

Review articles

Selected pathogenic characteristics of fungi from the genus *Candida**

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ABSTRACT. The prevalence of fungi from the genus *Candida* in humans is increasing, but the mere fact of their detection does not allow, in general, to diagnose a disease. In fact the development of fungal infection depends on several factors of the host-pathogen relationship. The occurrence of symptoms and the course of the disease are associated, inter alia, with general and immunological conditions of an infected person as well as the properties of strains. Differences between the strains responsible for asymptomatic and symptomatic invasion have been shown. Thus the determination of their pathogenicity parameters is an important element leading to proper identification, both mycological and clinical, which allows for the implementation of therapeutic intervention. There are several virulence factors that are essential for surviving in host's organism and play important role in each phase of fungal infection. This review provides an update on selected pathogenicity features: formation of hyphae and/or pseudohyphae, phenotypic switching, tropic reactions and biofilm production.

Key words: *Candida*, virulence factors, hyphae, phenotypic switching, tropism, biofilm

Invasion of host cells by fungi progresses in several phases. Initially, blastospores adhere to epithelial cells, then hyphae are formed which penetrate cells actively or by endocytosis (in the case of the oral epithelium both the ways are possible, and in the epithelium of the gastrointestinal tract – only active penetration), causing over time progressive damage to the tissue [1,2]. Several agents, the so-called virulence factors, are responsible for this process, among which the pivotal ones are the ability to grow at 37°C and physiological pH, the size enabling invasion of the human body; the others are: formation of hyphae and pseudohyphae, the ability of phenotypic switching, adherence to epithelial and endothelial cells, biofilm formation, secretion of hydrolytic enzymes (proteases, phospholipases, lipases), and tropisms. Each of these attributes influences the other, and all are essential for full pathogenicity of fungi from the genus *Candida* [3,4].

Pleomorphism, formation of hyphae and/or pseudohyphae

Candida albicans is characterized by polymorphism, i.e. the possibility of occurrence in many forms, namely blastospores (yeast forms), germ tubes, pseudohyphae, true hyphae and chlamydospores, of which the most important role during infection play the yeast and filamentous forms, and the fungus ability to switch from one form of growth to another (dimorphism) depending on environmental conditions, which allows colonization and invasion of various host organs [5–11].

Abundance of blastospores and deficiency of nutrient trigger their transition into hyphae that penetrate another organ and transform again into the form of yeast, or remain in the environment until nutrients are supplemented [10,12]. The formation of hyphae proceeds, depending on environmental signals, by pathways of mitogen-activated protein kinase (MAPK) or cAMP-dependent protein kinase A (cAMP-PKA) [13–15]. Important regulators of

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hyphae growth, the expression of genes requisite for fungal adherence in this phase and, consequently, virulence of strains are, among others, Efg1 (elongation factor G essential for the formation of a stable biofilm), Cph1, Eed1 (maintenance of cell polarity), Ras1, Rim101 (formation of hyphae in neutral and alkaline pH), Ssn6, Tec1, Ume6 and Gat2 [2,7,12,14–25]. The gene responsible for filamentation of *C. albicans*, while not affecting its viability, is CaMYO5 encoding myosin I which plays a role in organization of actin in the fungal cytoskeleton and changes distribution of chitin in the cell wall, hence contributing to disturbance in cell polarity required for growth [26–28]. It has been observed that myosin I does not affect formation of germ tubes and pseudohyphae but inhibits the growth of true hyphae, indicating that it is not essential for the initiation of polarized cell growth, but for its maintenance [28]. The composition of cell wall is also dependent on SMI1 gene, which triggers synthesis of glucans and biofilm matrix, determining hyphae elongation and invasion of infected cells [24]. In *C. albicans*, the genes SSK1, SKN7 and SRR1 encoding proteins that regulate response to stressors (e.g. oxidants, high salt content in the environment) contribute to hyphae formation, thereby ensuring the virulence of strains [29–30]. In addition, the SRR1 gene is critical to induce resistance to oxidative and osmotic stress [29]. Recent studies have shown that normal polarized growth and formation of *C. albicans* hyphae require the CaLAG1 gene, whose products catalyze the synthesis of inositol-containing sphingolipids. It stimulates expression of hypha-specific genes ECE1 and HWP1, thus securing high virulence of strains and capability to damage the host tissue. A similar task on solid substrates is performed by CaLAC1 gene encoding enzymes that mediate the formation of glucosylceramides; it is not required for filaments formation. However, in the absence of CaLag1p, inositol-containing sphingolipids essential for hyphal growth can be produced from ceramides synthesized by CaLac1p [31]. Mannosyltransferases (Pmt) constitute a family of five proteins which are also responsible for the formation of filamentous forms, as well as adherence and sensitivity to antifungal agents, mainly aminoglycoside antibiotics: hygromycin B and geneticin – G418 (Pmt1 and Pmt4 in particular) [32–34].

