers* and *dendrons*. Both belong to the family of dendritic polymers of controlled synthesis and monodispersity. Dendrimers are globular macromolecules formed by a central nucleus, formed by one or more atoms, from which branches emerge from which other ramifications can grow, creating radial layers called dendritic *generations*. Finally, on the periphery are the terminal functional groups [9, 27]. On the other hand, dendrons are the elementary unit of dendrimers that have a focal point from which branches grow (**Figure 2**).

The main dendritic families selected for studies in the biomedical field are poly (amino amide) (PAMAM), poly(propylamine) (PPI), poly(phosphorhydrazone) (PPH), poly(L-lysine) (PLL), carbosilane, and polyester dendrimers [9]. In this chapter, we will focus on the carbosilane dendritic family. The structure of carbosilane dendrimers is defined by carbon-carbon and carbon-silicon bonds. These molecules exhibit flexible, nonpolar, and thermally stable properties. In addition, they are usually formed by polar groups to favor their solubility.

The use of these dendritic systems has been studied in both gram-negative and gram-positive bacteria [28–31], including planktonic phase and biofilms [20, 22, 32, 33]. Its efficacy has also been evaluated in eukaryotes, such as yeasts (*Candida* spp.) and protozoa. Treatments against amoebae both in the trophozoite and cyst phases have been registered [34–38]. But in addition, its effectiveness against viruses has also been reported. Anionic carbosilane dendrimers have been shown to significantly

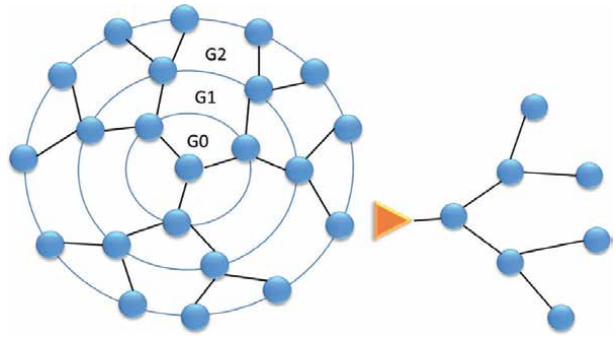

Figure 2.
Schematic representation of a dendrimer (left) and a dendron (right).

inhibit X4-HIV-1 infection [39]. In cell cultures, the effect of polyanionic carbosilane dendrimers has also been seen to prevent infection by the hepatitis C virus [40]. They have even been suggested as a possible strategy against SARS-CoV-2 [41]. Therefore, the use of dendritic systems is highly versatile and with promising results in these areas. On other occasions, dendrimers are associated with antimicrobials to improve their characteristics. For example, interesting results have been observed associating PAMAM dendrimers with amphotericin B to improve its toxicity and solubility [10].

6. Treatment of *Candida* biofilms with cationic dendritic compounds

The use of these dendritic compounds has been reported against both planktonic cells and biofilms. The *in vitro* biofilm studies are more complex, laborious, and critical. Besides, preceding biofilm treatment, the ideal is to test the production of biomass with dyes, such as crystal violet; therefore, verify the optimal conditions for its generation. In fact, it is important because it is not the same to treat a biofilm in formation, a mature biofilm, or an old biofilm.

The cell death caused by these dendritic compounds occurs by the interaction between the charges of the compounds with the cell membranes, destabilizing them. For this reason, the most frequently used dendritic compounds against bacteria and yeasts are the cationic systems because they have greater affinity; therefore, have much better activity again microorganisms. This fact has been achieved by different authors [42].

6.1 *In vitro* treatment with cationic dendrimers

In a study published in 2020, a dendrimer called BDSQ024 (**Figure 3**) was used against biofilms formed and in the formation of the *C. albicans* strain from the Spanish Type Culture Collection (CECT) 1002 and a clinical isolate of *C. albicans* from the Príncipe de Asturias University Hospital (Madrid, Spain) [19]. This highly stable dendrimer was a generation 0 compound with a tetrasiloxane core ([SiO] 4). From its nucleus grows four branches with -NMe_3^+ terminal groups that correspond to the periphery of the molecule. The BDSQ024 dendrimer showed antifungal activity, preventing the formation of *C. albicans* 1002 biofilms at 16–32 mg/L (MBIC) after 48 hours of treatment. In addition, it also presented a promising MBDC value of 16

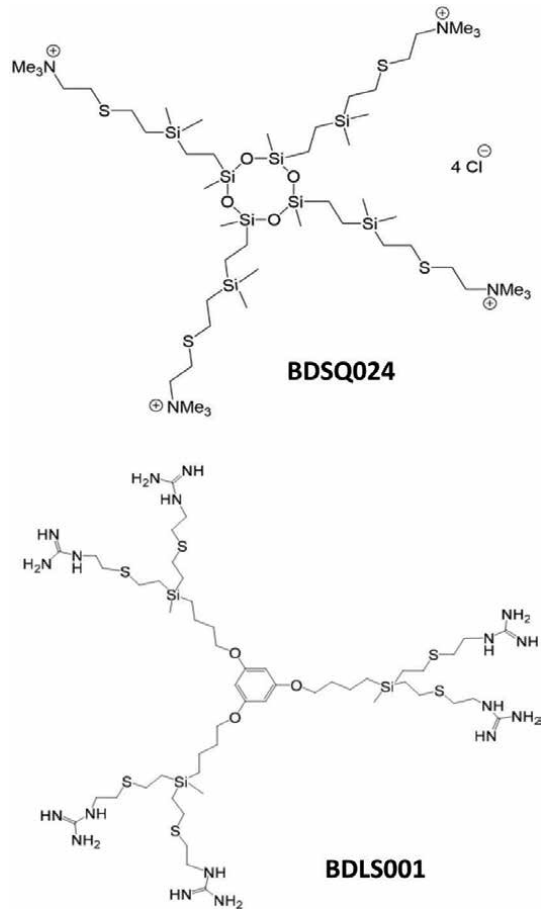

Figure 3.
Schematic structure of BDSQ024 dendrimer and BDLS001. Images have been obtained from the manuscript of [19, 43].

mg/L. However, it was not able to eradicate all the cells of a previously formed biofilm (MBEC). Notably, although the BDSQ024 dendrimer has greater activity against the clinical strain of *C. albicans* (MBIC value of 8 mg/L) than the one obtained against the CECT strain, the results of the MBDC and MBEC values were the same for both strains after 48 hours of treatment. Additionally, it was found that the addition of fresh compound after 48 hours of incubation did not improve its activity after incubating for another 24 hours (72 hours of treatment). In this article, it was also found another compound that was effective in preventing the formation of biofilms of *C. albicans* at 32 mg/L (MBIC). The compound was named BDLS001 and presented $C_6H_3O_3$ in its core and $(NHC(NH)NH_2)_6$ (6 Guanidine) as functional groups (**Figure 3**). The (BDLS001) dendrimer was tested in other studies against planktonic cells of *Escherichia coli*, *Staphylococcus aureus*, a strain of multi-resistant *S. aureus* (MRSA), and *Acanthamoeba polyphaga* trophozoites [43]. The results showed a clear efficacy against these microorganisms, both eukaryotes and prokaryotes. These data show the broad spectrum of this compound. In other studies, the antifungal and antibiofilm activity of oligostyrylbenzenes, poly(phenylene) vinylene dendrimers,

was also evaluated against *C. tropicalis*. Same as observed with other dendrimers, the cationic ones showed better antifungal activity. The compounds had antibiofilm activity, referring to the compound in the article as "compound 2" [42].

6.2 *In vitro* treatment with cationic dendrons

Interesting results of dendrons inhibiting the formation of *C. albicans* biofilms have also been reported in the literature, as is the case with compound YF017 (**Figure 4**) [19]. The dendron presented a maleimide group at the focal point and had positive charges on the surface (4 NMe_2H^+). This compound presented a MBIC and MFCB value of 32 mg/L. In addition, it has been evaluated against *S. aureus* biofilms. The results were somewhat worse than against *Candida*, detecting a MBIC value of 64 mg/L and a MFCB value of 128 mg/L [20]. Some of the dendritic compounds have been synthesized using existing compounds (non-antimicrobials) as molecules to place at focal point positions. In this way, we find molecules, such as carbosilane cationic dendrons with 4-phenylbutyric acid (PBA) located as the focal group. In a reported study, three compounds with PBA focal group were tested against *Candida* [21]. The $ArCO_2G_2(SNMe_3I)_4$ (**Figure 4**) dendron was the most active in preventing

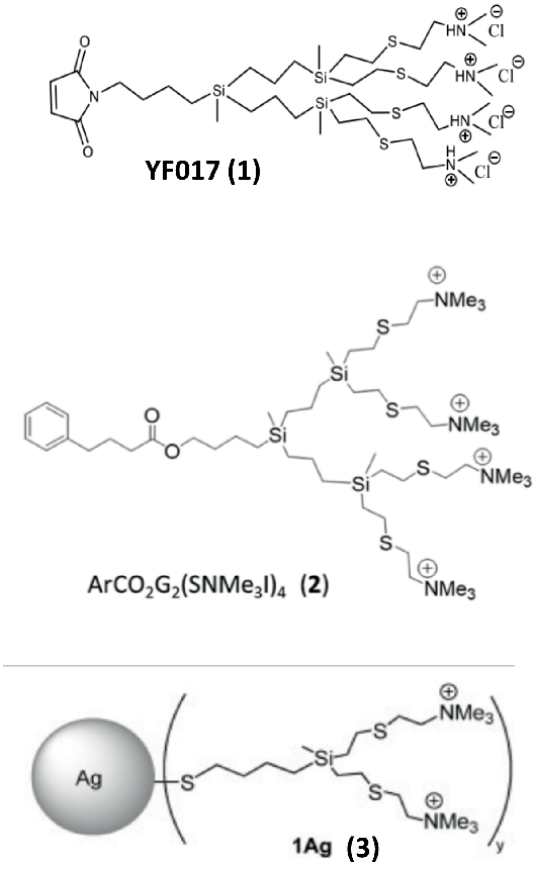

Figure 4.
Schematic structure of YF017 dendron (1) and $ArCO_2G_2(SNMe_3I)_4$ dendron (2), $AgNP(SG_1(S\text{-}NMe_3^+)_2$ (1Ag) (3). Images have been obtained from the manuscript of [20, 21, 28].

the formation of the *C. albicans* biofilm (MBIC value of 16 mg/L) and severely damaged a previously established biofilm (MBDC value of 64 mg/L). The beneficial effects of this compound against a NAC pathogenic species, *C. glabrata*, were also observed (data not shown).

6.3 *In vitro* treatment with dendron functionalized nanoparticles

The use of silver or gold nanoparticles to be functionalized with dendrons is another approach currently under study. The use of silver as an antimicrobial agent is well known. Also, the good activity of silver nitrate against biofilms [21] has been observed. Therefore, the good efficiency that a compound that presents these metals bound to dendritic systems would have to be expected. However, finding the perfect combination is complicated. Currently, dendronized silver nanoparticles (AgNPs), coated with cationic carbosilane dendrons, have been synthesized. Its activity has been tested against bacteria and yeasts. AgNP(SG_1(S-NMe_3^+)$_2$ (1Ag) (**Figure 4**) was highly active against *C. albicans* and *C. glabrata* with an MIC value of 1.8 mg/L in planktonic cells [28]. In another study, functionalized silver nanoparticles were also used: HSG1($SNMe_3^+$)2:HSHPEG (ratio 1:1), HSG2($SNMe_3^+$)4:HSHPEG (ratio 1:1) and HSG3($SNMe_3^+$)4:HSHPEG (ratio 3:1) [19]. None of the three compounds presented a MBIC value lower than 128 mg/L against *C. albicans* biofilm. Other interesting molecules are gold nanoparticles (AuNP), which can be also homofunctionalized with dendrons or heterofunctionalized, for example, with carbosilane cationic dendrons and (polyethylene) glycol (PEG) ligands [44]. Other paper reported a MIC value for *C. albicans* and *C. glabrata* of 47.6 mg/L using AuNP(SG_3(S-NMe_3^+)$_8$) (3Au) [28].

6.4 Synergistic studies between dendritic compounds and other molecules

In the clinical field, the use of antimicrobials in combination (combine therapy) is an interesting alternative, especially to overcome resistances or avoid the generation of resistance, and reduce the concentration of cytotoxic compounds. For this reason, many studies are carried out to find appropriate combinations that allow the reduction of the effective concentration of the drug to be administered. Synergy studies can determine whether there are synergistic, additive, antagonistic, or indifferent effects. The effect is synergistic when the activity of the two compounds together is much greater than when they are administered individually.

Synergistic studies have been carried out between dendritic systems and commercial antifungals, such as amphotericin and caspofungin. Amphotericin's mechanism of action is based on its binding to yeast membrane sterols, affecting membrane permeability and ultimately cell death. On the other hand, caspofungin inhibits the synthesis of beta (1,3)-D-glucan, a component of the yeast wall. In a study of the prevention of biofilm formation of *C. albicans*, three synergistic effects were determined using the dendrimer BDSQ024 (**Figure 3**), and these two mentioned antifungals [19]. In the case of amphotericin, a synergistic combination was achieved using the dendrimer at 8 mg/L and the antifungal at 0.06 mg/L. The results with caspofungin were even better. In this case, two synergistic effects were determined, the first using 8 mg/L of BDSQ024 dendrimer and 0.007 mg/L of caspofungin, and the second case, using 4 mg/L of BDSQ024 dendrimer and 0.03 mg/L of caspofungin. When the previously established biofilms were treated in a dendrimer-antifungal combination, the MBEC value was not reduced, however, the growth on agar plates was notably reduced with respect to the individual treatment.

Other studies reported the use of different substances to treat biofilms of *Candida* spp. In the combined therapy studies using $ArCO_2G_2(SNMe_3I)_4$ (**Figure 3**) and silver nitrate ($AgNO_3$), it was possible to reduce the viability of a biofilm in the formation of *C. albicans* to 11.6% when treated with the combination 8 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 4 mg/L of $AgNO_3$ [21]. In addition, it was also possible to determine a suitable combination capable of eradicating a previously established biofilm using concentrations of 32 mg/L $ArCO_2G_2(SNMe_3I)_4$ and 32 mg/L of $AgNO_3$ (MBEC). In this case, cell destruction was verified by scanning electron microscopy (SEM) (**Figure 5**). SEM approach is an interesting alternative that allows us to corroborate our results and to know the real damage that has been caused and how the compound affected the cell morphology or the cell membrane. Besides it also allows us to evaluate the thickness of the biofilm and the presence of cell dispersion.

Other combinations have been used, for instance, with ethylenediaminetetraacetic acid (EDTA), a chelating agent [21]. Combination therapy reduced the viability of a forming *C. albicans* biofilm to 24% using 8 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 32 mg/L of EDTA. Additionally, satisfactory results were found in the treatment of previously established biofilms. Total biofilm eradication (MBEC) was achieved using 256 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 16 mg/L of EDTA. The viability of a previously established biofilm was even reduced to 42.6% using 32 mg/L of $ArCO_2G_2(SNMe_3I)_4$ and 16 mg/L of EDTA.

