Virulence Traits Contributing to Pathogenicity of Candida Species

Abstract

Candida is the unique opportunistic mycotic pathogen that has adapted variety of mechanisms to establish itself both as commensal and pathogen in humans. This yeast like fungus presents in many clinical forms, ranging from superficial manifestations involving the skin, nails and mucosal surfaces to deep seated infections involving various internal organs and disseminated diseases. Although Candida spp. can initiate infection in both immunocompetent and immunocompromised hosts, the incidence of candidiasis is usually high in immunocompromised patients. Therefore the role of Candida in overall process of initiation and progression of infection was considered to be passive. However, recently this concept is revamped and it can be now stated that Candida actively participates in the pathophysiology of establishment and progression of infection through the mechanisms of aggression known as virulence factors. Some of these virulence factors help in colonization or initiation of infection while others aid in progression of infection or dissemination in host tissues. Adherence to host tissues, biofilm formation on medical devices and secretion of extracellular hydrolases are some of putative virulence traits of Candida spp. Although virulence factors contributing to pathogenicity of Candida albicans is well studied, the search through available literature has revealed a dearth of information on virulence factors of non albicans Candida (NAC) spp. The identification of virulence attributes unique to a particular Candida spp. is very important to understand the pathogenesis and epidemiology of candidiasis.

Keywords: Adhesion; Biofilms; Extracellular hydrolytic enzymes; Morphogenesis; Phenotypic switching; virulence traits

Introduction

The fungus belonging to genus Candida is the only opportunistic mycotic pathogen causing a wide spectrum of clinical manifestations ranging from mucocutaneous overgrowth to life threatening disseminated infections [1]. This opportunistic fungal pathogen is ubiquitous in nature and can exist as saprophyte and commensal. However, under certain conditions Candida may transit from a commensal to a potent pathogen.

The transition of Candida from a harmless commensal to potent pathogen is contributed by various host factors and pathogenicity of infecting Candida spp. As candidiasis is frequently encountered in immunocompromised and critically ill immunocompetent individuals the role of Candida in overall process of initiation and progression of infection was considered to be passive. Therefore, organic weakness or compromised immune status of the host was considered as the only mechanism responsible for establishment of this opportunistic mycotic infection [2]. However, recently this concept is revamped and it can be now stated that Candida actively participate in the pathophysiology of establishment and progression of infection through the mechanisms of aggression known as virulence factors [2,3]. Virulence factors can be defined as all traits produced by infecting strain of pathogen to establish the process of infection in the host.

The pathogenicity of Candida spp. can be attributed to various virulence traits like adhesion to host tissues and surface of medical devices, biofilm formation and secretion of extracellular hydrolases, phenotypic switching and thigmotropism [3, 4]. These virulence traits of infecting Candida spp. directly interact with host cells and leads to tissue damage [5]. Although virulence factors contributing to pathogenicity of Candida albicans is well studied, the search through available literature has revealed a dearth of information on virulence factors of non albicans Candida (NAC) spp. once ignored as commensal or disseminated as a mere contaminant in clinical specimens, have emerged as an important cause of infections [6]. Many researchers from different parts of the world have reported predominance of NAC spp. over C. albicans in different clinical types of Candida infections. In this research, various virulence factors contributing to the pathogenicity of Candida spp. with emphasis on ‘cryptic’ NAC spp. was investigated and concisely presented in this review.

Methodology

For preparation of this review article, relevant original and review articles were retrieved from search engines like ‘PubMed’ and ‘Google Scholar’. The search was made using MeSH terms like ‘virulence factors of Candida’, ‘pathogenicity of Candida species’ and ‘pathogenic features of Candida’. 

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Discussion

In *Candida* spp. combination of different virulence factors contributes at each stage of colonization and infection. At some stages a particular virulence factor may be dominant over the other.

Adhesion

The versatility and ability of *Candida* spp. to adhere and survive on a variety of human tissues makes it an important organism among fungal pathogens. Adhesion to the tissue is the primary and most important stage in the process of *Candida* colonization and infection [7]. *Candida* spp. can adhere to various host cells like epithelia, endothelia and phagocytes [8]. Adherence to host cells confers certain important properties to infecting *Candida* spp. Adhesion prevents or at least decreases the extent of clearance by defense mechanism of the host. It also ensures the delivery of Candidal enzymes and toxins to the host’s cell targeted [3]. Adhesion helps *Candida* cells to penetrate, disseminate and persist in host tissues [3].

