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Open Access Journal of Mycology & Mycological Sciences
ISSN: 2689-7822

Biofilm: Structure, Antifungal Resistance, Proteomics and Genomics of *Candida* Species

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Review Article

Volume 2 Issue 2

Received Date: October 14, 2019

Published Date: November 11, 2019

DOI: 10.23880/oajmms-16000108

Abstract

Candida species are fungal pathogens that may cause systematic or superficial infections in the human host and threaten human health. These pathogens are also known by their ability to construct a biofilm structure on biotic or abiotic surfaces. According to their high antifungal resistance, *Candida* biofilms are hardly eradicated. Actually, this biofilm forming ability, protects them from antifungal drugs as same as host immune system and helps them survive under unfavorable environmental condition. Recent researches have enlightened the biofilm formation mechanism and specified many biofilm markers; in order to prevent their development. Although these studies have been helpful in so many ways, but molecular mechanisms, controlling biofilm formation and pathogeniciy still remains unclear. This review focuses on information's which are already known of *Candida* biofilm development, antifungal resistance and genomics.

Keywords: *Candida*; Biofilms; Antifungal Resistance

Introduction

Biofilm, known as a biological membrane, is actually a complex, multifunctional, and multicellular structure consisting of one or more species of microorganisms, which is surrounded by a layer of inorganic and organic substances produced by the microorganisms adhered to both of the abiotic and biotic surfaces. The structure of biofilm improves the efficacy of microbial protection against the unfavorable environmental factors, such as antibiotics, decreases the efficacy of host defense mechanisms, promotes the risk of horizontal gene transfer via providing genetic and evolutionary variety, helps to facilitate the acquisition of the essential nutrients, and also enables the transmission of information between different microbial cells [1,2].

Microorganisms are able to form biofilm in different tissues in the human host or different medical devices, including intravascular catheters, joint replacements, and prosthetic heart valves, because of their ability to adhere to various types of surfaces, relating biofilms to persistent infections and colonization [3,4]. Biofilm formation becomes an extremely important field to investigate, regarding that 80% of all microorganisms are reported to

live in this form [5]. In accordance to these general features, biofilms can potentiate the establishment of some unyielding infections in their human host. This is the case of *Candida* biofilms, leading to systemic and superficial fungal infections in many immune compromised patients. These infections are usually very hard to treat because of the features of these species such as: expression of the virulence factors, resistance to antifungal compounds, and the potential to form biofilms. In fact, mucosal infections consist of biofilm formation, generally involving the interaction with host components and also commensal bacterial flora [6].

Candida biofilms are widespread and have been noticed in most of the medical devices currently used, including shunts, stents, implants, pacemakers, endotracheal tubes, cardioverter defibrillators, cardiac devices and different types of catheters, which hinders the eradication of the *Candida* infections. These infections might be caused by several *Candida* spp [7,8].

Candida albicans is the predominant species for Candidiasis, followed by *Candida glabrata* [9]. *Candida tropicalis* is usually responsible for urinary tract infections, *Candida parapsilosis* is often observed in the skin of healthy human hosts, making it the causative agent of some catheter-related infections [10,11].

Each *Candida* spp. reveals diversities regarding biofilm formation, mostly at the level of their features of the extracellular matrix (ECM), morphology and potential to grant antifungal resistance [2]. This diversity can increase the challenge of detecting an effective way to fight the threats of *Candida* biofilms for human health, as a unique problem. In fact, according to the emergence of these *Candida* infections, there is an urgent necessity to discover effective therapeutic approaches, which may have the ability to treat individuals more efficiently. The path to discover that therapeutics certainly consists of the study of the various pathogenic characteristics of these species including the biofilm formation. Although, it is a process present in all the *Candida* spp. mentioned above, biofilm formation varies markedly from species to species, dependent to host niche, surface, and some other factors [12]. These differences can highlight the complexity of the procedures underlying biofilm formation and also the challenge to discover a unique way that will lead to the eradication of all *Candida* biofilms. biofilms generally occur in the endothelium or mucosa evidenced to be involved in the development of common candidiasis, including oral and vaginal candidiasis, but also correlated with medical devices, including dentures and urinary and vascular catheters [2,6]. Since *Candida* biofilm-related infections exhibit many economic and clinical consequences, recent studies about the pathogenicity of *Candida* spp. have mainly focused on the management and prevention of biofilms. The current review defines the chief aspects of what is presently known about *Candida* biofilm regulation and development.

Biofilm Formation

Our conception of *Candida* biofilm development and structure is based on observations made employing different microscopic techniques such as fluorescence microscopy, confocal scanning laser microscopy, and scanning electron microscopy [2].

The procedure of biofilm formation is multistage and depends on the properties of the microorganisms, construction, and properties of the colonized materials or the host. There are four basic phases (A-D), as illustrated in Figure 1.

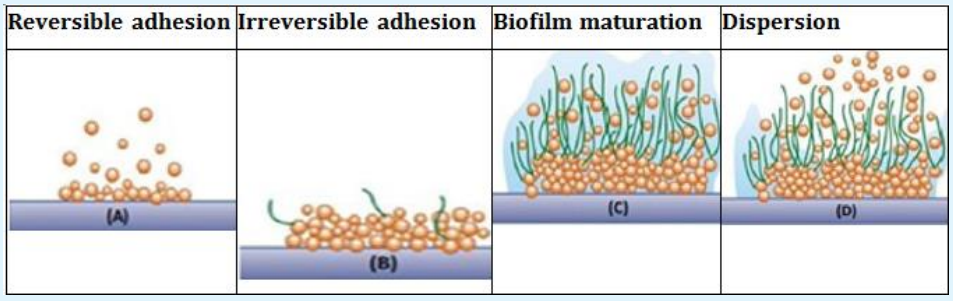

Figure 1: Biofilm formation phases.

