International Journal of Microbiological Research 5 (1): 01-05, 2014
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2014.5.1.8232

Distribution of *Candida albicans* and the Non-Albicans *Candida* Species in Different Clinical Specimens from South India

[1]K. Seshu Kumari, [1]Pendru Raghunath, [2]B. Harshavardhan and [3]Abhijit Chaudhury

[1]Department of Microbiology, Dr. VRK Women's Medical College Teaching,
Hospital & Research Centre, Aziznagar, R.R. District, 500075, India
[2]Department of Biotechnology, Sri Venkateswara Institute of
Medical Sciences (SVIMS), Tirupati, 517501, India
[3]Department of Microbiology,
Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, 517501, India

Abstract: Distribution of candida species in different clinical specimens was studied. As many as 100 isolates were recovered from 602 various clinical specimens. Among the 100 isolates of Candida recovered from various clinical specimens 66 were non-albicans Candida species and 34 were *C. albicans*. Out of the non-albicans isolates *C. glabarata* (30%) was the most dominant, followed by *C. tropicalis* (20%), and *C. kefyr* (16%). Out of 500 urine specimens, 66 (13.2%) yielded candida isolates. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were *C. albicans*. Among the 43 non-albicans *Candida* species isolated from urine, 16 were *C. glabrata*, 14 were *C. kefyr* and 13 were *C. tropicalis*. Out of 20 intravascular catheter tips, 8 (40%) were colonised with Candida species. Among the 8 isolates recovered from catheter tips, 5 were *C. albicans*, 2 were *C. glabrata* and one was *C. tropicalis*. In conclusion, the present study showed the distribution of Candida species in different clinical specimens and the predominance of non-albicans Candida species.

Key words: Distribution of Candida · Clinical Specimens · Non-Albicans Candida Species · *C. glabrata*

INTRODUCTION

Candidiasis is the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Number of candidiasis cases have been increased in the past few decades [1]. Excessive use of broad-spectrum antibiotics and the emergence of AIDS are important among the various contributing factors [2]. Until recently, *C. albicans* was considered as the predominant species in most countries, causing most of the cases of candidiasis. However, during last few decades, many countries around the world reported a change in the epidemiology of candida infections, characterized by a progressive shift from a predominance of *C. albicans* to non-albicans Candida species such as *C. tropicalis*, *C. glabrata* and *C. krusei* [3-7]. The clinical manifestations produced by various pathogenic Candida species are not distinguishable. Many of the non-albicans Candida species such as *C. glabrata* and *C. krusei* exhibit resistance to traditional triazole antifungals like fluconazole and may also demonstrate cross-resistance to newer triazoles [8]. Hence, identification of Candida isolates to the species level has become mandatory to aid the selection of appropriate antifungal agents in treatment.

In last few decades, there are many reports of candidiasis from India. However, in many of these reports, Candida isolates were not identified based on a detailed study of their biochemical characteristics. Because of considerable regional variability, local epidemiological knowledge is critical in the effective management of

Corresponding Author: Pendru Raghunath, Department of Microbiology VRK Women's Medical College Teaching Hospital & Research Centre R.R. District-500075, Hyderabad, India. Tel: +91 9618886504, Fax: 040-23732786, 08413-235071.

infections caused by Candida species. To our knowledge, there are not many reports of candidiasis from South India. The objective of the present study was to investigate the prevalence of different types of candida infections and to determine isolation pattern, species distribution of various isolates.

MATERIALS AND METHODS

Sample Collection: As many as 602 clinical specimens were collected from Department of Microbiology, Sri Venkateswara Institute of Medical Sciences, Tirupati during October 2011 to March 2012 for a period of six months. The clinical specimens investigated include urine-500, pus-30, intravascular catheter tips-20, respiratory tract secretions (sputum-10, bronchoalveolar lavage-10, Blood-10, stool-10, skin scrapings-06 and CSF-06). The 602 patients were grouped into 5 age groups, less than 20 years, 21-40 years, 41-60 years, 61-80 years and 81-100 years.