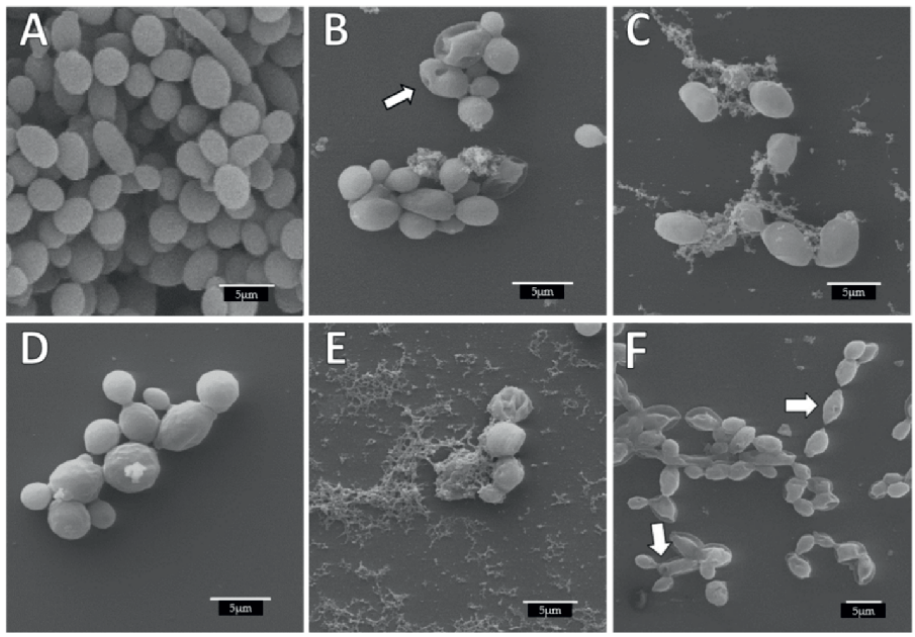

Figure 5.
Evaluation of cell damage of an established biofilm of C. albicans *treated with a dendron, $AgNO_3$ and EDTA observed by scanning electron microscopy (SEM). (A) Untreated; (B) 256 mg/L $ArCO_2G_2(SNMe_3I)_4$ (2) dendron; (C) 32 mg/L $AgNO_3$; (D) 256 mg/L EDTA; (E) 32:32 mg/L combination $ArCO_2G_2(SNMe_3I)_4$ (2):$AgNO_3$; (F) 256:32 mg/L combination $ArCO_2G_2(SNMe_3I)_4$ (2):EDTA White arrows: collapsed cells. SEM images were obtained from a previous study [21].*

7. Cytotoxicity, a key element

Finding new compounds with antifungal activity is a complicated task, especially, finding non-cytotoxic compounds. For example, the commercial antifungal amphotericin B is fungicidal with wide activity, however, it is toxic, affecting the kidney and the central nervous and hematopoietic systems [45]. The cytotoxicity of dendritic systems is often generation dependent, increasing the cytotoxicity with the generation. In this case, it also affects the number of groups on the surface and whether they are anionic, neutral, or cationic compounds. Positively charged dendrimers tend to show greater cytotoxicity [46, 47]. Despite this, it is possible to find references for effective compounds against microorganisms, especially eukaryotes, with a cytotoxic effect below the MIC and/or MBIC values. Furthermore, adding PEG ligands to dendritic systems structures may solve or improve the cytotoxicity problem as these ligands have been shown to improve biocompatibility as well as solubility [22, 44, 46]. Another alternative to reduce cytotoxicity is by using synergistic combinations of different compounds. These combinations will allow treatment of microorganisms at lower concentrations; therefore, the cytotoxicity will also decrease [19].

8. Conclusion

The generation of biofilms of *Candida* species is a major social problem, especially in clinical and hospital settings, and associated with immunocompromised patients. Therefore, finding new compounds capable of preventing the generation of biofilms and/or eliminating the viability of an established biofilm is strictly necessary. The use of new techniques, such as the resazurin colorimetric method, may allow us to evaluate *in vitro* the viability of a biofilm and to eliminate large costs in its process, providing reliability and security for the data obtained. Finally, the dendritic systems present a high activity against *Candida* biofilms. The summarized results are very promising, their high solubility in water and their low cytotoxicity make them a very interesting alternative as antifungals.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Gómez-Casanova N, Bellido A, Espinosa-Texis A, Cueva R, Ciudad T, Larriba G. *Candida tropicalis* isolates from Mexican Republic Exhibit high susceptibility to bleomycin and variable susceptibility to hydrogen peroxide. Microbial Drug Resistance. 2018;**24**(7):1031-1039

[2] Liu F, Zhong L, Zhou F, Zheng C, Zhang K, Cai J, et al. Clinical features, strain distribution, antifungal resistance and prognosis of patients with non-albicans Candidemia: A Retrospective Observational Study. Infect Drug Resistance. 2021;**14**:3233-3246

[3] Galia L, Pezzani MD, Compri M, Callegari A, Rajendran NB, Carrara E, et al. The Combacte Magnet Epi-Net Network. Surveillance of antifungal resistance in candidemia fails to inform antifungal stewardship in European Countries. Journal of Fungi (Basel). 2022;**8**:249

[4] Du H, Bing J, Hu T, Ennis CL, Nobile CJ, Huang G. *Candida auris*: Epidemiology, biology, antifungal resistance, and virulence. PLoS Pathogens. 2020;**16**(10):e1008921

[5] Pereira R, Dos Santos Fontenelle RO, de Brito EHS, de Morais SM. Biofilm of *Candida albicans*: Formation, regulation and resistance. Journal of Applied Microbiology. 2021;**131**(1):11-22

[6] Goyal P, Yoon K, Weck M. Multifunctionalization of dendrimers through orthogonal transformations. Chemistry. 2007;**13**(31):8801-8810. DOI: 10.1002/chem.200700129

[7] Galán M, Fuentes-Paniagua E, de la Mata FJ, Gómez R. Heterofunctionalized Carbosilane Dendritic Systems: Bifunctionalized Dendrons as Building Blocks versus Statistically Decorated Dendrimers. Organometallics. 2014;**33**(15):3977-3989. DOI: 10.1021/om500464k

[8] Apartsin E, Knauer N, Arkhipova V, Pashkina E, Aktanova A, Poletaeva J, et al. pH-sensitive dendrimersomes of hybrid triazine-carbosilane dendritic amphiphiles-smart vehicles for drug delivery. Nanomaterials (Basel). 2020;**10**(10):1899. DOI: 10.3390/nano10101899

[9] Ortega MÁ, Guzmán Merino A, Fraile-Martínez O, Recio-Ruiz J, Pekarek L, Guijarro G, et al. Dendrimers and dendritic materials: From laboratory to medical practice in infectious diseases. Pharmaceutics. 2020;**12**(9):874

[10] Jose J, Charyulu RN. Prolonged drug delivery system of an antifungal drug by association with polyamidoamine dendrimers. International Journal of Pharm Investigation. 2016;**6**(2):123

[11] Choudhary S, Gupta L, Rani S, Dave K, Gupta U. Impact of dendrimers on solubility of hydrophobic drug molecules. Frontiers in Pharmacology. 2017;**8**:261. DOI: 10.3389/fphar.2017.00261

[12] Czechowicz P, Nowicka J, Gościniak G. Virulence factors of *Candida spp*. and host immune response important in the pathogenesis of vulvovaginal candidiasis. International Journal of Molecular Science. 2022;**23**(11):5895

[13] Gulati M, Nobile CJ. *Candida albicans* biofilms: Development, regulation, and molecular mechanisms. Microbes

and Infection. 2016;**18**(5):310-321. DOI: 10.1016/j.micinf.2016.01.002

[14] Kim DJ, Lee MW, Choi JS, Lee SG, Park JY, Kim SW. Inhibitory activity of hinokitiol against biofilm formation in fluconazole-resistant *Candida species*. PLoS One. 2017;**12**(2):e0171244. DOI: 10.1371/journal.pone.0171244

[15] Nett JE, Cain MT, Crawford K, Andes DR. Optimizing a *Candida* biofilm microtiter plate model for measurement of antifungal susceptibility by tetrazolium salt assay. Journal of Clinical Microbiology. 2011;**49**(4):1426-1433. DOI: 10.1128/JCM.02273-10

[16] Xie Y, Liu X, Zhou P. *In vitro* antifungal effects of berberine against *Candida* spp. in planktonic and biofilm conditions. Drug Design, Development and Therapy. 2020;**14**:87-101. DOI: 10.2147/DDDT.S230857

[17] Dominguez E, Zarnowski R, Sanchez H, Covelli AS, Westler WM, Azadi P, et al. Conservation and divergence in the *Candida* species biofilm matrix mannan-glucan complex structure, function, and genetic control. mBio. 2018;**9**(2):e00451

[18] Lohse MB, Gulati M, Valle Arevalo A, Fishburn A, Johnson AD, Nobile CJ. Assessment and optimizations of *Candida albicans in vitro* biofilm assays. Antimicrobial Agents and Chemotherapy. 2017;**61**(5):e02749-e02716. DOI: 10.1128/AAC.02749-16

[19] Heredero-Bermejo I, Gómez-Casanova N, Quintana S, Soliveri J, de la Mata FJ, Pérez-Serrano J, et al. *In vitro* activity of carbosilane cationic dendritic molecules on prevention and treatment of *Candida albicans* biofilms. Pharmaceutics. 2020;**12**(10):918. DOI: 10.3390/pharmaceutics12100918

[20] Fernandez J, Martin-Serrano Á, Gómez-Casanova N, Falanga A, Galdiero S, Mata F, et al. Effect of the combination of levofloxacin with cationic carbosilane dendron and peptide in the prevention and treatment of *Staphylococcus aureus* biofilms. Polymers (Basel). 2021;**13**(13):2127

[21] Gómez-Casanova N, Lozano-Cruz T, Soliveri J, Gomez R, Ortega P, Copa-Patiño JL, et al. Eradication of *Candida albicans* biofilm viability: *In vitro* combination therapy of cationic carbosilane dendrons derived from 4-Phenylbutyric Acid with AgNO3 and EDTA. Journal of Fungi (Basel). 2021;**7**(7):574. DOI: 10.3390/jof7070574

[22] Quintana-Sanchez S, Gómez-Casanova N, Sánchez-Nieves J, Gómez R, Rachuna J, Wąsik S, et al. The antibacterial effect of PEGylated Carbosilane Dendrimers on *P. aeruginosa* alone and in combination with phage-derived endolysin. International Journal of Molecular Science. 2022;**23**(3):1873

[23] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathogens Global Health. 2015;**109**(7):309-318. DOI: 10.1179/2047773215Y.0000000030

[24] Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, et al. Tackling the emerging threat of antifungal resistance to human health. Nature Reviews. Microbiology. 2022;**29**:1-15. DOI: 10.1038/s41579-022-00720-1

[25] Roemer T, Krysan DJ. Antifungal drug development: Challenges, unmet clinical needs, and new approaches. Cold Spring Harbor Perspectives in Medicine. 2014;**4**(5):a019703. DOI: 10.1101/cshperspect.a019703

[26] Fuentes-Paniagua E, Sánchez-Nieves J, Hernández-Ros JM, Fernández-Ezequiel A, Soliveri J, Copa-Patiño JL, et al. Structure–activity relationship study of cationic carbosilane dendritic systems as antibacterial agents. RSC Advances. 2016;**6**:7022

[27] Caminade AM, Laurent R, Majoral JP. Characterization of dendrimers. Advanced Drug Delivery Reviews. 2005;**57**(15):2130-2146. DOI: 10.1016/j.addr.2005.09.011

[28] Peña-González CE, Pedziwiatr-Werbicka E, Martín-Pérez T, Szewczyk EM, Copa-Patiño JL, Soliveri J, et al. Antibacterial and antifungal properties of dendronized silver and gold nanoparticles with cationic carbosilane dendrons. International Journal of Pharmaceutics. 2017;**528**(1-2):55-61. DOI: 10.1016/j.ijpharm.2017.05.067

[29] Fernandez J, Acosta G, Pulido D, Malý M, Copa-Patiño JL, Soliveri J, et al. Carbosilane dendron-peptide nanoconjugates as antimicrobial agents. Molecular Pharmaceutics. 2019;**16**(6):2661-2674. DOI: 10.1021/acs.molpharmaceut.9b00222

[30] Rodríguez-Prieto T, Popp PF, Copa-Patiño JL, de la Mata FJ, Cano J, Mascher T, et al. Silver (I) N-heterocyclic carbenes carbosilane dendritic systems and their imidazolium-terminated analogues as antibacterial agents: Study of Their Mode of Action. Pharmaceutics. 2020;**12**(10):968. DOI: 10.3390/pharmaceutics12100968

[31] Quintana-SánchezS,Barrios-GumielA, Sánchez-Nieves J, Copa-Patiño JL, de la Mata FJ, Gómez R. Bacteria capture with magnetic nanoparticles modified with cationic carbosilane dendritic systems. Materials Science & Engineering. C, Materials for Biological Applications. 2021;**21**:112622. DOI: 10.1016/j.msec.2021.112622

[32] Llamazares C, Sanz Del Olmo N, Ortega P, Gómez R, Soliveri J, de la Mata FJ, et al. Antibacterial effect of carbosilane metallodendrimers in planktonic cells of gram-positive and gram-negative bacteria and *Staphylococcus aureus* biofilm. Biomolecules. 2019;**9**(9):405. DOI: 10.3390/biom9090405

[33] Llamazares C, Sanz Del Olmo N, Soliveri J, de la Mata FJ, Copa-Patiño JL, García-Gallego S. Insight on the structure-to-activity of carbosilane metallodendrimers in the fight against *Staphylococcus aureus* biofilms. Antibiotics (Basel). 2021;**10**(5):589. DOI: 10.3390/antibiotics10050589

[34] Heredero-Bermejo I, Copa-Patiño JL, Soliveri J, García-Gallego S, Rasines B, Gómez R, et al. *In vitro* evaluation of the effectiveness of new water-stable cationic carbosilane dendrimers against *Acanthamoeba castellanii* UAH-T17c3 trophozoites. Parasitology Research. 2013;**112**(3):961-969. DOI: 10.1007/s00436-012-3216-z

[35] Heredero-Bermejo I, Copa-Patiño JL, Soliveri J, Fuentes-Paniagua E, de la Mata FJ, Gomez R, et al. Evaluation of the activity of new cationic carbosilane dendrimers on trophozoites and cysts of *Acanthamoeba polyphaga*. Parasitology Research. 2015;**114**(2):473-486. DOI: 10.1007/s00436-014-4205-1

[36] Heredero-Bermejo I, Sánchez-Nieves J, Soliveri J, Gómez R, de la Mata FJ, Copa-Patiño JL, et al. *In vitro* anti-*Acanthamoeba* synergistic effect of chlorhexidine and cationic carbosilane dendrimers against both trophozoite and cyst forms. International Journal of Pharmaceutics. 2016;**509**(1-2):1-7. DOI: 10.1016/j.ijpharm.2016.04.075

[37] Martín-Pérez T, Lozano-Cruz T, Criado-Fornelio A, Ortega P, Gómez R, de la Mata FJ, et al. Synthesis and *in vitro* activity of new biguanide-containing dendrimers on pathogenic isolates of *Acanthamoeba polyphaga* and *Acanthamoeba griffini*. Parasitology Research. 2019;**118**(6):1953-1961. DOI: 10.1007/s00436-019-06341-7