Similar to other microbial pathogens, adhesion in *Candida* spp. is initiated and controlled by several cell-signaling cascades. The process of adherence to host cell is initiated by non-specific factors like hydrophobicity and electrostatic forces and further preceded by specific adhesins present on the surface of *Candida* cells [9]. Adhesins are surface proteins involved in specific adherence. ALS (agglutinin-like sequence) family, Hwp (hyphal wall protein) 1, Int (Integrin –like surface protein) 1, Mnt (α-1-2-mannosyltransferase) 1 and EPA (epithelial adhesin) 1 are examples of well characterized adhesins of *Candida* spp. [8,10]. Candidal adhesins recognizes and binds to ligands like proteins, fibrinogen and fibronectin. As adhesins like Als3 and Hwp1 are predominately expressed during the process of hypha formation, they play important role in the adhesion of *C. albicans* to host cells [8]. In *C. glabrata* (the only known haploid *Candida* spp. that lack ability to form hyphae or pseudohyphae) EPA gene family encodes for major group of adhesins. Epa proteins are that lack ability to form hyphae or pseudohyphae) EPA gene predominately expressed during the process of hypha formation, they play important role in the adhesion of *C. albicans* to host cells [8]. In *C. glabrata* (the only known haploid *Candida* spp. that lack ability to form hyphae or pseudohyphae) EPA gene family encodes for major group of adhesins. Epa proteins are. In case of *C. parapsilosis*, genes for five Als proteins and six for pga 30 (predicted glycosylphosphatidylinositol-anchored protein 30) have been identified [11]. At least three Als proteins have been identified in cell wall of *C. tropicalis* through western blot analysis [11].

The process of adhesion is influenced by various factors like the type of cell wall proteins and physicochemical properties of cell surface. Three types of interactions have been proposed for adhesions of *Candida* spp. to the host cells [12]. These include protein-protein interaction, lectin-like interaction and incompletely defined interactions [12]. Numerous assays like visual cell binding, radiometric binding, whole cell-ligand binding and Candidal adhesion-ligand binding are available for in vitro measurement of *Candida* adherence.

Biofilm formation

In recent years, *Candida* spp. is frequently isolated from nosocomial bloodstream infections, pneumonias and urinary tract infections [13]. Due to their versatility of adapting to a variety of different habitats including medical devices, *Candida* spp. have emerged as one of the important causes of health-care associated infections [14]. *Candida* rank 3rd among leading causes of catheter-associated infections [15]. Nearly half of all cases of nosocomial infections are associated with indwelling medical devices [16]. Medical devices are integral to a patient-centric care continuum, from screening to diagnosis to treatment and monitoring. *Candida* spp. can adhere, colonize and form biofilm on most, if not all, medical devices in current use [17]. Biofilms are surface-associated microbial communities which are firmly fixed within an extracellular matrix [13].

Biofilm limits the penetration of an antifungal agent through the matrix and protects the yeast cells from host defense mechanism. *Candida* biofilms are resistant to inhibitory action exerted by neutrophils [18]. The mortality rate of infections caused by biofilm forming *Candida* isolates is significantly higher than that of non biofilm forming strains. The mortality rate of medical device associated *Candida* infections is as high as 30% [2]. In *Candida* spp. the ability to form biofilm and extracellular matrix varies according to species, strain and environmental factors. Biofilm matrix of *C. albicans* composes of carbohydrates, proteins, phosphorus and hexosamines. *C. tropicalis* biofilm matrix has low levels of carbohydrates and proteins compared to *C. albicans* and other NAC spp [11]. *C. glabrata* biofilm matrix is made of high levels of proteins and carbohydrates. The extracellular matrix of *C. parapsilosis* biofilm is mainly contains carbohydrates and low levels of proteins [11]. Biofilm formation is noted in NAC spp. like *C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. dubliniensis*. In one of our study, we noted greater biofilm forming ability in *C. tropicalis* compared to *C. albicans* [6]. Various model systems like catheter disc, acrylic disc, microtiter plate, cylindrical cellulose filter; perfused biofilm fermenter and modified Robbins device have been used for characterization and antifungal susceptibilities of *Candida* biofilms.

Extracellular hydrolytic enzymes

Extracellular hydrolyses play an important role in the pathogenesis of *Candida* infections. These extracellular hydrolytic enzymes facilitate the invasion of host tissue by deranging constituents of host cell membrane [2]. Phospholipases, secreted aspartyl proteinases, lipases and hemolysins are the secreted hydrolases most commonly implicated in the pathogenicity of *Candida* [2]. In *Candida* spp., enzyme phospholipase hydrolyses phospholipids of host cell membrane and expose receptors to facilitate the adherence of yeast cells [19]. Phospholipase activity in *C. albicans* was first reported by Costa and Werner [3]. Till date, seven phospholipase genes have been identified in *C. albicans*. These include PLA, PLB1, PLB2, PLC1, PLC2, PLC3 and PLD1 [19].