The beginning of formation of biofilm is with the adhesion of free floating microorganisms to the biotic or abiotic surface. Reversible adhesion appears as the result of weak physical interactions causing the first cells to be attached to a solid surface such as electromagnetic surface charge, gravitational interaction, electrostatic, van

der Waals forces, and hydro/ thermodynamic forces (Brownian motion). These kinds of forces exert a vital role when there is large surface and distance between cells. Biofilms are impermanent and can be easily removed by both physical and chemical techniques. There is irreversible adhesion due to the formation of specific bonds, when there is less than 1.5 nm between cells and the surface. First microbial cells that are attached to the surface help other ones to attach by the formation of hydrophobic, non-specific or specific hydrogen bonds, additional to pairs and ionic complexes (carbon-carbon covalent bonds) [13-16].

An important place in the process of biofilm formation is the interaction of adhesives, specific receptors, and ligands on the cell surface of the microorganism or the extracellular ligand of the host cell. At first, a single layer of microbial cells covers the surfaces. In the process of making the basic EPS matrix, which gives the biofilm a specified structure and shape, an increase in the secretion and synthesis of extracellular biopolymers exerts an important role. Biofilms are produced by increasing the severity of cell proliferation. While, glycocalyx, which is a shell made of polysaccharide residues of glycoproteins and glycolipids of cell membrane, is expanded up to the whole surroundings of the micro colonies. At this stage, biofilm also includes dead cells, organic compounds, and mineral substances, in addition to living microorganisms. Microbial cells can join these parts. Irreversible adhesion allows the micro colonies formation and maturation of biofilm [14-16].

The microorganism reproduction, their gradual differentiation and the inhibition or activation of expression of certain genes can induce biofilm maturation. Biofilm cells obtain characteristics which are not demonstrated by planktonic cells and can transmit them to progeny and adjacent cells. When the membrane of biofilm reaches the critical thickness, we can have cell migration from peripheral parts of the mature biofilm to the surrounding environment and then the process of colonization begins. Due to a reaction to adverse environmental conditions, cells from biofilm disconnect. This dispersion is an intentional technique. Biofilm adapts to environmental pressures and the cells that have been detached start the process of colonization of new surfaces [1,14-18].

Candida biofilms forming in *in-vivo* systems seem to pursue the same sequence [19]. In spite of the fact that, thickness is greater and maturation is faster in these biofilms than in those grown in *in-vitro* models. The range of thickness of a biofilm formed in-vitro can alter from 25 mμ to 450 mμ whereas it usually exceeds 100 mμ in in-vivo systems [2,19-21].

Biofilms are different in their matrix and structure composition, differing among strains and species. Regarding *C. albicans*, the biofilm structure is typically made up of two layers; a basal deposit of blastopores, which is covered by a thick matrix film with hyphal forms. Moreover, biofilm production in this species, as previously mentioned, is correlated with the transition from yeast to hyphal growth. Comparing to *C. albicans*, *C. parapsilosis* biofilms are much less thick, composing of aggregated blastopores additional to yeast cells and pseudo hyphae. Regarding *C. tropicalis*, the mature biofilms are generally identified through their dense network of yeast cells with evident filamentous morphologies. Contrary to this species, biofilms of *C. glabrata* are distinguished via compact multilayer or monolayer with only blastopores, for the reason that this species is not capable of forming filamentous forms (Figure 2) [22].

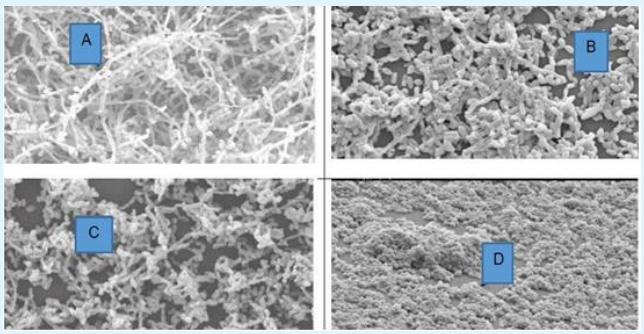

Figure 2: Scanning electron microscopy (SEM) images of 24 h *Candida* biofilm growth. A: *Candida albicans*; B: *Candida parapsilosis*; C: *Candida tropicalis*; D: *Candida glabrata*
http://dx.doi.org/10.1016/j.tim.2016.09.004

Factors Affecting Biofilm Architecture and Formation

Formation of biofilm is affected by various host and different *Candida*-derived variables, such as nutrients, fluid flow, microbial products, and host receptor.

Substratum

Numerous in vitro model systems, including silicone elastomer catheter disks, acrylic, cellulose cylindrical filters glass, polymethylmethacrylate, and plastic have been employed to develop *Candida* biofilms [12,23,24]. Biotic surfaces, including those in an engineered human oral mucosa model, have been reported to be employed as a substratum [25]. This reveals that various substrates can greatly affect the morphology, thickness, and architecture of biofilms. In a previous study, Douglas and Hawser evaluated different catheter materials and revealed that *C. albicans* biofilm formation was slightly increased on silicone or latex elastomer (P<0.05) comparing with polyvinylchloride but was substantially reduced on 100% silicone or polyurethane (P<0.001) [26]. Scanning electron microscopy exhibited that after 48 h, *C. albicans* biofilms involved a dense network of germ tubes, yeasts, hyphae, and pseudo hyphae; moreover, extracellular polymeric material was observed on the surfaces of some of those morphological forms.

In a recent investigation, Estivill, et al. [27] studied biofilm formation by 84 strains of 5 *Candida* spp. on 3 clinical materials and the results revealed that all of the tested *Candida* strains had the ability to form biofilms. Furthermore, all species demonstrated higher capacity to form biofilm on Teflon, except *C. glabrata that* exhibited greater capacity to form biofilm capacity on polyvinyl chloride. All together, these researches exhibited that the capacity of *Candida* to form biofilms is highly affected by the type of material on which it is growing and also on the species of *Candida*.