[38] Heredero-Bermejo I, Martín-Pérez T, Copa-Patiño JL, Gómez R, de la Mata FJ, Soliveri J, et al. Ultrastructural study of *Acanthamoeba polyphaga* Trophozoites and cysts treated *in vitro* with cationic carbosilane dendrimers. Pharmaceutics. 2020;**12**(6):565. DOI: 10.3390/pharmaceutics12060565

[39] Guerrero-Beltran C, Rodriguez-Izquierdo I, Serramia MJ, Araya-Durán I, Márquez-Miranda V, Gomez R, et al. Anionic carbosilane dendrimers destabilize the GP120-CD4 complex blocking HIV-1 entry and cell to cell fusion. Bioconjugate Chemistry. 2018;**29**(5):1584-1594. DOI: 10.1021/acs.bioconjchem.8b00106

[40] Sepúlveda-Crespo D, Jiménez JL, Gómez R, De La Mata FJ, Majano PL, Muñoz-Fernández MÁ, et al. Polyanionic carbosilane dendrimers prevent hepatitis C virus infection in cell culture. Nanomedicine. 2017;**13**(1):49-58. DOI: 10.1016/j.nano.2016.08.018

[41] Mignani S, Shi X, Karpus A, Lentini G, Majoral JP. Functionalized dendrimer platforms as a new forefront arsenal targeting SARS-CoV-2: An opportunity. Pharmaceutics. 2021;**13**(9):1513. DOI: 10.3390/pharmaceutics13091513

[42] Quinteros MA, Galera ILD, Tolosa J, García-Martínez JC, Páez PL, Paraje MG. Novel antifungal activity of oligostyrylbenzenes compounds on *Candida tropicalis* biofilms. Medical Mycology. 2021;**59**(3):244-252. DOI: 10.1093/mmy/myaa046

[43] Heredero-Bermejo I, Hernández-Ros JM, Sánchez-García L, Maly M, Verdú-Expósito C, et al. Ammonium and guanidine carbosilane dendrimers and dendrons as microbicides. European Polymer Journal. 2018;**101**:159

[44] Barrios-Gumiel A, Sanchez-Nieves J, Pérez-Serrano J, Gómez R, de la Mata FJ. PEGylated AgNP covered with cationic carbosilane dendrons to enhance antibacterial and inhibition of biofilm properties. International Journal of Pharmaceutics. 2019;**569**:118591. DOI: 10.1016/j.ijpharm.2019.118591

[45] Hong SY, Oh JE, Lee KH. *In vitro* antifungal activity and cytotoxicity of a novel membrane-active peptide. Antimicrobial Agents and Chemotherapy. 1999;**43**(7):1704-1707. DOI: 10.1128/AAC.43.7.1704

[46] Thakur S, Kesharwani P, Tekade RK, Jain NK. Impact of pegylation on biopharmaceutical properties of dendrimers. Polymer. 2015;**59**:67-92. DOI: 10.1016/j.polymer.2014.12.051

[47] Janaszewska A, Lazniewska J, Trzepiński P, Marcinkowska M, Klajnert-Maculewicz B. Cytotoxicity of dendrimers. Biomolecules. 2019;**9**(8):330. DOI: 10.3390/biom9080330

Chapter 7

Mass Spectrometry-Based Proteomics Study on *Candida* Infection of COVID-19 Patients to Discover New Antifungal Target

Debashis Dutta, Surendra Manuri, Vivek Kumar Sidhu and Prashant Kumar

Abstract

The molecular foundation of fungal disease can now be better understood and treated because to advances in mass spectrometry (MS) based proteomics technology. Numerous disease-related biomarkers and potential new drug targets have been discovered over the course of the past 30 years of proteomics research, which examines dynamic protein expression, post-translational modifications, cellular and sub-cellular protein distribution, and protein–protein interactions. Although MS proteomics was of paramount importance to understanding the molecular progression involved in their differential expressions but was challenging under invasive and non-invasive growth conditions of *Candida*. species but was challenging especially due to the lack of diagnostic morphological features for early prediction. The long-term goal of this chapter is to identify the biomarkers relevant for early prediction and future target molecules for drug discovery and to determine proteins linked to fungal action, made the identification of alterations in fungal physiology and host-pathogen interactions between cells and antibiotics during COVID-19 infection therapy. Here, we also discussed the developments of proteomic-driven interactions between the host and the fungal pathogens, clinical application of spectrometry-based *Candida.* proteome identification diagnosis, and treatment with antibiotics. Proteomic approach advancements open new pathways for effective prevention and medication development for infectious diseases brought on by fungi.

Keywords: mass spectrometry, proteomics, candidiasis, biomarker, drug–target interactions prediction, COVID-19

1. Introduction

The use of proteomics based on mass spectrometry (MS) for diagnosing infectious diseases relies on the type of pathogens being examined, such as bacteria or viruses. Laser desorption/ionisation time-of-flight with matrix assistance (MALDI-ToF) MS has been developed to identify bacteria in clinical microbiology. It is a quick,

straightforward, and affordable method of identification. High-throughput bacterial species identification is made possible by specialised mass spectrometers, standardised sample preparation methods, and spectrum libraries that the relevant authorities have approved. Utilising MALDI-ToF to biotype Protein MS is used most effectively by MS in clinical laboratories [1–4]. The third most common source of infections in healthcare is bloodstream infections (BSIs) [5]. BSIs often result from the urinary tract, intestinal or community-acquired pneumonia infections, mainly caused by bacteria or fungi [5, 6]. Hospitalised individuals frequently have "candidemia, "or the blood-borne fungus *Candida*. *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis*, and *Candida krusei* are the most commonly associated with candidemia [7].

In adults and children, *C. albicans* is the most prevalent fungus isolated from BSI and is linked to high mortality rates [8]. Recently, technologies based on genetic information and DNA sequencing have been utilised to find infections in the blood. Lipopolysaccharides for Gram-negative bacteria and galactomannan for fungus are two examples of serological techniques [8]. *C. albicans* is the primary source of invasive candidiasis (**Figure 1**), associated with considerable morbidity and mortality in immunocompromised and severely ill patients [9, 10]. Several circumstances predispose IC. These linked to altering the host's microbiota (for instance, through prolonged use of broad-spectrum antibiotics), (ii) rupturing the cutaneous and gastrointestinal barriers (for example, through organ transplantation or the use of central venous catheters), and (iii) weakening the host's defences (for instance, through cancer, neutropenia, chemotherapy, or corticosteroid therapy) [10, 11].

Over the past two decades, mass spectrometry (MS)-based proteomics has significantly contributed to microbiology. A search of PubMed papers revealed a steady increase in contributions due to the application of MS-based proteomics in fungal biology, host-fungal interactions, and the creation of antifungal medicines. Based on high-resolution MS, proteomics is a powerful method for measuring and profiling proteins in cells, organs, and tissues [12, 13]. It offers in-depth knowledge of the dynamics of cellular interactions, modifications, and processes.

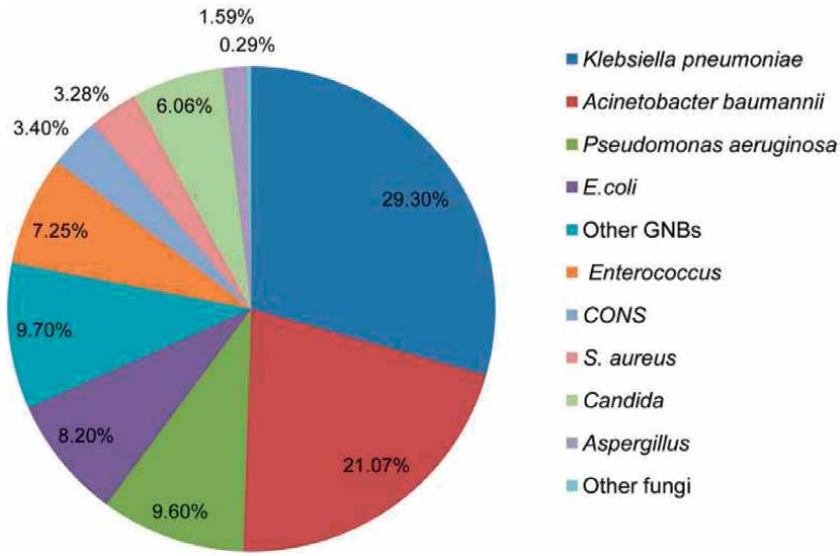

Figure 1.
Distribution of COVID-19 patient-isolated bacterial and fungal pathogens [9].

2. Antifungal resistance mechanisms

For instance, a quantitative proteomics study using iTRAQ labelling comparing haploid and diploid *C. albicans*, cultures looked for proteins linked to amphotericin B resistance, the "gold standard" antifungal drug [14]. The analysis also discovered 100 distinctively abundant proteins among the various fungal strains, focusing on those involved in the oxidative stress response, a key mechanism in Amphotericin B cytotoxicity. Alkyl hydroperoxide reductase 1 (ahp1) was crucial in determining antifungal susceptibility. This study revealed a unique antifungal resistance mechanism in *C. albicans*, linking Amphotericin B tolerance to ahp1 expression via preservation of persister cells' oxidative capability in biofilms. Profiling the proteome in response to drug treatment is another method for identifying antifungal resistance processes, as was recently shown in *Candida glabrata*. This study uses iTRAQ-MS from membrane-enriched samples to define a unique resistance mechanism for the azole antifungal Clotrimazole [15]. After clotrimazole treatment of *C. glabrata*, twelve proteins, including four multidrug resistance transporters—two previously characterised and linked to imidazole resistance—and two novel targets, were up regulated. By finding the novel targets (CgTpo1 2 and CgTpo1 1) by gene deletion, the mutant strains' greater susceptibility to various antifungals was discovered, highlighting the many functions played by these transporters in the survival of the fungus during treatment.

3. Principles of MS-based proteomics

Simple protein denaturation and solubilisation using a chaotropic agent, such as urea, to severe tissue destruction, such as probe sonication and boiling in a detergent are only a few sample preparation methods available for MS analysis (e.g., sodium dodecyl sulfate). Using sequence-specific proteases like trypsin or Lys-C, proteins are broken down into peptides for bottom-up and targeted research before being purified on C18 resin [16]. Specifically, metabolic (such as isotopically stable amino acid incorporation at the cellular level [17, 18] or chemical (such as the inclusion of mass tags or chemical derivatisation [19–21] or label-free [22] approaches are utilised for the absolute and relative quantification of proteins or peptides. Depending on size, mass, or charge, samples may be divided to streamline them further and promote excellent proteome coverage. The second MS scan (MS2) or MS/MS scan chooses and fragments peptides or ions for identification based on fragment masses after the first MS scan (MS1) records groups present at a particular instant. With the aid of LC-MS/MS, top-down proteomics analyses intact proteins to separate them based on size and peak isolation, as well as to identify and count various proteoforms. Bottom-up proteomics uses LC-MS/MS to objectively assess proteolytically digested proteins (peptides) to identify and quantify proteins in a sample. Targeted proteomics may identify, describe, and measure specific proteins and biomarkers using a predetermined set of peptides (isolation of parent ion by mass in Q1, collision of the ion in Q2, and mass filtering of synthesis in Q3).

4. Fungal pathogenesis by MS-based proteomics

The secretome and vesicles of the external environment, the complete proteome of the cellular compartment, and changed microbial states for survival could all be

molecular structures (i.e., biofilms and spores). Phosphorylation, glycosylation, and ubiquitination are a few of the most frequent post-translational modifications (PTMs) [8].

4.1 Cellular compartment

In addition to providing an overview of cellular regulatory mechanisms and signal transduction pathway alteration, the study of intracellular proteins also suggests connections between protein synthesis and disease. A subset of considerably elevated proteins focused on central carbon metabolism pathways (such as arabitol synthesis and glycerol formation) were found in *C. albicans* cells cultured under normal conditions (e.g., yeast potato dextrose (YPD) medium) and those under high osmolarity salt stress [23]. A closer look at this protein subset indicated strong selectivity for the accumulation of osmolytes during cellular stress adaptation during the osmotic stress response. This study highlights the significance of fungi adapting to shifting environmental conditions and illustrates how osmoregulation affects proteins and metabolic pathways. Proteomics can characterise the several growth states that thermodimorphic fungi like Paracoccidioides can alternate between [24]. Microbiological models of fungi are beneficial for the creation of MS-based proteomics methods. This was recently proven using *C. albicans* and enhanced stable isotope labelling in amino acid cell culture (SILAC). To properly quantify proteins in a sample, SILAC is a metabolic labelling technique that uses light- or heavy-labelled lysine and arginine. Traditionally, efficient labelling in SILAC requires auxotrophic mutants; however, Native SILAC (nSILAC), recently developed, offers excellent labelling throughout exponential cell growth without the need for auxotrophic strains [25]. Notably, strong proline conversion from heavy arginine causes issues with peak detection in *C. albicans*. To address this issue, the authors developed a computational strategy to modify the protein SILAC (heavy/light) ratios, which balanced the ratios of proline-containing to proline-free peptides.

4.2 Extracellular environment

The secretory apparatus and pathways also produce vesicles, which may affect the pathogenesis of fungi.

The extracellular vesicular fraction of *C. albicans* was proteomically profiled, and 75 proteins with highly varied biological activities were found to be exclusive to the vesicular fraction [26]. These 60% had a glycosylphosphatidylinositol (GPI)-anchor or a signal peptide, suggesting conventional secretion pathways. However, the other 40% lacked a signal peptide and most likely used other export mechanisms (e.g., vesicular pathways, non-classical secretion, or proteins capable of performing dual or multiple functions depending on cell localisation). Notably, the released immunogenic protein Bgl2 was present in the vesicular and supernatant fractions, and the isolated protein was examined as a possible vaccine against invasive candidiasis [26, 27].

4.2.1 Modifications and interactions

In order to build a list of targets, which is essential in understanding the function of proteins, including kinases and phosphatases, it is required to identify proteins that interact physically. In order to distinguish real interactors from

proteins that adhered non-specifically to the affinity matrix in *C. albicans*, SILAC labelling and affinity-purification MS (AP-MS) were used in conjunction with a substrate-trapping mutant of Cdc14, a crucial player in the regulation of mitosis and cell division [28]. One hundred twenty-six proteins were found to interact with Cdc14 due to the proteome study, of which 44% have a Cdc14 dephosphorylation motif and 80% are considered novel interactors. These proteins are necessary for DNA repair and cytokinesis during the cell cycle. This study provides an expanded list of Cdc14 interactors in *C. albicans*. It creates a reliable and quantitative mechanism for locating genuine kinase and phosphatase partners in a fungal system [8].

