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Research Article

Candida albicans in Patients with Pharyngitis Diagnostic

Rincón Cruz, María del Carmen and Imbert J Luis*

Institute of Health Sciences, Autonomous University of the State of Hidalgo, Mexico

Abstract

Objective: To know the isolation frequency of *Candida albicans* and other emerging *Candida* species in pharyngeal infections and to consider the predisposition to oral mycosis.

Materials and methods: A study of isolation and microbiological characterization was carried out in 145 outpatients, to whom the family doctor indicated the pharyngeal exudates test but no isolation and identification of yeast was requested. The clinical study that indicated the test included interrogation and medical examination evaluating signs such as pharyngitis, pain, cough, irritation, redness.

Results: The yeasts *Candida albicans* and probably *Candida dublinensis* were found in the cultures of the exudates, as well as other yeasts not identified. *Candida albicans* showed resistance to ketoconazole in a good proportion, 14 (53.8%) of 26 were resistant.

Conclusion: The proportion of patients whom were found *Candida albicans*, *Candida dublinensis* or another type of *Candida* was very high; this may indicate that the probability of having mixed infections by bacteria and by this type of yeast are not considered. It is alarming to find *Candida albicans* with ketoconazole resistance, so the diagnostic criterion should be highlighted that when suspecting Pharyngitis it includes culture for *Candida* spp and also antifungal susceptibility tests.

***Corresponding author**

Imbert J Luis, Institute of Health Sciences, Autonomous University of the State of Hidalgo, Mexico, Tel: 055-771-1441 7798; Email: imbertox@hotmail.com

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INTRODUCTION

Candida albicans and *Candida dublinensis* are opportunistic fungal pathogens that are closely related but differ in their epidemiology, clinical, pathology and diagnostic aspects as well as genetic and phenotypic characteristics, including certain virulence-related traits. For a long time it has been considered *Candida albicans* is a commensal microorganism that is found in the mouth, pharynx and gastrointestinal tract in 30 to 50% of most populations. It is therefore the main opportunistic pathogenic fungus and frequently causes cutaneous, mucosal and nail infections. Symptoms present in these cases may be considered mild or moderate; accompanying clinical entities such as diabetes leads to increased frequency of cases of onychomycosis, also known as "foot of diabetic" and whose final complication may be amputation [1,13,14].

The same does not happen with systemic infections with *Candida* species, since these have increased from 219% to 487% from 1980 to 1989; these microorganisms now account for 10% of the Nosocomial isolates found in systemic infections. This incidence is similar to that of *Escherichia coli* and exceeds that produced by the species of *Klebsiella spp*.

In addition to hematogenous spread, muco-cutaneous infections increase the problem, especially in patients who have acquired immunodeficiency syndrome (AIDS) because up to 80% of these have *Candida* infections; it also increases in cancer patients and immuno compromised and in post-surgical situations [2,3]. The high mortality rates associated with *Candida* infections have remained at a similar level despite better therapies with antifungal agents.

A better knowledge of the adhesive processes in candidiasis has not been obtained [4,5], thus the first stage in *Candida* yeast colonization is the adherence to the epithelial cells, later the establishment of the mucocutaneous or systemic infection can continue. Invasion to intravascular structures is another critical stage in the success of the fungus, which when transported by blood will infect other target organs. In these stages the transformation of levaduri form to mycelium and pseudo mycelium becomes a virulence factor [6,7].

When this fungus is cultivated in Sabouraud agar, the "yeast-like" form is obtained, it is unicellular and it reproduces by budding. The other two filamentous forms are the mycelium and the pseudomycelium [5-10]. The fourth form, called chlamydospora, appears when grown in a special medium such as Rice Cream Agar Tween (RAT) or Cornmeal agar (CmA) at 28°C in semianaerobiosis. Usually are produced from pseudomycelia after 24 h, and the test is used to distinguish *Candida albicans* and *Candida dublinensis* from other related species [9]. Both are the

unique species than forms true hyphae (germinal tubule growth).

Additional pathogenic and virulence characteristics have been searched for human *Candida* isolates. It is known that permutation phenomena called "switch yeast to hypha" and "switch white to opaque "are based on biochemical, physiological and genomic changes that have been correlated with virulence [11,12]. Thus, the virulence analysis is of clinical relevance, which allows a differentiation of the potentially pathogenic species.