Factors inhibiting formation of hyphae and regulating their conversion into blastospores are less well known and include, among others: Rbp1 and cooperatively acting Nrg1 and Tup1 [7,10,12,15, 20,22,23,35]. Nrg1 and Tup1 regulate the expression of gene PGA13 encoding GPI-anchored protein localized in the cell wall of *C. albicans* and responsible for its integrity; absence of this gene results in an extended period of hyphae formation [36]. Mutants lacking gene TUP1 grow exclusively in pseudohyphal form [22]. Sko1 represses filamentation ability in *C. albicans* and reduces expression of several hypha-specific genes [37]. The process of hyphae elongation is limited by HSL1 encoding protein kinase and HOG1 encoding the mitogen-activated protein kinase (MAPK) [24,30].

Both blastospores and hyphae are indispensable for adequately high pathogenicity of fungi from the genus *Candida* [3]. Both forms are observed in greater accumulation in different sites of the body (e.g. blastospores in the liver, spleen, gastro-intestinal tract and on the skin, and hyphae in the kidneys) and perform different roles depending on the stage of infection [7]. It is thought that blastospores are involved in dissemination of the fungus in the environment or via bloodstream, while hyphae, being larger and more elongated, contribute to increased adherence by inducing hypha-specific adhesins, e.g. Als3 and Hwp1 (no direct correlation was observed by Negri et al. [38] for *C. tropicalis*), invasion of tissues and plastic, activity of proteolytic enzymes Sap4-6 and antigenic modulation, making strains of fungi more pathogenic [5,7,11,23,35,39]. Additionally, hyphae are responsible for biofilm maturation and its three-dimensional structure, while dimorphism of *C. albicans* forms affects the biofilm quality by strengthening it [12,16,40]. Baillie et al. [40] observed that mutants incapable of hypha or blastospore formation produced only a single layer of biofilm (basal or outer, less adherent to the surface, respectively) in contrast to two layers formed by non-mutated strains, which weakened their virulence [7, 9]. The presence in *C. albicans* of genes SUV3, NUP85, MDs3 and KEM1 requisite for hyphae differentiation and biofilm formation proves that both the properties are interrelated [16]. In case of silicone materials used in medicine, hyphae damage them by penetrating deep, expanding and hardening the material [12]. Hyphae are the only form having the ability to bind ferritin (the receptor for it is Als3) from oral epithelial cells of the host, which is the source of iron for

C. albicans during infection [7,41].

Appropriate morphological form is also important during invasion to different organs. Active penetration of host cell membranes is carried out exclusively by hyphae, whereas endocytosis of filamentous forms through epithelial or endothelial cells occurs more efficiently compared with endocytosis of blastospores or cells of other shapes. Human serum stimulates the formation of hyphae which, in turn, activate the immune system; both invasion pathways are pivotal for dissemination of *C. albicans* via the bloodstream. The ability to induce endocytosis is associated with the possibility of damage to the infected host cells [7,12,22,42,43].

