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Original Article

Microbiology Section

Isolation, Identification and Antifungal Susceptibility Testing of *Candida* Species: A Cross-sectional Study from Manipur, India

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ABSTRACT

Introduction: Candidiasis is an opportunistic infection which occurs due to indiscriminate and prolonged use of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents, diabetes mellitus, Human Immunodeficiency Virus (HIV), chronic renal failure, haemodialysis, renal transplantation or indwelling urinary catheter. Recently, Non-*albicans Candida* (NAC) species have replaced *Candida albicans* and emerged as an important opportunistic pathogens exhibiting decreased susceptibility to commonly used antifungal agents. Early speciation of *Candida* isolates along with their antifungal susceptibility testing not only will restrict the empirical use of antifungal agent but also greatly influence the treatment options for the clinicians.

Aim: To speciate *Candida* isolates from various clinical specimens and to determine their antifungal susceptibility pattern.

Materials and Methods: This study was a cross-sectional study carried out in the Mycology Section, Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India, from September 2016 to August 2018. *Candida* isolates were identified using standard microbiological procedures and speciation was done following conventional and HiCrome differential media. Antifungal susceptibility testing was determined by using Clinical and Laboratory Standards Institute (CLSI) disk diffusion method. Data analysis was done using descriptive statistics and Chi-square test.

Results: A total of 100 isolates were identified from different clinical specimens, which included 43 (43%) from sputum, 34 (34%) from urine, in majority. Highest age was 92 years and lowest age was one year from whom the isolates were detected and females (57%) outnumbered males (43%) patients. Predominant *Candida* isolates were *Candida albicans* (44%), *Candida tropicalis* (32%). Among the azoles, the most sensitive agent was voriconazole (86%) and least was ketoconazole (56%), 81% of the total isolates were found sensitive to amphotericin B.

Conclusion: The present study demonstrated that NAC spp. have surpassed *Candida albicans* and there is an increase in the resistance of the *Candida* isolates to commonly used antifungal agents. Therefore, this study highlights the need for speciation of *Candida* isolates upto species level and to determine the antifungal susceptibility pattern to decrease the morbidity and mortality of the patients.

Keywords: Antifungal agents, *Candida albicans*, Non-*albicans Candida*, Speciation

INTRODUCTION

Candidiasis is the most common fungal disease found in humans affecting mucosa, skin, nails and internal organs of the body. It is caused by various species of yeast-like fungi belonging to genus *Candida* with *Candida albicans* as the representative species. Other pathogenic species include *Candida tropicalis, Candida krusei, Candida glabrata, Candida guilliermondii, Candida parapsilosis, Candida lusitaniae, Candida kefyr, Candida rugosa, Candida dubliniensis* and *Candida viswanathii* [1].

Candidiasis is an opportunistic infection occurring in presence of predisposing factors like extensive and prolonged administration of broad-spectrum antimicrobials, corticosteroids, immunosuppressive agents and cytotoxic drugs, diabetes mellitus, HIV, chronic renal failure, haemodialysis, renal transplantation or indwelling urinary catheter [2]. Till recently, *Candida albicans* was considered as the most frequently isolated *Candida* species accounting for 60-80% of the fungal infections but NAC have now become predominant [3]. NAC spp. such as *Candida glabrata*, *Candida krusei* and *Candida tropicalis* are emerging opportunistic pathogens and they exhibit varying degree of resistance, either intrinsic or acquired or both, to commonly used antifungal drugs [4]. Indiscriminate and widespread use of fluconazole for the prophylaxis and treatment of candidiasis has led to a reduction of infections due to *Candida albicans* but that has led to the emergence of *Candida* infections caused by fluconazole resistant NAC [5].

Hence, speciation and antifungal susceptibility of clinical isolates of *Candida* has gained significance in the management of *Candida* infections. Although, several studies regarding the speciation and antifungal susceptibility patterns of *Candida* isolates have been reported across the globe including different regions of India [6-11], such study is yet to be explored in Manipur, India. This study was taken up with the objective of generating data on different species of *Candida*, their characterisation upto the species level and to determine their antifungal susceptibility patterns.

MATERIALS AND METHODS

The study was a cross-sectional study which was carried out in the Department of Microbiology, Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal, Manipur, India, from September 2016 to August 2018. Informed written consent (prescribed format) was obtained from participating individuals. In case of minors, informed consent was taken from the parents/legal guardians. Privacy and confidentiality was maintained in all cases. Approval of ethical committee was obtained from the Institutional Ethical Committee (IEC) JNIMS vide no. Ac/06/IEC/JNIMS/2016(PGT) dated: Imphal, the 1st October, 2016.

Inclusion criteria: Patients of all age group and both sex with clinically suspected candidiasis, attending Outpatient and Inpatient Departments including intensive care units of JNIMS were included.

Exclusion criteria: Patients who were on antifungal treatment and refused to take part were excluded from the study.

Sample size calculation: Considering 95% confidence interval, 4% margin of error, Z score of 1.96 and prevalence rate of 4.03% [12], the sample size was taken 100 for the study using the following formula:

Sample size (n)=(z^2pq)/d^2 Where Z=Z-score, p=prevalence rate, q=(1-p), d=absolute allowable error.