A more precise identification of the *Candida* species that affect the pharyngeal tissue of patients is of paramount importance to resolve respiratory infections, and it represents so much for epidemiology, pathology, therapy and prevention. Constitute a priority for the public health.

Due to the aforementioned, the bacteriology laboratories of the hospitals must carry out more complete and precise studies in order to provide more reliable and accurate diagnoses. So, the main objective of the work was to identify the strains of *Candida* spp isolated from pharyngitis cases, and to associate them with the antifungal resistance and one virulence marker.

MATERIAL AND METHODS

Samples origin

The pharynx exudates were obtained from patients who attended the consultation from first level Hospital, at Pachuca, Hidalgo. Routinely they are made the cultures of pharyngeal exudates, and a number of 10 to 15 probable yeast strains per month are obtained, so generally after one year more than 100 of these fungi are obtained.

Other strains of *Candida spp*. used as reference were obtained from the collaboration with the mycology laboratory of the Center for Microbiological Research of the Benemerita Autonomous University of Puebla.

Identification of *Candida strains*

The yeasts were identified by the formation of germinal tube in serum and formation of chlamydospores in cornmeal agar as well as growth in the Chromagar® Biomerieux medium specific for *Candida spp*.

The "first isolation" was done by sowing with the swab of the pharyngeal exudates on Biggy agar, blood agar and Sabouraud agar and subsequent incubation at 37°C for 24 h. After identifying the typical colonial morphology and Gram staining of the yeasts, the *Candida* spp strains were re-plated on YPD agar [5,8].

Tubule germination test

A yeast colony grown on Sabouraud agar was taken and inoculated into a screw cap tube with 2 ml of YPD medium which was placed in the rotator-incubator (25 rpm/12 hours). Subsequently, 0.1 ml is transferred to another 2 ml of fresh YPD and incubated for 5 hours. To confirm the growth in the logarithmic phase, a 0.1 ml aliquot was taken from the second culture and a 1:10 dilution was made (culture: water), and the optical density was measured at 600 nm, which should be between 1 and 2. Confirmed the exponential growth, 0.1 ml of the culture was transferred to a sterile Eppendorf tube, after adding 0.1 ml of rabbit serum was incubated for three hours at 37°C. In this way the amount of yeast that is put to germinate is of the order of 10^2 to 10^4.

After incubation, the tubes were removed, centrifuged with a 20 seconds pulse at 11,000 rpm, then almost all of the supernatant is removed, and resuspended in 50 µl. From 5 or 10 µl were dripped on a slide and observed the yeast on phase contrast microscope. The number of yeasts present in this volume is adequate to count from 1 to 50 tubules in germination [5-7].

Test of chlamydospores production

It is generally considered that an environment of semi-anaerobiosis is a favorable factor in chlamydosporulation. As Sowing density is an important control factor. The Semi-anaerobiosis is obtained by cultivating the yeasts as *Candida albicans* under a coverslip (22 x 32 mm) in a special medium such as Rice Cream Agar Tween (RAT) or the Cornmeal agar at 28°C. In this way, chlamydospores occur near the edges and under the coverslip, after 24 or better yet 48h. Light, chloramphenicol and antimycin are inhibitors of chlamydosporulation due that those substances were absent from media [9].

Virulence test (yeast to hypha transition)

The mycelium production was induced in solid medium with 2% agar. The transition media was M199 containing Earle and glutamine salts, regulated with 150mM HEPES and 7.5 pH or also Yeast Phosphate Dextrose Agar with 10% fetal calf serum YPDA-FCS 10%. Filamentation was induced by inoculating $5x10^5$ cells in 10 µl of water and dripped onto the plates and incubated for 2 to 5 days at 37°C. After the growth they were observed with a stereoscopic microscope and photographs were taken [11,12].

Antifungal evaluation

To determine the antifungal sensitivity, were prepared Sabouraud agar plates with different concentrations of Ketoconazole 1.0, 10.0, 100.0 and 1,000.00 mg/L and on the surface were dripped 10 µl of the yeasts, growing in logarithmic phase, four times (quadrupled).

RESULTS AND DISCUSSION

Samples and yeasts isolation

145 pharyngeal exudates samples were obtained during 2004; February (32), March (29), September (46) and October (39). Corresponding to female (74/51%) and male (55/38%). In 16 samples the sex was not determined (11%).

