

CANDIDA SPECIES DISTRIBUTION IN CLINICAL SAMPLES

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RESUMO

Procedeu-se à identificação de *Candida spp.*, recolhidas no laboratório de Clínica Laboratorial Dr. Edgar Botelho Moniz, S.A., sediado em Santo Tirso, com o objectivo de avaliar a sua distribuição em Candidoses. Os 63 isolados foram obtidos a partir de diferentes amostras clínicas e identificados de acordo com a sua capacidade de crescimento a 42°C, aspecto das colónias em CAN2 e resultados do API ID 32C. *C. albicans* foi a espécie mais frequentemente isolada (90%), seguida de *C. glabrata* (5%), *C. tropicalis* (3%) e *C. parapsilosis* (2%). A maioria das amostras correspondia a exsudados vaginais.

PALAVRA-CHAVE

Candida spp., *C. albicans*, exsudados vaginais, candidose

ABSTRACT

We have identified *Candida spp.*, collected in the laboratory Clinica Laboratorial Dr. Edgar Botelho Moniz, S.A., located in Santo Tirso, in order to evaluate their distribution on diagnosed candidosis. The 63 isolates were recovered from different clinical samples and identified by their ability to growth at 42°C, colony color on CAN2 and by API ID 32C. *C. albicans* was the most frequently (90%) identified specie, followed by *C. glabrata* (5%), *C. tropicalis* (3%) and *C. parapsilosis* (2%). The majority of the strains were isolated from vaginal samples.

KEYWORDS

Candida spp., *C. albicans*, vaginal samples, candidosis

1. INTRODUCTION

Yeast, particularly *Candida*, was always identified as an important etiological agent of human infections (skin, mucous or systemic infections) (Anane *et al.*, 2007; Filioti *et al.*, 2007; Furuta, 2000; Marr, 2004; Pfaller and Diekema, 2004; Souza *et al.*, 2009).

The raise of systemic *Candida* infections might be related with several factors such as the increased of: (i) Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) infected individuals; (ii) neutropenic persons due to anticancer treatments; (iii) the abusive use of extended spectrum antibiotics; (iv) metabolic disorders, such as *Diabetes mellitus*. (Back-Brito *et al.*, 2009; Furuta, 2000; Imamura *et al.*, 2008; Kakabadze, 2008)

Historically, *C. albicans* accounted for 70 to 90% of the isolates recovered from infected patients while *C. glabrata* and *C. tropicalis* accounted for approximately 5 % each. Other *Candida* species were only rarely isolated from clinical specimens (Anane *et al.*, 2007; Fidel *et al.*, 1999; Filioti *et al.*, 2007; Furuta, 2000; Marr, 2004; Pfaller and Diekema, 2004). However, more recently, epidemiological data reveal a change in the patterns of infection with a shift from *C. albicans* to the non-*albicans Candida* species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* (Ahmad and Khan, 2009; Ferrer, 2000; Gutiérrez *et al.* 2002; Silva *et al.*, 2009). In fact, *C. glabrata* is frequently the second most common cause of candidosis, especially in vaginal infections (Ahmad and Khan, 2009; Ferrer, 2000; Fidel *et al.*, 1999).

Besides epidemiological pattern change, there is an increase of *Candida* spp. resistant to conventional therapy (Cappelletty e Eiselstein-McKitrick, 2007; Fidel *et al.*, 1999; Filioti *et al.*, 2007; Kakabadze *et al.*, 2008; Marr, 2004; Silva *et al.*, 2009; Tanabe *et al.*, 2008). *C. dubliniensis*, identified in 1995, have phenotypic similarities with *C. albicans*, such as the ability to form germ tubes and chlamydo spores (Anane *et al.*, 2007), which cause difficulties in *C. dubliniensis* identification by standard diagnostic procedures. Although preliminary studies showed that *C. dubliniensis* strains were susceptible to antifungal agents, fluconazole-resistant strains, showing an increased expression of multidrug resistance transporters (MDR1), have been detected (Gutiérrez *et al.* 2002).

Nevertheless, *Candida* spp. laboratorial identification is mostly directed to the differentiation between *C. albicans* and non-*albicans Candida* species (using limited standard diagnostic procedures) and antifungal prescription is almost entirely based on empirical knowledge. *Candida* species distribution, as well as antifungal resistance pattern, has shown to occur among health institutions, cities and countries probably by different antifungal prescription or control infection protocols (St-Germain *et al.*, 2001).

Due to the lack of knowledge about *Candida* spp. epidemiology in Portugal, the main objective of this work was to obtain data regarding the spectrum of *Candida* species responsible for community infections and provide a tool that might help antifungal prescription.

2. MATERIAL AND METHODS

2.1. CULTURE MEDIA

Sabouraud Dextrose Agar was acquired from BBL, Candida ID2 (CAN2) and Analytical Profile Index for yeasts identification (API ID 32C) from bioMérieux, Mycobiotic agar from Biogerm and tryptone soy broth (TSB) media from Difco.

2.2. MICROORGANISMS

Candida suspected colonies recovered from clinical samples were collected, isolated on Mycobiotic agar and frozen (TSB with 15% of glycerol) on Clínica Laboratorial Dr. Edgar Botelho Moniz, S.A. laboratory, during 2008.

2.3. CANDIDA ID2

All the yeasts were cultured on CAN2 chromogenic media. All the identification procedures were conducted in accordance with the manufacturer's instructions. Blue colonies were presumptively identified as *C. albicans* or *C. dubliniensis*. White colonies were identified as non-*C. albicans* or *C. dubliniensis* species.