Phenotypic differences are also important in the immune response of the host. Neutrophils may eliminate both fungal forms, although they are more active in the presence of hyphae; however, dendritic cells (also capable of phagocytizing hyphae) are more effective in controlling blastospores. Macrophages phagocytize yeast forms, which can survive and produce hyphae escaping out of the cell through the membrane [7]. Augmented resistance to phagocytosis of filamentous forms in relation to blastospores results, among others, from increased expression of gene SOD5 encoding superoxide dismutase [7,11]. Lewis et al. [9] compared the rate of macrophages migration towards *C. albicans* cells and then their engulfment depending on the form (studies on mutants deprived of genes EFG1 and HGC1). The authors observed no differences between migration to hypha-deficient strains and non-mutated but, after establishment of cell-cell contact, the internalization rate by macrophages was higher in relation to the wild type, and a portion of hypha-deficient strains was not at all absorbed; after a longer period they detached and a certain percentage was later ingested by other phagocytes. While comparing the yeast and filamentous forms of wild-type strains, it was found that blastospores were engulfed faster than hyphae, whose absorption rate depended on their length and was lower in case of hyphae exceeding 20 μm. Internalization of hyphae by macrophages takes place regardless of their spatial orientation, although it has an impact on the rate of the process by promoting end-on cell contact with macrophages as opposed to side-on orientation, or at an angle.

Both fungal forms stimulate cytokine production by dendritic cells, but blastospores stimulate Th1 lymphocytes, while hyphae the production of interleukin IL-4 and inhibit proliferation of Th1 lymphocytes [7]. This is related to differences in the cell wall composition of both the forms and present therein pathogen-associated molecular patterns (PAMP) which include, among others, cell wall polysaccharides, proteins on the cell surface, secreted enzymes and their waste products, and ATP released during lysis of the host cells; they are identified by different sets of pathogen recognition receptors (PRR) on host phagocytic cells [7,9,12]. Interaction between PAMP and PRR contributes also to the fungus adhesion to epithelial cells of the host [44].

During formation of filaments the hypha-specific genes (HSG) become expressed, which include: ALS3, SAP4-6 encoding proteinases, HWP1, HYR1, ECE1 (all three encode surface proteins) and FKH1 [2,5–7,45].

Transition from blastospores to true hyphae is caused by various environmental factors, e.g. increased temperature (37°C), concentration of CO_2 (5.5%), pH (≥7.0), the presence of serum, N-acetylglucosamines (GlcNAc), or a limited amount of nutrients (sources of carbon, nitrogen compounds). The opposite phenomenon – transformation into blastospores – occurs at lower temperatures, more acidic pH or in the presence of high glucose concentrations [3,12,14,17,19,35, 46–49].

The temperature increased to 37°C is required as a factor accompanying others which trigger the growth of filamentous forms (except for cells growing deep into the substrate), whereas the temperature of 39°C increases filamentation regardless of other agents. In response to elevated temperature the heat shock protein Hsp90 is activated, but global problems with folding proteins impair its functionality. The result is cell cycle arrest at various stages, transition from blastospores to hyphae (two types: binucleated which exhibit a delay in mitosis and multinucleated, bilobed with cytokinetic defects) via the RAS1-PKA cascade and attenuation of virulence. Pharmacological and genetic factors, and partly also conditions inducing hyphae formation, have a similar impact on Hsp90 [14,50,51].