Culture of Specimens: All clinical specimens were inoculated on MacConkey and Blood Agar. Biphasic blood culture bottles containing Brain Heart Infusion broth and agar (HiSafe™ Blood Culturing System, Hi Media, Mumbai) were used for blood samples. All the inoculated plates were incubated at 37°C. Urine was incubated overnight. CSF and pus samples were incubated for 48 hrs and blood culture bottles for 7 days. Intravascular catheters were cultured semi quantitatively after being cut with a sterile blade according to Maki *et al*. [9]. Cultures with > 15 CFU were considered positive. Skin scrapings for fungal culture were inoculated on slants of Sabouraud's dextrose agar (SDA) incubated for 2 weeks. Urine specimens which yielded Candida colony count > 10^5 CFU/ml were considered in the study. Other specimens were included in the study, if Candida species were isolated in pure culture.

Identification of Isolates: The colonies were initially studied for their morphological characters such as: colony appearance, colony colour, colony shape and colony texture. Colonies suggestive of Candida species were further identified and speciated by Gram's staining, germ tube test, chlamydospores formation on cornmeal agar, sugar fermentation test, sugar assimilation test and colour production on CHROM agar (HiMedia, Mumbai) [10, 11]. Gram's staining, sugar fermentation and sugar assimilation tests were performed using standard protocol.

For testing germ tube production, one or two colonies of candida isolates were suspended in individual tubes containing 1 ml of pooled human serum. The test tubes were incubated at 37°C for 2 hrs. One drop of each yeast-serum suspension was placed on a glass slide, covered with a cover slip and examined under the microscope for the presence of germ tubes.

For testing chlamydospores production, each isolate was picked up with a straight wire, streaked on corn meal agar plate and covered with a cover slip to produce a relative anaerobic condition. Then the plates were incubated at 25°C for 3 days. The plates were examined under microscope at every 24 hrs interval for the presence of chlamydospores.

RESULTS

Total number of Candida isolates that were identified from 602 various clinical specimens was 100. Among these 48 isolates were from males and 52 isolates were from females (Table 1). Among females, most number of Candida species were isolated from the age group 41-60 (Table 1). Where as in case of males, most number of Candida species were isolated from age groups 41-60 years and 61-80 years, each age group contributed 17 (32.6%) isolates (Table 1).

Out of 500 urine specimens, 66 (13.2%) yielded candida isolates. Among the 66 Candida species isolated from urine, 43 were non-albicans Candida species and 23 were *C. albicans* (Table 2). Among the 43 non-albicans Candida species isolated from urine, 16 were *C. glabrata*, 14 were *C. kefyr* and 13 were *C. tropicalis* (Table 2). Out of 20 intravascular catheter tips, 8 (40%) were colonised with Candida species. Among the 8 isolates recovered from catheter tips, 5 were *C. albicans*, 2 were *C. glabrata* and one was *C. tropicalis* (Table 2). Two out of ten blood samples yielded Candida species.

Among the 100 isolates of Candida species isolated from various clinical specimens 66 were non-albicans Candida species and 34 were *C. albicans* (Table 2). Out of the non- albicans isolates *C. glabarata* (30%) was the most common, followed by *C. tropicalis* (20%) and *C. kefyr* (16%) (Table 2).

