

SPUTUM COLONIZATION BY *Candida* IN PATIENTS OF THE PNEUMOLOGY UNIT OF GENERAL HOSPITAL IN RECIFE (BRASIL). II.¹

*Colonización del esputo por Candida en pacientes de la unidad de
neumología de un hospital general en Recife . (Brasil). II.*

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Palabras clave: Colonización, esputo, *Candida*

Key word: Colonization, sputum, *Candida*

RESUMEN

Se pesquisaron 322 pacientes internados en la Unidad de neumología del Hospital Geral Otavio de Freitas, Recife, PE, Brasil, para determinar la colonización por *Candida* en el esputo. De estos, 225 correspondían al sexo masculino y 97 al femenino. La colonización fue diagnosticada en 25 pacientes (7,7%) del sexo masculino y 9 (2,8%) del femenino. De los 25 casos detectados, 15 estaban asociados solamente a tuberculosis pulmonar, 5 a tuberculosis pulmonar y otra neumopatía, 2 a bronquiectasia, 1 a cáncer pulmonar, 1 a fistula arteriovenosa y 1 a infección respiratoria.

Fueron aisladas 13 cepas de *C. albicans*, 6 *C. parapsilosis*, 4 *C. membranaefaciens* y 2 *C. tropicalis*.

SUMMARY

Three hundred and twenty two patients hospitalized in the Pneumology Unit of the Otavio de Freitas General Hospital, Recife, PE, Brazil, were surveyed, being 225 males and 97 females. Amongst them *Candida* colonization was diagnosed in 25 (7.7%), 16 (4.9%) in male patient and 9 (2.8%) in female ones. Of the 25 cases, 15 were associated only with pulmonary tuberculosis; 5 with pulmonary tuberculosis and another pneumopathy; 2 with bronchiectasis; 1 with pulmonary cancer; 1 with an arteriovenous fistula and 1 with respiratory infection.

Among the isolated strains, 13 were *C. albicans*, 6 *C. parapsilosis*, 4 *C. membranaefaciens* and 2 *C. tropicalis*.

INTRODUCTION

Candida is a genus of yeast with several species which have been found in saprophytism and in parasitism.

C. albicans, *C. tropicalis*, *C. guilliermondii*, *C. parapsilosis*, *C. glabrata*, are associated with superficial and deep mycoses (Loder, 1970; Connant et al., 1971; Rippon, 1982; Kreger-van-Rij, 1984; Lacaz et al., 1991).

Candidiasis is the most common mycotic infection in man and can strike any tissue, organ or system. Among the systematic candidiasis, pulmonary, endocarditis, meningitis, renal, fungemia and other forms, can occur (Conant et al., 1971; Rippon, 1982; Wanke, 1984; Lacaz et al., 1991).

Various authors have identified the presence of *Candida* in patients with respiratory system problems, especially in pulmonary tuberculosis (Negroni & Daglio, 1948; Scharfing & Skinner, 1948; Osoagbaka, 1981; Wanke, 1984; Kim et al., 1988; Lacaz et al., 1991; Kumar et al., 1992; Bandele et al., 1993) neoplasias (Salerno et al., 1986; Vidotto et al., 1986;

Stokes et al., 1989; Pizzo & Walsh, 1990; Winthrop et al., 1990; Grillot et al., 1991; Elias et al., 1993), as well as in patients with acquired immunodeficiency syndrome (Joshi et al., 1986; Lazarrin et al., 1987; Kyriais & Kyriasis, 1993).

The objective of this study was to detect, isolated and identify fungi of the upper respiratory tract (sputum) in patients hospitalized in the Pneumology Unit of the Otavio de Freitas (SANCHO) General Hospital. Recife, Brasil.

MATERIAL AND METHODS

Sputum samples were collected from patients, hospitalized, with respiratory systems problems. Three hundred and twenty two patients were surveyed being 225 were male and 97 female.

The sputum samples were collected from each patient 3 times in sterilized Petri plates at intervals of 3 to 4 days. After collection, the samples were transported to the

¹ Part of the dissertation for getting a Master's degree with support of Coordenação do Aperfeiçoamento de Nível Superior (CAPES).

Department of Mycology where they were duly processed for direct examination and culture. The time between collection and manipulation of those did not exceed 2 hours.

The direct examination of the sputum was carried out on their native state (without colouring and without clarifier) and clarified with aqueous solution at 20% of potassium hydroxide.