5. Microbial competition demonstrating antifungal properties

Identifying proteins produced by each bacterium to ensure their survival through symbiosis, competition, or predation may be made possible by studying the interactions of microbial species coexisting in an environment. In support of the quickly expanding field of biocontrol agents, this strategy most recently resulted in the discovery of lugdunin, the first novel class of antibiotics discovered in more than 30 years [29]. In the nose, *Staphylococcus lugdunesis* competes with *Staphylococcus aureus* for lugdunin production. Researchers have discovered a connection between the activity of bacterial chitinase against fungal cell walls and the anti-pathogenic effects of *Bacillus safeness*, *Cryptococcus neoformans*, and *C. albicans* in the natural environment [30, 31]. The potential for new biocontrol agents can also be shown by comparing distinct fungal species. For instance, the mycoparasitic yeast *Saccharomycopsisschoeni* kills the growing multidrug-resistant *Corynebacterium auris* [32] despite the lack of clear explanations of the molecular mechanisms underlying this interaction. Here, quantitative live-cell microscopy assays and genomic, transcriptomic, and proteomic methods were combined to identify the genes and proteins overproduced by *S. schoenii* during its predation of model prey cells, *Saccharomyces cerevisiae* [33]. Proteome analysis of the interaction between *S. schoeni* and *S. cerevisiae* revealed an abundance of proteins related to catabolic processes and the regulation of sulphur metabolism. Predation, however, resulted in an enrichment of proteins related to cell walls. Additional investigation into predator–prey relationships indicated that aspartic protease overexpression was associated with predatory behaviour and that overall nutritional deficiencies were the main cause of predation [32]. When considered as a whole, this body of data offers a thorough and objective analysis of *S. schoeni's* predatory behaviours. It suggests *S. schoeni* yeasts as viable biocontrol agents as an alternative to the persistent misuse of antifungals, which encourages the emergence of resistant fungal strains [33].

6. Proteomics of host-fungal interactions using MS

The interaction between host and germ is essential for the early control and clearance of invasive microorganisms as well as the prognosis of the disease. Determining the conflicting functions of different biological systems from a global perspective is necessary to discover novel approaches to combat infection (i.e., taking into account both the host and microbial responses).

6.1 Host perspective

The host's immunological response, particular proteins or disease-fighting pathways, and potential treatment targets to boost the host's defence mechanisms can all be learned by profiling the impacts of fungal infection from the host's point of view. In *C. albicans*, a recent study found that protein abundance increased in correlation with reported transcript levels but that a decrease in transcript levels had no corresponding effect on protein abundance [21]. The relationship between reduced transcript levels and protein abundance depends on post-translational regulation and the intracellular stability of the protein (such as protein turnover rates) [27, 28].

6.2 Pathogen perspective

By characterising the host-pathogen relationship from the pathogen's point of view, it is feasible to identify new virulence factors, characterise new mechanisms of action for previously described fungal proteins, and discover anti-virulence strategies to avoid infection. According to an image-based high-throughput screening experiment to examine host-fungal interactions, a proteinS-acyltransferase (PFA4; involved in catalysing lipid modifications of proteins) influences fungal adherence and phagocytosis in human monocytic cells in *C. neoformans* [29]. Here, 72 Pfa4-specific host protein substrates were discovered using click chemistry, MS-based proteomics, and biorthogonal palmitoylome-profiling (metabolic labelling of fatty acids with a palmitic acid analogue containing an alkyne group).

6.3 Dual perspective

The development of MS technologies and bioinformatic platforms for integrated data processing has increased the accessibility of studying host-pathogen interactions. This is because it enables researchers to compare different points of view in a single experiment. In order to understand how *C. albicans* escapes from macrophages, dual proteome profiling is used [30]. This study discovered 1253 macrophage proteins, 227 *C. albicans* proteins, and 483 *C. albicans* proteins (5 showed differential regulations).

The scientists contrasted their mixed proteome analysis with conventional proteome analysis, which does not distinguish between different cell types but instead allows all the proteins in a sample set to be processed before being bioinformatically categorised as either fungal or mammalian. Traditional proteome analysis involves isolating and dividing cells according to their biological origin (for instance, fungal or mammalian), examining two databases that are particular to those two organisms.

7. Proteomics using MS to create new antifungal drugs

Antifungals face difficulties overcoming the close evolutionary link between eukaryotic fungal cells and the human host, in contrast to antibiotics that treat bacterial infections. Antifungal drugs must focus on the eukaryotic fungal cell while minimising cytotoxicity and injury to human biological processes [31, 32]. Currently,

polyenes, azoles, pyrimidine analogues, and echinocandins are the four antifungals frequently used in monotherapy or combination. Today, finding and creating new antifungal treatment methods to thwart the spread of fungal diseases is made possible by MS-based quantitative proteomics.

These techniques involve identifying the processes underlying antifungal resistance, identifying microbial interactions with antifungal qualities, and identifying fresh possibilities for developing antifungal medicines, vaccine candidates, and therapeutic repurposing.

8. New antifungal developments, vaccine design, and drug repurposing

The creative use of clinically approved drugs alone or in conjunction with other substances to fight infection is made possible by drug repurposing approaches. This strategy may lessen the amount or length of time that antifungals must be administered, which lowers the chance that fungus will acquire resistance. Recently, a novel therapeutic repurposing strategy was proposed to interfere with proteostasis utilising an FDA-approved anti-cancer drug to treat cryptococcosis caused by *C. Neoformans* [34]. *C. neoformans* is controlled by the cAMP/PKA signal transduction pathway, and quantitative proteomics investigation of this organism revealed protein clustering associated with translation and the ubiquitin-proteasome pathway. The ubiquitin-proteasome pathway, PKA activity, and protein degradation in neurological illnesses are connected. Further investigation of proteasome function using the inhibitor bortezomib revealed an impact on capsule creation and pathogenicity [35]. This study establishes synergistic drug studies combining bortezomib with commonly used antifungals to treat cryptococcal infection (such as fluconazole and amphotericin B). Designing vaccinations that can trigger protective immune responses against infection is an alternative strategy for halting the global spread of fungal illness. Th1-type CD4+ T cell-mediated immunity is essential for the host response because Th1 cytokines promote the recruitment of lymphocytes, phagocytes, and delayed-type hypersensitivity responses [36]. Proteomics and immunological techniques were used to identify three cytoplasmic proteins that could trigger a Th1 response and four immunogenic cell wall-associated proteins in *C. neoformans,* pointing to potential new vaccine candidates [37]. In another study, 13 species of medically necessary fungi were subjected to quantitative proteome analysis to create a pan-fungal or broad-spectrum vaccination to guard against infection by various fungal species [38].

These included Crf1, Ecm22, and EglC, 1,3-glucanosyltransferases (Gel1–4, Bgt1, and their homologues), Gel1, and Crf1. Gel1 and Crf1 had previously been identified as promising vaccination candidates, validating the use of proteomics in discovering potential candidates. These proteins were discovered to be prevalent in a wide variety of fungus species and to be unrelated to human proteins. Last but not least, it was found that two recently developed antifungal prototypes, thiosemicarbazide (TSC) and a camphene derivative of TSC (TSC-C), had advantageous medical properties, including the capacity to inhibit *P. lutzii* growth [39]; however, the targets of these antifungals have not yet been established. Here, the compounds interacting with TSC and TSC-C in *Paracoccidioidesbrasiliensis* were discovered using a chemoproteomics technique. After being immobilised on resin, the substances were treated with cell extracts [40]. Integrating multi-OMICs datasets (such as the transcriptome and proteome) revealed numerous targets of the medicines' activity,

including mitochondrial membrane damage, cell cycle arrest, and metabolic process inhibition. The findings of this study showed that TSC and TSC-C had no adverse effects on mammals while still having antifungal effectiveness against *Paracoccidioides brasiliensis*.

9. Antifungal-resistant Candida strains

Approximately 7% of all *Candida* blood samples evaluated at the CDC have fluconazole resistance.

Even though *Candida albicans*, one of the *Candida* species, is the main culprit behind severe *Candida* infections. Other species, particularly *Candida Auris, Candida glabrata*, and *Candida parapsilosis*, exhibit resistance the most frequently. It is especially alarming because echinocandins, a different type of antifungal medication, are becoming resistant. It appears that echinocandin resistance is rising, particularly in the species *Candida glabrata*. According to CDC monitoring data, the antifungal fluconazole is already highly resistant to *C. glabrata*, and this resistance has been broadly stable over the previous 20 years. Echinocandin resistance could significantly reduce the range of treatments available to individuals with *C. Glabrata* induced candidiasis. Echinocandin is the chosen treatment for *C. Glabrata* [41].

10. Mass spectrometry in COVID-19 infection

Immunological assays or RT-qPCR are the two most extensively used methods for following disease progression and diagnosing COVID-19. The former is a quick and inexpensive procedure but is limited for early diagnosis because the immune response is still building. However, the outcomes depend on several variables, including appropriate sample techniques and premium extraction kits [9, 11]. Early in the viral infection, SARS-CoV-2 is found in high concentrations in saliva [34]. According to a recent study, salivary glands are known to serve as a viral reservoir; as a result, this biofluid can be employed as a source for COVID-19 diagnosis and prognosis [35]. MALDI-MS and machine learning algorithms have been used in many protocols due to their relative affordability and speed, including for the diagnosis and prognosis of several types of cancer, the identification of fungi and bacteria [42, 43], detection of fungi and bacteria that are resistant to treatment, and diagnosis and prognosis of COVID-19.Tandem mass spectrometry (LC-MS/MS) is a technique that combines numerous mass spectrometers with liquid chromatography to provide a thorough analysis as well as the separation capabilities of liquid chromatography. It is a technique for conclusively detecting SARS-CoV-2 in human samples. Comparing LC–MS/MS to techniques like RT-PCR and quick testing has shown great sensitivity and specificity while reducing the possibility of false positives [32, 33].

11. Identification of proteomics in COVID-19 treatment

Several COVID-19 therapeutics has been identified by MS-based proteomics; the development of these therapies relies on identifying SARS-CoV-2 replication machinery. In order to identify therapeutic targets, Gordon et al. investigated the ligands of

SARS-CoV-2 interacting proteins [24]. Two successful therapeutic classes were identified after discovering 69 such compounds: agonists for the Sigma receptors and protein synthesis inhibitors (including ternatin-4 and zotatifin) (such as hydroxychloroquine and haloperidol). It was found that both classes inhibited SARS-CoV-2 reproduction over 8 hours by lowering the viral nucleoprotein. The identification of COVID-19 therapeutics has also been aided by discovering case severity-related protein biomarkers and host-virus protein interactions. Suvarna et al. used label-free quantitative MS to differentiate 38 proteins with different expression levels between mild and severe COVID-19 cases, and they found abnormalities in several cellular functions. In cases of severe COVID-19, proteins like alpha-2-macroglobulin and fibrinogen gamma chain are raised for the processes of blood coagulation and inflammation, respectively. Serpin Family A Member 3 is another protein upregulated for these activities [25]. Medical experts rely on medical history, symptoms, physical examinations, and laboratory tests to diagnose invasive candidiasis. Most often, invasive candidiasis testing entails sending a blood sample or sample from the affected body site to a lab to see if it will grow *Candida* in a culture. Due to its high contagiousness, SARS-CoV2 is only processed in specialised research facilities, which presents significant challenges for systems-level molecular analyses. In our developing world, finding more efficient solutions is a challenge. SARS-CoV2 treatment, as well as point-of-care testing (PoCT), may, in the future, play a far more significant role in this.

12. Conclusion

The primary therapeutic targets of today are proteins, which are important in the modern drug design process. Pathogens are especially interested in host proteins as targets that less likely to experience an obstruction-causing mutation to the therapy and create resistance. Development of therapeutic target usually requires several processes, including the building medication formulations based on a certain drug's structure target, confirmation of therapeutic effectiveness, testing for toxicity, followed by a clinical trial. High throughput MS based proteomics systems will provide a far more comprehensive, a thorough and focused approach to studying an infection and demonstrating the intricate actions that take place inside an infected cell while mitigating for a technique's shortcomings when used alone. Posttranslational modification such as phosphorylation, specific changes include acetylation and ubiquitination can change a certain protein target's activity which control of extensive signalling networks and pathways inside cells. In order to find new antifungal medications and host susceptibility factors, a thorough knowledge of the molecular mechanisms underlying viral infection continues to be a significant problem. It is anticipated that systems-level modelling and the merging of the genomic and proteomic fields with existing wet lab techniques would lead to new developments in the field. Here, we made an effort to skim through the significance and advantages of combining two potent proteomic approaches in order to better understand the molecular interactions primarily by taking a broad view of cellular signalling networks.

To sum up, advances in technology, platform development, and protein chemistry have significantly advanced the field of proteomics studies over the past thirty years. The identification of the molecular signatures of diseases based on protein pathways and signalling cascades offers enormous potential and utility for disease diagnosis and therapy development, combined with the use of microarrays and bioinformatics tool sets, with other omics based approach.

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References

[1] Nomura F. Proteome-based bacterial identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS): A revolutionary shift in clinical diagnostic microbiology. Biochimica et Biophysica Acta, Proteins and Proteomics. 2015;**1854**(6):528-537

[2] Schubert S, Kostrzewa M. MALDI-TOF MS in the microbiology laboratory: Current trends. Current Issues in Molecular Biology. 2017;**23**:17-20

[3] Seng P et al. Ongoing revolution in bacteriology: Routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Infectious Diseases. 2009;**49**(4):543-551

[4] Welker M et al. An update on the routine application of MALDI-TOF MS in clinical microbiology. Expert Review of Proteomics. 2019;**16**(8):695-710

[5] Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for Sepsis: For the third international consensus definitions for Sepsis and septic shock (Sepsis-3). JAMA. 2016;**315**(8):762-774

[6] Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe Sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Critical Care Medicine. 2001;**29**:1303-1310

[7] Dutta D, Debnath MD. Biosynthesis of low molecular weight antifungal protein from *aspergillus giganteus* in batch fermentation and in-vitro assay. Biocontrol Science. 2018;**23**(2):41-51

[8] Ball B, Bermas A, Carruthers-Lay D, Geddes-McAlister J. Mass spectrometry-based proteomics of fungal pathogenesis, host–fungal interactions, and antifungal development. Journal of Fungi. 2019;**5**:52

[9] Sonam V, Bansal N, et al. Secondary infections in hospitalized COVID-19 patients: Indian experience infect. Drug Research. 2021;**14**:1893-1903

[10] Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nature Reviews. Disease Primers. 2018;**4**:18026

[11] Pitarch A, Nombela C, Gil C. Diagnosis of invasive candidiasis: From gold standard methods to promising leading-edge technologies. Current Topics in Medicinal Chemistry. 2018;**18**:1375-1392

[12] Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. Nature. 2016;**537**:347-355

[13] Mann M, Kulak NA, Nagaraj N, Cox J. The coming age of complete, accurate, and ubiquitous proteomes. Molecular Cell. 2013;**49**:583-590

[14] Truong T, Zeng G, Qingsong L, Kwang LT, Tong C, Chan FY, et al. Comparative ploidy proteomics of *Candida albicans* biofilms Unraveled the role of the AHP1 gene in the biofilm persistence against amphotericin B. Molecular & Cellular Proteomics. 2016;**15**(11): 3488-3500

[15] Pais P, Costa C, Pires C, Shimizu K, Chibana H, Teixeira MC. Membrane proteome-wide response to the antifungal drug Clotrimazole in *Candida glabrata*: Role of the transcription factor CgPdr1 and the drug: H+ antiporters

CgTpo1_1 and CgTpo1_2. Molecular & Cellular Proteomics. 2016;**15**(1):57-72

[16] Rappsilber J, Mann M, Ishihama Y. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. Nature Protocols. 2007;**2**:1896-1906

[17] Ong S-E, Schenone M, Margolin AA, Li X, Do K, Doud MK, et al. Identifying the proteins to which small-molecule probes and drugs bind in cells. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:4617-4622

[18] Gouw JW, Krijgsveld J, Heck AJR. Quantitative proteomics by metabolic labeling of model organisms. Molecular & Cellular Proteomics. 2010;**9**:11-24