Nutrients

Nutrients in the growth media, such as lipids, sugars, and serum, are vital determinants of the *Candida* biofilm-forming capacity. In a previous study, the effects of sucrose on the colonization of acrylic by *C. albicans* in both mixed and pure culture in an artificial mouth was evaluated [28]. The results revealed that the number of *Candida* cells was markedly increased on acrylic which was exposed to sucrose, while the number of the salivary bacteria was not altered by sucrose. In another research, the growth of *C. albicans* biofilms in medium containing 50 mm glucose or 500 mm galactose reached the peak after 48 h and hence decreased; nonetheless, the cell yield was reported to be lower in the medium with low glucose [26].

Other investigations have demonstrated that fructose, lactose, and glucose favor the formation of *C. albicans* biofilm comparing with the other dietary sugars including maltose and sucrose [29]. In another study, Swindle, et al. [30] investigated the effect of parenteral lipid emulsion on *Candida* biofilms which were formed on the medical catheter surfaces. Biofilms, which were formed on silicone-elastomer catheter discs, were analyzed via confocal laser microscopy and scanning electron microscopy. Adding lipid emulsion to a standard growth medium leaded to an increase in the production of *C. albicans* biofilm and altered biofilm architecture and morphology. Moreover, lipid emulsion assisted in the growth of *C. albicans* and gave rise to germination. These results account for the increased risk of candidemia in individuals who receive lipid emulsion through medical catheters. A recent study conducted by Samaranayake, et al. [31] Confirmed that human serum can promote the growth of *C. albicans* biofilm on silicone biomaterial and it may also induce the expression of genes correlated with production of hydrolase (*PLB2*, *PLB1*,and *SAP*) and adhesion (*HWP1* and *ALS3*).

Fluid Flow

Physiological conditions such as fluid flow at the site of infection are major modulators of biofilm, because the liquid flow may affect structural integrity and the exchange of nutrient in biofilms [32]. Many researchers have tried to mimic these conditions in vitro, such as mimicking the flow of blood, saliva, and urine, additional to the application of continuous flow cells to investigate the fungal biofilms. In that regard a previous study evaluated the ability of *C. tropicalis* and *C. albicans* to form biofilms on silicone rubber voice prostheses in the presence or absence of a salivary conditioning film situated in a parallel-plate flow chamber, and exhibited that biofilms, which were formed under flow in the presence of a salivary film were more likely to detach faster than those, which were formed directly on the substratum [33]. In contrast to this finding Jin, et al. [24] has demonstrated that regardless of dietary sugar supplementation, the presence of a salivary coating does not markedly affect biofilm formation. These contrasting results can be because of method of sample collection, use of saliva, and diversities in the nature (for example the quality of saliva derived from different patients).

Other researchers employed the parallel-plate flow chamber to investigate the construction of *Candida*-

bacteria mixed biofilms on acrylic and glass. Zimmermann, et al. [34] in another study, employed the continuous flow culture to demonstrate that when testing under anaerobic conditions, voriconazole and fluconazole show cidal activity; however these agents were all static against *Candida* biofilms, under aerobic conditions. Tyler and Suci reported an in situ method to assess the function of chlorhexidine against *Candida* biofilms in a flow cell system via monitoring the penetration of the kinetics of propidium iodide (PI) into the cytoplasm of the individual cells while dosing with chlorhexidine [35]. This model permitted monitoring of the rate of propidium iodide penetration into different subpopulations (hyphae vs. yeast) of the biofilm. Researchers have also employed some airflow models to investigate voice prostheses, because the obstruction of airflow is an important cause of premature and early replacement of the mentioned devices [7].

Microbial Cohabitants

The ability of *Candida* to construct biofilm is influenced by the presence of other *Candida* spp. additional to the presence of different bacterial cohabitants. Reid, et al. [36], in a recent study revealed that the ability of *Candida* spp. to construct biofilms on epithelial cells and fibers is influenced by *Lactobacillus*. While *lactobacilli* exposure exhibited up to 91% displacement of preformed *C. albicans* biofilms, fibers, which were precoated with *lactobacilli,* actually inhibited the *Candida* adhesion by 0 to 67%. Experiments with epithelial cells demonstrated that the *lactobacilli* might markedly interfere with the adhesion of *Candida* to the cells. This suggests that members of normal female urogenital flora could interfere with infections, which are caused by *Candida*.

Holmes, et al. [37] proved that *C. tropicalis* and *C. albicans*, two frequent oral fungi, bind to *Streptococcus gordonii*, but the other two *Candida* species (*C. kefyr* and *C. krusei*) do not. Furthermore, there was a positive association between the ability of *Candida* to adhere to experimental salivary pellicle and adherence to *S. gordonii*. Depending on streptococcal growth conditions, whole saliva either slightly inhibited or stimulated adherence of *C. albicans* to *S. gordonii*. El-Azizi, et al. [38] investigated the interactions between *C. albicans* and twelve other species of *Candida* additional to bacteria in the biofilms and proved decreased biofilm production by *C. albicans* at the time the fungus was added to preformed biofilms of bacteria and non-albicans *Candida*. Although, when *Staphylococcus epidermidis* (a nonglycocalyx producer), *C. parapsilosis*, or *Serratia marcescens* was added to the preformed *C. albicans* biofilms, the number of the cells of the added microbes rose in the growing biofilms, exhibiting a dynamic interaction between biofilms of *C. albicans* and other fungi and bacteria.

In a recent study, Park, et al. [39] demonstrated that co-culturing with bacteria reduced the ability to form biofilms in *C. albicans*. In another study, van der Mei, et al. [40] investigated the ability of *C. tropicalis* and *C. albicans* to produce biofilms on silicone voice prostheses in the presence and absence of *Lactobacillus* strains and various commensal bacterial strains. They exhibited that biofilms, which consisted of combinations of a bacterial strain and *C. albicans* comprised markedly less viable organisms than those combinations that comprised *C. tropicalis*. Furthermore, high percentages of *Candida* were observed in biofilms that were grown in combination with *lactobacilli*. The procedures underlying the mentioned interactions within biofilms of *Candida* have been proposed to consist of microbial proteins (for example proteins produced by bacteria additional to *Candida* proteins) as well as the host products (for example salivary adhesins) Researches have also demonstrated that various *P. aeruginosa* virulence factors, such as phenazine (e.g., pyocyanin), and homoserine lactones are related to the inhibition of biofilms of *Candida* [41-43]. These Researches reveal that fungal-bacterial and fungal-fungal interactions exert key roles in modulating the ability of *Candida* to produce biofilms. How the mentioned interactions are related to varieties in microbial communities (*mycobiome* and *bacteriome*) in a biofilm is a topic that has not been well studied and demands a lot more investigations.