The sputum samples were seeded in duplicate, by spreading Sabouraud agar + 0.5% of yeast extract (YE) on their surface, to which 50mg of Chloramphenicol/l contained in a Petri plate was added. A plate was left at room temperature ($28^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and the other incubated at 37°C . The development of the cultures were lasted for up to 20 days.

The colonies which emerged after being purified were maintained in the above mentioned culture medium without antibiotic and held in a test tube.

To identify and classify the yeast strains Lodder, 1970; Kreger-van-Rij, 1984 and Barnett et al., 1990 methods were adopted.

The data about the patients were collected from their clinical records.

RESULTS

Candida colonization was diagnosed through the direct examination of the sputum of 25 (7.7%) patients, being 16 (4.98) male and 9 (2.8%) female. Direct examination revealed the presence of isolated yeast cells and pseudomycelium, both of which adhered to the epithelial cells present in the sputum (Figures 1 and 2).

In Sabouraud agar + YE + Chloramphenicol, both at room temperature ($28^{\circ}\text{C} \pm 1^{\circ}\text{C}$) and at 37°C , 25 *Candida* cultures were obtained, 13 were of *C. albicans*, 6 *C. parapsilosis*, 4 *C. membranaefaciens* and 2 *C. tropicalis* (Table 1).

C. albicans occurred in 7 male and 6 female patients; *C. parapsilosis* in 4 male and 2 female patients; *C. membranaefaciens* in 3 male and 1 female; *C. tropicalis* only in 2 male patients (Table 1; Figure 3).

The minimum age of the male patients was 29 and the maximum 85 years old. All of them had different occupations and all were born in the State of Pernambuco (Table 1).

The age of the female patients ranged from a minimum of 19 to a maximum of 78 years old, being all of them domestic servants. These patients were born in the State of Pernambuco; (Table 1).

Of the 16 male patients with colonization, *C. albicans* was isolated mainly in 5 patients with pulmonary tuberculosis and other species in another infections (Table 1)

Of the 9 cases of female patients with colonisation, *C. albicans* was isolated in 6 patients, mainly with pulmonary tuberculosis (3 cases). (Table 1).

The strains of yeasts were stored in the Mycotheca-URM of the Biological Sciences Centre of the Federal

University of Pernambuco. *Candida albicans* under the N (3622; 3623; 3625; 3626; 3628; 3629); *Candida parapsilosis* (3621; 3624; 3627); *Candida membranaefaciens* (3619) and *Candida Tropicalis* (3630).

DISCUSSION

Primary pulmonary candidiasis is rare, being more frequent the secondary attack of the lungs, because of various primary conditions such as tuberculosis and other bacterial and viral pneumopathies; neoplasias; suppurations; bronchiectasis and others (Wanke, 1984; Lacaz et al., 1991).

Due to the presence of *Candida* in such processes, its role is discussed in the quoted pneumopathies. Even as a secondary agent, this fungus can cause serious dangers (Lacaz et al., 1991).

In this study, the presence of *Candida* in the sputum, found in the direct examination and culture, was considered as "colonization" and not as candidiasis, based on considerations made by various authors. According to them, the presence of yeasts like *Candida*, *Trichosporon* and other opportunist fungi in the sputum, bronchial secretion or exudate, associated with other primary pathologies, is referred to by the terms colonization, secondary mycosis, opportunist pathogen and infection (Vidotto et al., 1986; Jain et al., 1991; Shigera et al., 1991; Kumar et al., 1992; Bandele et al., 1993).

The difficulties of diagnosing for pulmonary candidiasis are related to the clinical sample. What is considered of diagnostic value for primary pulmonary candidiasis is the demonstration of blastopores and/or pseudomycelium in samples obtained by pulmonary biopsy (Conant et al., 1971; Rippon, 1982; Browne et al., 1990; Pizzo & Walsh, 1990; Lacaz et al., 1991).

It is worth emphasizing that the importance of the direct examination is unanswerable to give evidence of *Candida* in the sputum. This has also been emphasized by Negroni & Daglio (1948); Schwarting & Skinner (1948); Conant et al., (1971); Osoagbaka (1981); as well as Meunier (1990) and Remington (1990), when they refer to the presence of *Candida* in clinical samples of the buccal cavity.

The results of this study with sputum samples demonstrate *Candida* colonization in patients with different pneumopathies. Taking into account men as well as women, the greatest number of cases occurred in patients in the age ranges of 32-49 and 72-78 years old (Table 1). Osoagbaka (1981) isolated the greatest number of *Candida* specimens from the sputum of patients with an age range of 41-80 years old without reference to sex.

