Distribution of *Candida* species in different clinical sources in Delhi, India, and proteinase and phospholipase activity of *Candida albicans* isolates

Somansu Basu[1], Harish C. Gugnani[1], Sangeeta Joshi[2] & Neera Gupta[3]

[1] Department of Medical Mycology, V. P. Chest Institute, University of Delhi, Delhi, [2] Department of Microbiology, Sir Ganga Ram Hospital, New Delhi and [3] Department of Microbiology, St. Stephen's Hospital, Delhi

Summary

Eighty-five isolates of *Candida* recovered from three hundred and fifty diverse clinical sources, viz. respiratory tract (sputum, bronchial washing, bronchoalveolar lavage, tracheal aspirate), blood, urine, high vaginal swab, skin and plastic devices, were studied in detail for their morphological and biochemical characters. Seven species of *Candida* were identified, viz., *C. albicans* (45.8%), *C. tropicalis* (24.7%), *C. parapsilosis* (10.5%), *C. krusei* (7.0%), *C. kefyr* (7.0%), *C. guilliermondii* (3.5%), and *C. glabrata* (1.1%). *C. albicans* was the predominant species isolated from all clinical specimens, except blood from which *C. krusei* was most frequently (38.4%) recovered. Out of 39 isolates of *C. albicans,* 26 (66.6%) and 19 (48.7%) exhibited strong proteinase and phospholipase activity respectively. There was a higher prevalence of proteinase producing strains amongst the vaginal and skin isolates than that in urinary and respiratory isolates. Also a greater number of phospholipase producing strains was observed in the vaginal and urinary isolates than that in the respiratory and skin isolates.

Key words *Candida* species, Clinical sources, Proteinase, Phospholipase, India

Distribución de especies de *Candida* en diferentes muestras clínicas en Delhi, India, y actividades fosfolipasa y proteinasa en aislamientos de *Candida albicans*

Resumen

Se estudiaron morfológica y bioquímicamente 85 aislamientos de *Candida* procedentes de 350 muestras clínicas de diversos orígenes: aparato respiratorio (esputo, lavado bronquial, lavado broncoalveolar, aspirado traqueal), sangre, orina, frotis vaginal, piel y materiales plásticos. Se identificaron siete especies de *Candida*: *C. albicans* (45,8%), *C. tropicalis* (24,7%), *C. parapsilosis* (10,5%), *C. krusei* (7%), *C. kefyr* (7%), *C. guilliermondii* (3,5%) y *C. glabrata* (1,1%). *C. albicans* fue la especie más aislada en todas las muestras clínicas, excepto en sangre, donde *C. krusei* se aisló en un 38,4%. De los 39 aislamientos de *C. albicans*, 26 y 19 respectivamente presentaron una alta actividad proteinasa y fosfolipasa. Los aislamientos vaginales y cutáneos presentaron una actividad proteinasa mayor que aquéllos procedentes de orina o de muestras respiratorias. De la misma manera, la actividad fosfolipasa de los aislamientos vaginales y de orina era mayor que la de los aislamientos de muestras respiratorias.

Palabras clave Especies de *Candida*, Origen clínico, Proteinasa, Fosfolipasa, India

Address for correspondence:
Prof. Harish C. Gugnani
Department of Medical Mycology
V. P. Chest Institute
University of Delhi, Delhi-110 007-India
E-mail: harishgugnani@hotmail.com

Aceptado para publicación el 17 de noviembre de 2003

1130-1406/01/10.00 Euros

Candidiasis has emerged as an alarming opportunistic disease with the increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation [1-3]. *Candida albicans* is considered to be the commonest and most virulent pathogenic species of the genus *Candida*.

In last few decades, there have been numerous reports of *Candida* infections in India. However, in many of these reports, identification of *Candida* isolates has not been based on a detailed study of their biochemical characteristics. Sengupta et al. [4] documented that out of sixty-three isolates of yeasts, 42 (66.6%) were *C. albicans*, 9 (14.3%) were *C. tropicalis*, 4 (6.3%) were *C. parapsilosis,* 2 each (3.2%) were *C. kefyr* and *C. krusei* and one (1.6%) was *C. guilliermondii*. In another study, Prasad et al. [5] reported that *C. albicans* was the predominant (47.6%) pathogenic species isolated from various clinical specimens followed by *C. tropicalis* (35.4%), *C. krusei* (4.9%), *C. glabrata* (3.7%), *C. zeylanoides* (2.4%), *C. guilliermondii* (2.4%), *C. kefyr* (1%) and *C. parapsilosis* (1%). In a 10 year study in a teaching hospital, Chakrabarti et al. [6] found an eleven fold increase of candidemia among the hospitalized patients between 1986 and 1990; the etiological agents were *C. albicans* (50%), *C. guilliermondii* (17%), *C. tropicalis* (15%), *C. parapsilosis* (8%), *C. glabrata* (3%), *C. krusei* (2%) and other *Candida* species (5%).