Several researchers have reported phospholipase activity in *C. albicans* and NAC spp. like *C. tropicalis* and *C. parapsilosis*. In contrary to *C. tropicalis* and *C. parapsilosis*, very few studies are available on phospholipase production in *C. glabrata* [19]. *C. dubliniensis* isolates often demonstrate low phospholipase activity. This could be one of the possible reason for minimal or no role of this NAC spp. in invasive infections [2]. As phospholipase activity is concentrated at the growing hyphal tip, this enzyme plays a vital role in invasion host tissue especially in disseminated *Candida* infections.
Several studies have reported association between phospholipase production and other factors of *Candida* spp. like antifungal resistance and biofilm production. Ying & Chungyang [20] reported co relation between high phospholipase activity in *Candida* isolates and resistance to fluconazole [20]. In one of our study, phospholipase activity was significantly high among biofilm forming *Candida* isolates [2]. Therefore screening of phospholipase activity in biofilm forming *Candida* spp. can serve as an important parameter to differentiate invasive strains from non invasive colonizers. Elifegy et al. [21] reported significantly high phospholipase activity among *Candida* spp. isolated from disseminated infections compared to mucocutaneous infections [21]. Egg yolk agar plate based method is the easy and widely used for screening of phospholipase production in *Candida* isolates.

Proteinase activity of *Candida* spp. is due to ten secreted aspartic proteinase (Sap) isoenzymes [22]. In *Candida*, Sap is encoded by ten genes SAP1-10. Sap deteriorates epithelial and mucosal barrier proteins such as collagen, keratin and mucin. Additionally, it also degrades complement, cytokines and immunoglobulins [3]. Sap1-6 is important for adherence, tissue damage and alteration in the host defense mechanisms [3]. The extracellular proteinase was first described by Staib [3]. Till date the role of Sap 7 in pathogenicity of *Candida* spp. is not completely understood whereas, Sap 9 and Sap 10 are important in maintaining the regulatory surface integrity of yeast cells [3]. Many studies have reported variable proteinase activity among different *Candida* spp. As compared to other *Candida* spp., very little is known about proteinase activity of *C. glabrata*, *C. krusei* and *C. kefyr*. In one of our study, *C. dubliniensis* isolated from HIV infected patients with oropharyngeal candidiasis showed higher proteinase activity compared to *C. albicans* [6]. Till date, three *Candida* genes (SAP1P-3) have been identified in *C. parapsilosis* [11]. Four SAP genes (SAP1P-4) have been identified in *C. tropicalis*. *C. tropicalis* demonstrates high proteinase activity in medium containing bovine serum albumin as the sole source of nitrogen [11].

Sometimes proteinase antigen is expressed on fungal elements engulfed by phagocytes. The proteinase production within the phagolysosome degrades proteins involved in the process of generation of candidaloidal oxygen radicals [23]. Various factors like strain type, type of infection, phenotypic switch type, environmental conditions and stage of infection affect the proteinase production. The role of Saps in pathogenesis of disseminated candidiasis appears to be less important compared to mucocutaneous infections [21].

In vitro proteinase activity of *Candida* spp. can be elicited in a culture medium containing exogenous protein like bovine serum albumin, hemoglobin, keratin, collagen, or complex peptide mixtures like peptone and tryptone as a sole nitrogen source. Sap can be also be detected by spectrophotometry, ELISA and western blotting technique. In *Candida* spp., hemolysins degrade hemoglobin and facilitate recovery of the elemental iron from host erythrocytes [3]. As hemolysins enable survival and persistence of *Candida* in the host, it is considered as one of the putative virulence traits contributing to pathogenicity.

Genetic mechanism for haemolysin production is not completely elucidated for *Candida* spp., however Luo et al. [24] reported hemolysin like protein (HLP) gene to be associated with hemolysin production in *Candida* spp. [24]. The extent of hemolysin production is dependent on both species and strain of *Candida* spp. Hemolysin activity is reported in both *C. albicans* and NAC spp. like *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis*. Blood agar plate assay is a simple and cost effective method for the detection of in vitro haemolytic activity of *Candida* spp.

As compared to other enzymes of *Candida* spp., very few studies are directed towards coagulase. Rodrigues et al. [25] first reported coagulase activity in *Candida* spp [25]. Enzyme coagulase binds to fibrinogen and activates a cascade of reactions that leads to clotting of plasma. Coagulase production in *Candida* spp. is slow as compared to *Staphylococcus* aureus. Coagulase activity is reported in *C. albicans* and NAC like *C. tropicalis* and *C. parapsilosis*. In one of our study, *C. glabrata* demonstrated high coagulase activity compared to other NAC spp. Similar finding was reported by Yigit et al. [26]. Rodrigues et al. [25] reported high coagulase activity in *C. tropicalis* isolates [25].