Of 20 patients with pulmonary tuberculosis, there were 15 with BAAR positive, 1 with BAAR not undertaken and 4 with BAAR negative. Amongst them, 15 were carriers of pulmonary tuberculosis only. The others presented different types of associations

Of patients who were not bearers of pulmonary

Table 1.- Aspects related to the cases of *Candida* colonization detected in patients hospitalized.

CASES	SEX	AGE	RESIDENCE (PE)	OCCUPATION	BEFORE DIAGNOSIS		MYCOLOGIC DIAGNOSIS		
					LAB.	CLIN.	C. S.	D.E.	CULTURE
1° SOS	M	29	Recife	Shop assistant	BAAR +	PT + bronchiectasis + AIDS	sputum	+	<i>Candida tropicalis</i>
2° ACA	M	34	Recife	Servant	BAAR NU	PT	sputum	+	<i>C. membranaefaciens</i>
3° HJN	M	34	Recife	Mechanic	BAAR +	PT + bronchiectasis	sputum	+	<i>C. albicans</i>
4° JGM	M	36	Barreiros	Businessman	BAAR -	Arteriovenous fistula	sputum	+	<i>C. parapsilosis</i>
5° CAB	M	39	Petrolândia	Rural	BAAR -	Pulmonary cancer	sputum	+	<i>C. albicans</i>
6° JLFL	M	39	Recife	Unemployed	BAAR -	Bronchiectasis	sputum	+	<i>C. parapsilosis</i>
7° JRBN	M	39	Recife	Unemployed	BAAR -	PT	sputum	+	<i>C. albicans</i>
8° ALM	M	43	Escada	Motorist	BAAR +	PT	sputum	+	<i>C. albicans</i>
9° CA	M	43	Recife	Receiver bus	BAAR +	TP	sputum	+	<i>C. albicans</i>
10° JDS	M	45	Itambé	Mechanic	BAAR +	PT	sputum	+	<i>C. albicans</i>
11° JHTL	M	53	Recife	Pensioner	BAAR -	PT	sputum	+	<i>C. parapsilosis</i>
12° JJS	M	64	Barreiros	Unemployed	BAAR +	PT	sputum	+	<i>C. albicans</i>
13° ASS	M	72	Recife	Pensioner	BAAR -	PT	sputum	+	<i>C. membranaefaciens</i>
14° SVF	M	73	Tracunhaém	Pensioner	BAAR +	PT	sputum	+	<i>Candida tropicalis</i>
15° VBS	M	73	Recife	Unemployed	BAAR +	PT+ bronchiectasis	sputum	+	<i>C. membranaefaciens</i>
16° MPS	M	85	Recife	Pensioner	BAAR -	Respiratory infection	sputum	+	<i>C. parapsilosis</i>
17° MLAS	F	19	Recife	Student	BAAR +	PT	sputum	+	<i>C. albicans</i>
18° RMS	F	23	Venturosa	Domestic	BAAR +	PT + AIDS	sputum	+	<i>C. albicans</i>
19° CMVS	F	32	Recife	Domestic	BAAR +	PT	sputum	+	<i>C. parapsilosis</i>
20° MFR	F	43	Recife	Domestic	BAAR +	PT	sputum	+	<i>C. parapsilosis</i>
21° AMAA	F	43	Recife	Domestic	BAAR +	PT	sputum	+	<i>C. albicans</i>
22° MNS	F	49	Recife	Domestic	BAAR -	Bronchiectasis	sputum	+	<i>C. albicans</i>
23° MJGS	F	61	Primavera	Domestic	BAAR +	PT + diabetes mellitus	sputum	+	<i>C. albicans</i>
24° MBO	F	72	Recife	Domestic	BAAR -	PT	sputum	+	<i>C. albicans</i>
25° SFS	F	78	Recife	Domestic	BAAR +	PT	sputum	+	<i>C. membranaefaciens</i>

M = Male sex
F = Female sex
LAB = Laboratory
CLI = Clinic

C. S. = Clinical sample
D. E. = Direct examination
BAAR = Bacillus alcohol-acid resistant
NU = Not undertaken

+ = positive
- = negative
PT = Pulmonary tuberculosis

Figure 1-2.- Pseudomycelium of *Candida* adhered to the epithelial cells in the sputum 650x.