In the Sabouraud or the Nickerson agar, 99% of the samples had growth; however, 47 samples (32%) did not grow or were contaminated. The total number of samples with yeast-like morphological growth was 98 (68%) (Figure 1).

Identification by germinal tubule production

The identification of possible *Candida albicans* through the germinal tubule production was for 59 strains (69%), other 26 yeasts were classified as *Candida* spp (31%) to be negative in this test, the total were 85 samples (58%), Table 1a and 1b.

Cornmeal Agar for Chlamydospores production

The identification of *Candida albicans* or *Candida* dublinensis by this test produced 59 strains (69%), with some kind of chlamydospores. In (Figure 2), the first 12 pictures are showing

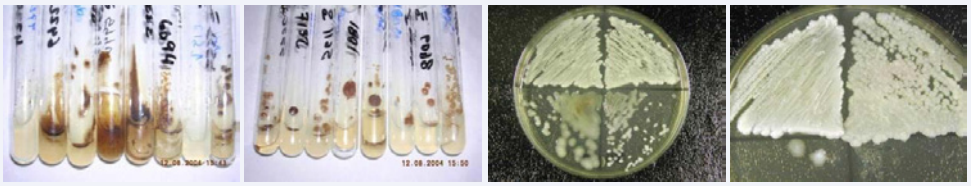

Figure 1 Typical colonial morphology of *Candida.* In Nickerson agar are shown 16 isolates and subsequent growth of six of them in Sabouraud medium. The colonies had brownish color in Nickerson and 6 different types of colonies in Sabouraud, can be described as white, creamy, whitish, mucoid-wet and filamentous and with a colony average size of 1 to 2 millimeters.

Table 1a: Characteristics of *Candida spp.* from patients with pharingitis and Ketoconazol susceptibility.

Name / No. strain	Sabouraud	Chlamidiospores	Tubule Germination		Ketoconazol mg/ml			
	G / M	CmA1 / CmA2	OD		1	10	100	1000
CANUAEH-1	N / T	>4+ / ++++	0.111	(100% Tb LT)	+	+	-	-
CANUAEH-3	N / T	+++	0.093	(100% Tb LT)	+	+	-	-
CANUAEH-4	N / T	++++	0.118	(100% Tb LT)	+	+	-	-
CANUAEH-5	N / T	++++	0.092	(100% Tb LT)	+	+	-	-
CANUAEH-6	N / T	-	0.107	(100% Tb LT)	+	+	-	-
CANUAEH-7	N / F	-	0.12	- (Tb, E, A)	+	+	-	-
CANUAEH-8	N / T	++++	0.084	(100% Tb LT)	+ d	+ d	-	-
CANUAEH-9	N / T	++++	0.108	(100% Tb LT)	+ d	+ d	-	-
CANUAEH-10	N / T	+++ / -	0.109	(100% Tb LT)	+ d	+ d	-	-
CANUAEH-15	N / T	++++	0.145	nd	-	-	-	-
CANUAEH-16	N / T	+++	0.101	(100% Tb LT)	+	+	-	-
CANUAEH-17	N / T	++	0.19	- (Tb, E)	nd			
CANUAEH-18	N / T	++++	0.28	- (Tb, E)				
CANUAEH-19	N / T	++++	0.209	(50% Tb LT)				
CANUAEH-20	N / T	++++	0.227	- (Tb, mE)				
CANUAEH-21	N / T	>4+	0.24	(50% Tb LT)				
CANUAEH-22	N / T	>4+	0.276	(50% Tb LT)				
CANUAEH-23	N / T	>4+	0.186	(50% Tb LT)				
CANUAEH-24	N / T	+++	0.289	(50% Tb LT)				
CANUAEH-25	N / T	+++ / +++	0.209	(50% Tb LT)				
CANUAEH-26	N / T	++ / >4+	0.172	(50% Tb LT)				
CANUAEH-28	N / F	- / -	0.191	- (Tb2, SthB)				
CANUAEH-29	N / F	- / -	0.007	- (Tb2, SthB)				
CANUAEH-30	N / T		0.159	(100% Tb LT)				
CANUAEH-31	N / T	+++	0.231	(100% Tb LT)				
CANUAEH-32	N / T	>4+	0.232	(100% Tb LT)				
CANUAEH-36	N / T	+	0.121	(100% Tb LT)	+	+	+ d	+ d
CANUAEH-37	N / T	+++	0.122	- (Tb2, ESThB)	+	+ d	-	-
CANUAEH-39	N / T	+++	0.155	- (Tb2, ESThB)	+	+ d	-	-
CANUAEH-41	N / T	++++	0.141	- (Tb2, ESThB)	+ d	-	-	-
CANUAEH-42	N / T	+	0.112	(100% Tb LT)	+ d	-	-	-
CANUAEH-44	N / T	- / -	0.131	(100% Tb LT)	+ d	-	-	-
CANUAEH-45	N / T	++ / ++	0.112	(100% Tb LT)	+ d	-	-	-
CANUAEH-46	N / T	+	0.143	- (Tb2, ESTh)	+ d	-	-	-
CANUAEH-47	N / T	+	0.129	- (Tb2, ESTh)	+ d	-	-	-