Variability of the Species

The ability to produce biofilms can differ widely among strains of *Candida*. In accordance to this, in a previous study, Branchini, et al. [44] produced pulsed-field gel electrophoresis and electrophoretic karyotyping to reveal slime production and genotypic variation among 31 isolates of *C. parapsilosis* derived from individuals with catheter infections or bloodstream. A total of fourteen DNA subtypes were detected among the 31 isolates, known that almost 80% of which constructed biofilms; the ability to form biofilm among the strains altered from moderate to strong (67%) and weak (13%). Kuhn, et al. [45], in a recent study compared the biofilms produced by *C. parapsilosis* and *C. albicans* on catheter surfaces employing dry weight assays and XTT, followed by confocal scanning laser microscopy and fluorescence microscopy. These researchers confirmed marked differences in biofilm formation between noninvasive and invasive isolates of *C. albicans*; *C. albicans* isolates formed more biofilm than *C. tropicalis, C. parapsilosis*, and *C.*

glabrata isolates. Furthermore, *C. albicans* biofilms involved a basal blastopore layer and a dense overlying matrix consisting of hyphae and exopolysaccharides, while *C. parapsilosis* biofilms had less volume than biofilms of *C. albicans* and were exclusively comprised of clumped blastopores and. In contrast to planktonically grown cells, biofilms of *Candida* rapidly (during 6 h) developed antifungal resistance (fluconazole resistance; MIC≥8μg/ml except of *C. glabrata* ≥ 64μg/ml). Douglas and Hawser [26] compared biofilm production by 15 isolates of *C. albicans* and confirmed some relation with pathogenicity: isolates of the less pathogenic *C. glabrata, C. parapsilosis* (Glasgow), and *C. pseudotropicalis*, produced markedly less biofilm compared to the more pathogenic *C. albicans*. In another study, biofilms that were formed by 3 non-*albicans Candida* species (*C. tropicalis*, *C. parapsilosis*, and *C. glabrata*) were reported to recover from different sources, employing crystal violet staining [46].

All non-*albicans Candida* species had the ability to form biofilms, in spite of the fact that these were less extensive for *C. glabrata* than *C. tropicalis* and *C. parapsilosis,* and *C. parapsilosis* biofilm formation was greatly strain dependent. Scanning electron microscopy showed that *C. parapsilosis* biofilm matrix consisted of large amounts of carbohydrate and less protein. The other way around, matrix, that were obtained from *C. tropicalis* biofilms consisted of low amounts of protein and carbohydrate. Interestingly, matrix of *C. glabrata* biofilm was high in both carbohydrate and protein content.

Biofilm production by ten clinical isolates each of *C. orthopsilosis, C. parapsilosis* and *C. metapsilosis* were characterized by Lattif, et al. [36]. They reported that as measured by XTT and biomass assays, all three species produced biofilms to the same extent. Although, variations dependent to strain in the metabolic function of produced biofilms was observed for all three tested species. Scanning confocal and electron microscopy exhibited that while the three species produced biofilms with similar architecture and topography, *C. metapsilosis* biofilms revealed a trend of lower thickness of biofilm comparing to *C. orthopsilosis* and *C. parapsilosis*. All together, these results exhibited that the ability to form biofilm, matrix composition, and structure are greatly depended to species. Generally, *C. albicans* forms more complex and larger biofilms than other the species.

Antifungal Resistance

Increased antifungal resistance of *Candida* spp., demonstrated in the biofilm mode of growth, was first discovered by Hawser and Dougla [2].

One of the most dangerous clinical aspects of main *Candida* spp. (*C. albicans, C. dubliniensis, C.glabrata, C. krusei, C. tropicalis, and C. parapsilosis*) is their capacity to form biofilm. The clinical importance of biofilm is growing with the increasing production of different medical devices that have been worked on the human body. Approximately all the contaminations of the devices mentioned before, is the result of their colonization by microorganisms. This can facilitate organization of biofilm structures [1].

The main classes of antifungal compounds, which are used to tackle *Candida* spp. infections, are azoles, polyenes, and echinocandins [47-50]. Azoles (e.g. voriconazole, fluconazole, and posaconazole) with a fungistatic effect, blocking ergosterol synthesis, targeting the enzyme lanosterol 14α-demethylase (related to the ERG11 gene) result in an accumulation of the intermediates of toxic sterol pathway. Polyenes (e.g; amphotericin B and nystatin) are fungicidal, attaching to membranes including ergosterol, creating pores that can destroy the proton gradient, which lead to the outflow of the cytoplasm and other cell substances. Echinocandins (e.g; micafungin, caspofungin, and anidulafungin) are also kind of a fungicidal, which can interfere with the synthesis of 1,3-β-glucan, a component of the cell wall of *Candida* spp. It is also important about azoles that the application of polyenes and echinocandins is suggested if the patient had prior azoles exposure and if the infection is severe for patients have been infected with *C. glabrata*, which is known as frequent azole-resistant. Echinocandins are most often and the first antifungal drug choice in these vigorous cases of candidemia, in accordance to the recent guidelines [51,52]. Several studies prove that prophylactic application of fluconazole may have positive effects for severe care unit, preterm neonates, transplant recipients patients, and other high-risk patient populations [53-55]. Though, because of some controversies, this is not considered as a general standard for all hospitals [21].