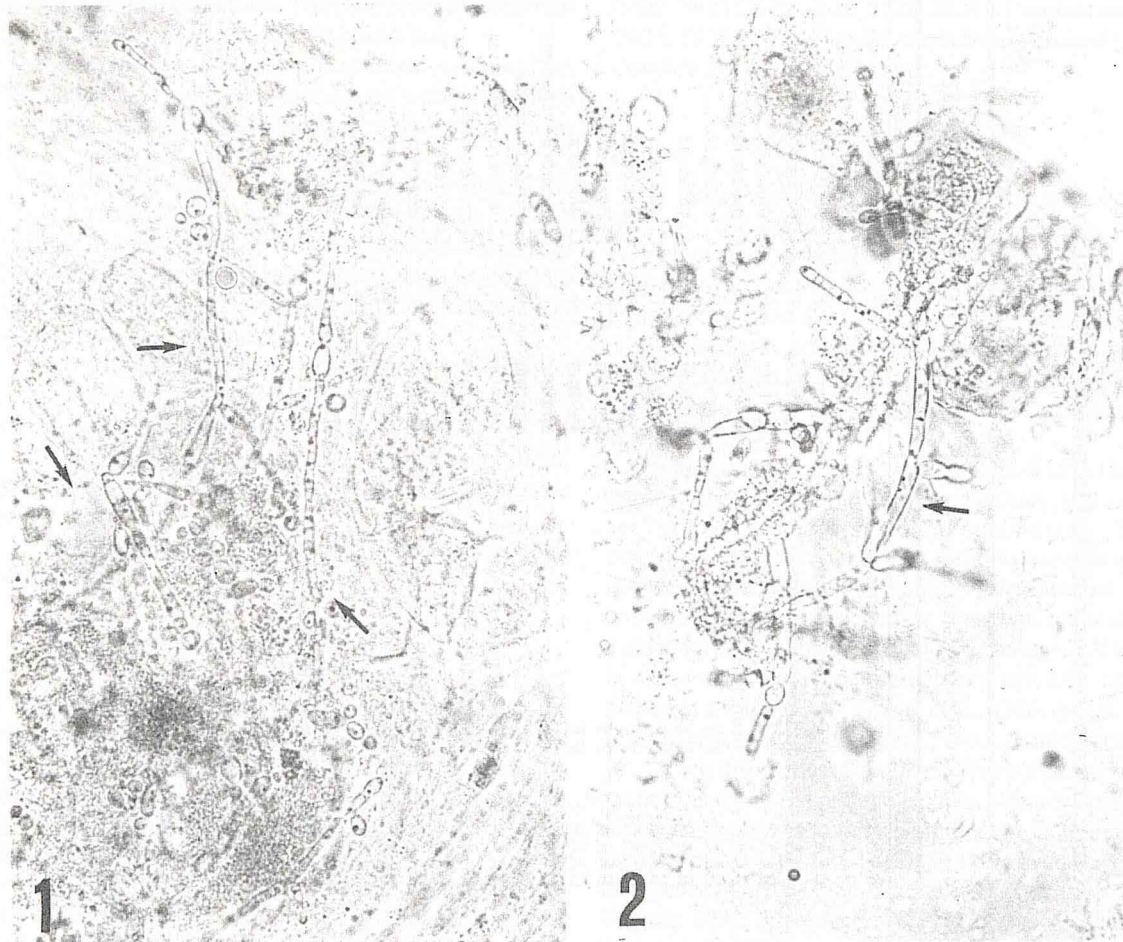
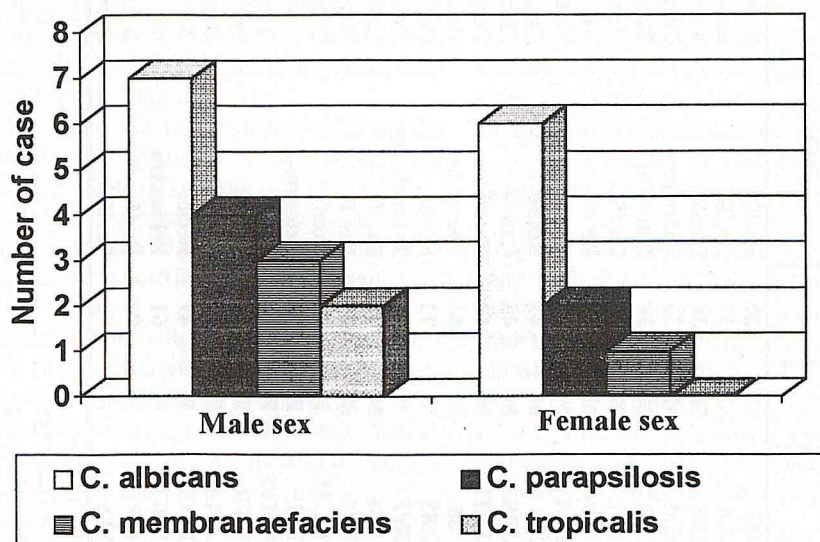