Identification and Speciation of *Candida* Isolates

Clinical samples such as urine, blood, sputum, central line tip, oral swab, vaginal swab, nail clipping or skin scrapping were processed following standard techniques [13,14].

Direct microscopy: Urine, sputum, oral swab, vaginal swab, nail clipping or skin scrapping were subjected to Potassium Hydroxide (KOH) wet mount for yeast cells with pseudohyphae and gram stain to look for gram positive, budding yeast cells with or without pseudohyphae, pus cells, epithelial cells or bacteria.

Culture: All the clinical samples were cultured on Sabouraud's Dextrose Agar (SDA) and incubated at 25°C and 37°C. Regarding blood culture, 5-10 mL of blood for adults, 2-5 mL of blood for children, and 1-2 mL of blood for infants and neonates was inoculated first in 50-100 mL, 20-50 mL and 10-20 mL of Brain Heart Infusion (BHI) broth respectively and incubated at 37°C for seven days, examined daily for microbial growth (turbidity) followed by subculture on SDA and incubated at 37°C. On SDA, *Candida* produced creamy, smooth, pasty and convex colonies within 24-72 hours. Some species required more than three days to appear on culture medium.

Gram staining: Isolated colonies obtained on SDA were further subjected to gram staining to identify the budding yeast cell and pseudohyphae.

Urease test [13]: A urease test was done to rule out *Cryptococcus neoformans* which is urease positive.

Criteria used to Indicate *Candida* Infection in Various Samples

- **Urine:** Quantitative culture with colony count of >10^5 Colony-Forming Unit (CFU)/mL of urine is associated with infection in patients without indwelling catheters and >10^3 CFU/mL for catheterised patients. Pyuria usually supports diagnosis of *Candida* infection. Low colony counts in presence of pyuria were considered significant. Repeat isolation in same patient was also considered significant [4,14].
- **Sputum:** Considered acceptable on gram stain when 25 or more polymorphonuclear leukocytes were seen per oil immersion (100x) field with few (<10) squamous epithelial cells [15].
- **Blood:** Candidemia is defined as presence of at least one positive blood culture containing pure growth of *Candida* species with supportive clinical features [14].
- **Central venous tip:** Greater than 15 CFU on roll plate culture was considered positive of Catheter-Related Bloodstream Infection (CRBSI) [16].
- **Oral and vaginal swabs:** Direct demonstration of pseudohyphae along with yeast cells using KOH wet mount or gram stain [16].

Speciation

Conventional methods: Germ tube test, demonstration of chlamydospore formation on Cornmeal agar with Tween 80, sugar fermentation test and sugar assimilation test were employed for speciation [1,13].

Temperature test (Growth at 45°C): This test was used to differentiate *Candida albicans* (growth) from *Candida dubliniensis* (no growth). The temperature test was performed using Yeast-Peptone-Dextrose (YPD) broth, BHI and SDA, and incubated at 45°C for 10 days [17].

HiCrome *Candida* differential agar: The *Candida* isolates were subcultured on HICROME *Candida* differential agar for species identification according to the manufacturer's instructions [18].

1) ***Candida albicans*:** Light green coloured smooth colonies.
2) ***Candida dubliniensis*:** Dark green coloured smooth colonies.
3) ***Candida tropicalis*:** Blue to metallic blue coloured raised colonies.
4) ***Candida glabrata*:** Cream to white smooth colonies.
5) ***Candida krusei*:** Purple fuzzy colonies.
6) ***Candida guilliermondii*:** Light pink to pink colonies.
7) ***Candida parapsilosis*:** Light pink colonies.

Antifungal susceptibility testing: This was done by disk diffusion method according to CLSI (formerly NCCLS), 2009, M44-A2 guidelines using commercially available 6 mm antifungal discs (Himedia, Mumbai, India) such as fluconazole 25 µg, voriconazole 1 µg, amphotericin B 20 µg, itraconazole 10 µg and ketoconazole 30 µg [19].

Due to the lack of defined breakpoints for itraconazole, ketoconazole and amphotericin B arbitrary values based on other studies and manufacturer (HIMEDIA, Mumbai) guidelines were employed [16,20].

Interpretive Categories

Susceptible (S): The susceptible category implied that an infection due to the strain might be appropriately treated with the dose of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.

Susceptible-Dose Dependent (S-DD): The susceptible-dose dependent category included isolates with antifungal agent Minimum Inhibitory Concentration (MIC) that approached usually attainable blood and tissue levels and for which response rates might be lower than for susceptible isolates.

Resistant (R): This category included those resistant strains which were not inhibited by the usually achievable concentrations of the agent with normal dosage schedules or when zone diameters had been in a range where clinical efficacy had not been reliable in treatment studies [Table/Fig-1] [16,19,20].