C. albicans tolerates a wide pH range of the environment, which can be significantly and rapidly altered by fungi [49].

Addition of certain chemicals to the medium may reduce hyphae development, as exemplified by diclofenac sodium – a nonsteroidal anti-inflammatory drug that inhibits the formation of germ tubes

and filaments and the expression of hyphae-specific genes ALS1, ALS3 and HWP1 [52]. Transition from blastospores to hyphal form of *Candida* sp. is also blocked by 4-hydroxycordoine (a natural secondary metabolite of plants belonging to chalcones), epigallocatechin gallate (a polyphenol with fungicidal properties found in green tea), thymol, geraniol, geranyl acetate and citronellol (organic compounds from the group of terpenes included in plant essential oils) [53–59]. In addition, 4-hydroxycordoine limits the formation of biofilm while not inhibiting the growth of *C. albicans*, which is done by other synthetic chalcones [56]. Another compound naturally occurring in essential oils that has the ability to lower the effectiveness of hyphae formation by *C. albicans* is cinnamaldehyde, causing also a significant weakening of hydrolytic enzyme secretion and shortening hyphae length [60]. The inhibitory effect on filamentation process and affecting it expression of SIR2 gene has also allicin, arising from alliin after crushing garlic (*Allium sativum*), which interferes with the integrity of the *Candida* sp. cell membrane [61]. Therefore, these substances may constitute effective agents impairing tissue invasion, targeting at one virulence attribute of strains, i.e. the ability to form filaments [56].

Blastospore-to-filament transition is also dependent on quorum-sensing molecules secreted by fungal cells [3,13–15]. To date, three signaling molecules (autoinductors) produced by *Candida* sp. have been described, of which the best known are two: farnesol and inversely acting tyrosol [11].

Farnesol inhibits development of new hyphae in dense populations (but does not prevent elongation of previously formed hyphae) and hence biofilm maturation. Transforming blastospores into hyphae is also prevented by secretion of dodecanol by *C. albicans.* Both molecules inhibit filamentation induced by serum or RAS1, but not caused by inhibition of Hsp90 activity; farnesol additionally reduces the expression of INF-γ and IL-2 in the kidneys [3,11,13–17,62–64].

Tyrosol secreted by blastospores stimulates filamentation after the phase of fungal adherence to the infected area [3,11,12,63].

The impact on the *Candida* sp. transition from one growth form to another may have bacteria present in the biofilm formed, for instance, in the oral cavity [63]. It has been shown that *Streptococcus gordonii* present therein is an agent increasing hyphal growth of *C. albicans* and attenuating the inhibitory effect of farnesol, whereas *Streptococcus mutans* and *Pseudomonas aeruginosa* slow down filamentation [13,65,66].

The presence of hyphae correlates with resistance of *Candida* sp. fungi to azole drugs. Costa et al. [67] observed that strains resistant to itraconazole and fluconazole produced more filamentous forms in their presence (depending on the drug concentration), and in susceptible strains the property was limited, probably due to ergosterol deficiency in the fungal cell membrane, the formation of which is inhibited by these substances.

C. glabrata incapable of creating true hyphae and germ tubes is a common cause of invasive candidosis, indicating that blastospores may be responsible for invasions [3,5,39]. In addition, numerous strains of this species, in contrast to *C. albicans*, are characterized by the ability of the so-called core switching, i.e. the transition from the white form (Wh), through the light brown (LB) and dark brown (DB) to very dark brown (vDB) observed on agar containing $CuSO_4$. Besides, each of these phenotypes can be transformed into irregularly wrinkled phenotype (IWr). While core switching does not change proportions or morphology of pseudohyphae and germ tube-like forms (budding cells, pseudohyphae and tubes are similar in each phenotype), the transformation into the wrinkled form reveals almost exclusively pseudohyphae. Most frequently observed and most virulent phenotype is dark brown [68–70].

C. tropicalis also demonstrates a weak ability to form pseudohyphae, hence its lesser capability to invade various tissues and organs compared to *C. albicans* [38]. The only fungi from the genus *Candida* capable of forming both pseudohyphae and true hyphae are *C. albicans* and their closest relative *C. dubliniensis* [6,46]. Less common human pathogens, such as *C. famata* and *C. lusitaniae*, produce only blastospores [23].