DISCUSSION

Candidiasis is the most common cause of fungal infections, leading to a range of life-threatening invasive to non-life-threatening mucocutaneous diseases. Until recently, *C. albicans* was considered as the predominant species causing candidiasis in most of the

Table 1: Isolation of Candida species from different age group male and female patients

	No. of patients yielding Candida species	
Age in years	Females	Males
>20 years	6	2
21-40	12	8
41-60	16	17
61-80	11	17
81-100	2	3
Total	48	52

Table 2: Species distribution of different Candida isolates among various clinical specimens

Isolate	Urine	Catheter tips	Sputum	Pus	CSF	Skin scrapings	Blood	BAL	Stool
C. albicans	23	5	5	1	Nil	1	1	1	Nil
C. tropicalis	13	1	2	2	Nil	1	1	Nil	Nil
C. glabarata	16	2	5	2	1	2	Nil	1	1
C. kefyr	14	Nil	Nil	1	Nil	1	Nil	Nil	Nil

countries. Over the past decade, there has been a significant increase in the number of reports of systemic and mucosal yeast infection with Candida species other than *C. albicans* [3-7]. The potential clinical importance of species level identification has been recognized as Candida species differ in the expression of putative virulence factors and antifungal susceptibility. Thus, it has become imperative to identify all yeast isolates up to the species level in routine microbiology laboratories. Molecular methods such as real-time PCR [12], multiplex-tandem PCR [13] and Ribosomal Intergenic Spacer Fingerprinting methods [14] are being increasingly employed for the identification of *Candida* isolates to the species level. However such methods are very expensive and not suitable for routine use in clinical laboratories. Hence, an attempt to identify clinical isolates of *Candida* up to species level based on biochemical methods such as Gram's staining, germ tube test, chlamydospores formation on cornmeal agar, sugar fermentation test, sugar assimilation test and colour production on CHROM agar (HiMedia, Mumbai), was under taken.

A total of 100 isolates from 602 various clinical specimens were studied. The predominant species identified in the present study was *C. albicans* and it was isolated from all kinds of clinical specimens taken into consideration. In the present study 66% of isolates belong to non- albicans Candida species and 34% were *C. albicans* (Table 2). Non-albicans Candida species are generally considered as normal flora of cutaneous and mucocutaneous surfaces and only rarely are incriminated as agents of infection. However, in last few decades many researchers have reported the increased isolation and significance of non-albicans *Candida* species [5, 15]. Out of the non-albicans isolates *C. glabarata* (30%) was the most common, followed by *C. tropicalis* (20%) and *C. kefyr* (16%) (Table 2). Several researchers have also reported that *C. glabrata* and *C. tropicalis* as the most common pathogens among non-albicans Candida species [16-19].

Candida species in measurable quantities in the urine (candiduria) are found in <1% of clean voided specimens in healthy persons [20], but account for 5% of all urine culture results in the general hospital setting and 10% of urine isolates in tertiary care facilities [21]. In the present study, 13.2% of urine samples yielded Candida isolates. Bouza and colleagues reported that the incidence of candiduria in non-catheterized subjects was 6.6% [22]. Among the 66 Candida species isolated from urine, 43 were non-albicans *Candida* species and 23 were *C. albicans* (Table 2). Non-albicans Candida species appear better adapted to the urinary tract environment with many studies reporting that >50% of urinary Candida isolates belong to non-albicans species [23, 24]. Among the 43 non-albicans Candida species isolated from urine, 16 were *C. glabrata*, 14 were *C. kefyr* and 13 were *C. tropicalis* (Table 2). Kauffman [24] reported *C. glabrata* as the dominant species among urinary isolates of Candida. Our result is in agreement with this report. *C. glabrata* appears to be adapt well to selected urine properties such as substrate availability, osmolality and pH [25]. Paul and colleagues have identified *C. tropicalis* as the most prevalent fungal isolate from urine specimens [26]. This study reports that *C. tropicalis* as the third most dominant fungal isolate from urine.

In the present study, 40% of intravascular catheter tips were colonised with *Candida* species. Previous reports suggest that Candida colonization of intravascular devices is associated with candidemia [27, 28]. Leenders and colleagues [29] suggested pre-emptive antifungal treatment based on catheter tip cultures. Previous reports suggest that 8% of cases of nosocomial blood stream infections were caused by

Candida species and nearly half of the isolates were non-albicans Candida species [30, 31]. In this study, 20% (2/10) of blood samples yielded Candida species. Out of 2 isolates, one was *C. albicans* and the other one was *C. tropicalis* (Table 2).