[19] Thompson A, Schäfer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, et al. Tandemmass tags: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS. Analytical Chemistry. 2003;**75**:1895-1904

[20] Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, et al. Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents. Molecular & Cellular Proteomics. 2004;**3**:1154-1169

[21] Dayon L, Hainard A, Licker V, Turck N, Kuhn K, Hochstrasser DF, et al. Relative quantification of proteins in human cerebrospinal fluids by MS/MS using 6-plex isobaric tags. Analytical Chemistry. 2008;**80**:2921-2931

[22] Cox J, Hein MY, Luber CA, Paron I, Nagaraj N, Mann M. Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. Molecular & Cellular Proteomics. 2014;**13**:2513-2526

[23] Jacobsen MD, Beynon RJ, Gethings LA, Claydon AJ, Langridge JI, Vissers JPC, et al. Specificity of the osmotic stress response in *Candida albicans* highlighted by quantitative proteomics. Scientific Reports. 2018;**8**(1):14492

[24] Soares CM, Araújo DS, Pereira M, Portis IG, dos Santos Junior AD, Fontes W, et al. Metabolic peculiarities of Paracoccidioidesbrasiliensis dimorphism as demonstrated by iTRAQLabeling proteomics. Frontiers in Microbiology. 2019;**10**:555

[25] Fröhlich F, Christiano R, Walther TC. Native SILAC: Metabolic Labeling of proteins in prototroph microorganisms based on lysine synthesis regulation. Molecular & Cellular Proteomics. 2013;**12**(7):1995-2005

[26] Gil-Bona A, Llama-Palacios A, Parra CM, Vivanco F, Nombela C, Monteoliva L, et al. Proteomicsunravels extracellular vesicles as carriers of classical cytoplasmic proteins in *Candida albicans*. Journal of Proteome Research. 2015;**14**(1):142-153

[27] Wolf JM, Espadas-Moreno J, Luque-Garcia JL, Casadevall A. Interaction of Cryptococcus neoformans extracellular vesicles with the cell wall. Eukaryotic Cell. 2014;**13**(12):1484-1493

[28] Kaneva IN, Sudbery IM, Dickman MJ, Sudbery PE. Proteins that physically interact with the phosphatase Cdc14 in *Candida albicans* have diverse roles in the cell cycle. Scientific Reports. 2019;**9**:6258

[29] Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, Weidenmaier C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. Nature. 2016;*535*:511-516

[30] Mayer FL, Kronstad JW. Discovery of a novel antifungal agent in the pathogen box. mSphere. 2017;**2**

[31] Mayer FL, Kronstad JW. Disarming fungal pathogens: *Bacillus safeness* inhibits virulence factor production and biofilm formation by *Cryptococcus neoformans* and *Candida albicans*. MBio. 2017;**8**(5):e01537-17

[32] Junker K, Bravo Ruiz G, Lorenz A, Walker L, Gow NAR, Wendland J. The mycoparasitic yeast *Saccharomycopsisschoenii* predates and kills multi-drug resistant *Candida Auris*. Scientific Reports. 2018;**8**:14959

[33] Junker K, Chailyan A, Hesselbart A, Forster J, Wendland J. Multi-omics characterization of the necrotrophic mycoparasite *Saccharomycopsis Schoeni*. PLOS Pathogens. 2019;**15**(5):e1007692

[34] Geddes JMH, Caza M, Croll D, Stoyanov N, Foster LJ, Kronstad JW. Analysis of the protein kinase a-regulated proteome of *Cryptococcus neoformans* identifies a role for the ubiquitin-proteasome pathway in capsule formation. MBio. 2016;**7**:1-15

[35] Huang Q, Figueiredo-Pereira ME. Ubiquitin/proteasome pathway impairment in neurodegeneration: Therapeutic implications. Apoptosis. 2010;**15**(11): 1292-1311

[36] Huffnagle GB, Lipscomb MF. Cells and cytokines in pulmonary cryptococcosis. Research in Immunology. 1998;**149**:387-396

[37] Dutta D, Debnath MD. Optimization and partial characterization of intracellular anticandidalprotein from *aspergillus giganteus* MTCC 8408 using taguchi DOE. Bioengineering. 2017;**8**(5):536-548

[38] Dutta D, Debnath MD. Effect of carbon and nitrogen sources on mycelial biomass and biosynthesis of antifungal protein from *aspergillus giganteus* MTCC 8408 in submerged fermentation. Journal of Scientific and Industrial Research. 2017;**76**(06):369-375

[39] Do Carmo Silva L, Ossa DP, da Conceição Castro SV, Pires LB, de Oliveira CM, da Silva CC, et al. Transcriptome profile of the response of Paracoccidioides spp. to a camphene Thiosemicarbazide derivative. PLoS One. 2015;**10**(6):e0130703

[40] Borba JVVB, Tauhata SBF, De Oliveira CMA, Marques MF, Bailão AM, De Almeida Soares CM, et al. Chemoproteomic identification of molecular targets of antifungal prototypes, thiosemicarbazide and a camphene derivative of thiosemicarbazide, in *Paracoccidioidesbrasiliensis*. PLoS One. 2018;**13**(8):e0201948

[41] Kilburn S et al. Antifungal resistance trends of *Candida auris* clinical isolates in New York and New Jersey from 2016 to 2020. Antimicrobial Agents and Chemotherapy. 2022;**66**:3

[42] Arastehfar A, Carvalho A, Nguyen MH, Hedayati MT, Netea MG, Perlin DS, Hoenigl M. COVID-19-associated candidiasis (CAC): An underestimated complication in the absence of immunological predispositions? Journal of Fungi. 2020;**6**(4):211. DOI: 10.3390/jof6040211

[43] Nazari T, Sadeghi F, Izadi A, Sameni S, Mahmoudi S. COVID-19-associated fungal infections in Iran: A systematic review. PLoS One. 2022;**17**(7):e0271333. DOI: 10.1371/journal.pone.027133

Chapter 8

Antifungal Activity of Propolis against *Candida* Species: Propolis and Antifungal Action

Emine Kucukates

Abstract

Candida species live as commensal in humans and cause candidiasis in the presence of some predisposing factors. They are the most common among systemic mycosis agents. Currently, existing drugs used in the treatment of *Candida* infections may develop resistance, especially azole group compounds, and may lead to serious side effects and problems that may occur in therapy. Therefore, alternative natural treatment methods with very low side effects or no side effects should be considered. Propolis is one of the most natural products which has been used as a natural drug in traditional medicine for the treatment of various diseases for thousands of years. Propolis is a sticky resinous substance collected and deposited by bees from plant buds, leaves, and stems. Propolis has a wide spectrum of biological activities such as antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, immunomodulatory, and antioxidant. The compounds responsible for the biological activity of propolis are thought to be flavonoids, caffeic acid and esters, phenolic compounds, aromatic acid and esters. In this chapter, I aimed to investigate the antifungal activity of propolis against *Candida* species. Considering the safety, low cost, and usefulness of propolis, it should be considered as an alternative natural treatment method.

Keywords: propolis, antifungal activity, *Candida* species

1. Introduction

1.1 *Candida* species

Candida species are diploid, dimorphic, asexual fungi, and common in nature and humans and they live harmless, commensal in humans [1]. Yeasts of the genus *Candida* are found in the skin, respiratory system, genito-urinary system, and gastrointestinal tract mucous membranes of humans. Although there are approximately 200 *Candida* species, at least 30 species have been identified as infectious agents in humans and continue to increase in three decades and the notional numbers of non-albicans *Candida* infections have increased. *Candida albicans* is the most frequently isolated from *Candida* infections, followed by *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida lusitanie*, *Candida dubliniensis*, and

Candida guilliermondii and several other less often obtained species. This genus is the largest in which medically important yeasts are found [1–4]. When the body's normal microbiota or immunity is impaired, overgrowth of *Candida* species can cause host damage and opportunistic infection may occur [5, 6]. The disease caused by *Candida* is called candidiasis. *Candida* infections can be acute, chronic, localized, or systemic. These opportunistic pathogens are a major cause of morbidity and mortality worldwide, and therefore, poses a great threat to public health [3, 4]. Risk factors for *Candida* infections are broad-spectrum antibiotic use, advanced age, *Candida* colonization in the oral mucosa, burn, malignancy, central venous catheter, steroid use, total parenteral nutrition, prosthetic devices, HIV/AIDS patients, diabetes mellitus, indwelling devices, hyperalimentation fluids, organ transplantation, immunosuppressive drugs, hemodialysis, surgical interventions, and invasive applications [1,4–7]. *Candida* species was declared to be the fourth in 2004 and third in 2014 in the United States as the most common cause of bloodstream infections (BSIs). Most of these BSIs are caused by foreign body (implant) such as catheters (peritoneal dialysis or hemodialysis, and/or intravascular catheters; central lines, arterial catheters, or peripheral intravenous catheters), prosthetic valve, endotracheal tube and joint prosthesis [3, 8–10]. Oropharyngeal candidiasis is the most common oral evidence in HIV patients. It is also common in patients with head and neck cancer [5, 11].

1.2 *Candida* virulence factors

The virulence factors of *Candida* species declerated, to cause infections based on the site of infection, the type of infection, the stage of infection, and the host reply. The primary virulence factors of *Candida* species are biofilm formation, production of acid proteinase, phospholipases, and lipases, the ability to change its morphology from yeast to hyphal forms, and its metabolic adaptability [1, 3–12]. *Candida* first attaches to the host cells with adhesins on the surfaces of fungal cells. They have to invade the host after adherence. Many *Candida* species can form biofilm. Biofilm formation of *Candida* includes first the reproduction of yeasts on a surface contact to host cells or biomaterial, and then these are followed by filamentation. Biofilms consist of a community of microorganisms that adhere to a living or biomaterial surface, embedded in the organic exopolysaccharide matrix, they produce, and irreversibly attached to each other, to one another, to a solid surface or to an interface, and biofilms are important for the development of infection. Biofilm-forming *Candida* species can occur with conditions that clinicians do not want to encounter, such as antifungal resistance, chronic infection, and foreign body infections. Biofilm-associated *Candida* infections are very difficult to treat. The biofilm production is related to the antifungal resistance of *Candida* species [1, 4, 5, 10, 13].

1.3 Antimicrobial resistance of *Candida* species

Antimicrobial resistance is a threat worldwide and is a major public health problem. Antifungal selection is very important in several fungal infections, because in universal treatment, various problems such as side effects, drug interaction and toxicity, and antifungal resistance such as azole and polygenic derivatives may occur related to conventional treatments. The rapidly increasing antimicrobial resistance in the world results in an increase in the number of diseases and deaths [5, 7, 9, 13]. Therefore, alternative treatment methods with natural products due to very low side effects or no side effects should be considered. In recent years, the increase in

antimicrobial resistance and its side effects and the increase in cancer cases have led scientists to seek alternative natural treatments in modern medicine.

2. Apitherapy

Bee products have been used for treatment in folk medicine for centuries. Apitherapy is a type of alternative therapy that bee products are used that obtained directly from honeybees. Bee products such as honey, royal jelly, beeswax, bee venom, propolis, and pollen are thought to be beneficial to humans due to their biological and pharmacological properties [14–16]. It has been used all over the world, especially in China, Japan, and Korea in recent years. Apitherapy has been used for thousands of years. Bee venom therapy used since ancient times in Egypt, Greek, China, and Central Asia for various types of pain and arthritis pain and it is anti-inflammatory and helps relieve pain. Clinical studies have shown that bee venom treatment reduces the need for medication and reduces the risk of pain and recurrence. Diseases such as infections, wounds, burn, lupus, arthritis, shingles, pain, and muscle and joint disorders are some of the areas of apitherapy [16, 17]. Modern herbalists recommend propolis for the treatment of gastrointestinal ulcers and to increase the body's natural defense mechanisms against infections due to its antibacterial, antifungal, antiviral, anti-inflammatory, and liver protective properties [16]. Today, there are extensive apitherapy centers in China, Romania, and some Eastern European countries where diseases are treated with bee products [16]. With the regulation on traditional and complementary medicine practices published in 2014, in Turkey, apitherapy courses have been organized and apitherapy centers have begun to be established.

3. Biological properties of propolis

Propolis is a useful and versatile natural, non-toxic, low-cost bee product that has been used to cure diseases, and only bees can make propolis. It is used in ancient times in Egypt, Greece, Rome, Europe, and North Africa in the treatment of various diseases or to reduce their side effects. The Greeks used propolis as an antiseptic and in wound healing, while the Assyrians used it in wound and tumor treatments. Egyptians used propolis to mummify the dead [16, 18]. Propolis in Greek "pro" means "in defense for" and "polis" means "city" [19]. Propolis is a resinous and waxy substance collected by bees from the leaves, stems, buds, and similar parts of plants, which has a nice and pungent smell and does not dissolve in water. Propolis wax at 15–20°C, at 30–40°C sticky and gum-like. Generally, it melts at 60–70°C, when frozen times take a hard and brittle structure. Propolis gains a strong and sticky property due to the change of the structure of the collected plant resin by the bees [17, 18]. It is extremely rich in antioxidant content. Bees use propolis as an agent with antibacterial, antiviral, and antifungal activities to maintain a sterile environment in the hive and to protect the health of bees. Bee propolis is used the coat the inside of the hive, close the cracks, harden and repair the edges of the honeycombs, make the hive entrance hole easily defendable, and clean and polish the cells before the queen lays her eggs. It is brittle and hard in the cold but can become sticky when in hot environment. The structure of propolis consists of 50% resin and herbal balm, 30% wax, 10% essential and aromatic oils, 5% pollen, 5% protein, and other substances [19–23] (**Figure 1**). There are more than 300 compounds in the content of propolis.