The ability of phenotypic switching

The virulence potential of a given *Candida* strain results from, among others, the possibility of frequent change of morphological form, e.g. from white, round or oval cells, with a smooth surface (white-phase) to gray, elongated, rough, twice as large (opaque-phase) and vice versa, depending on the changes in environmental conditions during invasion (temperature, pH, zinc concentration or UV radiation) [3,5,35,71]. It has been noted that

each of these forms differs in their ability to colonize, e.g. white phase is more frequently detected in intravenous infections and in kidneys, and the dark phase – on the skin [3,5]. Such change occurs more frequently in pathogenic strains of *C. albicans* and is associated with the ability to adapt to another cell during mating [3,7,12].

High frequency of morphological transformations (approximately one colony changed per $10–10^4$) contributes to antigenic modification due to frequent changes on the cell surface [3,11,35]. Phenotype switching affects also adherence ability of strains (differences in the cell wall components of particular forms) and biofilm formation [3,7]. Their enzymatic activity is also varied (white-phase cells secrete aspartyl proteases Sap2, and the opaque-phase: Sap1 and Sap3), as well as resistance to antifungal agents, phagocytosis and oxidants [3,35]. It has been demonstrated that the white phase has a greater ability to survive in the host organism and is more virulent than the opaque phase, whereas the latter has a greater ability to fuse with another cell during mating [5,35,72].

The capability of other species of the genus *Candida* to switch phenotype is less known, but it has been reported that in various parts of the host body different forms of *C. glabrata* may dominate, while different phenotypes of *C. lusitaniae* are characterized by a greater, compared with others, resistance to amphotericin [5].

Tropisms

The direction of polarized hyphal growth is dependent on environmental stimuli, such as imposed electric field and surface configuration which hyphae encounter during invasion, or lack of oxygen [3,73]. The abilities to change growth orientation are galvanotropism, thigmotropism and aerotropism, respectively [3,12,73,75]. In the first case, germ tubes form on the cathode side and developing hyphae orientate their growth in that direction, which during germ tubes formation is additionally determined by the influx of Ca^{2+} ions into the cell and the presence of calcineurin – phosphatase activating transcription factor CRZ1, which affects the activity of Cch1 and regulates the influx of Ca^{2+} [3,46,73]. Thigmotropism also depends on CRZ1, CCH1 i Ca^{2+}, but not on calcineurin [46,73]. Having encountered an obstacle or deficiency of nutrients, the influx of Ca^{2+} sets a new direction of hyphae elongation and contributes to laying them on solid and semi-solid mediums in the form of a two-dimensional sinusoid or three-dimensional right-handed helix [3,12,74–76]. These attributes, observed among commensal and pathogenic fungi, facilitate the invasion of host tissues through sites where surface integrity is disturbed (e.g. pores) [76]. Tropisms, in response to environmental stimuli, allow hyphae to spread evenly on the substrate making full use of available nutrients, enhance the penetration of cells, the degree of their damage by fungi and, consequently, the virulence of the strain. For tropic reactions are responsible proteins determining cell polarity, such as Bud2, Rsr1/Bud1, or its GTPase activating protein (GAP) and Ras-GTPase, and this mechanism is independent from the hyphal elongation process [75].

Tropisms are also utilized during mating to reach another cell and fuse with it [75].

Biofilm

Biofilm, i.e. a structure consisting of adherent cells of microorganisms embedded within a self-produced extracellular matrix, may be formed from one or more species on the surface of the host tissue (e.g. mucosal epithelium of the oral cavity, vagina, gastrointestinal tract), as well as plastics (e.g. contact lenses, urinary, peritoneal and intravascular catheters, hemodialysis fistulae, artificial valves, pacemakers, implants, articular and pharyngeal prostheses, dentures) [11,64,77–79].