Since fluconazole is used as the main antifungal drug for HIV/AIDS patients, fluconazole resistance has been explored in several previous articles. In vitro fluconazole resistance of *Candida* biofilms is in the range of 250 to 400 times that of planktonic *Candida* [20]. In vivo, *Candida* biofilms also show an increase in fluconazole resistance; biofilms of *Candida* had a minimum inhibitory concentration (MIC) for fluconazole, which was 128 times as high as that of the planktonic *Candida* [20,56]. This feature has clinical implications, but a huge hope has been raised with the development of newer antifungal components, such as echinocandins and liposomal forms of amphotericin B. Some studies have explained that the

latter antifungals can be effective against *Candida* biofilms [2,57].

Studies have demonstrated that *Candida* species. Biofilm has some resistance to fluconazole at a concentration of 2000 times higher than the value of MIC for the planktonic form. Echinocandin and the liposomal formulation of amphotericin B are the most active antibiotics against *Candida* sp, biofilm. These agents demonstrate anti-biofilm function in concentrations 2-25 times higher than the MIC values against the planktonic forms [56,58] There is also the possibility of producing different mechanisms of drug resistance by each cell in the biofilm [1].

Factors Affecting Antifungal Resistance

Multiple factors have been suggested for increasing antifungal resistance of *Candida* biofilms. These involve metabolic /growth ratio of the biofilm cells, presence of the persisted cells, expression of the resistance genes, and also presence of the extracellular matrix [2].

Biofilm Growth

Although there is connection between growth rate and resistance of bacterial biofilms, Baillie and Douglas showed that growth rate is not related to *Candida* biofilm resistance to amphotericin B. But, the susceptibility of planktonic cultures is the result of their growth rate [59]. As a result, antifungal resistance of *Candida* biofilms is not significantly correlated with the reduction of the cell growth rate [20].

A previous study did compared the MIC values of nystatin, amphotericin ,B fluconazole, and chlorhexidine antifungals during early, intermediate, and maturation phases of *Candida* biofilms. Development of drug resistance of the *Candida* biofilm was correlated with huge increase in metabolic function of the expanding biofilm. This conception also proved that increased drug resistance is not basically a result of lower metabolic function of cells in maturing biofilms, but is more associated with the maturation procedures [12].

Extracellular Matrix

Generally, the extracellular polymeric substances (EPS) can act as a physical barrier that prevents the access of antimicrobials to cells imbedded in the biofilm community, in turn leading to advanced drug resistance. This barrier depends mostly on the nature and amount of the EPS, and also the physicochemical characteristics of the drug. In bacterial biofilms, EPS enzymes digest drugs, too. The role of the EPS in the resistance of *Candida* biofilm is obscure. In one of the studies in this field, the survival rate of *Candida* cells in biofilms that had been treated with amphotericin B decreased about 20% when the EPS was removed [60].

In another study, *C. albicans* biofilms that are formed under a permanent liquid flow were being stimulated to increase synthesis of matrix to an extent that improved the resistance against amphotericin B markedly [61]. This field of research is surely a valuable source for future studies and has several implications as EPS in clinical fields or its components may be employed as possible drug goals [2].

Genetic Basis of Antifungal Resistance

Molecular mechanisms, which confer superior antifungal resistance in *Candida* biofilms, are not completely elucidated. Studies have displayed the involvement of ATP-binding cassette and main facilitator superfamily pumps of drug efflux in increased resistance to azole antifungals. However, efflux pumps lead to azole resistance only in the early stages, but not in the later phases, of *Candida* biofilm growth. Moreover, membrane sterol compounds may lead to azole resistance in the mature and intermediate phases. In this connection, *CDR1, CDR2*, and *MDR1* genes are being up-regulated in the biofilm growth [2].

Persisted Cells

Rather than mutants, persister cells are among phenotypic variants of wild-type cells and continue to live despite the presence of antibiotics at some concentrations more than the MIC [59]. Bacterial biofilms produce recumbent persister cells known to be responsible for the tolerance against drugs, and have been observed in biofilms of *Staphylococcus aureus*, *Escherichia coli* and, *Pseudomonas aeruginosa*. A subpopulation of highly antifungal tolerant cells was found in *Candida* biofilms; however, these cells could not be found in cultures of *Candida* planktonic [62].

In the recent studies, *Candida* biofilms demonstrated a biphasic killing pattern responding to microbial factors. The surviving subpopulation of persister cells have demonstrated multidrug tolerance, while others were susceptible to the antifungals studied. Reaggregation of surviving cells can construct a new biofilm with a new persister cells subpopulation, hence proving that persister cells are phenotypic variants of the wild type with a genotype, which has the ability of heritage. This area of study about biofilm biology requires a significant

focus and a lot more studies has to be conducted to explore this phenomenon [2].

Proteomics and Genomics

Proteomics and genomics are broad disciplines that have appeared as necessary sciences for understanding molecular basis of any pathophysiological phenomenon. However, the exact genetic discussions are beyond the purpose of this article [2]. In this regard, we decided to outline the most relevant genomic and proteomic findings related to *Candida* biofilm. Genomic researches on *Candida* biofilm have gathered invaluable information about mechanisms, which are controlling biofilm formation. Several methods have attempted to determine the genetic mechanism behind *Candida* biofilm formation. Researchers have investigated the result of single deletion mutation, by performing systemic searches using mutant collections running transcriptomic analyses or running individualized transcription factor analyses [63-67].

The specific hypotheses related to biofilm formation have prompted the study of some genes [68]. For example, hyphae are important factors in *C. albicans* biofilms, hence it was assumed that hyphae are essential to form a biofilm and the transcription factor genes *CPH1* and *EFG1* are positive factors in hyphae morphogenesis regulation; while *efg1/efg1 cph1/cph1* mutant is incapable of forming a normal biofilm [67,69]. Likewise expression profiling confirmed that many amino acid biosynthetic genes were regulated, in higher orders, during biofilm development, and also the fact that amino acid activator GCN4 mutation decreases biofilm biomass claiming that expression increase of those genes is practically considerable for biofilm formation [64].