Antifungal agent	Disk content	Sensitive (S)	Susceptible-Dose Dependent (S-DD)	Resistant (R)
Fluconazole	25 µg	≥19 mm	15-18 mm	≤14 mm
Voriconazole	1 µg	≥17 mm	14-16 mm	≤13 mm
Amphotericin B	20 µg	≥15 mm	13-14 mm	≤12 mm
Itraconazole	10 µg	≥17 mm	14-16 mm	≤13 mm
Ketoconazole	10 µg	≥28 mm	27-21 mm	≤20 mm

[Table/Fig-1]: Breakpoint zone diameter (mm) for *Candida* spp [16,19,20].

Quality control: Every batch of media prepared was checked for sterility by incubating at 37°C for 24 hours. *Candida albicans* American Type Culture Collection (ATCC) 90028 was used as quality control strain for the antifungal susceptibility testing.

STATISTICAL ANALYSIS

Data collected was entered in Microsoft excel sheet. Data was analysed using descriptive statistics. Analytical statistics such as Chi-square (χ^2) was done to test for association. A p-value <0.05 was taken as significant.

RESULTS

During the study period of two years, 100 isolates were identified from different clinical specimens, which included 43 (43%) from sputum and 34 (34%) from urine as shown in [Table/Fig-2]. A 28 isolates were collected from Outpatient and 72 from Inpatient Department.

Samples	No. of isolates
Urine	34
Sputum	43
Blood	7
Oral swab	5
Nail clipping	6
Catheter tip	1
Skin scrapping	1
Vaginal swab	2
Pus from diabetic foot	1
Total	100

[Table/Fig-2]: Distribution of samples.

Of the 100 isolates, 27 (27%) were obtained from the age group of >70 years and least number of 5 (5%) isolates was seen in age <10 years (5%). Mean±standard deviation age was 49.49±24.04 years median was 51.5 years. Highest age, from whom the isolate was detected, was found to be 92 year and lowest age to be one year. Female (57%) outnumbered male (43%) participants as shown in [Table/Fig-3].

Age (years)	Culture positive cases		Total
	Male (%)	Female (%)	
0-10	3 (7.0)	2 (3.5)	5
11-20	0	9 (15.8)	9
21-30	2 (4.7)	11 (19.3)	13
31-40	3 (7.0)	6 (10.5)	9
41-50	6 (14.0)	8 (14.0)	14
51-60	8 (18.6)	5 (8.8)	13
61-70	6 (14.0)	4 (7.0)	10
>70	15 (34.9)	12 (21.1)	27
Total	**43 (43%)**	**57 (57%)**	**100**

[Table/Fig-3]: Distribution of patients according to age and sex.
χ^2=16.506 with 7 d.f.; p-value=0.021 (significant), Mean±SD=49.49±24.04, Median (Minimum-Maximum)=51.50 (1-92)

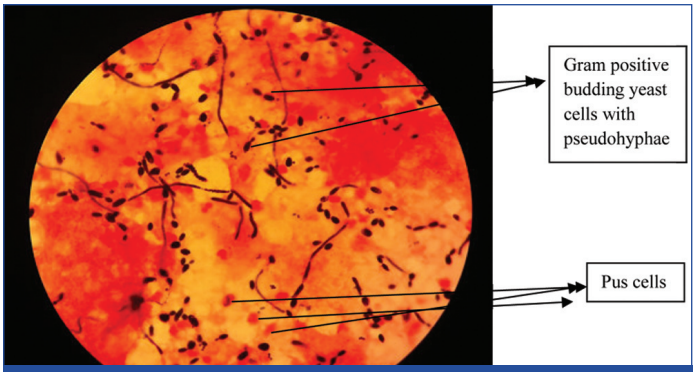
The most common risk factor was found to be prolonged antibiotic therapy (26%) followed by pregnancy (23%), diabetes (21%), HIV (16%) as depicted in [Table/Fig-4]. Gram stain smears and various isolates of *Candida* spp. on HICROME agar are shown in [Table/Fig-5-7].

Candida albicans comprised of 44% of the total isolates whereas the NAC spp. comprised of 56% of the total isolates. Among the NAC, *Candida tropicalis* (32%) was the most predominant species and least was *Candida glabrata* and *Candida parapsilosis* (4%) as shown in [Table/Fig-8].

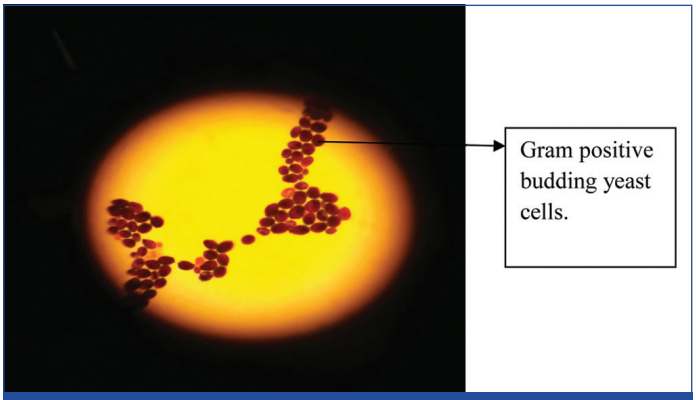
Maximum isolate of *Candida albicans* were from sputum 20 (46.5%) out of 43 and that of *Candida tropicalis* were from urine 14 (41.2%) out of 34 as shown in [Table/Fig-9].