In conclusion, the present study showed the distribution of Candida species in different clinical samples and the predominance of non-Candida albicans such as *C. glabrata, C. tropicalis* and *C. kefyr.*

REFERENCES

1. Ying, S. and L. Chunyang, 2011. Correlation between phospholipase of Candida albicans and resistance to fluconazole. Mycoses., 55: 50-55.
2. Enoch, D.A., H.A. Ludlam and N.M. Brown, 2006. Invasive fungal infections: a review of epidemiology and management options. J. Med. Microbiol., 55: 809-818.
3. Krcmery, V. and A.J. Barnes, 2002. Non-albicans 5. Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J. Hosp. Infect., 50: 243-60.
4. Hickey,W.F., L.H. Sommerville and F.J. Schoen, 1983. Disseminated Candida glabarata: report of Am. J. Clin. Pathol., 80: 724-727.
5. Wingard, J.R., W.G. Merz and R. Saral, 1979. Candida tropicalis: A major pathogen in immunocompromised patients. Ann. Intern. Med., 91: 539-543.
6. Prasad, K.N., J. Agarwal, A.K. Dixit, D.P. Tiwari, T.N. Dhole and A. Ayyagri, 1999. Role of yeasts as nosocomial pathogens and their susceptibility to fluconazole and amphotericin B. Ind. J. Med. Res., 110: 11-17.
7. Latha, R., R. Sasikala, N. Muruganandam and R. Venkatesh Babu, 2011. Study on the shifting patterns of Non Candida albicans Candida in lower respiratory tract infections and evaluation of the CHROMagar in identification of the Candida species. J. Microbiol. Biotech. Res., 1: 113-119.
8. Magill, S.S., C. Shields, C.L. Sears, M. Choti and W.G. Merz, 2006. Triazole 6. cross-resistance among Candida spp.: case report, occurrence among bloodstream isolates and implications for antifungal therapy. J. Clin. Microbiol., 44: 529-535.
9. Maki, D.G, C.E. Weise and H.W. Sarafin, 1977. A semiquantitative culture method for identifying intravenous-catheter-related infection. N. Engl. J. Med., 296: 1305-1309.
10. Nadagir, S.D., S.K. Chunchanur, L.H. Halesh, K. Yasmeen, M.R. Chandrasekhar and B.S. Patil, 2008. Significance of isolation and drug susceptibility testing of non-Candida albicans species causing oropharyngeal candidiasis in HIV patients. Southeast. Asian. J. Trop. Med. Public. Health., 39: 492-495.
11. Rippon, J.W., 1988. Candidiasis and the pathogenic yeasts. In: Wonseiwicz M, editor. Medical mycology. 3rd ed. Philadelphia: W.B. Saunders., 531-581.
12. Frickie, S., C. Frickie, C. Schimmelpfenning, C. Oelkrug, U. Schonfelder, R. Blatz, C. Zilch, S. Faber, N. Hilger, M. Ruhnke and A.C. Rodloff, 2010. A real-time PCR assay for the differentiation of Candida species. J. Appl. Microbiol., 109: 1150-1158.
13. Lau, A., T.C. Sorrell, S. Chen, K. Stanley, J. Iredell and C. Halliday, 2008. Multiplex tandem PCR: a novel platform for rapid detection and identification of fungal pathogen from blood culture specimens. J. Clin. Microbiol., 46: 3021-3027.
14. Cornet, M., B. Sendid, C. Fradin, C. Gaillardin, D. Poulain and H.V. Nguyen, 2011. Molecular identification of closely related Candida Species using two ribosomal intergenic spacer fingerprinting methods. J. Mol. Diagn., 13: 12-22.
15. Mujika, M.T., J.L. Fingnelievich, Jewchowiczv and Lovannittica, 2004. Prevalance of Candida albicans and Candida nonalbicans in clinical samples during 1999-2001. Rev. Argent. Microbiol., 36: 107-112.
16. Deorukhkar, S.C. and S. Saini, 2012. Species distribution and antifungal susceptibility profile of Candida species isolated from blood stream infections. Journal of Evolution of Medical and Dental Sciences 1: 241-249.
17. Hitchcock, C.A., G.W. Pye, P.F. Troke, E.M. Johnson and D.M. Warnock, 1993. Fluconazole resistance in Candida glabrata. Antimicrob. Agents. Chemother., 37: 1962-1965.
18. Kothavade, R.J., M.M. Kura, A.G. Valand and M.H. Panthaki, 2010. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. J. Med. Micobiol., 59: 873-880.
19. Shivaprakasha, S., K. Radhakrishnan and P. Karim, 2007. Candida spp. other than Candida albicans: A major cause of fungaemia in a tertiary care centre. Indian. J. Med Microbiol., 25: 405-407.
20. Guze, L. and L. Haley, 1957. Fungus infections of the urinary tract. Yale. J. Biol. Med., 22: 292-305.

