Anita et al. | Article 7837
J Pure Appl Microbiol. 2023;17(1):427-433. doi: 10.22207/JPAM.17.1.34
Received: 11 May 2022 | Accepted: 17 January 2023
Published Online: 02 March 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Speciation and Antifungal Susceptibility Testing of *Candida* isolated from Immunocompromised Patients of a Tertiary Care Centre in Gujarat, India

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Abstract

***Candida* species are one of the most common causes of opportunistic infections in immunocompromised patients. An upsurge in incidence of less common species of *Candida* have been documented since last few years as the major cause of candidemia all over the world and they are also less susceptible to azoles, particularly fluconazole, than *C. albicans.* The purpose of study was speciation and antifungal susceptibility testing of *Candida* isolates, obtained from immunocompromised patients. The study included 150 consecutive immunocompromised patients and was initiated after the ethical approval from Sumandeep Vidyapeeth Institutional Ethical Committee (SVIEC). Informed consent was obtained from all and a Detailed Questionnaire regarding the Patient's history and clinical findings were noted. All the specimens were subjected to a battery of microbiological examination for isolation, identification, and antifungal susceptibility testing. In our study, the incidence of candidiasis in immunocompromised patients was43.1%.C.tropicalis(n=23,37.09%) was the most common species isolated followed by *C. albicans* (n=20, 32.25%), *C. glabrata* (n=15,24.19%) and *C. parapsilosis* (n=4, 6.45%). The isolates showed 100% sensitivity to Amphotericin B and Nystatin, whereas 37.09% sensitivity to Ketoconazole, 20.9% sensitivity to clotrimazole, 19.35% sensitivity to itraconazole and 14.5% sensitivity to Fluconazole. The study undoubtedly indicates a substantial move in the species from *C. albicans* to Non-albicans *Candida* and with added resistance to common antifungal drugs.**

Keywords: *Candida albicans*, Non-*albicans Candida*, Immunocompromised Patients, Resistance

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Citation: Anita, Kumar S, Pandya HB. Speciation and Antifungal Susceptibility Testing of *Candida* isolated from Immunocompromised Patients of a Tertiary Care Centre in Gujarat, India. *J Pure Appl Microbiol.* 2023;17(1):427-433. doi: 10.22207/JPAM.17.1.34

INTRODUCTION

Candida species are the constituent of normal flora of human body commonly found in skin, mucous membranes, throughout the gastrointestinal tract and female genital tract, mainly in the vagina of pregnant women.[1] Candidiasis is a term used for fungal infection affecting, mucosa, skin, internal organs of the body and nails. It mainly occurs as a secondary infection in an individuals with some underlying immunocompromised condition, resulting in the high mortality.[1] *Candida* species are the 4th most common primary blood stream organism and constitute 8th most common pathogen to cause health care associated infections.[2]

Among *candida* species, *Candida albicans* is the most common cause of *candidiasis* accounting for about 60-80% of infections.[3] During the last century, two significant predisposing factors have been perceived; first was the advent and indiscriminate use of antibiotics and second was the emergence of AIDS pandemic, and along with that, the changing trend of *Candida* species from non-pathogenic to pathogenic organism was facilitated by various virulence factors, such as adherence to host tissues, medical devices, formation of biofilms, and secretion of extracellular hydrolytic enzymes.[4] This explains the fact that with the escalation of immunocompromised patients, candidiasis has become an emerging, alarming opportunistic disease. It has been noted in the recent years that there is a remarkable shift from *Candida albicans* to *Non-albicans Candida* (NAC) as a major pathogen causing infection in human, also there is change in the antifungal drug susceptibility pattern and it is mostly due to over and random use of antifungal agents.[5,6] Many new antifungal drugs are available nowadays. The various manifestations of candidiasis often create dilemma for the physician, both in diagnosis and treatment, and are frequently the major cause of death in the patient. Therefore, it is very important to find out the species of *Candida*, and their antifungal drug susceptibility pattern in specimens for better management of the patients and also to reduce mortality. Hence, this study was conducted to identify the different species of *Candida* isolates and to explore its antifungal susceptibility pattern.