Other genes were also identified through genetic screens. As an example, a random insertional mutagenesis confirmed that KEM1, MDS3, NUP85 and SUV3 are necessary for in vitro biofilm formation [63]. Although there was no previous functional connection between these genes, they all turned to be necessary for hyphal development, which confirms the hypothesis that hyphal formation is necessary for biofilm formation. For second example, it was revealed that nonsex genes in mating type locus of *C. albicans* are related to a/α biofilm formation. The mating type locus (MTL) of *C. albicans* includes the mating type genes playing a particular role in the mating process; but this locus also includes 3 seemingly unrelated "nonsex" genes (NSGs) such as PIK, PAP and OBP, the first 2 are necessary for growth. By mutational analysis, it is illustrated that bothering the nonsex and mating type genes in MTL are important in a/α biofilm formation, and that OBP is necessary for fluconazole resistance and impermeability [70].

There were also some studies focusing on biofilms in particular kinds of candidiasis. For example, ocular *C. albicans* isolated from patients with microbial cellulitis and keratitis showed different susceptibility to antifungal agents, and some of them had ability to form a biofilm. Multiple genes related to biofilm formation, pathogenicity and drug resistance were regulated in biofilm-forming *C. albicans*, in comparison with non-biofilm-forming *C. albicans*. Temporal gene expression in biofilm-forming species was helpful in order to find potential necessary genes in different stages of biofilm formation; these genes may serve as probable targets for biofilm formation and resistance to antifungal disturbance [71].

Proteomics is a branch of biology science that studies "protein complements of genome" [72]. *Candida* proteomic studies have mostly been restricted to cell wall analysis and assessment of proteomic changes related to drug responses, change in pathogenicity of mutants, and serological response to candida infection [73]. While, there have been less studies on *Candida* biofilms in comparison. Interestingly, according to former studies, there is a remarkable similarity between biofilm and planktonic proteomic [74].

In one study, it was noticed that non-glucan attached proteins of the extracellular matrix of *C. albicans* biofilms and the cell surface are usually similar to planktonic yeast proteins [75]. There also have been some studies focusing on some specific proteomic aspects. For example, proteomic analysis of ergostrol, sphingolipid and oxidative stress pathway modulation by myristic acid (MA) have been conducted. According to this article, proteins related to glucosyl ergostrol and oxidative stress pathway is necessary for *C. albicans* to conflict with host immune system in order to survive. The negative regulation of those proteins proofed the antifungal activity of MA against *C. albicans*. As a result, MA can be considered as an ideal candidate for combination therapy with fluconazole [76]. In the end, we can briefly say that getting familiar with genomic and proteomic basis of biofilm formation in candida species can be extremely helpful with developing further treatment strategies.

Conclusion

The main pathogenic characteristic that helps *Candida* spp. to cause disease in human host is biofilm formation. Biofilm development, architecture and antifungal resistance mechanisms have been the major purpose of

former researchers. In general, biofilm cells in comparison with planktonic cells are less sensitive to antifungal agents and have a higher survival rate. Increasing development of microscopic, genomic and proteomic tools are really helpful with picturing a vivid overview of molecular basis governing pathogenic mechanism of *Candida* biofilm formation. Such information can give us invaluable clues, in order to help us combat this ubiquitous fungus.

References

1. Targalska M, Kunicka Styczynska A (2018) Candida Biofilms: Environmental and Clinical Aspects. Open access peer-reviewed chapter 2, pp: 14-28.

2. Seneviratne CJ, Jin L, Samaranayake LP (2008) Biofilm lifestyle of Candida: a mini review. Oral diseases 14(7): 582-590.

3. Douglas LJ (2003) Candida biofilms and their role in infection. Trends microbial 11(1): 30-36.

4. Potera C (1999) Forging a link between biofilms and disease. Science 283(5409): 1837-1839.

5. d'Enfert C, Janbon G (2016) Biofilm formation in Candida glabrata: What have we learnt from functional genomics approaches?. FEMS Yeast Res 16(1): fov111.

6. Cavalheiro M, Teixeira MC (2018) Candida Biofilms: Threats, Challenges, and Promising Strategies. Front med 5: 28.

7. Elving GJ, van der Mei HC, Busscher HJ, van Weissenbruch R, Albers FW (2002) Comparison of the microbial composition of voice prosthesis biofilms from patients requiring frequent versus infrequent replacement. Ann Otol Rhinol Laryngol 111(3): 200-203.

8. Ramage G, Martinez JP, Lopez Ribot JL (2006) Candida biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res 6(7): 979-986.

9. Tscherner M, Schwarzmuller T, Kuchler K (2011) Pathogenesis and Antifungal Drug Resistance of the Human Fungal Pathogen *Candida glabrata*. Pharmaceuticals 4(1): 169-186.

10. Rho J, Shin JH, Song JW, Park MR, Kee SJ, et al. (2004) Molecular investigation of two consecutive nosocomial clusters of Candida tropicalis candiduria using pulsed-field gel electrophoresis. J microbial 42(2): 80-86.

11. Yapar N (2014) Epidemiology and risk factors for invasive candidiasis. Ther Clin Risk manag 10: 95-105.

12. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, et al. (2001) Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J bacterial 183(18): 5385-5394.

13. Kolwzan B (2011) Analiza zjawiska biofilmu-warunki jego powstawania i funkcjonowania. Ochrona Srodowiska 33(4): 3-14.

14. U N (2013) Znaczenie biofilmu w patogenezie i leczeniu grzybic. Zakazenia 4: 56-59.

15. Czaczyk K (2004) Czynniki Warunkujace Adhezje Drobnoustrojow Do Powierzchni Abiotycznych. Post Microbiol 43(3): 267-283.

16. Karatan E, Watnick P (2009) Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiol Mol Biol Rev 73(2): 310-347.

17. Budzynska A, Rozalska B (2012) Potencjalne wykorzystanie roslinnych olejkow eterycznych w zwalczaniu zakazen z udzialem biofilmow drobnoustrojow. Zycie Weterynaryjne 87(3): 213-215.