CANUAEH-48	N / T	+++	0.11	- (Tb2, ESTh)	-	-	-	-
CANUAEH-50	N / T		0.118	(100% Tb LT)	+	+	+ d	-
CANUAEH-52	N / T	+	0.125	- (Tb2, A,aT,B)	+	+	+	+ d
CANUAEH-53	N / T	-	0.043	- (Tb2, A,aT,B)	+ d	+ d	-	-
CANUAEH-56	N / T	+	0.09	- (Tb2, A,aT,B)	+	+ d	+ d	-
CANUAEH-57	N / T	++ / psh2+ (++)	0.018	- (Tb2, E,aT,B)	-	-	-	-
CANUAEH-61	N / T	+ / psh2+ (++)	0.11	- (Tb2, A,aT,B)	+	+ d	+ d	-
42 samples	39 N / 3 F	34 + / 7- / 2 ND	Average 0.144	(25)Tb, (17)Tb2 = 42	+ (14)	+ (10)	+ (1)	

Abbreviations: G / M: Growth / Morphology; N normal; T typical; A atypical; F Filamentouse; Tb LD, Tubule long and thin; Tb2-SthB Tubule type 2-Short-thick-Branched; CmA Cornmeal Agar; OD optical density; nd not determined; + positive; +d positive weak; (-) negative; (+, 10-20), (++, 20-50), (+++, 50-100), >4+ / microscopic field 40X.

Table 1b. Characteristics from Candida spp. from patients with pharingitis without susceptibility test.

Name / No. strain	Sabouraud	Chlamidiospores (pseudohiphae)	Tubule Germination
CANUAEH-62	N / T	psh+ (-)	+ (Tb LT 10-20/c)
CANUAEH-64	N / T	pshl (-)	+ (Tb LT 0-1-2/c)
CANUAEH-65	N / T	pshe (-)	+ (Tb LT 10-20/c)
CANUAEH-66	N / T	pshe (-)	+ (Tb LTth 40-50/c)
CANUAEH-67	N / T	psh3+ (-)	+ (Tb LTth 10-20/c)
CANUAEH-68	N / T	psh4+ (+E)o/e	+ (Tb LTthBP 40-50/c)
CANUAEH-69	N / T	psh3+ (+?)	+ (Tb LTthBP 40-50/c)
CANUAEH-71	N / T	psh1+ (+)	+ (Tb LTthBP 10-20/c)
CANUAEH-72	N / T	pshe (+E)	+ (Tb LTth 10-20/c)
CANUAEH-74	N / T	psh2+ (+E)	+ (Tb LTth 10-20/c)
CANUAEH-75	N / T	psh1+ (+E)	+ (Tb LTthBP 10-20/c)
CANUAEH-76	N / T	psh1+ (++)o/e	+ (Tb LTth 5-10/c)
CANUAEH-77	N / T	psh1+ (++)	+ (Tb LT 2-10/c)
CANUAEH-78	N / T	psh1+ (+E)o/e / psh- (-)	+ (Tb LT 10-20/c)
CANUAEH-79	N / T	(-) / psh- (-)	+ (Tb LT 40-50/c)
CANUAEH-83	N / T	psh2+ (++)o/e/ psh+ (+)	+ (Tb LTth 1-5/c)
CANUAEH-84	N / T	(-) / psh- (-)	+ (Tb LTSthB >50/c)
CANUAEH-85	N / T	psh3+ (2+)/psh-(-)	+ (Tb LT 40-50/c)
CANUAEH-90	N / T	(-) / psh- (-)	+ (Tb LT 10-20/c)
CANUAEH-92	N / T	psh+E (-)/ psh- (-)	+ (Tb LT 10-20/c)
CANUAEH-97	N / T	psh1+ (+)/psh+ (+)	+ (Tb LT 5-10/c)
CANUAEH-102	N / T	pshE (-) / psh- (-)	+ (Tb LTBPrCC+)
CANUAEH-107	N / T	psh- (+)	+ (Tb LT 20-30/c)
CANUAEH-110	N / T	psh- (-)	+ (Tb LT 20-30/c)
CANUAEH-112	N / T	psh- (-)	+ (Tb LT 20-30/c)
CANUAEH-115	N / T	psh- (-)	+ (Tb LT 20-30/c)
CANUAEH-119	N / T	psh- (+++)	+ (Tb LT 20-30/c)
CANUAEH-120	N / T	psh- (+++)	+ (Tb LT 50-100/c)
CANUAEH-123	N / T	psh- (+)	+ (Tb LT 50-100/c)
CANUAEH-128	N / T	psh- (+)	+ (Tb LT >90/c)
CANUAEH-130	N / T	psh- (++)	+ (Tb LT >90/c)
CANUAEH-131	N / T	psh- (-)	+ (Tb LT >90/c)
CANUAEH-133	N / T	psh+ (-)	+ (Tb LT >90/c)
CANUAEH-137	N / T	psh- (-)	+ (Tb LT >90/c)
34 samples			34 +