MATERIALS AND METHODS

This Prospective cross-sectional study was undertaken for a duration of 19 months from January 2015 to July 2016 at the Tertiary Care Centre, Gujarat. Approval of Sumandeep Vidyapeeth Institutional Ethical Committee was taken prior to the initiation of work (SVIEC NO. 108). A total of 150 consecutive patients with varied immunocompromised conditions like HIV seropositivity, Diabetes mellitus, Transplant recipient, Tuberculosis, Malignancy, and haemodialysis with clinical manifestations were included in the study. Those patients, not willing to participate were excluded from the study. Different specimen like sputum, urine (Midstream and Catheterized), blood, pus, and oral swab were aseptically collected and were processed for the identification of yeast.

Methodology

Direct Microscopy

The clinical sample was subjected to Gram's staining, for the identification of yeast cells and pus cells. Presence of hyphae and pseudohyphae were examined under the light microscope (100X magnification). The Staining was done according to the standard protocols.[7]

Culture

All the samples were inoculated on Sabouraud Dextrose Agar (SDA), with chloramphenicol and chlorhexidine, (Procured from HiMedia) and Blood Agar (BA) The plates were incubated at 37°C for 24- 48 hours, after which growth was identified by the following characteristics; rapidity of growth, color and morphology of the colony on the obverse and pigmentation on the reverse.

Blood culture

For blood culture, 8-10 ml(adult) or 3-5 ml(children) venous blood was collected aseptically and inoculated in BD BACTEC aerobic plus/F (Becton-Dickinson, New Jersey, USA) bottles in BD BACTEC FX40, Series FF 7004 automated blood culture system was used. The bottles were inserted either immediately or within 30 minutes inside the machine. Whenever the machine gave

an alert signal, or sound, the respective bottle were removed and were sub cultured on Blood agar and Saboraud dextrose agar with chloramphenicol and chlorhexidine, (Procured from HiMedia)

Identification of the isolates

The identification of the growth as *Candida* was done by colony morphology (cream colored, smooth and pasty colonies) and Gram's stain. The Speciation of *Candida* was done by performing various conventional tests such as Germ tube test,[8] chlamydospore formation, color change on HiCromeAgar [Hichrome *Candida* Differential Agar (HiMedia Pvt. Ltd., Mumbai)].[9] Sugar fermentation and Assimilation tests.[10] HiCrome Agar is very easy and low cost method for identification of *Candida* species. One important advantage of HiCrome Agar is ability to detect mixed cultures.[11]

Antifungal Susceptibility Testing

Antifungal Susceptibility Testing was done by Disc diffusion method, which is very easy to perform and results can be interpreted by 24 hours. Therefore it can be used for diagnostic purposes on routine basis.[12] MHA (Muller Hinton Agar) supplemented with glucose and methylene blue, was prepared.[13,14] Following antifungal discs were used amphotericin B (100 units), fluconazole (25mcg), clotrimazole (10mcg), itraconazole (10mcg), ketoconazole (10 mcg), Nystatin (100 units) (procured from HiMedia, Mumbai). Antifungal susceptibility test was performed according to CLSI guidelines documented in M44-A.[15]

Table 1. Association of *Candida* infection with Age and Gender

Variables		With *Candida* infection	Without *Candida* infection
Gender	Male (n=81)	34(41.9%)	47(58.02 %)
	Female(n=69)	28(40.57 %)	41(59.42 %)
Age-Group	0-10(n=15)	5(33.3 %)	10(66.6 %)
	11-20(n=10)	2(20 %)	8(80%)
	21-30(n=20)	6(30%)	14(70%)
	31-40(n=30)	10(33.3%)	20(66.6%)
	41-50(n=40)	15(37.5%)	25(62.5%)
	51-60(n=12)	10(83.3%)	2(16.6%)
	61-70(n=17)	9(53 %)	8(47 %)
	71-80(n=4)	3(75 %)	1(25 %)
	81-90(n=2)	2(100 %)	0(0 %)

RESULTS

150 clinical samples were collected from 150 immunocompromised patients (3-85yrs) admitted at our institute. Out of which 62 were positive for *Candida* species which shows the incidence of 43.1 % in immunocompromised patients. *C.tropicalis*(n=23,37.09%) was the commonest isolate followed by *C. albicans* (n=20, 32.25%), *C. glabrata* (n=15,24.19%) and *C. parapsilosis* (n=4, 6.45%) (Figure).