Proteinase and phospholipase enzymes secreted by isolates of *C. albicans* are regarded as virulence factors [7,8]. There are a few reports on proteinase activity of Indian strains of this species [9,10]. However, to the best of our knowledge, no investigations have been carried out on phospholipase activity of Indian strains of *C. albicans*. The present investigation deals with a detailed study of morphological and biochemical characteristics of 85 isolates of *Candida* species recovered from diverse clinical sources in some hospitals in Delhi. All the 39 isolates of *C. albicans* recovered during the study were also tested for proteinase and phospholipase activities.

Materials and methods

Source of clinical specimens and isolates. The clinical specimens investigated included respiratory tract secretions (sputum, bronchoalveolar lavage, bronchial washing and tracheal aspirate) - 116, blood- 83, urine - 54, high vaginal swab - 39, skin scrapings -22, and plastic devices (Foley's catheters tip, endotracheal tube tip, intravascular catheter tip and renal stent) - 36 samples. These samples were collected from Sir Ganga Ram Hospital, New Delhi, St. Stephen's Hospital, Delhi, and Clinical Research Centre, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi. Eighty-five isolates of *Candida* recovered from these specimens, and 50 isolates obtained from throat swab of normal healthy individuals were included in the study.

Identification of isolates. The isolates were studied for their colony morphology on Sabouraud dextrose agar (SDA), and direct microscopic features were observed in lactophenol cotton blue stain. For germ tube formation, loopfuls of the test isolates were inoculated in small quantities of serum and incubated at 37 °C for 2 h. The isolates were tested for chlamydoconidia formation in cut streak cultures on rice meal agar supplemented with 1% Tween-80 after 48 h of incubation at 25 °C.

The biochemical tests were carried out essentially according to the procedure of Beneke et al. [11]. The carbohydrate assimilation pattern of the isolates was studied on yeast nitrogen base (Difco, USA) medium by auxanographic method, using filter paper discs impregnated with different carbohydrate sources (Hi-media, India). API 20 Candida (bioMérieux, France) was used for determining the specific identity of those *Candida* isolates, which could not be identified by auxanographic method. Three reference control isolates of *C. albicans* viz. ATCC 36801, 44505 and 44506 were included as controls.

Testing in vitro proteinase activity. The method employed for this test was that of Rüchel et al. [12] with minor modifications recommended by Chakrabarti et al. [9]. After 5 days incubation at 37 °C, the plates were stained with 0.5% amidoblack solution to record the clear zone around the discs. All tests were run in duplicate and an uninoculated disc was used as negative control.

Testing in vitro phospholipase activity. This test was done according to the method described by Ibrahim et al. [13] and the phospholipase activity (Pz) value was calculated from the zone of opacity accordingly.

Results and discussion

The predominance (45.8%) of *C. albicans* in this study approximates to that reported by Kao et al. [14], Sengupta et al. [4] and Prasad et al. [5]. The other species recovered in order of frequency were *C. tropicalis* represented by 21 (24.7%) isolates and *C. parapsilosis* represented by 9 (10.5%) isolates. As is evident in the Table 1,

Table 1. Prevalence of *Candida* species in various clinical sources

Sources of clinical isolates	No. of isolates % of different species from various clinical sources							
	C. albicans	*C. tropicalis*	*C. parapsilosis*	*C. krusei*	*C. guilliermondii*	*C. kefyr*	*C. glabrata*	Total
Respiratory tract (Sputum, BAL*, BW**, TA***)	18 (45.0)	8 (20.0)	7 (17.5)	–	3 (7.5)	4 (10.0)	–	40
Blood	1 (7.6)	4 (30.7)	1 (7.6)	5 (38.4)	–	1 (7.6)	1 (7.6)	13
High Vaginal Swab	6 (66.6)	3 (33.3)	–	–	–	–	–	9
Skin scraping	2 (100)	–	–	–	–	–	–	2
Urine	5 (45.5)	3 (27.2)	1 (9.0)	1 (9.0)	–	1 (9.0)	–	11
Plastic device	7 (70.0)	3 (30.0)	–	–	–	–	–	10
Total	39	21	9	6	3	6	1	85