Abbreviations: G / M: Growth / Morphology; N normal; T typical; A atypical; F Filamentouse; Tb LD, Tubule long and thin; Tb2-SthB Tubule type 2-Short-thick-Branched; CmA Cornmeal Agar; OD optical density; nd not determined; + positive; +d positive weak; (-) negative; (+, 10-20), (++, 20-50), (+++, 50-100), >4+ / microscopic field 40X.

some yeasts producing these structures, it is noted that in addition to the amount (in someone there is a single chlamydospora surrounded by dozens of yeasts) the circular or oval shape distinguishes them.

In the last four photographs, below, there are not chlamydospores, the translucent and elongated structure at the end as well as the rest of the body of the mycelium even longer does not correspond to that of *Candida albicans* or dublinensis, and cause confusion in the diagnosis. These yeasts then could be another kind of *Candida* spp.

Virulence test

After inoculation in M199 medium, someone of the spotted yeasts grew as independent colonies with variable filamentous morphology, (Figure 3). The exchange phenomena called "yeast to hypha" of *Candida albicans* was not present in most strains, this test is not a clear sign indicative of someone virulence characteristic (13,14).

Antifungal sensitivity

The tests of antifungal sensitivity of 26 (61.9%) of 42 *Candida albicans* strains shown that 14 (53.8%) were resistant to 1 mg/ml, 10 (38.4%) were to 10 mg/ml and 2 were to 100 mg/ml. All strains were sensitive to 1,000 mg /ml, Table 1a, Table 1b shows 34 more *Candida albicans* strains which only two identification tests were done; the total of *Candida albicans* was 76 (89.4%), from the initial number of isolated yeasts (85).

CONCLUSIONS

It is important to improve the mycological diagnosis in the pharyngeal exudates.

The germinal tubule tests together to chlamydospore test are quite reliable to presumptively identify *C. albicans* and *C. dublinensis*.

The switch test was variable in most of the isolates

The main limitations of the study were cannot make correlations between yeast isolates and pathogenic bacteria, and cannot to document in more detail the diagnosis and clinical symptoms of patients. All of them which originates a management incomplete of pharyngeal disease, and therefore cannot be associated with morbid-mortality and Public Health.

Ethical considerations

The authors declare that no experiments have been conducted on humans or animals for this research.