Figure 3. - Occurrence of *Candida* species in relation to sex.



tuberculosis, the species *C. parapsilosis* and *C. albicans* were isolated. *C. albicans* was the species which occurred in the largest number of cases; on the other hand, pulmonary tuberculosis was the most prevalent pneumopathy. The presence of *C. albicans* in these patients was also found by Osoagbaka (1981), Kumar et al. (1992), Bandele et al. (1993).

Patients with AIDS, represent a serious risk group for opportunist infections, amongst them those of a fungal nature. Lazzarin et al. (1987) also report the isolation of *C. albicans* in patients only with AIDS.

Cases of association between bronchiectasis and *Candida* are reported by Wanke (1984), Lacaz et al. (1991) and Kumar et al. (1992).

Patients with neoplasias under treatment present serious risks of infections, these are fungals which are difficult to diagnose and treat (Vidotto et al., 1986; Pizzo & Walsh, 1990). Vidotto et al. (1986) apart from *C. albicans* isolated *C. tropicalis*, *C. pseudotropicalis* and *C. glabrata* (= *Torulopsis glabrata*) from patients with cancer in the respiratory tract.

The presence of *Candida* and *Trichosporon* in patients with cancer has been found through clinical samples obtained by brush, bronchial washing, exudation, biopsy and blood culture (Stokes et al., 1989; Browne et al., 1990; Shigehara et al., 1991; Elias et al., 1992). These last authors report a fatal case of septicemia by *Candida* in a patient with cancer.

The presence of *Candida* species in the sputum of patients with diabetes mellitus was reported by Bandele et al. (1993), as well as being reported in this study, however the patient had pulmonary tuberculosis too (Table 1).

Candida parapsilosis was isolated in an arteriovenous fistula and respiratory infection. Was not found reference on the isolation of this species with backgrounds such as these.

All the authors referred to in this study, mention pulmonary tuberculosis as the most frequent pneumopathy; this information is analogous to the results contained in Table 1; since the same pathology was reported in 20 patients.

The process of colonization by *Candida*, verified in this study, is corroborated by Nwobu et al. (1989), who isolated *Candida* strains from the sputum of patients with primary pneumopathy.

Bandele et al. (1993) did not consider the high percentage of pulmonary "infections" caused by species of *Candida* in patients with pulmonary tuberculosis due to the nutritional, depressive and immunological state of these patients.

An interest in infections by *Candida* has increased in recent years, due to the ageing population, those with cancer under treatment, with transplants, AIDS patients and with prolonged use of antibiotics and intensive care units. Colonization by species of *Candida* can evolve to a disseminated illness, mainly in immuno-compromised patients (Gibbs, 1990; Meunier, 1990).

Some gastroenterologists argue that unless these organisms are seen in biopsy, there is no reason why they have

to be treated as candidiasis. This is incorrect, because esophagitis by *Candida* can be ulcerative, being it possible to have lesions also with a negative biopsy. On the other hand there can occur cases in which *Candida* invades the submucosa from where it enters the blood stream and is fully disseminated; consequently, the serious problem of oral and esophageal candidiasis is then evident (Remington, 1990).

Almost 50% of the patients with cancer die of infections from *Candida*, being the patients with altered immunological systems who demonstrate vulnerability to the infections. Diagnosing pneumonia by *Candida* without a pulmonary biopsy, but in autopsy, 61% of the patients with profound candidiasis presented pulmonary compromise. "This highlights the sad fact that nowadays we are not capable of making a diagnosis premortem in the majority of patients with deep candidiasis" (Remington, 1990).

Thus we can conclude that in all the cases of colonization by *Candida*, they were associated with pneumopathies and that mainly one species of yeast was isolated; *C. albicans* prevailed amongst the other species of the cases of colonization; it is worth emphasizing that direct examination is indispensable for the diagnosis of colonisation by *Candida*.

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