Risk factors	Numbers
Prolonged antibiotic therapy	26
Pregnancy	23
Diabetes	21
HIV	16
Urinary Tract Infection (UTI)	6
Chronic Obstructive Lung Disease (COPD)	3
Corticosteroid therapy	3
Vaginitis	1
Onychomycosis	1
Total	**100**

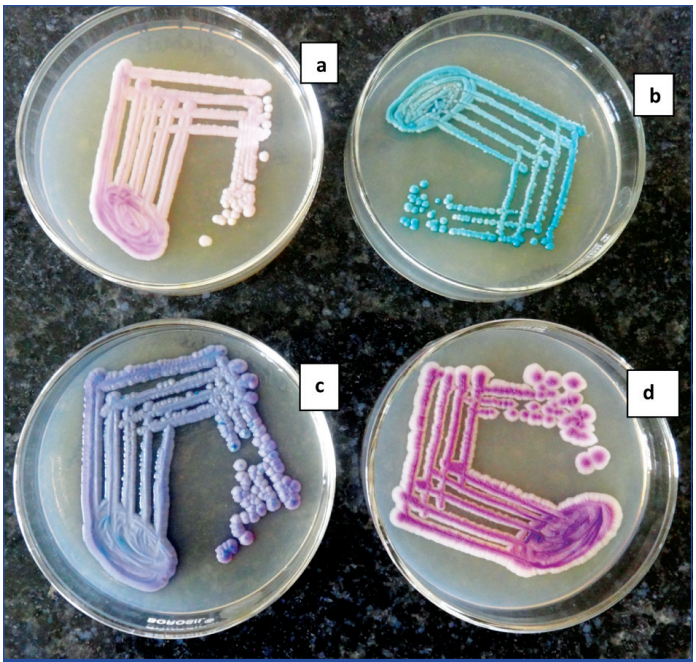
[Table/Fig-4]: Distribution of risk factors associated with candidiasis.



[Table/Fig-5]: Direct gram stain smear of sputum sample showing gram positive budding yeast cells with pseudohyphae and pus cells (100X).



[Table/Fig-6]: Gram stain smear from isolated colonies of SDA showing gram positive budding yeast cells (1000X).



[Table/Fig-7]: Various species of *Candida* on HICROME agar. a) *Candida glabrata;* b) *Candida albicans;* c) *Candida tropicalis;* d) *Candida krusei*.
Images for the rest of rest three species are not available as they are not well developed on HICHROME but differentiated by conventional methods

Candida species	Numbers
Candida albicans	44
Candida tropicalis	32
Candida guilliermondii	6
Candida krusei	5
Candida dubliniensis	5
Candida glabrata	4
Candida parapsilosis	4
Total	**100**

[Table/Fig-8]: Distribution of *Candida* spp and Non-*albicans Candida* (NAC) spp.

Species	Samples								
	Urine	Oral swab	Sputum	Blood	Pus from diabetic foot	Nail clipping	Catheter tip	Skin scrapping	Vaginal swab
C. albicans	11	4	20	2	0	4	1	0	2
C. tropicalis	14	0	11	4	1	2	0	0	0
C. krusei	3	0	1	1	0	0	0	0	0
C. glabrata	3	0	1	0	0	0	0	0	0
C. parapsilosis	1	1	2	0	0	0	0	0	0
C. gulliermondi	2	0	3	0	0	0	0	1	0
C. dubliniensis	0	0	5	0	0	0	0	0	0
Total	34	5	43	7	1	6	1	1	2

[Table/Fig-9]: Distribution of different species of *Candida* among various clinical specimens.
χ^2=47.46 With 48 d.f; p-value=0.495 (Insignificant)

Among the antifungals, the most sensitive agent was voriconazole (86%) and least was observed with ketoconazole (56%) as shown in [Table/Fig-10]. Chi-squares (χ^2) of fluconazole, voriconazole, itraconazole, ketoconazole and amphotericin B are 37.556, 13.546, 25.157, 13.546 and 24.189, respectively with degrees of freedom of 12 in all the antifungal agents. The findings were significant for fluconazole (p-value <0.001), itraconazole (p-value=0.014) and amphotericin B (p-value=0.019), and insignificant for voriconazole (p-value=0.331) and ketoconazole (p-value=0.331).

The present study showed female preponderance (53%) which might be attributed to more number of cases in female with UTI, pregnancy, vaginitis, prolonged contact with water in housewives as in case of onychomycosis. Similar findings were observed by Amar CS et al., and Khandari KC and Rama KM [25,26]. However, male preponderance has been reported by Patel LR et al., [27].