Extracellular lipases facilitate degradation of lipids for nutrient acquisition and adhesion to host tissues. Lipases also nonspecifically initiate inflammatory processes by enhancing cell mediated immunity [27]. This group of enzymes also alters host defense by lysing competing microflora. Extracellular lipase production in *C. albicans* was first described by Werner (1965) [27]. *C. albicans* lipases are encoded by 10 genes (LIP1 to LIP10) [27]. Similar sequences are also identified in *C. tropicalis*. However till date no studies have been conducted to investigate the role of lipase genes in the virulence of *C. tropicalis*.

**Polymorphism**

Among various pathogenic yeasts *Candida* spp. is the only polymorphic fungus. *C. albicans* can either grow as budding yeast, as elongated ellipsoid cells with constrictions at the septa (pseudohyphae) or as parallel-walled true hyphae [18]. The production of hyphae is considered as one of the mechanism of virulence as it facilitate tissue invasion and also resists phagocytosis. Apart from *C. albicans*, the novel species *C. dubliniensis* is also capable of growing isotropically (yeast form) or apically (hyphal or pseudohyphal form). Although *C. tropicalis* demonstrates similar morphological forms like *C. albicans* only few studies have explored its role in virulence. Silva et al. [28] reported that only apical forms of *C. tropicalis* are capable of invading oral epithelium [28].

The transformation of unicellular yeast to multicellular hyphal forms is known as dimorphism. Both yeast and hyphal forms play an important role in the virulence. Although hyphal forms are more invasive than yeast forms, yeast form is believed to be primarily involved in dissemination. This mechanism may explain the involvement of *C. glabrata* in disseminated candidiasis. *C. glabrata* is the only *Candida* spp. that is haploid and lack the ability to produce both true and pseudohyphae.

Many strains of *C. albicans* are capable of switching spontaneously, reversibly and at high frequencies between numerous general phenotypes distinguishable by colony morphology [2]. Phenotypic switching is one of the putative
virulence attributes of \textit{C. albicans}. Soll et al. [29] described phenotypic switching in \textit{C. albicans} [29]. Phenotypic switching is defined as the ability of microorganisms of a single strain to switch reversibly and at high frequency among different colony phenotypes. It regulates various phenotypic characteristics involved in pathogenesis such as adherence to host cells, expression of cell surface hydrophobicity and secretion of proteases. Phenotypic switching in \textit{Candida spp.} due to the regulation of pathogenic phase-specific genes is observed during response to antifungal therapy and alteration in host immune mechanism [30]. Mane et al. [31] noted greater phenotypic switching in \textit{Candida} isolates from HIV infected individuals compared to those from HIV non infected individuals [31]. Phenotypic switching could be considered analogous to the ‘Phase transition’ described for bacteria. It also allows rapid adaptation of \textit{Candida spp.} to different stressful environments. The molecular mechanism involved in phenotypic switching is yet to be elucidated.

In addition to \textit{C. albicans}, \textit{NAC spp.} like \textit{C. dubliniensis} also exhibits high frequency of phenotypic switching and produces numerous colony morphologies. Csank & Haynes [32] reported phenotypic switching in \textit{C. glabrata} [32]. \textit{C. glabrata} demonstrated spontaneous, reversible and high frequency phenotypic switching on CuSO4-containing indicator agar plates [32]. Till date, there is no study available on the phenotypic switching of \textit{C. tropicalis} and other \textit{NAC spp.}

**Thigomotropism**

The directional hyphal growth demonstrated by \textit{Candida spp} on surfaces with specific topologies is known as thigomotropism [18]. Thigomotropism is one of the important mechanisms contributing to the virulence of \textit{Candida spp.} This process helps in biofilm formation on various abiotic surfaces like medical devices and dissemination into host tissues. Thigomotropism of \textit{C. albicans} hyphae is regulated by extracellular calcium uptake via the calcium channels [18]. Further studies are needed to explore the role of this virulence trait in the pathogenicity of \textit{NAC spp.}

**Conclusion**

Virulence factors are all traits required for establishment and progression of infection. The pathogenicity of \textit{Candida spp.} is attributed to the number of virulence factors like adhesion to host tissue, biofilm formation and secretion of ectoenzymes. The different virulence factors contribute at each stage of infection. However at some stages one virulence factor may be dominant over other or may act as an antagonist. Therefore, the identification of virulence attributes unique to a particular \textit{Candida spp.} is very important to understand the pathogenesis and epidemiology of candidiasis.

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