The formation of a biofilm composed of fungal cells, e.g. *Candida albicans,* is a complex, long-term process (developing over at least 48 hours), which runs in three phases. Initially, (early phase) fungal cells adhere to the colonized surface, then (intermediate phase) hyphae develop and matrix is formed, consisting primarily of cell wall polysaccharides (predominantly β-glucan) but also proteins, hexosamine, phosphorus and uronic acid. Subsequently, (maturation phase) the whole structure grows spatially, assuming the appearance of a network of blastospores, hyphae and pseudohyphae suspended in extracellular matrix [11,16,62, 77–81]. The resulting structure *in vitro* consists of two layers: a thin base composed of blastospores, and a thicker, less compact, outer layer made of fungal hyphae [64]. The biofilm *in vivo* is not so orderly – blastospores randomly intersperse with hyphae, and the matrix additionally includes erythrocytes, platelets, neutrophils, macrophages

and other cells of the host organism [77,79].

The most frequently reported biofilm-forming fungi from the genus *Candida*, apart from *C. albicans*, are *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei* [38,39,78,81,82]. It has been observed that they form under *in vitro* conditions a thinner biofilm layer (composed only of blastospores), with lower mass and lesser metabolic activity than *C. albicans* considered to be a more pathogenic species [64,78,82]. Some strains of *C. parapsilosis* have the ability to develop hyphae, producing increased biofilm mass and being more pathogenic. In addition, the biofilm formed by *C. parapsilosis* isolated from blood exhibits a distinct accumulation of blastospores in irregular clumps and a small amount of extracellular matrix compared to *C. albicans* [64,82]. In case of the above species the correlation between biofilm density and its metabolic activity has been found, with the exception of *C. tropicalis*, whose activity was much lower than density [81]. *C. glabrata* biofilm compared with *C. albicans* produces smaller amounts of polysaccharide matrix; the major component in *C. tropicalis* matrix is hexosamine, while in *C. albicans* glucose, which may be of importance for fungal resistance to drugs [11,62].

A number of factors influence the process of biofilm formation and its structure, which include both the type of the involved fungus with morphogenesis, as well as chemical composition, surface structure and the degree of hydrophobicity of the material to which it adheres. Roughness and hydrophobicity of material are factors facilitating biofilm formation; it is easily formed on methyl polyacrylate, which is the basic material used for the production of dentures, or silicone elastomer used as soft material lining such prostheses, and latex; more difficult – on polyurethane and 100% silicone, and PVC in comparison with latex [11,78,79]. Different biofilm structure is observed on cellulose filters and catheter discs [40,78]. Environmental factors also play a role, such as medium containing high glucose concentration (especially for *C. parapsilosis* and *C. albicans*, compared with mediums with galactose), cell surface hydrophobicity dependent on growth conditions, mucus production by *C. parapsilosis*, or gentle shaking which increases the production of extracellular matrix that surrounds fungal cells with thick layer – all enhancing the described *in vitro* process [39,64,78,83–85]. However, of additional importance *in vivo* is availability of nutrients and space (not occupied by other components of microflora), the ability of the host organism to elicit immune response, changes in the expression of genes involved in processes of adherence, biosynthesis and metabolism of amino acids accompanying biofilm maturation [11].

Enhancement of biofilm is conditioned by fungal cells and their ability to secrete quorum sensing molecules that regulate the expression of specific genes coordinating processes within the biofilm, such as growth rate, the possibility of detachment, resistance or pathogenicity. Farnesol, dodecanol, phenylethanol and tryptophol slow down the growth of biofilm by suppressing the formation of germ tubes and hyphae, whereas tyrosol acts inversely because it can stimulate the formation of filamentous forms. The accumulation of farnesol and/or dodecanol in a mature biofilm (resulting from their production *in situ* by the biofilm) causes detachment of individual yeast cells, thus contributing to fungal dissemination and colonization of new organs [11,12,16,17,62–64].

