Factors Influencing the Virulence of *Candida Spp*

HDM Coutinho

ABSTRACT

Background: *Diseases produced for Candida species (spp) are called candidiasis and this includes settling, superficial infection, deep tissue invasion and haematogenic dissemination (eyes, the skin, the kidneys and the brain). The species that more usually cause candidiasis are* C albicans, C tropicalis *and* C glabrata. *These species usually are found in the normal microbial populations of the mucosae and can be pathogenic due the vulnerability of the host.*

Objective: *The objective of this paper is to review the factors that influence infection by* Candida spp *in order to empower health-workers in the management of such infections.*

Methods: *Data were retrieved from the bibliographical data banks of SCIELO, HIGHWIRE, PUBMED, LILACS and SCIRUS.*

Conclusions: *Knowledge about factors such as environment, exoenzymes, preferred infection sites and other factors that are essential for combating Candida infection and the risks associated with infection, will enhance efficient management.*

Factores que Influyen en la Virulencia de *Candida Spp*

HDM Coutinho

RESUMEN

Introducción: *Las enfermedades producidas por las especies de Cándida se denominan* candidiásis, *e incluyen asentamiento, infección superficial, invasión del tejido profundo y diseminación hematogénica (en los ojos, la piel, los riñones y la piel). Las especies que causan candidiásis con mayor frecuencia son* C albicans, C tropicalis *y* C glabrata. *Estas especies usualmente reencuentran en las poblaciones microbianas normales de las mucosas y pueden tornarse patogénicas debido a la vulnerabilidad del huésped.*

Objetivo: *El objetivo de este trabajo es examinar los factores que influyen en la infección por Candida spp, a fin de empoderar a los trabajadores de la salud en el tratamiento de este tipo de infección.*

Métodos: *Los datos fueron tomados de los bancos de datos bibliográficos, tales como SCIELO, HIGHWIRE, PUBMED, LILACS y SCIRUS.*

Conclusiones: *El conocimiento acerca de factores tales como el medio ambiente, las exoenzimas, los sitios preferidos de la infección y otros factores esenciales para combatir las infecciones por Cándida y los riesgos asociados con la infección, contribuirá a hacer el tratamiento más eficiente.*

West Indian Med J 2009; 58 (2): 160

INTRODUCTION

There is a high incidence of candidiasis in immunodeficient patients and knowledge of the virulence factors is important to comprehend the several ways by which *Candida spp* avoid antifungal therapy and the immune response to the host causing infection. Infection by *Candida spp* can be the cutaneous form (affecting the mucosa) or generalized, affecting deep tissues as heart, lung, liver and brain, causing clinical problems. The pathogenic pattern of candidiasis is affected by adherence, cell multiplication at the mucosal surface (with formation of filaments and the germinative tube in *Candida albicans*). This process is followed by the production of proteinases and phospholipases which damage tissues and cause an inflammatory response. This process can lead to

From: Departamento de Ciencias Fisicas e Biologicas- DCFB; Centro de Ciencias Biologicas e da Saude-CCBS; Universidade Regional do Cariri-URCA.

Correspondence: Dr HD Melo Coutinho, Universidade Federal da Paraíba – UFPB; Centro de Ciências Exatas e da Natureza – CCEN; Departamento de Biologia Molecular – DBM; Laboratório de Genética de Microrganismos – LGM. CEP:58051-900. João Pessoa – PB – Brasil. E-mail: hdmcoutinho@

systemic infection. The damage to the host tissues can be extensive (1).

One of the most important trait of the *Candida* genus is multiplication to form blastospore, pseudohypha and septate hypha. *Candida glabrata* is the one species of this genus that have only yeast cells (2).

C albicans is a dimorphic fungi that grow on the surface of solid medium as germinative and oval yeast. Both morphological forms can exist. The growth of this micro-organism on culture medium is best at 37◦C. The culture using maize agar can induce the formation of great spores with large walls, with large and dense chlamydospore coat and inside of which is a granulated mass, 8–12 ?m, that is at the extreme of pseudomycelium, being a characteristic for identification. The chlamydospores are specific to *C albicans*. The transformation of blastospore to hypha could be equivalent to the change of the trophic state of commensal to parasitic. The hypha can penetrate the oral tissue but only on the epithelial surface.

The penetration of hypha at straight angle was observed on fragments of tissues infected by Candida. The reason for this invasion form is not clear and it has been suggested that this invasion form is one way to obtain nutrients and avoid the loss of adherence from epithelial desquamation (1).