These are polyphenols (flavonoids, phenolic acid, and its esters), terpenoids, steroids, aromatic acid, and its esters, alcohols, aldehydes, chalcones, hydrocarbons, quinones, amino acids, coumarin, ketones, essential fatty acids, vitamins (B1, B2, B6, A, C, and E), and minerals (calcium, magnesium, potassium, sodium, manganese, selenium, iron, zinc, and copper). Polyphenols and terpenoids are considered the most active compounds. The flavonoids are antimicrobial effect, and they include chrysin, pinocembrin, apigenin, galangin, pinostrobin, quercetin, kaempferol, tectochrysin, and other similarly structured compounds. The beneficial feature of propolis is usually due to the flavonoid groups it contains [19, 20, 23–25]. Flavonoids are generally found in photosynthesizing cells. Since they are secondary plant metabolites and cannot be synthesized by humans, they are important for human nutrition. Aromatic acids are among the other important components of propolis, and the most important ones are caffeic acid, cinnamic acid, ferulic acid, benzoic acid, and coumaric acid [24, 25]. Propolis has different and richer content than bee pollen. Propolis supports the immune system with its antimicrobial, anti-inflammatory, and antioxidant properties [23]. Propolis can be protective against tumor formation by preventing the structural change of the cell by neutralizing free oxygen radicals with its antioxidant property in cellular damages caused by oxidant. The effects on the cardiovascular system and eye health are also based on this feature [26–29]. Cinnamic acid and its active ingredients, galangin, pinocembrin, and cumaric acid in propolis are involved in a wide therapeutic spectrum. It shows antimicrobial, antifungal, and antibacterial effects [30–32]. Propolis and its extracts have numerous procedures in treating various diseases due to its antiseptic, antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antitumor, antiulcer, anticancer, scar-forming, tissue regeneration, local anesthetic, immunomodulatory and cytostatic activity. Therefore, it has been used in foods and beverages for the prevention of cancer, heart diseases, and diabetes [19, 20, 23, 33]. There are also harmful effects of propolis besides the

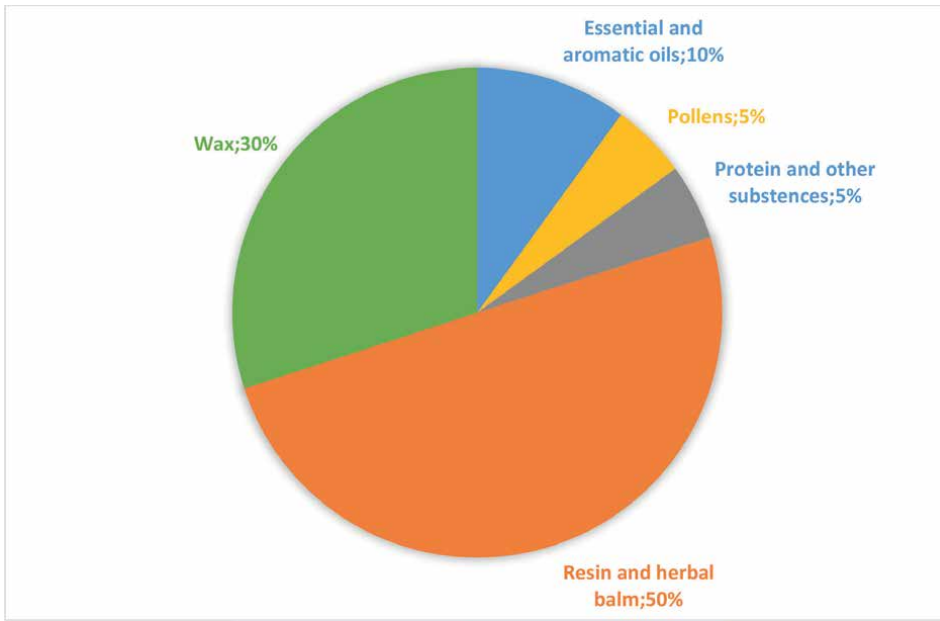

Figure 1.
The structure of propolis.

beneficial effects. Sensitive as a result of allergic reactions in humans occurring in different parts of the body such as mouth, tongue, hand, back, feet such as eczema, dermatitis (skin crusting, watery picking, itching, pain, etc.) cough, etc. symptoms can be seen [18, 27].

The structure of propolis varies according to the type of plant it is collected from, the way it is collected and used by the bee, and the methods used. Propolis should not be consumed as it is produced in the hive. Since raw propolis contains unwanted parts such as bee dead, larval remains, and plant parts, it is pre-purified by extraction with suitable solvents before use. In order for people to benefit from this product, it must be processed. Since it is a natural product and has a characteristic smell [19].

Since the chemical structure of propolis changes according to the plant source from which it is collected, different plant species can be propolis sources. Particularly in continental climate regions, mainly Populus spp., Abies spp., Acer spp., Alnus spp., Ulmus spp., Tilia spp., Pinus spp., Betula spp., Salix spp., Corylus spp., *Castanea sativa*, Eucalyptus spp., Quercus spp., and Prunus spp. are shown as botanical sources of propolis [17]. Properties such as the content, color, and smell of propolis may differ according to the vegetation around the hive, climate, season, geographical region, collection time and the source plant obtained. At the same time, these factors also affect the color of propolis. Propolis that is usually dark brown can be yellow, green, red, or transparent if these properties change. But the basic composition ratios are similar to each other [34]. However, it is reported that the season of harvesting in the same region does not have a significant effect on the composition of propolis [34].

4. Collection time of propolis

The best time to harvest propolis from the hives is between September and October. Because before the bees enter the winter months, the market holes in the hive should be as small as possible with the help of propolis in order to protect themselves in the best way possible, and they are harvested by beekeepers when the time comes. It is reported that propolis production may be more active with the onset of the rainy season in tropical climates. Phenolic compounds containing flavonoid and cinnamic acid derivatives are predominantly found in propolis obtained from temperate regions. While diterpenes and prenylated compounds are very rare in temperate zone propolis, it has been reported that they are found together with lignans, flavonoids, and other group compounds in tropical propolises obtained from South America [27].

4.1 Extraction methods of propolis

The composition of the propolis varies according to the type of solvent used and the extraction method. Solvents that do not pose a threat to health should be used in propolis extracts offered for human consumption. There may be variations in the biological effects of different solvents depending on the solubility properties of different components in propolis [35, 36]. Ethanol is the most preferred propolis extract, besides water, methanol, glycerol, methylene chloride, hexane, acetone, olive oil, and propylene glycol are other preferred solvents for the extraction of propolis. One of the solvents in the legislation, water is not preferred because it cannot dissolve the components in propolis sufficiently. Solubility in water-based propolis is only 1%. Like the tween used to dissolve chemicals with detergent, properties are harmful to health. In the case of using water as a solvent, the expected benefits cannot be

achieved due to the low rate of penetration of caffeic acid, phenyl ester, and some important flavonoids (chrysin, galangin, pinobanksin, pinocembrin) in the content. Ethanol, glycerol, and propylene glycol alcohol- derived solvents. The reason why ethanol is mostly preferred in propolis extraction is that it dissolves more bioactive substances. High-pressure liquid chromatography (HPLC) with a diode array detector (DAD) (HPLC-DAD), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and many chromatographic methods are used to examine the chemical structure of propolis. It is reported that HPLC-DAD and high-pressure liquid chromatography-mass spectrometry (HPLC-MS) give good results due to the polar nature of propolis (the molecular structure generally contains in OH– groups) [17, 35, 36]. Therefore, when interpreting the results of the studies, which solvent is used should be considered.

5. Studies on the chemical composition and antimicrobial activity of propolis in the world

There are many studies on the chemical structure and antimicrobial effect of propolis in the world [21, 23–29, 33–35, 37–53] (**Table 1**). Popova et al. [21] searched 114 propolis specimens from different countries to determine the chemical characteristics of poplar propolis and formed two groups according to the data obtained. Central and Southern Europe, Turkey, Syria, and other locations in continental climate (97 samples) first group, in the same zone as colder regions (Northern and mountains regions; Baltic Countries, England, Ukraine, Siberia, Canada, and Sweden) the second group. Groups varied considerably in phenolic and flavonoid content. Northern and mountainous region propolises were found to have lower values of 25% phenolic, 38% flavone and flavonol, and 17% flavonone/dihydroflavonol compared with the first group propolis. However, no significant differences were observed between the antimicrobial activities of all specimens. AL-Ani et al. [23] studied the chemical components, biological activities, and synergistic effects of antibiotics in different plant-derived propolis samples collected from various regions of Europe; Ireland, Germany, and Czech Republic. The chemical components of the ethanol extract of propolis (EEP) and water extract of propolis (WEP) were analyzed by gas liquid chromatography-mass spectrometry (GLC-MS) and high-performance liquid chromatography (HPLC), and more than 100 different phytochemicals were obtained from the ethanol and water extracts. In Irish propolis identified many flavonoids like pinocembrin, chrysin, and galangin were as well as significant amounts of nonacosane, heptacosane, pentacosane, guaiol, alpha-bisabolol, and caffeic acid. German propolis contained several acids such as benzoic acid, cinnamic acid, salicylic acid, myristic acid, 4-methoxyphenyl propanoic acid, hexadecanoic acid, and dodecanoic acid. Also, in Czech propolis detected as dominant compounds flavonoids such as galangin, pinocembrin, and chrysin and phenyl carboxylic acids such as benzoic acid, caffeic acid, cinnamic acid, and *p*-coumaric acid. All ethanol extracts displayed a free radical scavenging effect (IC_{50} ranging between 26.45 ± 3.4 μg/mL and 36.40 ± 3.2 μg/mL). Irish and Czech Republic propolises showed the highest free radical scavenging effect (IC_{50} 26.45 ± 3.8 μg/mL and 27.72 ± 5.2 μg/mL). Also, water extract of propolis samples demonstrated moderately antioxidant activity (IC_{50} 36.40 ± 3.2 μg/mL). In the investigation of antimicrobial effect, Irish propolis displayed noteworthy antimicrobial activity against

Region	Main compounds	Activity	References
First group: Central and Southern Europe, Turkey, Syria, and other locations in continental climate	First group: phenolic, flavone and flavonol, flavonone/dihydroflavonol	Same antimicrobial activity; Gram-positive bacteria	Popova et al. [21]
Second group: Northern and mountains regions; Baltic Countries, England, Ukraine, Siberia, Canada, and Sweden.	Second group: lower values than first group 25% phenolic, 38% flavone, and flavonol, 17% flavonone/ dihydroflavonol	Same antimicrobial activity; Gram-positive bacteria	
Ireland	Flavonoids, galangin, and caffeic acid	Gram-positive bacterial and antifungal effect	AL-Ani et al. [23]
Germany	Several acids, benzoic acid, cinnamic acid, and salicylic acid	Gram-positive bacterial and antifungal effect	
Czech Republic	Flavonoids	Gram-positive bacterial and antifungal effect	
Germany	All of samples have similar flavonoids and phenolic esters	Highest antimicrobial activity on *S. aureus* and *E. Coli*	Hegazi et al. [33]
France		Low effect on all pathogens	
Austria		Highest activity to *C. albicans*	
Six different regions of Turkey:		–	Popova et al. [37]
Artvin, Erzurum, Adana	Low phenolic and very low flavonoid compounds		
İzmir, Kayseri, Yozgat	Very similar phenolic and flavonoid content		
Bulgarian propolis Turkish propolis	Similar features: The samples are rich in caffeic acid and ferulic acid	Antibacterial and antifungal	Velikova et al. [38]
Iran	Pinocembrin, acetate, pinobanksin, pinobanksin-3, pinostrobin flavonones, and flavones	–	Mohammadzadeh et al. [39]
Iran	Pinocembrin, caffeic acid, kaempferol, galangin, and chrysin	Gram-positive bacterial and antifungal activity	Yaghoubi et al. [40]
Egypt	–	Antibacterial and high antifungal effect	Gharib et al. [41]
Lebanon	–	Antibacterial (bacteriostatic) and antifungal (fungicidal) effect	Chamandi et al. [42]
Brazilian propolis	Cinnamic acid derivatives and flavonoids	Antibacterial effect	Moncla et al. [43]
Uruguayan propolis	Flavonoids, phenolic acid esters, and aromatic acids	–	Kumazawa et al. [44]
Japan	Total phenolic compounds	Antioxidant activity	Hamasaka et al. [45]

Table 1.
Chemical characterization and antimicrobial activity of propolis in the world.

Gram-positive bacteria, the other Czech and German propolis extracts. All propolis specimens observed antifungal effects on reference and *Candida* species obtained from clinics. Excellent fungicidal effect was observed in Irish and Czech ethanol extracts with a minimum fungicidal concentration between 0.1 µg/mL and 2.5 µg/mL, also, the other propolis origins displayed mostly fungistatic effect; MIC values between 0.6–5 µg/mL. *C. tropicalis*, *Candida glabrata* and *C. parapsilosis* were the most susceptible *Candida* species. Synergism was determined in the combination of ethanol extract of propolis and vancomycin on *Streptococcus pyogenes*. Furthermore, combination of EEP and levofloxacin was found on *Streptococcus pneumoniae* and *Haemophilus influenzae*. Hegazi et al. [33] researched the chemical properties and antimicrobial effects of three propolis specimens collected from Germany, France, and Austria by GC–MS method. The main plant sources of all of these propolises were poplar buds. Flavonoids and phenolic esters were similar in all the propolises samples, but the flavonoid and phenolic ester amounts of German and French propolises were higher than that of Austrian propolis. A total of 41 components were isolated from these propolises, 11 of which defined for the first time. In the specimens searched in this study, few polar compounds were found that are characteristics of poplar buds. Compounds like aromatic acid found in all researched specimens are in the first group. *Trans-p-* coumaric was the greatest in all samples. In German propolis, galangin, benzylferulate, and phenylethyl-*trans*-caffeate were dominant. Benzyl caffeate was dominant in the French propolis specimen. Also, in French and Austrian propolis was dominant, and pinocembrin and *trans-p*-cumaric acid were dominant in all specimens. Among the studied propolises, German propolis had the highest antimicrobial effect on *S. aureus* and *E. Coli*, also Austrian propolis displayed the highest effect to *C. albicans*. French propolis was demonstrated less effective studied on all pathogens than German and Austrian propolis. Popova et al. [37] investigated the composition and antimicrobial effect of propolis specimens from six different regions of Turkey (Adana, Artvin, Erzurum, İzmir, Kayseri and Yozgat). They detected that Western (İzmir) and Central (Kayseri and Yozgat) Anatolia propolis specimens showed very similar phenolic and flavonoid ingredient. They found low phenolic and very low flavonoid concentrations in propolis samples from Adana (Central Anatolia), Artvin and Erzurum (Eastern Anatolia). Velikova et al. [38] performed a chemical analysis of a Bulgarian and two Turkish propolis types by using GC–MS. The chemical components of the propolis of both countries were similar. They reported that they probably showed the characteristics of poplar propolis. The samples were found particularly rich in caffeic acid and ferulic acids and also, they showed antibacterial, antifungal, and cytotoxic effects. Mohammadzadeh et al. [39] analyzed the chemical components of propolis collected from Tehran by GC–MS method and it has been reported that the botanical source of Iranian propolis may be popular due to the presence of acetate, pinocembrin, pinobanksin, pinobanksin-3, pinostrobin flavonones and flavones such as galangin and chrysin. Yaghoubi et al. [40] investigated the antimicrobial activity of ethanol extract of Iranian propolis (EEP) against Gram-positive, Gram-negative, and fungi by disc diffusion method. EEP was only Gram-positive and fungi but, not Gram-negative. They identified pinocembrin, caffeic acid, kaempferol, phenethyl caffeate, chrysin, and galangin in Iran propolis. The total phenolic and flavonoid ingredients were 36% and 7.3%. They suggested that the powerful antimicrobial effect of Iranian propolis may be due to rich sources of flavonoid and phenolic composites. Gharib et al. [41] analyzed antimicrobial effect of propolis on some bacteria and fungi. Egyptian propolis was higher effective *S. aureus* than *E. coli*. They found high antifungal effect on tested fungi. Chamandi et al. [42]

searched the antimicrobial effect of propolis obtained from different regions of Lebanon. These microorganisms are multi-drug resistant bacteria (MDR), Extended Spectrum Beta Lactamases (ESBL) positive *Klebsiella pneumoniae*, *Methicillin Resistant S. aureus* (MRSA), and *C. albicans*. Ethanol Extract Propolis (EEP) specimens against ESBL-positive *K. pneumoniae* and MRSA displayed bacteriostatic activity. Also, it showed fungicidal effect against *C. albicans*. Moncla et al. [43] examined chemical content and antibacterial activity of Brazilian propolis against *Enterococcus* spp. They found flavonoids, prenylated compounds, and cinnamic acid derivatives as the main constituents in Brazil propolis. Kumazawa et al. [44] studied the structure of Uruguayan propolis. They found 33 compounds that are 18 flavonoids including two new compounds, 11 phenolic acid esters including one new compound, and four aromatic carboxylic acids. Components obtained from Uruguayan propolis were similar to propolis of European and Chinese origin. They suggested that Uruguayan propolis has a plant origin and is similar to those of propolis from Europe and China. Hamasaka et al. [45] investigated the chemical compositions and antioxidant activity of propolis collected from various regions of Japan (Tokyo, Nagano, Okinawa, Okayama, Tottori, Kanagawa, Akita, Fukushima, Gifu, Fukuoka, Shizuoka, and Hokkaido). They detected that the components and quantitative values of propolis vary according to geographic origin. Ethanol Extract Propolis (EEP) from Akita (Minamiakita) and Okinawa was almost potent antioxidant activity and connected with total polyphenol ingredient. Also, in propolis collected from Akita (Minamiakita) was found a large amount of antioxidant components.