18. Reslinski A, Mikucka A, Szczesny W, Szmytkowski J, Gospodarek E, et al. (2008) Wykrywanie biofilmu in vivo na powierzchni siatki chirurgicznej-Opis przypadku. Chirurgia Polska 10(3-4): 181-188.

19. Andes D, Nett J, Oschel P, Albrecht R, Marchillo K, et al. (2004) Development and characterization of an in vivo central venous catheter Candida albicans biofilm model. Infect immune 72(10): 6023-6031.

20. Ramage G, Vandewalle K, Wickes BL, Lopez Ribot JL (2001) Characteristics of biofilm formation by Candida albicans. Rev Iberoam Micol 18(4): 163-170.

21. Chandra J, Mukherjee PK, Leidich SD, Faddoul FF, Hoyer LL, et al. (2001) Antifungal resistance of candidal biofilms formed on denture acrylic in vitro. J Dent Res 80(3): 903-908.

22. Araujo D, Henriques M, Silva S (2017) Portrait of Candida Species Biofilm Regulatory Network Genes. Trends Microbial 25(1): 62-75.

23. Baillie GS, Douglas LJ (1999) Role of dimorphism in the development of Candida albicans biofilms. J Med Microbiol 48(7): 671-679.

24. Jin Y, Samaranayake LP, Samaranayake Y, Yip HK (2004) Biofilm formation of Candida albicans is variably affected by saliva and dietary sugars. Arch Oral Biol 49(10): 789-798.

25. Mukherjee PK, Mohamed S, Chandra J, Kuhn D, Liu S, et al. (2006) Alcohol dehydrogenase restricts the ability of the pathogen Candida albicans to form a biofilm on catheter surfaces through an ethanol-based mechanism. Infect immune 74(7): 3804-3816.

26. Hawser SP, Douglas LJ (1994) Biofilm formation by Candida species on the surface of catheter materials in vitro. Infect Immun 62(3):915-921.

27. Estivill D, Arias A, Torres Lana A, Carrillo Munoz AJ, Arevalo MP (2011) Biofilm formation by five species of Candida on three clinical materials. J microbiol Methods 86(2): 238-242.

28. Richards S, Russell C (1987) The effect of sucrose on the colonization of acrylic by Candida albicans in pure and mixed culture in an artificial mouth. J Appl Bacteriol 62(5): 421-427.

29. Parahitiyawa NB, Samaranayake YH, Samaranayake LP, Ye J, Tsang PW, et al. (2006) Interspecies variation in Candida biofilm formation studied using the Calgary biofilm device. APMIS 114(4): 298-306.

30. Swindell K, Lattif AA, Chandra J, Mukherjee PK, Ghannoum MA (2009) Parenteral lipid emulsion induces germination of Candida albicans and increases biofilm formation on medical catheter surfaces. J Infect Dis 200(3): 473-480.

31. Samaranayake YH, Cheung BP, Yau JY, Yeung SK, Samaranayake LP (2013) Human serum promotes Candida albicans biofilm growth and virulence gene expression on silicone biomaterial. PloS one 8(5): e62902.

32. Chandra J, Mukherjee PK (2015) Candida Biofilms: Development, Architecture, and Resistance. Microbiol Spectr 3(4).

33. Busscher HJ, Geertsema Doornbusch GI, van der Mei HC (1997) Adhesion to silicone rubber of yeasts and bacteria isolated from voice prostheses: influence of salivary conditioning films. J Biomed Mater Res 34(2): 201-209.

34. Zimmermann K, Bernhardt J, Knoke M, Bernhardt H (2002) Influence of voriconazole and fluconazole on Candida albicans in long-time continuous flow culture. Mycoses 45(S1): 41-46.

35. Suci PA, Tyler BJ (2002) Action of chlorhexidine digluconate against yeast and filamentous forms in an early-stage Candida albicans biofilm. Antimicrob Agents Chemother 46(11): 3522-3531.

36. Lattif AA, Mukherjee PK, Chandra J, Swindell K, Lockhart SR, et al. (2010) Characterization of biofilms formed by Candida parapsilosis, C. metapsilosis, and C. orthopsilosis. Int J Med Microbiol 300(4): 265-270.

37. Holmes AR, Cannon RD, Jenkinson HF (1995) Interactions of Candida albicans with bacteria and salivary molecules in oral biofilms. J Ind Microbiol 15(3): 208-213.

38. El Azizi MA, Starks SE, Khardori N (2004) Interactions of Candida albicans with other Candida spp. and bacteria in the biofilms. J Appl Microbiol 96(5): 1067-1073.

39. Park SJ, Han KH, Park JY, Choi SJ, Lee KH (2014) Influence of bacterial presence on biofilm formation of Candida albicans. Yonsei medical journal 55(2): 449-458.

40. van der Mei HC, Buijssen KJ, van der Laan BF, Ovchinnikova E, Geertsema Doornbusch GI, et al. (2014) Voice prosthetic biofilm formation and Candida morphogenic conversions in absence and presence of different bacterial strains and species on silicone-rubber. PloS one 9(8): e104508.

41. Hogan D, Kolter R (2002) Pseudomonas-Candida Interactions: an Ecological Role for Virulence Factors. Science 296(5576): 2229-2232.

42. Lindsay AK, Morales DK, Liu Z, Grahl N, Zhang A, et al. (2014) Analysis of Candida albicans mutants defective in the Cdk8 module of mediator reveal links between metabolism and biofilm formation. PLoS genet 10(10): e1004567.

43. Morales DK, Grahl N, Okegbe C, Dietrich LEP, Jacobs NJ, et al. (2013) Control of Candida albicans metabolism and biofilm formation by Pseudomonas aeruginosa Phenazines. mBio 4(1): e00526-00512.

44. Branchini ML, Pfaller MA, Rhine Chalberg J, Frempong T, Isenberg HD (1994) Genotypic variation and slime

production among blood and catheter isolates of Candida parapsilosis. J clin Microbiol 32(2): 452-456.