In the saprophytic state, *Candida albicans* can be found in the yeast state, with round and egg-form cells, 2– 4 ?m, with thin walls and asexual reproduction by blastospores in the buds. In the parasitic state, these are filaments with round tips, 3–5 ?m of diameter. The buds are detached from the mother-cell and take a rod form, forming pseudomycelium. The colonization and infection by *Candida* starts with its adherence to the epithelial cells. The specific receptors at the cytoplasmic membrane are necessary for the fixation and penetration of the micro-organism into the cell. These receptors are genetically determined (1).

Toxins Production and Change of Environment

The pathological reaction is mediated by endotoxins produced during the fast growth and death of the micro-organisms on the skin surface. These toxins could be in two groups: high-molecular-weight-toxins (canditoxin) and low-molecular-weight-toxins. Both toxins affect mechanisms of humoral immunological response affecting the interaction between macrophages and fungi and the accuracy of this cell to destroy hypha or blastoconidia (3).

The adherence to the epithelium and the production of carboxylic acids of short chain as a product of sugar metabolism by the growing organism produce an acid environment that could affect the pathological process in several ways: 1) producing irritation at the mucosal surface, starting the inflammatory process; 2) activating the *Candida* acid proteins, making possible their action at the mucosal surface and the cleavage of secreted IgA, an important factor to prevent the *Candida* adherence; 3) activating phospholipase production, that will destroy the membranes of the host cells; 4) stimulating the growth of aciduric organisms and inhibiting the commensal populations of micro-organisms. The commensal populations are important to prevent the yeast adhesion mechanisms to the cells (1). In the beginning of the infectious process, the yeasts can penetrate the tissues and make themselves more resistant to the phagocytic action of the defense cells by the selection of more resistant yeasts. During this moment, the germinative tubes will be developed and the infection could be disseminated to other tissues (1).

Filamentation Mechanism

The filamentation process is regulated in multiple ways, including kinase activation by the mitogenic mediator Cph1, by cAMP/PKA and by Cph2. Two genes, *DDR48* and *YPL184*, regulate the hyphal growth in these ways. The signaling filamentation ways show the same regulation by these genes with different expressions patterns. Maybe *C albicans* uses this system because it is a very important characteristic of virulence, essential to the survival and pathogenicity of yeast (4).

Toxin Production

Candida albicans can produce several extracellular enzymes, mainly proteinases, phospholipases and lysophospholipases. These hydrolytic enzymes can modify the membrane components, causing a misfunctioning. Other non-pathogenic species of *Candida*, although with a similar structure to *C albicans*, do not produce these phospholipases, indicating the importance of these enzymes in the pathogenicity (5, 6).

The presence of phospholipases and lysophospholipases are very important to *Candida*. These enzymes are important to growth of yeasts and to remodelling of cell parasitic membrane. They are also implicated in the mechanism of invasion of host tissue. But the most important trait of these enzymes is their relationship with pathogenicity (7). The production of proteinases was detected on all analysed yeast strains but the presence of phospholipases was detected only on *Candida albicans* (5). Besides, many other factors affect the pathogenicity of *Candida albicans*: hyphal formation, expression of genes to drug resistance, adherence properties and the production and secretion of extracellular aspartic proteinases (SAP). Data indicate SAP as an important virulence factor of *C albicans* (8).

Environmental Influence

Studies with bacterial pathogens show that several genes involved with virulence are regulated by signals of the host infection site. These signals include temperature, pH, osmotic strength and iron and calcium concentrations (9). The comprehension of this situation could reveal how this micro-organism could adapt itself and survive on this host site. *C albicans* may cause infection at several sites of the host and

one or more of these signals could be important to regulate the virulence of this micro-organism. A previous identification of *Candida* genes regulated by pH is very important because pH affect the expression of any virulence factors as SAP (10). When the pH of saliva of any patient is more acid (*eg* patients with cancer) there is a growth increase of yeasts and a higher enzymatic activity (7).

Host – parasite relationship

The interactive mechanisms – host-parasite – take place on the contact zone between micro-organism and host cell surface and affect the microbial virulence. The microbial cell surface has five functions that are very important to pathogenicity: penetration inside the host tissue, multiplication *in vivo*, modification of host defense, specificity host – tissue and host damage (7).