In this study, the NAC spp. (56%) had predominance over *Candida albicans* (44%). Among the NAC spp., *Candida tropicalis* was the

Species (No.)	Fluconazole			Voriconazole			Itraconazole			Ketonazole			Amphotericin B		
	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)	S (%)	SDD (%)	R (%)
C. albicans (44)	35 (79.5)	0	9 (20.5)	37 (84.1)	0	7 (15.9)	35 (79.5)	1 (2.3)	8 (18.2)	28 (63.6)	1 (2.3)	15 (34.1)	41 (93.2)	1 (2.3)	2 (4.5)
C. tropicalis (32)	10 (31.2)	3 (9.4)	19 (59.4)	26 (81.3)	1 (3.1)	5 (15.6)	18 (56.2)	8 (25)	6 (18.8)	16 (50)	2 (6.2)	14 (43.8)	21 (65.6)	8 (25)	3 (9.4)
C. krusei (5)	0	0	5 (100)	5 (100)	0	0	2 (40)	3 (60)	0	2 (40)	0	3 (60)	3 (60)	0	2 (40)
C. glabrata (4)	4 (100)	0	0	4 (100)	0	0	3 (75)	0	1 (25)	3 (75)	0	1 (25)	4 (100)	0	0
C. parapsilosis (4)	2 (50)	1 (25)	1 (25)	4 (100)	0	0	1 (25)	1 (25)	2 (50)	0	1 (25)	3 (75)	2 (50)	1 (25)	1 (25)
C. gulliermondi (6)	5 (83.3)	0	1 (16.7)	6 (100)	0	0	4 (66.7)	0	2 (33.3)	4 (66.7)	0	2 (33.3)	6 (100)	0	0
C. dubliniensis (5)	4 (80)	0	1 (20)	4 (80)	0	1 (20)	4 (80)	0	1 (20)	3 (60)	1 (20)	1 (20)	4 (80)	1 (20)	0
Total (100)	60 (60)	4 (4)	36 (36)	86 (86)	1 (1)	13 (13)	67 (67)	13 (13)	20 (20)	56 (56)	5 (5)	39 (39)	81 (81)	11 (11)	8 (8)

[Table/Fig-10]: Antifungal susceptibility pattern of *Candida* species.
S: Sensitivity, SDD: Susceptible dose dependent, R: Resistant

DISCUSSION

Candida spp. has been increasingly emerged as principal pathogens of opportunistic infections in healthcare settings. Therefore, early isolation, speciation and antifungal susceptibility testing are essential for the clinicians to choose the best therapeutic approach for the patients to reduce morbidity and mortality. Majority of the isolates in this study were obtained from sputum (43%) followed by urine (34%). This might be due to the fact that the presence of fungi (both yeasts and moulds) in sputum has been of increasing interest since the advent of antibiotics and steroids as common therapeutic agents. Moreover, *Candida* spp. are reported as seventh most common nosocomial pathogen in hospital settings causing 25% of all Urinary Tract Infections (UTI) in some of the previous studies [21]. Gopi A and Murthy NS observed that predominant isolates were from sputum (41.6%) and urine samples (20.4%) [22]. However, studies by Shaik N et al., and Joseph K et al., recovered maximum number of isolates from urine (60% and 46.9%, respectively) followed by respiratory samples (17.3% and 20.4%, respectively) [7,23].

Majority of the *Candida* isolates was obtained from age group of >70 years (27%) and least seen in <10 years (5%) of age. Similar findings were found by Joseph K et al., and Goel R et al., [23,24]. Predominance of *Candida* spp. in elderly group in current study might be due to the presence of significant co-morbid conditions like diabetes, chronic obstructive pulmonary disease and prolonged antibiotic therapy.

most common accounting for 32%. Similar findings were observed by previous literature [6,10,28,29], as shown in [Table/Fig-11]. However, some authors in their studies [8,9,30,31], also observed a significant predominance of *Candida albicans* over NAC spp [Table/Fig-11] [6-11,28-34].

In the present study, it was observed that prolonged antibiotic therapy was the most common predisposing risk factor accounting for 26% followed by diabetes (21%) and HIV (16%). Chakrabarthi A and Shivaprakash MR observed higher rate of *Candida* infections in those patients with antibiotics administration of more than seven days and receiving three or more antibiotics [35]. Administration of broad spectrum antibiotics suppresses the endogenous micro flora, permitting fungal overgrowth and any impairment of mucosal immunity is a potential threat for dissemination of *Candida*. Similarly Kandhari KC and Rama KM found higher occurrence of candidiasis in those individuals with diabetes and HIV [26]. The occurrence of *Candida* infections in diabetic patients might be due to hyperglycaemic environment which favours immune dysfunction thereby increasing the susceptibility to infections.