5.1 Studies on the chemical analysis and antimicrobial activity of propolis in Turkey

Numerous studies are carried out on the chemical composition and antimicrobial activity of propolises produced in Turkey [15, 30, 32, 36, 54–64] (**Table 2**). Bayram et al. [15] investigated the chemical analysis and antimicrobial action of propolis from Hakkari region (Eastern Anatolia) of Turkey on some pathogens by GC–MS. They detected more the total flavonoid amount than the other compounds such as terpenes, ketones, alcohols, hydrocarbons, aromatic acids, cinnamic acids, and their esters and aliphatic acids and esters. They found pinocembrin (9.16%), pinostrobin chalcone (8.85%), ethyl oleate (8.15%), and chrysin (5.82%) as major flavonoids. Kartal et al. [54] investigated the antimicrobial activities of propolis samples collected from Ankara (Kazan) and Muğla (Marmaris) regions of Turkey, by GC–MS chromatography method and detected 24 different compounds in the samples. They prepared four different ethanol extracts (using 30%, 50%, 70%, and 96%) from propolis samples and examined the effects of these extracts on seven Gram-positive, four Gram-negative, and fungal culture. They stated that the samples taken from Ankara-Kazan showed stronger antimicrobial activity compared with the Muğla-Marmaris samples, and they stated that the chemical content of Ankara-Kazan propolis was similar to the bud secretions of Populus species. They explained that the observed activity was mostly due to caffeic acid and its esters. Also, the active components of the Mugla-Marmaris samples were determined as isopimaric acid. The other study on the chemical content of Turkish propolis was conducted by Sorkun et al. [55] and in this study, samples from different regions of Turkey (Bursa, Erzurum-Askale, Gumushane-sogutagil and Trabzon-Caglayan) were collected and their chemical analyzes were performed by GC–MS. According to the results of this study, propolis samples taken from Trabzon and Gumushane regions have similar chemical content, and the basic

Region	Main compounds	Activity	References
Hakkari	Total flavonoids		Bayram et al. [15]
Ankara (Kazan)	Caffeic acid and esters	Higher antimicrobial activity	Kartal et al. [54]
Muğla (Marmaris)	Isopimaric acid	Antimicrobial activity	
Bursa	Flavonones, terpenoids, flavones, aromatic acid, and their esters and ketones	–	Sorkun et al. [55]
Erzurum-Askale	Aromatic acid esters and alcohols		
Gumushane-Sogutagil	Aromatic acids, aliphatic acids, and their esters and ketones		
Trabzon-Caglayan	Aromatic acids, aliphatic acids, and their esters and ketones		
Erzurum	High aromatic acid esters and amino acid contents	Strong Gram-positive effect (*S. aureus*), weak Gram-negative bacteria efficacy (*E. Coli* and *P. aeruginosa*) and yeast (*C. albicans*)	Silici and Kutluca [56]
Trabzon	Flavonoids, crysin, apigenin, and flavonones	All of propolis specimens high antibacterial effect on *S. aureus* and *E. coli*, also, no showed inhibitory activity to *K. pneumoniae* and *Morganella morgagni*	Katırcıoglu and Mercan [57]
Erzurum	Flavonoids, crysin, apigenin, and flavonones		
Tekirdag	Flavonoids, crysin, apigenin, flavonones, and naringenin		
Different geographic region of Turkey	Flavonoids, aromatic alcohols, aromatic acids and esters, terpens, aliphatic carboxylic acids, and esters	High antibacterial activity to *Salmonella enteritidis* and *Listeria monocytogenes*	Temiz et al. [58]

Table 2.
Chemical structure and antimicrobial efficiency of propolis in Turkey.

components are aromatic and aliphatic acids and their esters and ketones, Erzurum propolis has a different structure and aromatic acid esters and alcohols are the basic components, and it is better than other samples. It was found to contain more amino acids. In the samples collected from Bursa region, it was determined that flavonones, terpenoids, flavones, aromatic acid esters, and ketones were the main amounts. In the samples from Bursa region, flavones, flavonones, and ketones were detected in very rich amounts. Silici and Kutluca [56] investigated the chemical profile and antimicrobial effect of propolis collected by three different honeybees in the same region and the same season in Erzurum (East Anatolia) with the GC–MS method and 48 compounds were identified 32 being new for propolis. They found high aromatic acid esters and amino acid contents and strong Gram-positive activity in *S. aureus*, weak Gram-negative efficiency in *E. coli* and *P. aeruginosa,* and yeast in *C. albicans*. Katırcıoglu and Mercan [57] studied the chemical components and antimicrobial activity of propolis samples obtained from different regions of Turkey. They collected

propolis samples from Trabzon, Erzurum, and Tekirdağ in 2004 and analyzed them by GC–MS chromatography method. They determined as major compounds flavonoids, crysin, apigenin, and flavonones in Trabzon propolis, crysin, flavonoids, apigenin, and flavonones in Erzurum propolis, and also crysin, apigenin, naringenin, flavonoids and flavonones in Tekirdağ propolis. These propolis specimens displayed a strong inhibitory effect against *Escherichia coli* and *Staphylococcus aureus* isolates, but they did not show inhibitory action on the *K. pneumoniae* and *Morganella morgagni* strains. Temiz et al. [58] researched the antimicrobial activities of 25 propolis specimens obtained from different geographical regions of Turkey on two food-borne pathogens, *Salmonella enteritidis* ATCC 13076 and *Listeria monocytogenes* ATCC 1462. The chemical compounds of ethyl alcohol extracts of propolis (EEP) specimens were identified by GC–MS. The main constituents were flavonoids, aromatic alcohols, aromatic acids and esters, terpenes, aliphatic carboxylic acids, and esters. Flavonoids were the only shared compound determined in all samples with different levels. Antimicrobial actions of the propolis samples were investigated at two different dilutions 10% and 1%. All propolis specimens at 10% dilution displayed high activity against both bacteria. Also, 1% dilution ratios were found high antimicrobial effect on *L. monocytogenes*.

5.2 Antifungal properties of propolis and mechanisms of action

The mechanisms of action of propolis are shown in **Figure 2**. The studies have reported that compounds such as flavonoids (especially pinocembrin) and phenolic compounds present in honey and propolis are responsible for their antifungal activity by affecting cytoplasmic membrane permeability and resulting in total leakage of cell components and inorganic ions, leading to complete cell death [46–48]. The ability of *C. albicans* to switch from yeast form to hyphae form plays an important role in its virulence. Recently, propolis has been used in the treatment of oral fungal diseases. Germ tube formation contributes to adherence in *C. albicans*. Propolis inhibits germ tube formation. The inhibition of *C. albicans* growth and germ tube formation by propolis is probably due to interaction with cellular sulphydryl compounds [49, 50]. Flavonoids, which are components of propolis, contain subgroups such as flavones, flavonols, flavanols (flavan-3-ol), isoflavones, anthocyanins, and chalcones [65]. The antifungal effect of flavonols such as kaempferol, quercetin, and myricetin has been defined to inhibit the growth and cell division of *C. albicans* [66]. Also, the flavanols subclass flavan-3-ol and gallotannin indicated an inhibitory effect on the growth and cell division of *C. albicans* [67]. Serpa et al. [68] found that flavones induced apoptosis in *C. albicans*. Terpenes showed an antibiofilm efficacy in the treatment of *Candida* infections related to device use in the hospital. Mechanism of action of terpenes; alteration of the cellular cytoplasmic membrane and induction of apoptosis [69]. Resistance to antifungal drugs used in the recent years has been considered as a health problem. Treatment difficulties are experienced especially in infections associated with biofilm formation in implanted medical devices such as cardiac, urinary, dental prosthesis, and catheters. *Candida* species are associated with biofilm-related infection. They are capable of colonizing medical implantable devices and mucosal membranes and develop resistance to commonly used conventional antifungal agents. *Candida* species may develop resistance to fluconazole and other azole groups during treatment or prophylaxis and high-level cross-resistance to azole groups often develops and also echinocandins [10, 70–72].

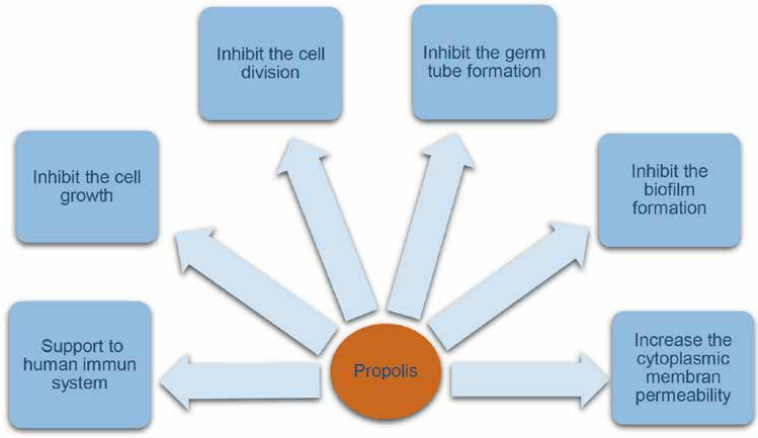

Figure 2.
The mechanisms of efficacy of propolis.

5.3 Studies on the antifungal properties of propolis

Investigation on the antifungal efficacy of propolis is shown in **Table 3**. Hegazia et al. [33] found the highest antifungal activity of propolis on C. albicans, which was detected in Austrian propolis. Ota et al. [51] investigated the antifungal effect of Brazilian propolis against *Candida* species isolated from the saliva of patients with denture stomatitis. They reported that the antifungal activity of propolis was caused by phenolic constructions containing phenolic acids and their esters. In this *in vitro* study, the highest fungicidal activity showed C. albicans and others respectively were *C. tropicalis, C. krusei*, and *C. guilliermondii*. Also, in the *in vivo* study with propolis in these patients, a decrease in the number of *Candida* species was observed in the mouth rinse. Yarfani et al. [52] investigated the antifungal activity of Iranian propolis specimens to fluconazole-resistant C. albicans isolates obtained from HIV patients with oropharyngeal candidiasis. They found higher fungicidal activity in all fluconazole-resistant *C. albicans* specimens and they detected high amounts of phenolic acid and aromatic acids (especially caffeic acid) in each propolis specimen. These components of propolis have antifungal and antibacterial activities. Oliveira et al. [53] reported a high activity of propolis on *C. albicans, C. tropicalis, C. parapsilosis*, and other species obtained from patients with onychomycosis lesions. Fernandez-Calderon et al. [72] examined the activity of a new Spanish Ethanol Extract of Propolis (SEEP) on growth, cell surface hydrophobicity, adherence, and biofilm formation of *C. glabrata* to azole-resistant isolates obtained from different clinical specimens. The Spanish propolis had good antifungal activity within the range of 0.1–0.4% (60–240 μg/mL), with a MIC_{50} and MIC_{90} of 0.2% (120 μg/mL). SEEP displayed perfect fungicidal effect on *C. glabrata* strains, with an MFC_{50} of 0.4% (240 μg/mL), an MFC_{90} of 0.8% (480 μg/mL) and range 0.4–>1.5%. SEEP did not exhibit a clear activity on surface hydrophobicity and adhesion, but an inhibitory activity on biofilm formation was displayed at sub-inhibitory concentrations (0.1 and 0.05%) with a significant decrease in biofilm formation. Capoci et al. [73] observed the antifungal effect of propolis and inhibition of biofilm production in strains of *C. albicans* isolated from patients with vulvovaginal candidiasis (VVC). The MIC of propolis extract ranged from 68.35 to 546.87 μg/mL of total phenol ingredient in gallic acid. Propolis

Region	Mechanism of action	Activity	References
Austria	All of propolis specimens inhibit the growth	Perfect effect against *C. albicans*	Hegazi et al. [33]
Germany		Antifungal action	
France		Low antifungal activity	
Brazilian propolis	–	Highest fungicidal action	Ota et al. [51]
Iran	–	Higher fungicidal effect	Yarfani et al. [52]
Brazil	–	Fungicidal activity	Oliveira et al. [53]
Spain	Inhibit the biofilm formation	Excellent fungicidal activity	Fernandez-Calderon et al. [72]
Brazil	Inhibit the biofilm formation	Antifungal activity	Capoci et al. [73]
Brazil	Inhibit the biofilm formation	Predominantly fungistatic effect	Freires et al. [74]
Thailand	Inhibit hyphal adhesion, invasion, and biofilm formation	Anticandidal effect	Iadnut et al. [75]

Table 3.
Mechanisms of action and antifungal activity of propolis.

solution inhibited biofilm formation by *C. albicans* in patients with VVC. Freires et al. [74] analyzed the chemical properties and antifungal effect of Brazilian propolis (type 3 and type 13) on *Candida* species (*C. albicans, C. dubliniensis, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei*). Propolis ethanol extracts were examined by GC–MS. In the EEP 3 were found the phenolic compounds *p*-coumaric acid, caffeic acid phenetyl ester, kaempferol, and quercetin. In the EEP 13 were identified isoflavonoids such as vestitol and formononetin. All extracts inhibited biofilm formation. Brazilian propolis has frequently fungistatic effect. Ladnut et al. [75] examined the antifungal and antivirulence effects of biologically synthesized Ethanolic Extract of Propolis-Loaded poly (lactic-co-glycolic acid) PLGA Nanoparticles (EEP-NPs) on *C. albicans.* EEP-NPs showed a higher antifungal effect than EEP in free form and reduced the effect of virulence factors such as adhesion, hyphal germination, biofilm formation, and invasion.

In conclusion, resistance to antifungals used in traditional treatment such as azole groups and also echinocandins in *Candida* species has increased due to the adhesion capacity, germ tube, and biofilm formation of *Candida*. Propolis is a resinous natural, non-toxic product collected by honeybees. There are many in vitro studies on the antimicrobial and antifungal properties of propolis in the world. However, clinical studies examining its effects on animals and humans are very limited. For the safe use of propolis, further and more clinical studies should be performed to develop alternative therapies with natural products like propolis that complement conventional treatments.