21. Rivett, A.G., J.A. Perry and J. Cohen, 1986. Urinary candidiasis: a prospective study in hospital patients. Urol. Res., 14: 183-186.
22. Bouza, E., R. San Juan, P. Munoz, A. Voss and J. Kluytmans. 2001. A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI-004 study). European Study Group on Nosocomial Infection. Clin. Microbiol. Infect., 7: 532-542.
23. Alvarez-Lerma, F., J. Nolla-Salas, C. Leon, M. Palomar, R. Jorda, N. Carrasco and F. Bobillo, 2003. Candiduria in critically ill patients admitted to intensive care medical units. Intensive. Care. Med., 29: 1069-1076.
24. Kauffman, C.A., J.A. Vazquez, J.D. Sobel, H.A. Gallis, D.S. McKinsey, A.W. Karchmer, A.M. Sugar, P.K. Sharkey, G.J. Wise, R. Mangi, A. Mosher, J.Y. Lee and W. E. Dismukes, 2000. Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin. Infect. Dis., 30: 14-18.
25. Fisher, J.F., K. Kavanagh, J.D. Soebel, C.A. Kauffman and C. A. Newman. 2011. Candida Urinary Tract Infection: Pathogenesis. Clin. Infect. Dis., 52: 437-451.
26. Paul, N., E. Mathai, O.C. Abraham, J.S. Michael and D. Mathai. 2007. Factors associated with candiduria and related mortality. J. Infect., 55: 450-455.
27. Ostrosky-Zeichner, L. and P.G. Pappas, 2006. Invasive candidiasis in the intensive care unit. Crit. Care. Med., 34: 857-63.
28. Richet, H., P. Roux, C. Des Champs, Y. Esnault and A. Andremont, 2002. Candidemia in French hospitals: incidence rates and characteristics. Clin. Microbiol. Infect., 8: 405-412.
29. Leenders, N.H.J., J.J. Oosterheert, M.B. Ekkelenkamp, D.W. De Lange, A.I.M. Hoepelman and E.J.G. Peters, 2011. Candidemic complications in patients with intravascular catheters colonized with Candida species: an indication for preemptive antifungal therapy?. Int. J. Infect. Dis., 15: 453-458.
30. O'grady, N.P., M. Alexander, E.P. Dellinger, J.L. Gerberding, S.O. Heard, D.G. Maki, H. Masur, R.D. McCormick, L.A. Mermel, M.L. Pearson, I.I. Raad, A. Randolph and R.A. Weinstein, 2002. Guidelines for the prevention of intravascular catheter-related infections. Centers for Disease Control and Prevention. MMWR. Recomm. Rep., 51: 1-29.
31. Galbán. B. and F. Mariscal, 2006. Epidemiology of candidemia in ICU. Rev. Iberoam. Micol., 23: 12-15.