In the present study, disc diffusion method for antifungal susceptibility testing of *Candida* isolates was used. Among the azoles, voriconazole showed the maximum sensitivity of 86%, was the most sensitive and least in ketoconazole (56%). It has also been observed that all the *C. krusei* were resistant to fluconazole as they are intrinsically resistant to fluconazole. However, amphotericin B in the present study showed a sensitivity of 81% and resistance of 8%. Similar sensitivity

Authors, places and year of publication / *Candida* species	*C. albicans* (%)	*C. tropicalis* (%)	*C. krusei* (%)	*C. glabrata* (%)	*C. parapsilosis* (%)	*C. gulliermond* (%)	*C. dubliniensis* (%)
Pahwa N et al.,Indore, 2014 [6]	42.19	22.36	3.38	3.8	6.33	0.84	0.84
Shaik N et al., Guntur, 2016 [7]	20	57.3	4.7	1.3	-	3.3	8
Khadka S et al., Nepal, 2017 [8]	56	20	10	14	-	-	-
Jaycharan AL et al., Chennai, 2018 [9]	59.6	19.2	7.05	6.41	5.76	-	1.92
Pandita I et al., Aurangabad, 2019 [10]	40.8	29.3	0.6	1.2	9.7	-	-
Bhaskaran R et al., Thrissur, 2020 [11]	31.25	28.75	15	1.25	16.25	-	-
Talukdar A et al., Guwahati, 2020 [30]	53.33	9.33	33.33	4	-	-	-
Shukla R et al., Telangana, 2020 [32]	25	61.33	1	8	5.33	2.66	-
Deepthi KN et al., Kerala, 2020 [33]	31	47	6	-	16	-	-
Shwetha DC and Venkatesha D, Mandya, 2021 [28]	48.7	21	11.9	18.4	-	-	-
Chakraborty M et al., Kolkata, 2021 [34]	27	24.5	14	1.5	8.5	2	-
Verma S et al., Mumbai, 2021 [29]	42	27	1	4.3	8	1	-
Chen J et al., China, 2021 [31]	36.36	19.09	-	8.18	-	-	-
Present study, Manipur, 2022	44	32	5	4	4	6	5

[Table/Fig-11]: Comparison of distribution of *Candida* species among various studies [6-11,28-34].

Candida spp. with antifungals / Authors with places and years of publication		Rajeevan S et al., Tamil Nadu, 2016 (%) [36]	Sabhapandit D et al., Shillong, 2017 (%) [37]	Jaycharan AL et al., Chennai, 2018 (%) [9]	Gade N et al., Chhattisgarh, 2019 (%) [38]	Bhaskaran R et al., Thrissur, 2020 (%) [11]	Talukdar A et al., Guwahati, 2020 (%) [30]	Shukla R et al., Telangana, 2020 (%) [32]	Deepthi KN et al., Kerala, 2020 (%) [33]	Shwetha DC and Venkatesha D, Mandya, 2021 (%) [28]	Chakraborty M et al., Kolkata, 2021 (%) [34]	Verma S et al., Mumbai, 2021 (%) [29]	Chen J et al., China, 2021 (%) [31]	Present study, Manipur, 2022 (%)
C. albicans	F	70	100	88.2	89.8	100	85	80	96.7	85.1	85	77	92.5	79.6
	V	-	100	-	100	100	100	88	100	85.1	100	78	92.5	84.1
	It	24	-	89.2	-	-	-	-	-	-	-	94	-	79.6
	Kt	27	-	-	-	-	-	-	-	-	-	-	-	63.6
	AmpB	100	100	98.9	97.9	96	100	84	-	100	56	97	-	93.2
C. tropicalis	F	89	100	83.3	89.8	100	28.5	65.2	91.5	93.8	43	79	80.9	31.3
	V	-	100	-	100	100	85.7	82.6	100	96.9	100	79	61.9	81.3
	It	82	-	76.6	-	-	-	-	-	-	-	93.5	-	56.3
	Kt	92	-	-	-	-	-	-	-	100	-	-	-	50
	AmpB	100	60	99	97.9	100	71.4	89.1			100	94.5	-	65.6
C. krusei	F	0	-	-	89.9	0	28	0	0	0	100	-	-	0
	V	-	-	-	100	100	28	100	83.3	66.7	100	100	-	100
	It	25	-	-	-	-	-	-	-	-	-	-	-	40
	Kt	16	-	-	-	-	-	-	-	-	-	-	-	40
	AmpB	100	-	-	100	0	64	100	-	100	93	66	-	60
C. parapsilosis	F	29	80	100	-	100	-	100	87.5	-	93	60	91.9	50
	V	-	80	-	-	92.3	-	100	100	-	100	80	94.6	100
	It	21	-	100	-	-	-	-	-	-	-	-	-	25
	Kt	29	-	-	-	-	-	-	-	-	-	-	-	0
	AmpB	100	60	100	-	100	-	50	-	-	93	95	-	50

[Table/Fig-12]: Comparison of sensitivity patterns of *Candida* species to antifungals among various studies [9,11,28-34,36-38].
F: Fluconazole, V: Voriconazole, It: Itraconazole, Kt: Ketoconazole, AmpB: Amphotericin B

patterns were found by other studies as depicted in [Table/Fig-12] [29,30,32,36]. Better susceptibility patterns have been observed by Bhaskaran R et al., Chen J et al. Deepthi KN et al., Chakraborty M et al., Sabhapandit D et al., and Gade N et al., [Table/Fig-12] [9,11,28-34,36-38].