Candida infections may cause a wide range of invasive and superficial illnesses, affecting patients exposed to several types of risk factors (11). On the immunological aspect, these commensal micro-organisms can be pathogenic when the host defense mechanisms is modified or by failure of the anatomical barriers (as burning or invasive medical procedures). Modifications of the host defense mechanisms may occur after physiological changes of infancy (prematurity) and ageing or, more commonly, associated with degenerative diseases as cancer, innate or acquired immunodeficiency and immunodepression induced by medical procedures (11, 12). The cellular mechanisms of defense, mainly the immune response mediated by T cells that directly act on the fungal infections, are very important in host resistance against *Candida* invasion. By this fact, this infection is more common in persons with cellular immunodeficiency but not in patients with humoral deficiency. The secreted IgA is very important to the host defense against oral candidiasis. The salivary IgA inhibit the adherence of *Streptococcus* to the human epithelial cells. This IgA action may help in the regulation of oral *Candida albicans* population of patients with Sjögrens-Larsson syndrome (7).

Factors Affecting the *Candida* Infections

All these factors cited cause an environmental instability and the fungi, using this situation, could infect the site. Several situations where these factors cited may occur are: systemic illnesses such as lymphomas, leukaemia, uncontrolled diabetes mellitus, tumours and others (12). Several epidemiological data show the prevalence of candidiasis in patients with HIV. The infection maybe a marker of the disease and a predictive factor to the immunosuppressions mediated by T lymphocytes. The xerostomy (low secretion of salivary fluid) is a symptom observed in patients with HIV, causing tissue damage and undersecretion of antimicrobial proteins, important conditions for *Candida* infection (7, 13, 14).

In the last decade, the incidence of infections caused by other species of *Candida,* as *C tropicalis, C glabrata, C krusei* and other genus such as *Trichosporum* and *Cryptococcus* has been related (2). These micro-organisms are found all over the world and make up part of the normal human and animal microbial populations, colonizing the mucosa of the digestive tract (50 – 70%), mouth (30 – 50%), vagina (5–30%) and skin (4 –7%) [6].

Research with patients with cancer at the Hospital Araújo Jorge, in the city of Goiânia-GO (Brazil), showed that 56.8% of these patients presented with *Candida lesions*, 96% with *C albicans* and 4% with *C krusei.* All these isolates were phospholipase – producing and were classified as the biotype 811 (95, 8%) and 511 (4, 2%) due to the susceptibility to the killer toxins (15). Nutritional factors as hypovitaminosis, mainly vitamins A and B_{12} in diets rich in carbohydrates as saccarose and therapeutic factors such as prolonged antibiotic and corticosteroid therapy or hormonal contraceptives in high dosages affect positively *Candida* infection (12, 16). All factors with the capacity to reduce the resistance of the mucosa make possible the invasion of commensal micro-organisms, like gingival infections through deficiency of vitamin C or *Candida* infection (stomatitis) by a modification of the resident microbial populations after wide spectrum antibiotic therapy. When the salivary flux is low (as between meals) there is a four times increase in the number of the Unit Forming Colony (UFC) in saliva. Dehydrated patients have low salivary flux and there is increase oral bacterial growth. All body surfaces have a delicate equilibrium between resident microbial populations and tissue invading ones. The difference between both depends on the host defense capacity (2).

The extreme of ages may affect the immune system both in children and older people, contributing to the *Candida* infection. This feature may be enhanced by other factors as smoking, radiotherapy and periodontal diseases (1). Oral colonization by yeasts depends on the capacity to adhere to the epithelial cells. This capacity may be influenced by dental prosthesis because the resin increase the capacity of yeasts to adhere (17).

Other factors can increase candidiasis, mainly vaginal, as pregnancy, use of very tight pants, use of clothes of synthetic material (nylon and Lycra), daily use of absorbents and other conditions that make more difficult the local ventilation (16). Correlation analysis identified the association between the production of exoenzymes by *Candida* strains and the results of *in vitro* sensitivity tests with antifungal drugs (5). But this clinical correlation with sensitivity tests is difficult because the techniques of these tests may not reproduce the dynamics of the biological complex fungi *x* antifungic drug *in vivo*. The growth, the characteristic of virulence of *Candida spp* and the specific factors of the host are not considered on these tests. In addition, other factors not directly related to the *in vitro* sensitivity, as pharmacokinetic properties of drug, site of infection and immune response of host have a great importance over the activity of antifungal drugs

in vivo. Resistant infection in anatomically protected areas such as meninges, heart valves, eyes, bones and prostate are directly related to the resistance to antifungal therapy because they may act as sites of protection to the pathogens (4, 18, 19).