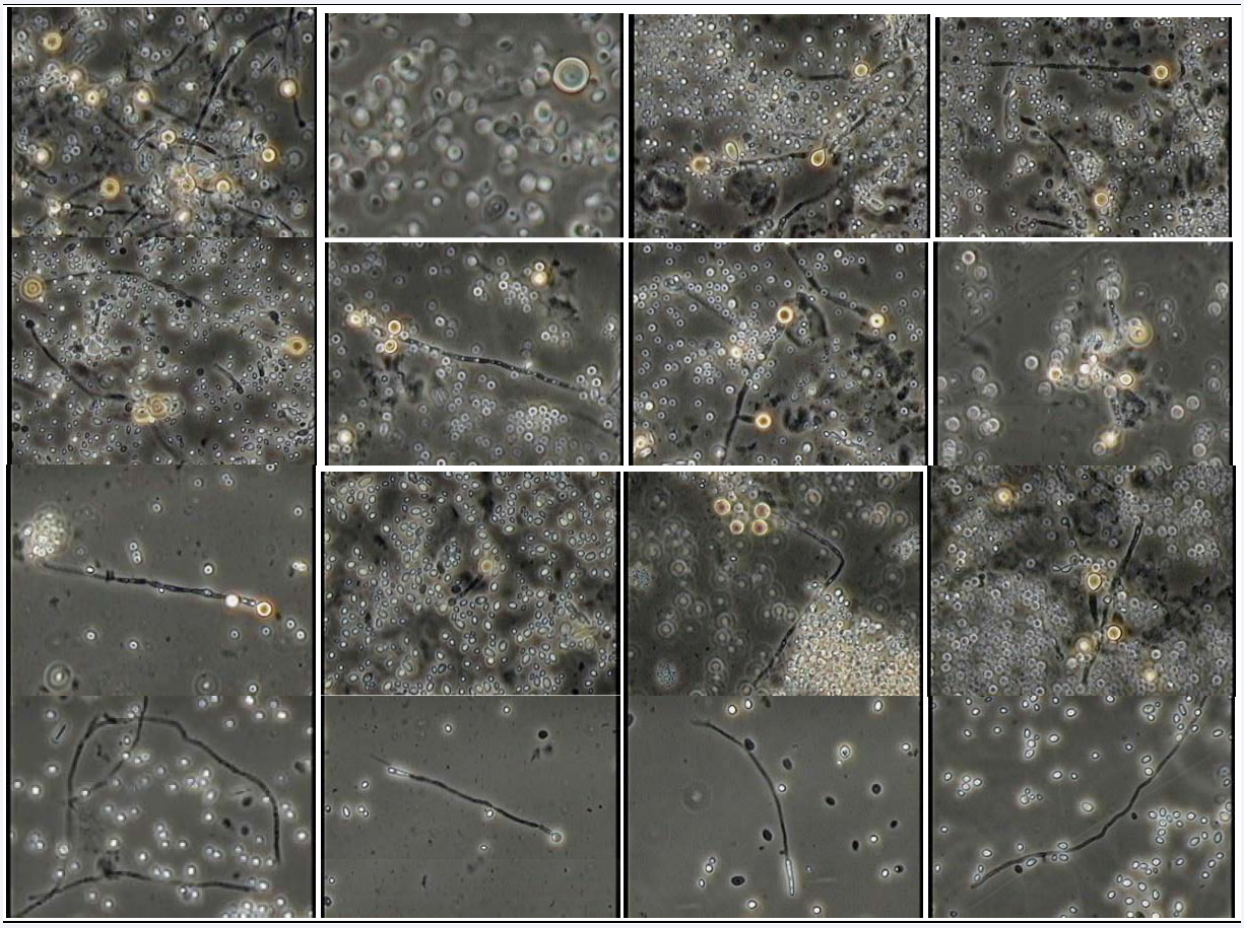

Figure 2 Round, thick-walled vesicles or ellipsoidal forms on Cornmeal Agar. Chlamydospores were observed with phase contrast microscope; note the yellowish appearance and refraction light of terminal vesicles. Numerous small blastoconidia and pseudohyphae are also present too.

Figure 3 [Confirmatory test for switch yeast to hypha transition].
The pictures are from eleven colonies of *Candida* strains and were taken with reflected and transmitted light. The different colonial morphology goes through smooth to rough, smooth or filamentary circular edges, the filamentation is short or long, uniform or discreet, the branch corresponding to the hypha yeast transition is simple or varied only in the first three colonies, they are also observed with radial or diffuse and random folds, finally the surfaces have a smooth, creamy or wet appearance.

Data confidentiality right to privacy Informed consent

The authors declare that patient data do not appear in this article, so it was not necessary to sign an informed consent letter.

This research was conducted during the years from 2003 to 2004. Most of the strains, although were stored at -20°C, are no longer viable for complementary and defining studies such as PCR.

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