2.4. GROWTH AT 42°C

In order to distinguish between *C. albicans* and *C. dubliniensis* all the isolates that produced blue colonies on CAN2 were incubated on Sabouraud dextrose agar at 37°C and 42°C. Strains growing at 42°C and 37°C were identified as *C. albicans* and those growing only at 37°C as *C. dubliniensis*. (Sullivan e Coleman, 1998).

2.5. API ID 32C

Non-*C. albicans* and non-*C. dubliniensis* species were identified by API ID 32C. All the identification procedures were conducted in accordance with the manufacturer's instructions. API ID 32C plastic strips were incubated at 29°C and results automatically registered (Mini/API; bioMérieux) after 24 and 48 hours incubation.

3. RESULTS AND DISCUSSION

Clínica Laboratorial Dr. Edgar Botelho Moniz, S.A. laboratory is located in Santo Tirso and its patients are from this city or cities nearby, namely Guimarães, Trofa, Lousada and Vila Nova de Famalicão (data not shown).

From the 63 collected samples only two were obtained from masculine patients while the rest were collected from feminine patients.

The age of the patients showed an asymmetric distribution with more patients included in the age group of 20 to 49 years (Figure 1), with a mean of 39 ± 17 years and a median of 34 years, ranging from 11 to 88 years old.

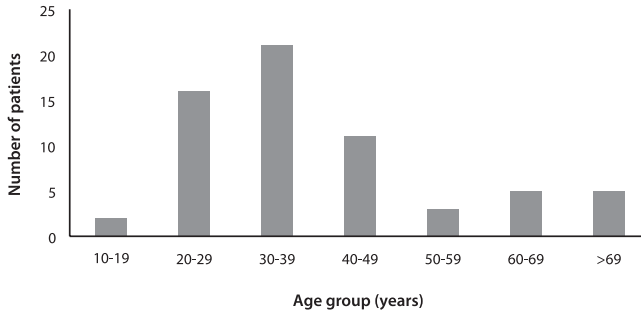


Figure 1. Characterization of the population included in the study by age group.

Candida spp. were mainly isolated from vaginal samples (Figure 2), corresponding the other samples to urine, feces and sputum.

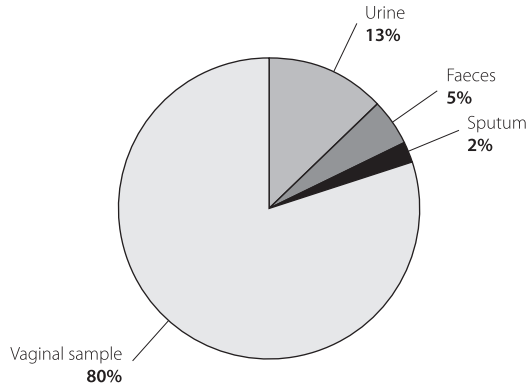


Figure 2. Characterization of clinical samples used for *Candida* isolation.

Two of the samples were obtained from patients in hospitalar facilities, corresponding to urine collected from women with 87 and 88 years old. All the other clinical samples were obtained from outpatient. This fact can justify the nonexistence of samples collected from deep candidosis, as blood samples.

As already referred, the majority of the patients were in the age group of 21 to 49 years and this might be related with the fact that this is the age group with higher hormonal variations, sexual activity and rates of pregnancy. This theory is supported by the percentage of vaginal samples included in our study. *Candida* urinary tract infections probably reflect endogenous infection due vaginal colonization and anatomical proximity to urethra or the presence of urinary catheters.

C. albicans was the most frequently recovered specie (90%) from all the clinical samples (Table 1), as already expected (Ahmad and Khan, 2009; Anane *et al.*, 2007; Filioti *et al.*, 2007; Furuta, 2000; Marr, 2004; Pfaller and Diekema, 2004).

Table 1. Distribution of *Candida* species.

Species	Number (%) of isolates in:	
	All clinical samples	Vaginal samples
<i>C. albicans</i>	57 (90)	47 (92)
<i>C. glabrata</i>	3 (5)	3 (6)
<i>C. tropicalis</i>	2 (3)	1 (2)
<i>C. parapsilosis</i>	1 (2)	-
Total	63	51

Candida spp. distribution is changing (Cappelletty e Eiselstein-McKitrick, 2007; Colombo e Guimarães, 2003; Ferrer, 2000; Krcmery e Barnes, 2002; Li *et al.*, 2007; Neppelenbroek *et al.*, 2006; Schorling *et al.*, 2000; Tanabe *et al.*, 2008; Trofa *et al.*, 2008). *C. albicans* is still the most frequently recovered specie from vaginal infection but, nowadays, is common the isolation of *C. glabrata* and other *Candida* species as infection agents (Ferrer, 2000; Ringdahl, 2006), a fact confirmed by our results (Table 1).

The growing importance of non-*albicans Candida* species as infectious agents is reflecting in the failure of candidosis empirical treatment, prescribed without the support of a correct laboratory diagnosis. Some of those species are naturally or potentially resistant to classical antifungal treatment (Pfaller and Diekema, 2004; Tanabe *et al.*, 2008).

C. dubliniensis colonies weren't identified in our study. However, our result can't totally exclude its presence because, due to their phenotypic proximity, this test might not be conclusive. In these cases, molecular biology might be a very useful method to discriminate between *C. albicans* and *C. dubliniensis*, although expensive (Anane *et al.*, 2007; Filioti *et al.*, 2007; Neppelenbroek *et al.*, 2006).

Co-infection cases with more than one *Candida* spp. were also not registered.

4. CONCLUSION

This work contributes for a better understanding of Portuguese reality concerning *Candida* infections. The knowledge of specie distribution among clinical samples and their antifungal resistance profiles might influence prescription, decreasing the rates of empiric antifungal treatment failure.

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