Association of *Candida* infection with age and gender (Table 1)

Majority of the patients with *Candida* infection belonged to the younger category

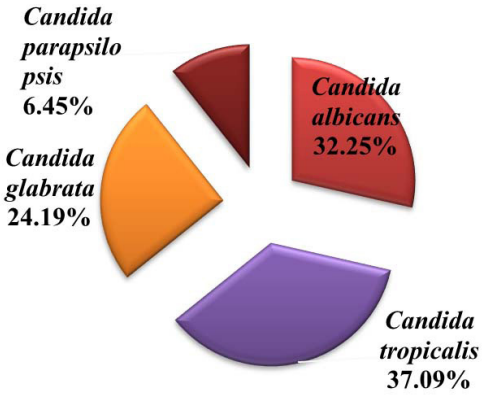

Figure. Speciation of *Candida* isolated from various immunocompromised patients

Table 2. Ward wise distribution of *Candida* infection

Ward	Total Sample	With Candida n=62	Without Candida N=88
ICU	80	42	38
Medicine	32	13	19
Paediatric ward	15	02	13
Surgery ward (n=6)	12	03	09
Neuro ward	01	00	01
TB ward	08	02	06
Ortho ward	02	00	02
Total	150	62	88

Table 3. Association of *Candida* species with various clinical specimens

	Culture positivity				Total positive
	C.albicans	*C.glabrata*	*C.tropicalis*	*C.parapsilosis*	
Urine (n=87)	14	7	15	4	40(45.9%)
Sputum (n=45)	4	6	6	-	16(35.5%)
Blood (n=12)	-	2	2	-	4(33.3%)
Pus (n=4)	-	-	-	-	0
Oral Swab (n=2)	2	-	-	-	2(100%)
Total	20(32.2%)	15(24.1%)	23(37.05%)	4(6.45%)	28(48%)

Table 4. Antifungal Susceptibility Pattern of *Candida* Species

Candida species	Antifungal	Sensitivity	(%)	Resistance	(%)
C. tropicalis (n=23)	Fluconazole	2	8.69%	21	91.30%
	Ketoconazole	3	13.04%	20	86.95%
	Itraconazole	-		23	100%
	Clotrimazole	2	9.52%	21	91.30%
	Amphotericin-B	23	100%	-	
	Nystatin	23	100%	-	
C.albicans (n=20)	Fluconazole	20	100%	-	
	Ketoconazole	6	30%	14	70%
	Itraconazole	-		20	100%
	Clotrimazole	2	10%	18	90%
	Amphotericin-B	20	100%	-	
	Nystatin	20	100%	-	
C.glabrata (n=15)	Fluconazole	3	20%	12	80%
	Ketoconazole	10	66.66%	5	33.33%
	Itraconazole	7	46.66%	8	53.33%
	Clotrimazole	10	66.66%	5	33.33%
	Amphotericin-B	15	100%	-	
	Nystatin	15	100%	-	
C.parapsilosis (n=4)	Fluconazole	3	75%	1	25%
	Ketoconazole	3	75%	1	25%
	Itraconazole	2	50%	2	50%
	Clotrimazole	2	50%	2	50%
	Amphotericin-B	4	100%	-	
	Nystatin	4	100%	-	

followed by the middle age group. Gender-wise, we found slight male predominance (54%) than female (46%).

Ward wise emergence of *Candida* infection (Table 2)

Most of the *Candida* species were isolated from ICU (42/80, 52.5 %) followed by Medicine ward (13/32, 40.6 %). TB ward (2/8, 25%), Surgery ward (3/12, 25%) and Paediatric ward (2/15, 13.3%).

Sample wise distribution of *Candida* species (Table 3)

Most of the *Candida* species were isolated from urine sample (40/87, 45.9%). *C tropicalis* (15/87) was the most common isolates followed by *C. albicans* (14/87), *C. glabrata* (7/87) and *C. parapsilosis* (4/87). From sputum sample, *C. glabrata* (6/45) and *C. tropicalis* (6/45) were predominant followed by *C.albicans* (4/45). *C. glabrata* (2/12) and *C. tropicalis* (2/12) were isolated from blood sample. From oral swab *C. albicans* (2/2) was isolated.