The strength of this study was speciation of seven species of *Candida* employing conventional and HiCrome differential media, and their antifungal susceptibility testing using disk diffusion method as per CLSI guidelines could be carried out.

Limitation(s)

Advance automated systems like Vitek 2 Compact and molecular methods for molecular characterisation of *Candida* isolates at the subspecies level could not be accessed due to lack of infrastructure. There is no interpretative zone size for antifungal drugs by disc diffusion method other than fluconazole and voriconazole as per CLSI guidelines. Hence susceptibility zone size was followed and was interpreted based on manufacturer's guidelines and previous studies for ketoconazole, itraconazole and amphotericin B.

CONCLUSION(S)

The present study showed that even in Manipur, there is a changing trend of increased incidence of NAC over *Candida albicans*. An increase in the predisposing conditions in the recent years has resulted in an increasing incidence of *Candida* infections. Therefore early speciation of *Candida* isolates not only will restrict the empirical use of antifungal agent but also greatly influence the treatment options for the clinicians and thus will be beneficial for the patients as some *Candida* species are intrinsically resistant to some antifungal. Extensive study is required in our state to know the prevailing *Candida* spp. which may in turn help to develop guidelines on empiric therapy for invasive fungal infections.

REFERENCES

[1] Chander J. Textbook of Medical Mycology. 3rd edition. New Delhi: Mehta publisher; 2009.
[2] Kashid RA, Belawadi S, Devi G, Indumati. Incidence of Non *Candida albicans* in patients with urinary tract infection with special reference to antifungal sensitivity. J Evolution Med Dent Sci. 2014;1(4):572-77.
[3] Manjunath V, Vidya GS, Sharma A, Prakash MR, Murugesh. Speciation of *Candida* by Hicrome agar and sugar assimilation test in both HIV infected and non infected patients. Int J Biol Med Res. 2012;3(2):1778-82.
[4] Deorukhkar SC, Saini S, Mathew S. Non-*albicans Candida* infection: An emerging threat. Interdiscip Perspect Infect Dis. 2014;2014:615958.
[5] Gupta N, Mittal N, Sood P, Kumar S, Kaur R, Mathur MD. Candidaemia in neonatal intensive care unit. Indian J Pathol Microbiol. 2001;44(1):45-48.
[6] Pahwa N, Kumar R, Nirkhiwale S, Bandi A. Species distribution and drug susceptibility of *Candida* in clinical isolates from a tertiary care centre at Indore. Indian J Med Microbiol. 2014;32(1):44-48.
[7] Shaik N, Penmetcha U, Myneni RB, Yarlagadda P, Singamsetty S. A study of identification and antifungal susceptibility pattern of *Candida* species isolated from various clinical specimens in a tertiary care teaching hospital, Chinakakani, Guntur, Andhra Pradesh, South India. Int J Curr Microbiol App Sci. 2016;5(7):71-91.
[8] Khadka S, Sherchand JB, Pokhrel BM, Parajuli K, Mishra SK, Sharma S, et al. Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal. BMC Res Notes. 20178;10:218. https://doi.org/10.1186/s13104-017-2547-3.
[9] Jayacharan AL, Katragadda R, Ravinder T, Vajravelu L, Manoranjan L, Hemalatha S, et al. Antifungal susceptibility pattern among *Candida* species: An evaluation of disc diffusion and micro broth dilution method. J Microbiol Infect Dis. 2018;8(3):97-101.
[10] Pandita I, Wyawahare AS, Mulay MV, Hussain T, Kulkarn S. Speciation and antifungal susceptibility pattern of *Candida* isolates from various clinical specimens at a tertiary care hospital, Maharashtra. Int J Adv Res. 2019;7(10):244-50.
[11] Bhaskaran R, Valsan C, Sathiavathy KA. Molecular speciation and antifungal susceptibility profile of *Candida* species in a tertiary care centre in Central Kerala. J Evolution Med Dent Sci. 2020;9(6):357-62.
[12] Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016;2(2):20-27. Doi: 10.18869/acadpub.cmm.2.2.5.
[13] Indian Council of Medical Research. Standard Operating Procedures for Fungal Identification and Detection of Antifungal Resistance- National Antifungal Resistance Surveillance. New Delhi: Indian Council of Medical Research; 2019.
[14] Deorukhkar SC, Saini S. Laboratory approach for diagnosis for candidiasis through ages. Int J Curr Microbiol App Sci. 2014;3(1):206-08.
[15] Jha BJ, Dey S, Tamang MD, Joshy ME, Shivananda PG, Brahmadatan KN. Characterisation of *Candida* species isolated from cases of lower respiratory tract infection. Kathmandu Univ Med J. 2006;4(3):290-94.
[16] Gupta J, Shah H. Isolation, speciation and antifungal drug susceptibility of *Candida* species from clinically suspected infections in a tertiary care hospital. Trop J Path Micro. 2016;2(3):183-91. Doi: 10.17511/jopm.2016.i03.18.
