ORIGINAL ARTICLE

Characterisation and antifungal susceptibility profile of Candida species isolated from a tertiary care hospital

Prasadini Guru, Geetha Raveendran

Department of Microbiology, Government Medical College, Thiruvananthapuram, Kerala, India

ABSTRACT

Background: An increase in the prevalence of infections caused by non-albicans Candida (NAC) has been reported from many parts of the world. The increased isolation rates of NAC and a gradual shift in the antifungal susceptibility profile underlines the need for early and accurate diagnosis of Candida infections along with antifungal susceptibility testing. **Aim:** The aim of this study is to characterise Candida spp. isolated from various clinical samples and determine the antifungal susceptibility pattern. **Settings and Design:** This study was conducted in the Department of Microbiology, at a tertiary care referral centre, over a period of one year and included 200 Candida spp. **Materials and Methods:** Candida isolates were characterised by conventional techniques and CHROMagar. Antifungal susceptibility test was performed using disc diffusion method. Clinical details and risk factors were recorded and analysed. **Results and Conclusions:** *Candida albicans* was the most common species isolated, followed by *Candida tropicalis*. The Candida isolates were more susceptible to Amphotericin B than other antifungal agents tested. Diabetes mellitus appeared to be the most common predisposing factor for the Candida infections, followed by indiscriminate drug usage.

Key words: Antifungal susceptibility testing, *Candida albicans*, non-albicans Candida, risk factors

INTRODUCTION

Candidiasis is the most common fungal disease in humans. Candida species is endogenous and the disease represents opportunistic infection. The clinical manifestations of the disease are extremely varied, ranging from acute, subacute and chronic to an episodic involvement. It may be localised or may become systemic. They cause diseases with severity ranging from benign to potentially life-threatening infection. The increase in the predisposing conditions in recent years has resulted in a concurrent increase in the number of patients who suffer from candidiasis. *Candida albicans* (CA) remains the predominant spp. causing over the half of all the yeast infection cases in the world.[1] Increase in the prevalence of yeast infections caused by non-albicans Candida (NAC) has been reported in many parts of the world. Accurate species identification is important for the treatment of the Candida infections as the NAC continues to be increasingly documented with decreased susceptibility to antifungal agents.[2] Hence, this study was undertaken to isolate and speciate Candida from cases where candidiasis was suspected clinically, to determine the antifungal susceptibility, and to analyse the predisposing conditions for candidiasis.

MATERIALS AND METHODS

A total of 200 Candida isolates from various clinical specimens were taken up for the study. A detailed clinical history was taken to determine the associated risk factors. The various clinical specimens were collected and

Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/0972-1282.184753

Address for correspondence: Dr. Prasadini Guru, E-mail: drpgopal@gmail.com

How to cite this article: Guru P, Raveendran G. Characterisation and antifungal susceptibility profile of Candida species isolated froma tertiary care hospital. J Acad Clin Microbiol 2016;18:32-5.

processed as per the standard microbiological procedures.[3] The Candida isolates which were obtained were further speciated by the standard protocol for yeast identification which included the following tests: Gram's stain, culture on Sabouraud's dextrose agar, germ tube test, cornmeal agar morphology (Dalmau technique), sugar fermentation test and sugar assimilation test (auxanographic plate method). In addition, CHROMagar (BioMérieux) morphology was also studied.

Antifungal susceptibility test was done using the Clinical Laboratory Standards Institute, 2010 method for antifungal disc diffusion susceptibility for yeasts with approved guideline M44-A. We used the following antifungal discs: Fluconazole (25 μg) (HiMedia), Itraconazole (10 μg) (HiMedia) and Amphotericin B (20 mcg) (HiMedia).

RESULTS

A total of 200 clinical isolates of Candida from various clinical specimens were processed during the study. The rate of isolation of the Candida species was more in females than in males [Table 1].

Table 2 shows the age distribution in the culture positive cases. The highest incidence was seen in the age group above 60 years 48 (24%).

Table 3 shows the various clinical samples from which Candida species were isolated.

Table 4 shows the distribution of various Candida species among the isolates. CA was the predominant isolate.

Table 5 shows the distribution of various Candida species among the different samples received.

An analysis of the risk/predisposing factors in patients from whom the Candida species were isolated showed that 45 (22.5%) had underlying diabetes mellitus, 32 (16%) were on long-term multiple antibiotics and 4.5% had immunosuppression due to other causes [Table 6].