n = Total number of isolates.
*Bronchoalveolar lavage
**Bronchial washing
***Tracheal aspirate

the frequency of different species varied with the source of clinical specimens. However, *C. albicans* was the predominant species recovered from all the specimens except for blood, which yielded *C. krusei* as the most frequent species. Among the 50 isolates from throat swab of normal healthy individuals *C. albicans* was again predominant, represented by 30 (60%) isolates and the rest comprised of 13 isolates of *C. tropicalis*, and 4 of *C. parapsilosis* and 3 of *C. kefyr.* Sputum samples in 12 (30%) patients yielding *C. albicans* in culture were positive for direct microscopy. These patients were aged more than 65 years and had symptoms of productive cough and dyspnea. They had probably secondary infection due to *Candida*. Ten out of 11 specimens of mid-stream urine samples showed positive microscopy for *Candida* pseudohyphae and blastoconidia. These patients, including four diabetics, had symptoms of urinary tract infection, and no pathogenic bacteria were recovered from urine samples of these patients. *C. albicans* was the most frequent species (45.5%) among urinary isolates, followed by *C. tropicalis* (27.2%); and one isolate each of *C. kefyr, C. parapsilosis* and *C. krusei*. All nine patients giving positive cultures from high vaginal swab had symptoms of *Candida* vaginitis; direct microscopy was positive in eight of them. The vaginal swabs yielded *C. albicans* (66.6%) and *C. tropicalis* (33.3%). Both the two isolates from skin were *C. albicans* and they exhibited pseudohyphae in microscopy.

The different blood isolates were recovered from the neonates admitted in intensive care unit with high fever and respiratory distress. Nine of them received prolonged parenteral nutrition while two were on fluconazole prophylaxis. Three neonates died in spite of an adequate antifungal therapy. The isolation of non-albicans *Candida* has been frequently encountered from candidemic patients in the past few decades [14-16]. It is, therefore, not surprising that 12 out of 13 isolates of *Candida* recovered from blood were non-albicans, predominantly represented by *C. krusei* and *C. tropicalis*. The emergence of *C. krusei* as a common etiological agent of candidemia has important clinical implication due to its innate resistance to fluconazole [17].

The recovery of *C. albicans* and *C. tropicalis* from plastic devices is noteworthy as there has been no previous report of isolation of *Candida* from plastic devices from India. Dorko et al. [18] documented that *C. tropicalis* possess the abilility to adhere to PVC materials. Scrapings from six plastic devices also showed yeast cells in microscopy. The adherence of microrganisms to polymer surface of plastic devices such as catheters, cannulas etc. predisposes the patient to opportunistic infections and consequent colonization of these biomaterials allows the establishment of a reservoir of chronic inoculation and dissemination of fungal cells [19].

Proteinase and phospholipase are regarded as important virulence factors for *C. albicans* [9,13]. In the present study, presence of proteinase and phospholipase activity has been investigated in the 39 isolates of *C. albicans* from different clinical sources. The proteinase activity was detected in 26 (66.6%) of the isolates from clinical sources and in four (13.3%) of 30 isolates from throat swab of normal healthy individuals (Table 2). It is worth mentioning that as many as five out of six vaginal swab isolates, three out of five urinary isolates and both the skin isolates exhibited proteinase activity. On the other hand, 13 (72.3%) respiratory and two (28.5%) isolates from plastic devices demonstrated proteinase activity.

Table 2. Proteinase and phospholipase activity exhibited by *C. albicans* isolated from different clinical sources

Sources of clinical isolates	No. of isolates showing	
	Proteinase activity	Phospholipase activity (Pz value)
Respiratory (n=18)	13	10 (0.88 to 0.95)
Blood (n=1)	1	1 (0.64)
High vaginal swab (n=6)	5	4 (0.82 to 0.86)
Skin (n=2)	2	1 (0.92)
Urine (n=5)	3	3 (0.84 to 0.89)
Plastic device (n=7)	2	–
Control (n=30)	4	2 (0.92 to 0.95)
Total (clinical isolates excluding controls)	26	19

n= total number of *C. albicans* isolates

There is no previous study of phospholipase activity of Indian strains of *C. albicans*. In the present study, 48.7% clinical isolates of *C. albicans* demonstrated phospholipase activity while only two isolates from normal healthy persons were positive for this enzyme (Table 2). However, Mayser et al. [20] documented that 85.9% of *C. albicans* isolates were positive for phospholipase. Interestingly, phospholipase activity with higher Pz values seems to be more commonly present in vaginal *Candida* isolates (66.6% isolates with Pz value 0.82 - 0.86) and urinary (60% with Pz value 0.84 - 0.89) than in respiratory *Candida* isolates (55.5% isolates with Pz value 0.88 - 0.95). Further it is noteworthy that four of 13 isolates negative for proteinase showed phospholipase activity whereas 11 out of 20 isolates negative for phospholipase activity exhibited proteinase activity. Thus an isolate of *C. albicans* may not produce both proteinase and phospholipase at the same time.