CONCLUSIONS AND PERSPECTIVES

Candidiasis is a very ubiquitous infectious disease, mainly infecting immunodeficient patients, and can become a serious public health problem. So, all information about this micro-organism is of importance to health-workers, mainly information about the factors that affect the virulence of the *Candida* strains. Knowledge of such factors such as environment, production of exoenzymes, main infection sites and others are essential to combat this infection and the risks associated.

REFERENCES

1. Batista JM, Birman EG, Cury AE. Susceptibility to antifungal drugs of *Candida albicans* strains isolated from patients with denture stomatitis. Rev Odontol Univ São Paulo 1999; **13:** 343–8.
2. De Bernardis F, Mondello F, San Millàn R, Pontòn J, Cassone A. Biotyping and Virulence Properties of Skin Isolates of *Candida parapsilosis*. J Clin Microbiol 1999; **37:** 3481–6.
3. De Bernardis F, Mühlschlegel FA, Cassone A, Fonzi WA. The pH of the Host Niche Controls Gene Expression in and Virulence of *Candida albicans.* Infect Immun 1998; **66:** 3317–25.
4. Boatto HF, Moraes MS, Machado AP, Girão MJBC, Fischman O. Relationship of laboratory results with clinical signs and symptoms of patients with vulvovaginal candidiasis and the significance of the sexual partners for the maintenance of the infection. Rev Bras Ginecol Obstet 2007; **29:** 80–4.
5. Cavassani VGS, Andrade-Sobrinho J, Homem MGN, Rapoport A**.** Oral candidiasis as prognostic marker of HIV-infected patients. Rev Bras Otorrinolaringologia 2002; **68:** 630–4.
6. Colombo AL,Guimarães T. Epidemiology of hematogenous infections due to *Candida spp*. Rev Soc Bras Med Trop. 2003; **36:** 599–607. Epub 2003 Oct 21.
7. Coutinho HDM, Bezerra DAC, Lôbo K, Barbosa IJF. Antimicrobial activity of natural products. Conceitos 2004; **10:** 77–85.
8. Henning MM, Perrone M. Factores Determinantes de Patogenicidad em Relacion a la Ecologia de *Candida albicans* em cavidad bucal. Acta Odontol. Venez 2001; **39:** 1–15.
9. Kontoyiannis DP, Lewis RE. Antifungal drug resistence of pathogenic fungi. Lancet 2002; **359:** 1135–44.
10. Lane S, Birse C, Zhou S, Matson R, Liu H. DNA Array Studies Demonstrate Convergent Regulation of Virulence Factors by Cph1, Cph2, and Efg1 in *Candida albicans*. J Biol Chem 2001; **276:** 48988–990.
11. Mims C. Medical Microbiology. 2ª ed. São Paulo:Editora Manole Ltda, 1999.
12. Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical Microbiology. 3ª ed. Rio de Janeiro: Guanabara Koogan, 2000.
13. Neves RP, Cavalcanti MAQ, Chaves GM, Magalhães OMC. Yeasts isolated from clinical samples of AIDS patients. Braz J Microbiol 2002; **33:** 363–4.
14. Oliveira EE, Silva SC, Soares AJ, Attux C, Cruvinel B, Silva MRR. *Killer* toxin sensitiviy and production of enzymes by *Candida albicans* isolated from the oral mucosa of patients with cancer. Rev Soc Bras Med Trop 1998; **31:** 1–2.
15. de Holanda AA, Fernandes AC, Bezerra CM, Ferreira MA, de Holanda MR. Vulvovaginal candidiasis: symptomatology, risk factors and concomitant anal colonization. Rev Bras Ginecol Obstet 2007; **29:** 3–9.
16. Silva VV, Díaz MCJ, Febré N. Vigilancia de la resistencia de levaduras a antifúngicos. Rev Chi Infectol 2002; 19(**suppl. 2):** 1–3.
17. Uip DE, Strabelli TMV. Candidíase Esofagiana. Rev Bras Med Trop 2000; **57:** 1294.
18. Weinfeld I, Birman EG, Paula CR. Macrophage phagocytosis of *Candida albicans*. An in vitro study. Rev Odontol Univ São Paulo, 1999; **13:** 1–10.
19. Wu T, Wright K, Hurst SF, Morrison CJ. Enhanced Extracellular Production of Aspartyl Proteinase, a Virulence Factor, by *Candida albicans* Isolates following Growth in Subinhibitory Concentrations of Fluconazole. Antimicrob Agents Chemother, 2000; **44:** 1–2.