45. Kuhn DM, Chandra J, Mukherjee PK, Ghannoum MA (2002) Comparison of biofilms formed by Candida albicans and Candida parapsilosis on bioprosthetic surfaces. Infect Immun 70(2): 878-888.

46. Silva S, Henriques M, Martins A, Oliveira R, Williams D, et al. (2009) Biofilms of non-Candida albicans Candida species: quantification, structure and matrix composition. Med Mycol 47(7): 681-689.

47. Gutierrez Correa M, Ludena Y, Ramage G, Villena GK (2012) Recent advances on filamentous fungal biofilms for industrial uses. Appl Biochem Biotechnol 167(5): 1235-1253.

48. Arendrup MC (2010) Epidemiology of invasive candidiasis. Curr Opin Cri Care 16(5): 445-452.

49. Mikolajewska A, Schwartz S, Ruhnke M (2012) Antifungal treatment strategies in patients with haematological diseases or cancer: from prophylaxis to empirical, pre-emptive and targeted therapy. Mycoses 55(1): 2-16.

50. Cowen LE (2008) The evolution of fungal drug resistance: modulating the trajectory from genotype to phenotype. Nat Rev Microbiol 6(3): 187-198.

51. White TC, Marr KA, Bowden RA (1998) Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11(2): 382-402.

52. Patterson TF, Thompson GR 3rd, Denning DW, Fishman JA, Hadley S, et al. (2016) Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis 63(4): 1-60.

53. Weinstein RA, Rex JH, Sobel JD (2001) Prophylactic antifungal therapy in the intensive care unit. Clinical infectious diseases 32(8): 1191-1200.

54. Manzoni P, Arisio R, Mostert M, Leonessa M, Farina D, et al. (2006) Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. Pediatrics 117(1): 22-32.

55. Blumberg HM, Jarvis WR, Soucie JM, Edwards JE, Patterson JE, et al. (2001) Risk factors for candidal bloodstream infections in surgical intensive care unit patients: the NEMIS prospective multicenter study. The National Epidemiology of Mycosis Survey. Clini Infect Dis 33(2): 177-186.

56. Cannon RD, Lamping E, Holmes AR, Niimi K, Tanabe K, et al. (2007) Candida albicans drug resistance another way to cope with stress. Microbiology 153(10): 3211-3217.

57. Kojic EM, Darouiche RO (2004) Candida infections of medical devices. Clin Microbiol Rev 17(2): 255-267.

58. Heizmann P, Klefisch F, Heizmann WR (2011) Basic Research–Significance of Detection and Clinical Impact of Candida albicans in Non-Immunosuppressed Patients. Pharmacology & Pharmacy 2(4): 354-360.

59. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K (2004) Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett 230(1): 13-18.

60. Al Fattani MA, Douglas LJ (2004) Penetration of Candida biofilms by antifungal agents. Antimicrob Agents chemother 48(9):3291-3297.

61. Baillie GS, Douglas LJ (1998) Effect of growth rate on resistance of Candida albicans biofilms to antifungal agents. Antimicrob Agents Chemother 42(8): 1900-1905.

62. Lafleur MD, Kumamoto CA, Lewis K (2006) Candida albicans biofilms produce antifungal-tolerant persister cells. Antimicrob Agents Chemother 50(11): 3839-3846.

63. Richard ML, Nobile CJ, Bruno VM, Mitchell AP (2005) Candida albicans biofilm-defective mutants. Eukaryot Cell 4(8): 1493-1502.

64. Garcia Sanchez S, Aubert S, Iraqui I, Janbon G, Ghigo JM, et al. (2004) Candida albicans biofilms: a developmental state associated with specific and stable gene expression patterns. Eukaryot Cell 3(2): 536-545.

65. Murillo LA, Newport G, Lan CY, Habelitz S, Dungan J, et al. (2005) Genome-wide transcription profiling of the early phase of biofilm formation by Candida albicans. Eukaryot Cell 4(9): 1562-1573.

66. Ramage G, VandeWalle K, Lopez Ribot JL, Wickes BL (2002) The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in Candida albicans. FEMS Microbiol Lett 214(1): 95-100.

67. Nobile CJ, Mitchell AP (2006) Genetics and genomics of Candida albicans biofilm formation. Cellular Microbiology 8(9): 1382-1391.

68. Lewis RE, Lo HJ, Raad, II, Kontoyiannis DP (2002) Lack of catheter infection by the efg1/efg1 cph1/cph1 double-null mutant, a Candida albicans strain that is defective in filamentous growth. Antimicrob Agents Chemother 46(4): 1153-1155.

69. Srikantha T, Daniels KJ, Pujol C, Sahni N, Yi S, et al. (2012) Nonsex Genes in the Mating Type Locus of Candida albicans Play Roles in a/α Biofilm Formation, Including Impermeability and Fluconazole Resistance. PLOSPathog 8(1): e1002476.

70. Ranjith K, Kalyana Chakravarthy S, Adicherla H, Sharma S, Shivaji S (2018) Temporal Expression of Genes in Biofilm-Forming Ocular Candida albicans Isolated From Patients With Keratitis and Orbital Cellulitis. Invest Ophthalmol Vis Sci 59(1): 528-538.

71. Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphery Smith I, et al. (1996) Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev 13: 19-50.

72. Pitarch A, Jimenez A, Nombela C, Gil C (2006) Decoding serological response to Candida cell wall immunome into novel diagnostic, prognostic, and therapeutic candidates for systemic candidiasis by proteomic and bioinformatic analyses. Mol Cell Proteomics 5(1): 79-96.

73. Nett JE, Zarnowski R, Cabezas Olcoz J, Brooks EG, Bernhardt J, et al. (2015) Host contributions to construction of three device-associated Candida albicans biofilms. Infect Immun 83(12): 4630-4638.

74. Vediyappan G, Chaffin WL (2006) Non-glucan attached proteins of Candida albicans biofilm formed on various surfaces. Mycopathologia 161(1): 3-10.

75. Prasath KG, Sethupathy S, Pandian SK (2019) Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in Candida albicans. J Proteomics 208: 103503.