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Frequency and enzymatic activity of *Candida albicans* isolated from the oral cavity of HIV-positive patients at Fortaleza, Ceará

Freqüência e atividade enzimática de Candida albicans *isolado da cavidade oral de pacientes HIV-positivos em Fortaleza, Ceará*

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key words

Candida albicans

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Phospholipase

Proteinase

abstract

Candida albicans and other species are usually involved in opportunistic infections in patients with acquired immunological deficiency syndrome (AIDS). The virulence mechanisms by which this yeast expresses its pathogenicity include adherence patterns, ability to form pseudomycelia and production of extracellular enzymes, among others. The objective of this research was to verify the frequency of *Candida* and the production of proteinase and phospholipase in 52 strains of *Candida albicans* from the oral cavity of patients infected by HIV treated at Hospital São José, AIDS reference and training center in Fortaleza, Ceará. Samples were collected of patients, with or without oral lesions characteristic of candidosis. From 100 patients, 80% presented positivity for *Candida*: 65% (52) were identified as *C. albicans*, 27.5% (22) as *C. tropicalis*, 2.5% (2) as *C. glabrata*, 2.5% (2) as *C. krusei* and 2.5% (2) as *C. guilliermondii*. Among the strains of *C. albicans* isolated from the oral cavity, proteinase and phospholipase were detected in 69.2% and 73%, respectively. The results suggested that *C. albicans* was the most frequent species observed, with intermediate expression of proteinase and phospholipase.

unitermos

Candida albicans

HIV

Fosfolipase

Proteinase

resumo

Candida albicans *e outras espécies são usualmente envolvidas em infecções de pacientes com a síndrome da imunodeficiência adquirida (AIDS). Os mecanismos de virulência pelos quais a levedura expressa sua patogenicidade incluem padrões de aderência, habilidade por formar pseudomicélio, produção de enzimas extracelulares e outros. O objetivo deste trabalho foi verificar a freqüência de* Candida *e a produção de proteinase e fosfolipase em 52 cepas de* Candida albicans *da cavidade oral de pacientes infectados pelo HIV atendidos no Hospital São José, hospital de referência e centro de treinamento em AIDS em Fortaleza, Ceará. Neste trabalho foram coletadas amostras de pacientes com ou sem lesões características de candidose. Dos cem pacientes 80% apresentaram positividade para* Candida*, sendo 65% (52) identificados como* C. albicans*, 27,5% (22) como* C. tropicalis*, 2,5% (2) como* C. glabrata*, 2,5% (2) como* C. krusei *e 2,5% (2) como* C. guilliermondii*. Entre as cepas de* C. albicans *isoladas da cavidade oral foram detectadas proteinase e fosfolipase em 69,2% e 73%, respectivamente. Os resultados sugerem que a* C. albicans *foi a espécie mais freqüentemente observada, com intermediária expressão de proteinase e fosfolipase.*

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Candida albicans has frequently been isolated from several infectious processes among patients with immunosuppressive illnesses, patients submitted to transplants and/or treatment with antibiotics and human immunodeficiency virus infected (HIV-positive) individuals. Candidosis refers to several diseases caused by *Candida albicans* and related species. *Candida albicans* and other species are usually involved in deep infections in HIV patients. In a significant proportion of the population, it is part of the normal human microbiota[5, 20].

HIV-positive individuals are predisposed to a great number of fungal infections due to the profound functional alterations that take place in their T-cell immune compartment[6].

The microbiota of the oral cavity comprises, among other microorganisms, yeasts of the genus *Candida* that are typically opportunistic and can be found in 10% to 50% of healthy individuals. Favorable conditions can transform these yeasts into pathogens[9, 11].

Among various species of the genus, *Candida albicans* plays a relevant role as a causative agent of mycotic diseases, of which the mucosal forms are the commonest and earliest. Oral candidosis develops in 90% to 95% of symptomatic HIV-infected individuals and its prevalence increases in parallel with the severity of the immune dysfunction. The occurrence of oral candidosis was described since the first cases of acquired immunodeficiency syndrome (AIDS) were reported. It constitutes an important clinical sign for diagnosis, as well as an indicator of the evolution of immunodeficiency among HIV carriers[12].

Virulence mechanisms, like proteinase and phospholipase produced by *C. albicans*, have been evaluated. These enzymes are capable of invading tissues, leading to dysfunction or even rupture of cellular membranes, and to a higher grade of adherence and colonization[2, 12, 14, 16].

Considering that the presence of *Candida albicans* in the oral cavity occurs in patients with HIV causing candidosis, and that the proteinase and phospholipase production can interfere in the pathogenic potentiality of the yeast, the aim of this study was to evaluate the frequency of *Candida* and the enzymatic activity of *Candida albicans*.