[17] Marinho SA, Teixeira AB, Santos OS, Cazanova RF, Ferreira CA, Cherubini K, et al. Identification of *Candida* spp. by phenotypic tests and PCR. Braz J Microbiol. 2010;41(2):286-94. Doi: 10.1590/S1517-83822010000200004.
[18] Mehta R, Wyawahare AS. Evaluation of Hicrome *Candida* differential agar for species identification of *Candida* isolates from various clinical samples. Int J Cont Med Res. 2016;3(4):1219-22.
[19] Clinical Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing of yeasts; Approved Guideline. CLSI document 2009;29(17):M 44-A2.
[20] Jayalakshmi L, RatnaKumari G, Samson SH. Isolation, speciation and antifungal susceptibility testing of *Candida* from clinical specimens at a tertiary care hospital. Sch J App Med Sci. 2014;2:3193-98.
[21] Roy RC, Sharma GD, Barman SR, Chanda S. Trend of *Candida* infection and antifungal resistance in a tertiary care hospital of northeast India. Afr J Microbiol Res. 2013;7:3112-16.
[22] Gopi A, Murthy NS. Presumptive identification of *Candida* species by using Chromogenic agar in comparison with yeast identification protocols in a tertiary care hospital. J Evid Based Med Healthc. 2014;1(13):1604-13.
[23] Joseph K, Ameena KK, George AT. A study on proportion, speciation and antifungal resistance pattern of the *Candida* isolates in a tertiary care hospital of North Kerala. Int J Curr Microbiol App Sci. 2017;6(5):434-39.
[24] Goel R, Singh S, Gill AK, Kaur A, Kaur I. Speciation, characterisation and antifungal susceptibility pattern of *Candida* species. Int J Contemp Med. 2018;5(5):01-04.
[25] Amar CS, Ashish J, Hajare V, Shreekantha, B Yogesh, Vinodchandran. Study of prevalence and antifungal susceptibility of *Candida*. Int J Pharm Bio Sci. 2013;4(2):361-81.
[26] Khandari KC, Rama KM. Clinical and laboratory studies on cutaneous Candidiasis. Indian J Dermatol Venereol Leprol. 1969;35(2):102-07.
[27] Patel LR, Pethani JD, Bhatia P. Prevalence of *Candida* infections and its antifungal susceptibility pattern in a tertiary care hospital, Ahmedabad. Natl J Med Res. 2012;2(4):439-41.
[28] Shwetha DC, Venkatesha D. Identification and antifungal susceptibility of *Candida* species isolated from various clinical samples at a tertiary care hospital. Int J Curr Microbiol App Sci. 2021;10(03):527-32.
[29] Verma S, Patil E, Nerurkar V. Speciation and antifungal susceptiblity profile of *Candida* species– A study from a private diagnostic reference lab in Mumbai. IAR J Med Sci. 2021;2(4):48-55.
[30] Talukdar A, Barman R, Sarma A, Krishnatreya M, Sharma JD, Kataki AC. Fungal profile and antifungal susceptibility pattern of candidiasis in esophageal cancer patients. J Can Res Ther. 2020;16:S209-12.
[31] Chen J, Hu N, Xu H, Liu Q, Yu X, Zhang Y, et al. Molecular epidemiology, antifungal susceptibility, and virulence evaluation of *Candida* isolates causing invasive infection in a tertiary care teaching hospital. Front Cell Infect Microbiol 2021;11:01-12. Doi: 10.3389/fcimb.2021.721439.
[32] Shukla R, Reddy SG, Bilolikar AK. A study of *Candida albicans* and non-*albicans Candida* species isolated from various clinical samples and their antifungal susceptibility pattern. J Med Sci Res. 2020;8(1):01-11. Doi: http://dx.doi.org/10.17727/JMSR.2020/8-1.
[33] Deepthi KN, Menon AR, Nair PK. Identification of *Candida* species from clinical isolates and their antifungal susceptibility pattern. J Evolution Med Dent Sci. 2020;9(24):1813-17. Doi: 10.14260/jemds/2020/396.
[34] Chakraborty M, Banu H, Gupta MK. Epidemiology and antifungal susceptibility of *Candida* species causing blood stream infections: An eastern India perspective. J Assoc Physicians India. 2021;69(8):11-12. PMID: 34472809.
[35] Chakrabati A, Shivaprakash MR. Microbiology of systemic fungal infections. J Postgrad Med. 2005;5:16-20.
[36] Rajeevan S, Thomas M, Appalaraju B. Characterisation and antifungal susceptibility pattern of *Candida* species isolated from various clinical samples at a tertiary care centre in South India. Indian J Microbiol Res. 2016;3(1):53-57.
[37] Sabhapandit D, Lyngdoh WV, Bora I, Prasad A, Debnath K, Elantamilan D. Prevalence of non-albicans candidaemia in a tertiary care hospital in Northeast India. Int J Med Sci Public Health. 2017;6(11):1620-25.
[38] Gade N, Neral A, Monga N, Aradhey P, Singh R, Barapatre R, et al. Antifungal susceptibility pattern of clinical isolates of *Candida* from a tertiary care hospital in Chhattisgarh, India. Saudi J Pathol Microbiol. 2019;4(12):906-13.

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