Antifungal Susceptibility Pattern of Candida Species (Table 4)

All the isolated species of *Candida* were 100% susceptible to amphotericin B, and nystatin. All the isolated species of *C. albicans* (100%), *C. parapsilosis* (75 %) and *C. glabrata* (20%) were susceptible to fluconazole whereas *C. tropicalis* isolates showed 91 % resistance to fluconazole. All the *Candida* isolates showed varying degree of resistance to ketoconazole, such as *C. albicans* (70), *C. tropicalis* (86.9), *C. glabrata* (33.3) and *C. parapsilosis*. The sensitivity of Clotrimazole for *C. albicans* was 10%, for *C. tropicalis* was 9.52%, for *C. glabrata* was 66.6% and for *C. parapsilosis* was 50%. *C. tropicalis* and *C. albicans* showed 100% resistance to Itraconazole, while *C. glabrata* showed 53.3% and *C. parapsilosis* showed 50% resistance to Itraconazole.

DISCUSSION

In recent years, *Candida* spp. have emerged as a main pathogen of a variety of human infections which clearly proves that there is an extreme change in model of infectious disease. Those microorganisms previously non-pathogenic are now the commonest cause of morbidity and mortality. Among *candida* species, *C. auris* have emerged as a pandrug resistant organism which lead to prolong hospital stay and healthcare cost.[16] In the present study we found 62 isolates of *Candida* species from 150 clinical specimens. About 80% of the *Candida* species were isolated from urine and sputum sample, which indicates the higher incidence and distribution of *Candida* species causing urinary tract and respiratory tract infections. Among *Non-albicans Candida*, *Candida* tropicalis was the most common species isolated followed by *C. albicans, C. glabrata and C. parapsilosis* respectively. Our results were in accordance with the studies done by D. Kumar et al.[17] Lata R Patel et al.[18] and Ayushi Jain et al.[19] which revealed *C. tropicalis* as the most prevalent species. among the *Candida* isolates. There were many studies which showed that the incidence of NAC *(Non-albicans Candida*) ranges from 54-75%. Golia et al.[20] A study done by Vijaya et al.[21] showed *C. albicans* (45.9%) have a lower incidence than *Non- albicans Candida* (54.1%). A study done by Manchanda et al.[22] also presented *Non-albicans Candida* (72.4%) with a higher incidence than *C.albicans* (27.5%). Recently a study done by Urvashi Chongtham et al.[23] showed that *Non-albicans Candida* (NAC) species have replaced *Candida albicans* with decreased susceptibility to commonly used antifungal agents. In the latest decade, increasing incidence of candidemia caused by *Non-albicans Candida* (NAC) species have been reported, which has led to the rise of NAC investigations. Their ability to cause recurrent infections, higher resistance to antifungal drugs, and potential to cause outbreaks infections has led to further research. In the present study, the prevalence of *Non-albicans Candida* was significantly high, this might be due to random use of antifungal drugs, which lead to elimination of more sensitive *C. albicans* and selection of azole resistant *Non-albicans Candida*. The antifungal susceptibility pattern was different for different *Candida* spp Such as, all *Candida* isolates tested were susceptible to Amphotericin B and Nystatin. All the *Candida* isolates were tested for Azole group showed reduce susceptibility. Whereas all *C. albicans* were found sensitive to fluconazole. D. Kumar et al.[17] reported the susceptibility profile of *Candida* isolates as 100% sensitive to Amphotericin B and Voriconazole and 89.3% were sensitive to fluconazole. Sonu Panwar et al.[24] reported the susceptibility profile of *Candida* isolates as 94.1% were sensitive to AMB, 58.3% to Fluconazole, 72.5 % to Ketoconazole, and 85 % to Nystatin which is very much similar to our study. From above listed study it is clear that these NAC species are resistance to most of the commonly prescribed antifungal drugs. Therefore, The emergence of multidrug and pandrug-resistant *C. albicans* and NAC noted in several parts of the world necessitates continuous antifungal susceptibility testing and monitoring.[25]

CONCLUSION

In present study, the incidence of candidiasis was 43.1 % with *C. tropicalis* as the most common species isolated. Identification of *Candida* species helps to start early suitable antifungal dugs and also it helps to reduces the morbidity and mortality. The antifungal drugs fluconazole and ketoconazole were most commonly used drugs but least effective against

NAC species, higher resistance against them were shown by *C. tropicalis* and *C. glabrata.* This Changing trends in the antifungal susceptibility pattern recommends for antifungal susceptibility testing of *Candida* isolates on routine basis in clinical microbiology laboratories.

ACKNOWLEDGMENTS

The authors would like to thank Sumandeep University, India, for giving permission to conduct this research study successfully.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Sumandeep Vidyapeeth Institutional Ethical Committee with reference number SVIEC NO-108.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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