It is thought that interaction between fungi and bacteria co-existing in the environment, e.g. in the oral cavity, is important in biofilm formation [63]. *C. albicans* can form aggregates with such microorganisms as *Streptococcus gordonii*, *S. oralis* and *S. sanguinis,* involving fungal adhesins, cell-wall proteins and polysaccharides of streptococci. *Porphyromonas gingivalis* may delay surface colonization by *C. albicans*. Farnesol produced by *Candida* sp., while acting on the cell membranes of *Staphylococcus aureus* and *Escherichia coli*, can inhibit bacterial biofilm formation process and increase its sensitivity to drugs, thereby indicating that it can also control mixed biofilms (*Candida*-bacteria) [63,86]. It is thought to reduce the production of quinolones – molecules signaling *Pseudomonas aeruginosa* density, thereby increasing the chance of *Candida* sp. maintenance in the biofilm composed of both species. On the other hand, the bacterial signal molecules (e.g. homoserine lactone, dodecanoic acid) may influence the fungal biofilm. In case of *Pseudomonas aeruginosa* and *C. albicans*, this collaboration (involving homoserine lactone) increases the resistance of both species to pharmaceuticals [63]. In commonly observed biofilm *in vivo* composed of *C. albicans* and *Staphylococcus epidermidis* a slower penetration of antifungal drugs is detected [16].

Organisms forming biofilm in the oral cavity are thanks to it resistant to mechanical flushing by

saliva and gingival fluid, and to the immune response of the host [11,64,79]. This indicates a direct relationship between the ability of biofilm formation and invasiveness of strains, ability to infect tissues and host mortality [11,64,82]. No difference in mortality rate among patients with biofilm-related candidosis and people infected with *Candida* unable to produce biofilm was found by Tobudic et al. [81]. Interestingly, researchers additionally discovered that larger quantities and higher activity of biofilm are produced by non-invasive strains in comparison with isolates responsible for candidaemia. Several studies, not showing significant difference in biofilm formation by invasive and non-invasive strains, were cited by Kojic et al. [78].

Higher pathogenicity of biofilm-forming fungi is associated with their higher (similar in monolayer and more complex biofilms) resistance to commonly used drugs, primarily azoles, induced by many factors, e.g. adsorption of antifungal substances by extracellular matrix impeding their expansion and access to fungal cells, slow growth and reduced activity of persister cells, and changes in cell wall components (1.3-β-glucan in the wall of fungi present in the biofilm to a higher degree binds antifungal agents in comparison with planctonic forms) [5,11,16,63,64,77–80,87,88]. Other factors generating the biofilm resistance to antifungal agents are: presence in the biofilm of ATP-binding cassette transporter proteins having properties of multidrug efflux pump, recognizing and removing the drug from the cytoplasm, which is particularly important in the early stages of biofilm formation, and increased expression of drug resistance genes (CDR1, CDR2, MDR) [11,16,63,80]. Cross-resistance resulting from increased tolerance to stress caused by growth of cells under conditions that prevail in the biofilm environment (low content of nutrients and oxygen) may also be involved [63]. Comparatively high resistance of *C. albicans* and *C. parapsilosis* biofilms to such drugs as fluconazole, ketoconazole, itraconazole, voriconazole, ravukonazole, flucytosine, amphotericin B (also in higher concentrations than MIC for planctonic cells), nystatin (including its lipid form), terbinafine and chlorhexidine has been demonstrated; *C. glabrata* is also resistant to azole drugs. Echinocandins (caspofungin, micafungin, anidulafungin), which block the production of 1.3-β-glucan and lipid formulations of amphotericin B (of reduced nephro- and hepatotoxicity with regard to the standard amphotericin B) inhibiting the metabolic activity of biofilm *in vitro*, have proved to be effective in combating *C. albicans* and *C. parapsilosis* infections [39,62–64,77–80,87]. Non-steroidal anti-inflammatory drugs, such as aspirin, etodolac, diclofenac, celecoxib, nimesulide, ibuprophen and meloxicam, suppress biofilm formation by affecting prostaglandin synthesis and secretion by *C. albicans*, wherein the strongest effect was observed for the first three substances. Such properties have not been demonstrated for indomethacin and piroxicam [62].

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