CA was isolated in more numbers when compared to NAC in all samples, except from immunosuppressed patients. Table 7 shows species distribution according to associated risk factors.

All the isolates of Candida were subjected to antifungal susceptibility testing. A total of 196 (98%) isolates were found to be sensitive to Amphotericin B, whereas only 147 (73.5%) were sensitive to Fluconazole [Table 8].

Table 1: Gender-wise distribution of patients

Gender	Number	Percentage
Female	109	54.5
Male	91	45.5
Total	200	100

Table 2: Age distribution of patients

Age in years	Number
≤10	43
11-20	18
21-30	31
31-40	25
41-50	18
51-60	17
>60	48

Table 3: Clinical specimens from which Candida were isolated

Specimens	Number
Urine	106
Exudate	31
High vaginal swab	27
Respiratory tract samples	15
Blood	15
Catheter tip	6
Total	200

Table 4: Distribution of Candida species

Species	Number	Percentage
Candida albicans	90	45
Candida tropicalis	50	25
Candida glabrata	32	16
Candida parapsilosis	28	14
Total	200	100

Table 5: Distribution of Candida species in various samples

Specimen	Isolate			
	Candida albicans	*Candida tropicalis*	*Candida glabrata*	*Candida parapsilosis*
Urine	25	34	23	24
Exudate	26	3	1	1
High vaginal swab	18	3	6	0
Respiratory tract samples	10	4	0	1
Blood	9	4	1	1
Catheter tip	2	2	1	1
Total	90	50	32	28

DISCUSSION

Due to the variable clinical presentation of Candida infections, it becomes very important to identify these pathogens from all the routine culture specimens received at the laboratory irrespective of clinical diagnosis. Candida

spp. differ in their antifungal susceptibility and virulence factors. Thus, identification of Candida up to species level along with antifungal susceptibility becomes very essential. Early identification and speciation of Candida is important in selecting appropriate antifungal treatment since resistance to polyenes has been reported in Candida spp., especially *Candida tropicalis*, *Candida glabrata*, and CA.[4]

Although candidiasis can occur at all ages, studies by Dalal and Kelkar showed the highest incidence of candidiasis to be in the age group of 21–40 years.[5] These findings were in concurrence with those of our study where the age group of >18 years to <45 years was that which had the highest incidence of candidiasis. This might be because this age group is associated with higher hormonal variation, sexual activity and rates of pregnancy.

Female patients contributed 54.5% and male patients 45.5%. Similar female predominance by noted by Kandhari *et al.* (61.2%), Dharwad and Saldanha (64%).[6] This could be due to the higher number of samples which were collected from female patients.

Table 6: Predisposing factors in various patients

Predisposing factors	Number of patients	Percentage
Diabetes mellitus	45	22.5
Chronic drug therapy	32	16
Pregnancy	24	12
Sepsis	9	4.5
Immunosuppression	16	8
Preterm/low birth weight	8	4
Inferior vena cava, intrauterine device	8	4
No specific predisposing factor	58	29
Total	200	100

Table 7: Species distribution according to associated risk factors

Risk factor	*Candida albicans*	*Candida tropicalis*	*Candida glabrata*	*Candida parapsilosis*
Diabetes mellitus	22	14	3	6
Chronic drug therapy	20	8	3	1
Pregnancy	12	8	2	2
Immunosuppression	4	8	3	1
Sepsis	5	1	3	0
Preterm/low birth weight	4	2	1	1
Devices *in situ*	5	1	1	1
Total	72	42	16	12

In this study, among the 200 isolates, CA (45%) was the major species isolated. A similar prevalence was noted by many other researchers; Price *et al.*[7] found CA to be the most common isolate. However, we observed that when put together, NAC (55%) are more frequently encountered than CA (45%). This is in agreement with the studies conducted by Chakrabarti *et al.* and Mokaddas *et al.*[8-10] These findings seem to suggest that NAC are emerging as important pathogens. The predominant NAC isolated in our tertiary care centre was *C. tropicalis*.