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References

[1] Mohandas V, Ballal M. Distribution of *Candida* species in different clinical samples and their virulence: Biofilm formation, proteinase and phospholipase production: A study on hospitalized patients in Southern India. Journal of Global Infectious Diseases. 2011;**3**(1):4-8

[2] Brandt ME, Lockhart SR. Recent taxonomic developments with *Candida* and other opportunistic yeasts. Current Fungal Infection Reports. 2012;**6**(3):170-177

[3] Pfaller MA, Andes DR, Diekema DJ, Horn DL, Reboli AC, Rotstein C, et al. Epidemiology and outcomes of invasive candidiasis due to non-albicans species of *Candida* in 2,496 patients: Data from the Prospective Antifungal Therapy (PATH) registry 2004-2008. PLoS One. 2014;**9**:e101510. DOI: 10.1371/journal.pone.0101510

[4] Naglik JR, Challacombe SJ, Hube B. *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. Microbiology and Molecular Biology Reviews. 2003;**67**:400-428

[5] Vila T, Romo JA, Pierce CG, McHardy SF, Saville SP, Lopez-Ribot JL. Targeting *Candida albicans* filamentation for antifungal drug development. Virulence. 2017;**8**(2):150-158

[6] Kadosh D, Lopez-Ribot JL. *Candida albicans*: Adapting to succeed. Cell Host & Microbe. 2013;**14**:483-485

[7] Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, et al. Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008-2013: Results from population-based surveillance. PLoS One. 2015;**10**(3):e0120452

[8] Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clinical Infectious Diseases. 2004;**39**:309-317

[9] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clinical Microbiology Reviews. 2007;**20**:133-163

[10] Kojic EM, Darouiche RO. *Candida* infections of medical devices. Clinical Microbiology Reviews. 2004;**17**(2):255-267

[11] Thompson GR, Patel PK, Kirkpatrick WR, Westbrook SD, Berg D, Erlandsen J, et al. Oropharyngeal candidiasis in the area of antiretroviral therapy. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010;**109**:488-495

[12] Sudbery P, Gow N, Berman J. The distinct morphogenic states of *Candida albicans*. Trends in Microbiology. 2004;**12**(7):317-324

[13] Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence. 2013;**4**(2):119-128

[14] Albayrak S, Albayrak S. Propolis: Natural antimicrobial matter. Ankara Üniversitesi Eczacılık Fakültesi Dergisi. 2008;**37**(3):201-215

[15] Bayram S, Bayram NE, Gercek YC, Aydogan MN. Chemical analysis and

antimicrobial effect of propolis from Hakkari province of Turkey against some pathogenic microorganisms. European Journal of Biology. 2017;**76**(2):74-78

[16] Castaldo S, Capasso F. Propolis an old remedy used in modern medicine. Fitoterapia. 2002;**73**:51-56

[17] Silici S. Honeybee products and therapy. Turkish Journal of Agriculture-Food and Science Technology (TURJAF). 2019;**7**(9):1249-1262

[18] Bankova VS, Castro DLS, Marcucci MC. Propolis: Recent advances in chemistry and plant origin. Apidologie. 2000;**31**:3-15

[19] Przbylek I, Karpinski TM. Antibacterial properties of propolis. Molecules. 2019;**24**:2047. DOI: 10.3390/molecules24112047

[20] Pasupuleti VR, Sammugam L, Ramesh N, Honey GSH. propolis and royal jelly: A comprehensive review of their biological actions and health benefits. Oxidative Medicine and Cellular Longevity. 2017:21. DOI: 10.1155/2017/1259510

[21] Popova MP, Bankova VS, Bogdanov S, Tsvetkova I, Naydenski C, Marcazzan GL, et al. Chemical characteristics of poplar type propolis of different geographic origin. Apidologie. 2007;**38**:306-311

[22] Stepanovic S, Antic N, Dakic I, Svabic-Vlahovic S. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiological Research. 2003;**158**:353-357

[23] AL-Ani I, Zimmermann S, Reichling J, Wink M. Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. Medicine. 2018;**5**(2):1-17

[24] Kedzia B. Pochodzenie propolisu w'swietle teori i bada'n naukowych. The origin of propolis in the theories and scientific research. Herba Polonica. 2008;**54**:179-186

[25] Zabaiou N, Fouache A, Trousson A, Baron S, Zellagui A, Lahouel M, et al. Biological properties of propolis extracts: Something new from an ancient product. Chemistry and Physics of Lipids. 2017;**207**:214-222

[26] Bufalo MC, Candeias MG, Sforcin JM. In vitro cytotoxic effect of Brazilian green propolis on human laryngeal epidermoid carcinoma (HEp-2) cells. Evidence-Based Complementary Alternative Medicine (eCAM). 2009;**6**(4):483-487

[27] Banskota AH, Nagaoka T, Sumioka LY, Tezuka Y, Awale S, Midorikawa K, et al. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. Journal of Ethnopharmacology. 2002;**80**:67-73

[28] Orsolic N, Terzic S, Mihaljevic Z, Sver L, Basic I. Effect of local administration of propolis and its polyphenolic compounds on tumor formation and growth. Biological & Pharmaceutical Bulletin. 2005;**28**(10):1928-1933

[29] Demestre M, Messerli SM, Celli N, Shahhossini M, Kluwe L, Mautner V, et al. CAPE (Caffeik Acid Phenethyl Ester)-based propolis extract (Bio 30) suppresses the growth of human Neurofibromatosis (NF) tumor xenografts in mice. Phytotherapy Research. 2009;**23**:226-230

[30] Kartal M, Yıldız S, Kaya S, Kurucu S, Topçu G. Antimicrobial

activity of propolis samples from two different regions of Anatolia. Journal of Ethnopharmacology. 2003;**86**:69-73

[31] Tawata S, Taira S, Kobamoto N, Zhu J, Ishihara M, Toyama S. Synthesis and antifungal activity of cinnamic acid esters. Biotechnology and Biochemistry. 1996;**60**:909-910

[32] Duran GG. Investigation of antibacterial, antifungal, and Leishmaniacidal effects of propolis in vitro conditions. [Master Thesis], Hatay, Turkey: Mustafa Kemal University, Institute of ScienceTechnology, 2007

[33] Hegazi AG, Abd El Hady FK, Abd Allah FAM. Chemical composition and antimicrobial activity of European propolis. Zeitschrift für Naturforschung. 2000;**55**:70-75

[34] Pietta PG, Gardana C, Pietta AM. Analytical methods for quality control of propolis. Fitoterapia. 2002;**73**(1):S7-S20

[35] Cunha IBS, Sawaya ACHF, Caetano FM, Shimizu MT, Marcucci MC, Drezza FT, et al. Factors that influence the yield and composition of Brazilian propolis extracts. Journal of the Brazilian Chemical Society. 2004;**15**(6):964-970

[36] Bakkaloglu Z, Arici M. Effect of propolis extraction with different solvents on total phenolic content, antioxidant capacity and antimicrobial activity. Akademik Gıda. 2019;**17**(4):538-545

[37] Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine. 2005;**12**(3):221-228

[38] Velikova M, Bankova V, Sorkun K, Popov S, Kujumgiev A. Chemical and biological activity of propolis from Turkish and Bulgarian origin. Mellifera. 2001;**1**:57-59

[39] Mohammadzadeh S, Shariatpanahi M, Hamedi M, Ahmadkhaniha R, Samadi N, Ostad SN. Chemical composition, oral toxicity and antimicrobial activity Iranian propolis. Food Chemistry. 2007;**103**:1097-1103

[40] Yaghoubi MJ, Ghorbani GH, Soleimanian Zad S, Satari R. Antimicrobial activity of Iranian propolis and its chemical composition. DARU. 2007;**15**(1):45-48

[41] Gharib AA, Omnia YAE, Taha M. Antimicrobial activity of propolis against some bacteria and fungi. The Zagazig Veterinary Journal. 2013;**41**(1):81-97

[42] Chamandi G, Olama Z, Holail H. Antimicrobial effect of propolis from different geographic origins in Lebanon. International Journal of Current Microbiology and Applied Sciences. 2015;**4**(4):328-342

[43] Moncla BJ, Guevara PW, Wallace JA, Marcucci MC, Nor JE, Bretz WA. The inhibitory activity of typified propolis against *Enterococcus* species. Zeitschrift für Naturforschung. 2012;**67**:249-256

[44] Kumazawa S, Hayashi K, Kajiya K, Ishii T, Hamasaka T, Nakayama T. Studies of the contituents of Uruguayan propolis. Journal of Agricultural and Food Chemistry. 2002;**50**:4777-4782

[45] Hamasaka T, Kumazawa S, Fujimoto T, Nakayama T. Antioxydant activity and constituents of propolis collected in various areas of Japan. Food Science and Technology Research. 2004;**10**(1):86-92

[46] Shehu A, Ismail S, Rohin MAK, Harun A, Aziz AA, Haque M. Antifungal properties of Malaysian tualang honey

and stingless bee propolis against *Candida albicans* and *Cryptococcus neoformans*. Journal of Applied Pharmaceutical Science. 2016;**6**(2):44-50

[47] Montero JC, Mori GG. Assessment of ion diffusion from a calcium hydroxidepropolis paste through dentin. Brazilian Oral Research. 2012;**26**(4):318-322

[48] Farnesi AP, Aquino-Ferreira R, De Jong D, Bastos JK, Soares AE. Effects of stingless bee and honey bee propolis on four species of bacteria. Genetics and Molecular Research. 2009;**8**(2):635-640

[49] Mello AM, Gomez RT, Lara SR, Silva LG, Alves JB, Cortes ME, et al. The effect of Brazilian propolis on the germ tube formation and cell wall of *Candida albicans*. Pharmacology. 2006;**3**:352-358

[50] Santos VR, Pimenta FJGS, Aguiar MCF, do Carmo MAV, Naves MD, Mesquita RA. Oral candidiasis treatment with Brazilian ethanol propolis extract. Phytotherapy Research. 2005;**19**:652-654

[51] Ota C, Unterkircher C, Fantinato V, Shimizu MT. Antifungal activity of propolis on different species of *Candida*. Mycoses. 2001;**44**:375-378

[52] Yarfani R, Khosravi AR, Shokri H. The antifungal activity of Iranian propolis samples against fluconazole-resistant *Candida albicans* strains isolated from HIV^+ patients with oropharyngeal candidiasis. Journal of ApiProduct and ApiMedical Science. 2010;**2**:161-166

[53] Oliveira ACP, Shinobu CS, LonghiniR FSL, Svidzinski TIE. Antifungal activity of propolis extract against yeast isolated from onychomycosis lesions. Memórias do Instituto Oswaldo Cruz. 2006;**101**(5):493-497

[54] Kartal M, Kaya S, Kurucu S. GC-MS analysis of propolis samples from two different regions of Turkey. Zeitschrift für Naturforschung. 2002;**57**:905-909

[55] Sorkun K, Süer B, Salih B. Determination of chemical composition of Turkish propolis. Zeitschrift für Naturforschung. 2001;**56c**:666-668

[56] Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology. 2005;**99**:69-73

[57] Katircioglu H, Mercan N. Antimicrobial activity and chemical compositions of Turkish propolis from different region. African Journal of Biotechnology. 2006;**5**(11):1151-1153

[58] Temiz A, Sener A, Ozkok Tuylu A, Sorkun K, Salih B. Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, *Salmonella enteritidis* and *Listeria monocytogenes*. Turkish Journal of Biology. 2011;**35**:503-511

[59] Ozcan M. Antifungal properties of propolis. Grasas y Aceites. 1999;**50**(5):395-398

[60] Ozcan M, Ceylan A, Unver A, Yetişir R. Antifungal effect of polen and propolis extracts collected from different regions of Turkey. Uludağ Arıcılık Derg. 2003;**3**:33-36

[61] Koc AN, Silici S, Ayangil D, Ferahbas A, Cankaya S. Comparison of in vitro activities of antifungal drugs and ethanolic extract of propolis against *Trichophyton rubrum* and *T. mentagrophytes* by using a microdilution assay. Mycoses. 2005;**48**:205-210

[62] OzbilgeH KEG, Albayrak S, Silici S. Anti-leishmanial activities of ethanolic extract of Kayseri propolis. African Journal of Microbiology Research. 2010;**4**(7):556-560

[63] Duran N, Muz M, Culha G, Duran G, Ozer B. GC-MS analysis and antileishmanial activities of two Turkish propolis type. Parasitology Research. 2011;**108**(1):95-105

[64] Kanbur M, Eraslan G, Silici S. Antioxidant effect of propolis against exposure to propetamphos in rats. Ecotoxicology and Environmental Safety. 2009;**72**(3):909-915

[65] Seleem D, Pardi V, Murata RM. Review of flavonoids: A diverse group of natural compounds with anti-*Candida albicans* activity in vitro. Archives of Oral Biology. 2017;**76**:73-83

[66] Herrera CI, Alvear M, Barrientos I, Montenegro G, Salazar IA. The antifungal effect of six commerical extracts of Chilean propolis on *Candida* spp. Ciencia e Investigación Agraria. 2010;**37**:75-84

[67] Mulaudzi RB, Ndhlala AR, Kulkarni MG, Van Staden J. Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. Journal of Ethnopharmacology. 2012;**143**:185-193

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

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

[70] Nobile CJ, Joshson AD. *Candida albicans* biofilms and human disease. Annual Review of Microbiology. 2015;**69**(1):71-92

[71] Bisvas C, Chen SCA, Halliday C, Martinez E, Rocket RJ, Wang Q, et al. Whole genom sequencing of *Candida glabrata* for detection of markers of antifungal drug resistance, e5674. Journal of Visualized Experiments. 2017;**130**

[72] Fernandez-Calderon MC, Hernandez-Gonzales L, Gomez-Navia C, Blanco-Blanco MT, Sanchez-Silos R, Lucio L, et al. Antifungal and anti-biofilm activity of a new Spanish extract of propolis against *C. glabrata*. BMC Complementary Medicine and Therapies. 2021;**21**:147

[73] Capoci IRG, Bonfim-Mendonca PS, Arita GS, Pereira RRA, Consolaro MEL, Bruschi ML, et al. Propolis is an efficient fungicide and inhibitor of biofilm production by vaginal *Candida albicans*. Evidence-Based Complementary and Alternative Medicine. 2015, 2015:287693. DOI: 10.1155/2015/287693

[74] Freires IA, Queiroz VCPP, Furletti VF, Ikegaki M, de Alencar SM, Duarte MCT, et al. Chemical composition and antifungal potential of Brazilian propolis against *Candida* spp. The Journal of Medical Mycology. 2016;**26**:122-132

[75] Iadnut A, Mamoon K, Tammasit P, Pawichai S, Tima S, Preechasuth K, et al. In vitro antifungal and antivirulence activities of biologically synthesized ethanolic extract of propolis-loaded PLGA nanoparticles against *Candida albicans*. Evidence Based Complementary and Alternative Medicine. 2019, 2019:3715481. DOI: 10.1155/2019/3715481

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This book collates up-to-date information about *Candida* and candidiasis, covering the following topics: diversity and genetic structure of the genus *Candida*, invasion biology of candidiasis, pathogenicity, drug resistance, antifungal susceptibility, antifungal-resistant *Candida* strains, antifungal-resistant genes among resistant isolates, molecular mechanisms of antifungal resistance, diagnosis and targeted therapy of candidiasis, cytotoxicity, biofilms, *Candida albicans* and non-*C. albicans*, Covid-19 patients and proteomics, pustules, systemic therapies, topical therapies, new effective compounds against *Candida* spp. cutaneous candidiasis, oropharyngeal candidiasis, oesophageal candidiasis, vulvovaginal candidiasis, intestinal candidiasis, invasive candidiasis, molecular methods in the diagnosis of *Candida* strains, host-pathogen interactions, interactions between *Candida* and cancer, antifungal drug discovery, natural compounds effective against *Candida* strains, and novel active compounds for candidiasis treatment.

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