The authors are very grateful to Mr. Amit Kumar, Mr. Mahipal, Mr. Sultan Singh and other laboratory staffs for their help.

References

1. Iwama A, Yoshida M, Miwa A, Obayashi T, Sakamoto S, Miura Y. Improved survival from fungaemia in patients with haematological malignancies: Analysis of risk factor for death and usefulness of early antifungal therapy. Eur J Haematol 1993; 51: 156-160.
2. McCullough MJ, Ross BC, Reade PC. *Candida albicans:* a review of its history, taxonomy, epidemiology, virulence attributes and methods of strain differentiation. Int J Oral Maxillofacial Surg 1996; 25: 136-144.
3. Merlino J, Tambosis E, Veal. Chromogenic tube test for presumptive identification or confirmation of isolates as *Candida albicans.* J Clin Microbiol 1998; 36: 1157-1159.
4. Sengupta P, Ohri VC. Study of yeast species isolated from clinical specimens. Med J Armed Forces Ind 1999; 55: 319-321.
5. Prasad KN, Agarwal J, Dixit AK, Tiwari DP, Dhole TN, Ayyagri A. Role of yeasts as nosocomial pathogens and their susceptibility to fluconazole and amphotericin B. Ind J Med Res 1999; 110: 11-17.
6. Chakrabarti A, Chander J, Kasturi P, Panigrahi D. Candidemia: a 10 year study in an Indian Teaching Hospital. Mycoses 1992; 35: 47-51.
7. Cutler JE. Putative virulence factors of *Candida albicans.* Annu Rev Microbiol 1991; 45: 187-218.
8. Odds FC. *Candida* species and virulence. ASM News 1994; 60: 31-38.
9. Chakrabarti A, Nayak N, Talwar P. In vitro proteinase production by *Candida* species. Mycopathologia 1991; 114: 163-168.
10. Kakru DK, Thoker MA, Shah A. Correlation of morphotyping and extraceulluar proteinase production as virulence markers in *Candida albicans.* Ind J Med Microbiol 1999; 17: 74-77.
11. Beneke ES, Rogers AL. Opportunistic Infections – Yeasts. In: Medical Mycology and Human Mycoses. California, Star Publishing Co, 1996: 149-160.
12. Rüchel R, Tegeler R, Trost MA. A comparison of secretory proteinases from different strains of *Candida albicans.* Sabouraudia 1982; 210: 233-244.
13. Ibrahim AS, Mirbod F, Filler S, et al. Evidence implicating phospholipase as a virulence factor of *Candida albicans.* Infect Immun 1995; 63: 1993-1998.
14. Kao AS, Brandt ME, Pruit WR, et al. The epidemiology of candidemia in two United States cities: Result of a population based active surveillance. Clin Infec Dis 1999; 29: 1164-1170.
15. Pfaller MA. Nosocomial candidiasis: emerging species, reservoirs and modes of transmission. Clin Infec Dis 1996; 22: S89-S94.
16. Segal E, Elad D. *Candida* species and *Blastoschizomyces capitatus,* In: Ajello L, Hay RJ (Eds.) Microbiology and Microbial Infection. New York, Arnold, 1998: 423-460.
17. Ng KP, Saw TL, Na SL, Soo-Hoo TS. Systemic *Candida* infection in University Hospital 1997-1999: The distribution of *Candida* biotypes and antifungal susceptibility patterns. Mycopathologia 2000; 149: 141-146.
18. Dorko E, Kmet'ova M, Marossy A, Frantisek D, Molokacova M. Non-*albicans Candida* species isolated from plastic devices. Mycopathologia 1999; 148: 117-122.
19. Kennedy MJ, Rogers ALK, Yancey RJ Jr. Environmental alteration and phenotypic regulation of *Candida albicans* adhesion to plastics. Infect Immun 1989; 57: 3876-3881.
20. Mayser P, Laabs S, Heuer K, Gründer K. Detection of extracellular phospholipase activity in *Candida albicans* and *Rhodotorula rubra.* Mycopathologia 1996; 135: 149-155.