The association of various risk factors was studied in all the 200 patients. Diabetes mellitus was the most frequently associated risk factor. In this group, the most commonly isolated species was CA. A history of multiple antibiotics usage was the second most frequently associated risk factor (22%). The drugs which were incriminated were mainly antibiotics, corticosteroids and contraceptive pills. Pregnancy was the third most common predisposing factor. In this group, CA was the most common species isolated. Occurrence of candidal vulvovaginitis in pregnant women causes a risk of transmission to new-born infants.[11] Other significant risk factors were immunosuppression, sepsis, preterm/low birth weight and indwelling devices.

The antifungal susceptibility pattern showed that 53 (26.5%) of the Candida isolates were resistant to Fluconazole, 19 (9.5%) were resistant to Itraconazole and 4 (2%) were resistant to Amphotericin B.[12,13] It was also observed that resistance was more in the NAC group when compared to CA.

CONCLUSIONS

Over the last few decades, fungal infections are increasing at an alarming rate. This is a great challenge to health-care professionals. It is important to monitor the resistance trends and distribution of Candida spp. in the face of increasing usage of potent, broad-spectrum antibacterial agents in hospitals across India. We should develop local guidelines on treatment based on the epidemiology of infection in the Indian subcontinent. Continued surveillance will be important to document changes in epidemiological features of candidiasis and antifungal

Table 8: Distribution of isolates according to anti-fungal susceptibility pattern

Isolates	Fluconazole, *n* (%)		Itraconazole, *n* (%)		Amphotericin B, *n* (%)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Candida albicans	72 (80)	18 (20)	84 (93.33)	6 (6.67)	90 (100)	0 (0)
Candida tropicalis	35 (70)	15 (30)	46 (92)	4 (8)	49 (98)	1 (2)
Candida glabrata	20 (62.5)	12 (37.5)	27 (84.37)	5 (15.63)	29 (90.63)	3 (9.37)
Candida parapsilosis	20 (71.4)	8 (28.6)	24 (85.7)	4 (14.3)	28 (100)	0 (0)
Total	147 (73.5)	53 (26.5)	181 (90.5)	19 (9.5)	196 (98)	4 (2)

susceptibilities. Therefore, the description of Candida infections at the species level and research on antifungal sensitivity will be very useful in the treatment and prevent the development of resistance.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20:133-63.
2. Page BT, Kurtzman CP. Rapid identification of Candida species and other clinically important yeast species by flow cytometry. J Clin Microbiol 2005;43:4507-14.
3. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. Netherlands: Elsevier; 2007. p. 696-712.
4. Spampinato C, Leonardi D. Candida Infections, Causes, Targets, and Resistance Mechanisms: Traditional and Alternative Antifungal Agents. BioMed Research International 2013. Article ID 204237, 13 pages.
5. Dalal PJ, Kelkar SS. Clinical patterns of Candida infections in Bombay. Indian J Dermatol Venereol Leprol 1980;46:31-2.
6. Dharwad S, Saldanha DR. Species identification of Candida isolates in various clinical specimens with their antifungal susceptibility patterns. J Clin Diagn Res 2011;5 Suppl 1:1177-81.
7. Price MF, LaRocco MT, Gentry LO. Fluconazole susceptibilities of Candida species and distribution of species recovered from blood cultures over a 5-year period. Antimicrob Agents Chemother 1994;38:1422-4.
8. Mokaddas EM, Al-Sweih NA, Khan ZU. Species distribution and antifungal susceptibility of Candida bloodstream isolates in Kuwait: A 10-year study. J Med Microbiol 2007;56(Pt 2):255-9.
9. Chakrabarti A, Ghosh A, Batra R, Kaushal A, Roy P, Singh H. Antifungal susceptibility pattern of non-albicans Candida species & distribution of species isolated from candidaemia cases over a 5 year period. Indian J Med Res 1996;104:171-6.
10. Mini PN. Fungal isolates 2014. J Acad Clin Microbiol 2015;17:36-9.
11. Narain S, Shastri JS, Mathur M, Mehta PR. Neonatal systemic candidiasis in a tertiary care centre. Indian J Med Microbiol 2003;21:56-8.
12. Passos XS, Costa CR, Araújo CR, Nascimento ES, e Souza LK, Fernandes Ode F, *et al.* Species distribution and antifungal susceptibility patterns of Candida spp. bloodstream isolates from a Brazilian tertiary care hospital. Mycopathologia 2007;163:145-51.
13. Knechtel SA, Klepser ME. Amphotericin B resistance: Epidemiology, mechanisms, and clinical relevance. J Invasive Fungal Infect 2007;1:93-8.