The sample consisted of 100 patients infected by the human immunodeficiency virus (HIV) treated at Hospital São José, AIDS reference and training center in Fortaleza, Ceará. Patients were of both genders, without distinction of race or skin color, aging from 23 to 45 years old.

Using sterile swabs, samples for the test were collected from oral cavity (jugal mucosa, lateral edge and dorsum of tongue), with or without oral lesions characteristic of candidosis[15]. They were immediately inoculated on Petri dish of Sabouraud dextrose agar (Difco) with chloramphenicol. Incubation was made at 35°C, for up to seven days. The strains were identified according to the methodology described by Kreger-Van Rij[7]. The *Candida* species were identified on the basis[7]: production of germ tubes, the microscopic anatomy of cultures grown on cornmeal Tween 80 agar, carbohydrate assimilation and carbohydrate fermentation.

The study of proteinase and phospholipase were carried out based on Ruchel *et al.*[17], Price *et al.*[16] and Samaranayake *et al.*[16], respectively; the lecture was performed after 72 hours. The samples producing enzymes presented an opaque zone of precipitation around the yeast inoculation point, being the enzymatic activity obtained by measuring the colony diameter and dividing it by the diameter plus the precipitation zone (P_z). In both cases, when P_z was 1, the enzymatic activity was zero. Between 0.64 and 0.99, this activity was considered positive; when lower than 0.64, it confirmed a strong positivity.

From 100 patients infected by HIV treated at Hospital São José, 80% (80) presented positivity for *Candida*, being 65% (52) identified as *C. albicans*, 27.5% (22) as *C. tropicalis*, 2.5% (2) as *C. glabrata*, 2.5% (2) as *C. krusei* and 2.5% (2) as *C. guilliermondii*. The *C. albicans* was the most frequent and submitted to enzyme production assays.

The enzymatic evaluation demonstrated that 69.2% (36/52) of *C. albicans* colonies produced proteinase, P_z between 0.54 and 0.85, presenting intermediate enzymatic activity. Phospholipase was detected in 73% (38/52), presenting intermediate enzymatic activity, P_z between 0.68 and 0.95.

Within the genus *Candida*, the yeast *Candida albicans* is the most frequently associated with lesion development. This species is part of the normal microbiota of man, found in the oral cavity of half of the healthy carriers and in association with a wide array of microorganisms, including bacterial species. The coexistence of such microbial population with an individual starts at birth. It involves the action of immunological mechanisms in a continuous process of adaptation and readaptation that ensures the saprophyte condition of the microorganism and a state of balance between it and the human carrier[10, 15].

A higher frequency of isolation of *Candida* in patients infected by HIV has already been observed[10, 11, 13, 19]. It was confirmed in this study, which revealed positivity for *Candida* in 80% of the samples.

From 80 isolated samples of the research 52 were *Candida albicans* (65%); 22 were *Candida tropicalis* (27.5%); two were *Candida glabrata* (2.5%); two were *Candida krusei* (2.5%) and two were *Candida guilliermondi* (2.5%). In our results, *Candida albicans* was the most predominant yeast, similarly to the results of Delgado and Aguirre[4], Boerlin *et al.*[1], Silva[19] and Oliveira[13], that reported the isolation of *C. albicans*, most predominant, from the oral cavity, as well as *C. tropicalis*, *C. krusei* and *C. glabrata* in patients infected by HIV.

In relation to the enzymatic activity of *C. albicans*, 69.2% of the analyzed samples produced the exoenzyme proteinase, P_z between 0.54 and 0.85, presenting intermediate enzymatic activity. Other studies carried out in Brazil showed that this activity was present in about 53% to 100% of *Candida albicans* cultures[13, 14, 19]. According to international data, proteinase activity was detected in 16% to 100% of the studied samples[2, 3, 6, 8, 12]. Our study did not find a correlation between *in vitro* proteolytic activity and the severity of the lesion. The lesion was visually evaluated by a physician.

Phospholipase activity was detected in 73% of the examined samples, presenting low or intermediate enzymatic activity, P_z between 0.68 and 0.95, also not suggesting defined activity related to the severity of the lesion. Price *at al.*[16] described values ranging from 46% to 100%. Penha *et al.*[14] reported that the enzymatic production of phospholipase was present in 83.3% of the samples, with strong positivity in 36.6%. De Bernardes et al.[3] described a relationship between the activity of phospholipase in the mucosa and the severity of the lesions.

Thus, *C. albicans* was the most frequent yeast in patients infected by HIV treated at Hospital São José. Proteinase from *C. albicans* presented intermediate level of positive production, and phospholipase presented low or intermediate level of activity.

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