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Original Research Article

A Clinico-Mycological Study on the Distribution of Species and Antifungal Susceptibility Profile of Candida Isolates from a Tertiary Care Hospital of a North India

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Abstract:

Candida species are opportunistic pathogens responsible for a range of infections in healthcare settings, posing a significant clinical challenge due to their increasing resistance to antifungal agents. The study aimed to understand the distribution of Candida species and their susceptibility to antifungal agents, providing valuable insights into local epidemiology and treatment strategies. Results revealed a diverse distribution of Candida species, with varying levels of susceptibility to antifungal drugs. These findings underscore the importance of continuous surveillance and tailored management approaches to combat fungal infections effectively in the region.

Introduction

In comparison to bacterial pathogens, fungi were less frequently the cause of infectious diseases in humans. However, with the increased number of immunosuppressed patients fungal infections have gained enormous medical importance.

During last century two important pre-disposing factors have been noticed. One was the advent of antimicrobial antibiotics and their indiscrimination use led to the increased incidence of candidiasis and secondly the emergence of pandemic of acquired immune deficiency syndrome (AIDS). Other risk factors include underlying malignant diseases, organ transplantation, hospital stay, and exposure prolonged to invasive procedures. Candidiasis is undoubtedly the most common fungal infection in HIV-infected individuals. [1,2] Candida species are component of normal flora, as they are commonly found on the skin throughout gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy. This has been observed that most of the times it is found as an endogenous infection due to its commensal nature.[1]

The clinical manifestation of candidiasis among humans depending upon their immune system and underlying predisposing factors are mucocutaneous manifestations like oral candidiasis, angular chelitis, alimentary, vulvovaginitis, balanitis, balanoposthitis, and ocular candidiasis. Cutaneous manifestations include intertrigo, paronychia and onychomycosis, diaper dermatitis, Candidal granuloma. Systemic manifestations include urinary tract candidiasis, candiduria, endocarditis and pericarditis, pulmonary candidiasis, meningitis, candidemia and septicaemia.[1]

The yeasts of genus Candida have been the fourth most common primary blood stream organisms in the United States and the seventh most common pathogens to cause health-care associated infections during the last four decades.[1]

Only few studies from India have reported candidemia rates (6-18%) and increase in isolation of non-albicans Candida from BSIs.(8) In a recent study, the incidence rate of candidemia has been reported to be 6.9 per 1000 in intensive care unit (ICU) patients, and 7.5% of ICU patients receiving antifungal therapy.[3]

Although Candida albicans remains the most common causative agent of both superficial and deep fungal infections, an increasing incidence of less common species of Candida has also been documented in the last few years. These species include C.tropicalis, C. krusei, C. glabrata and C. parapsilosis and tend to be less susceptible to azoles, particularly fluconazole, than C. albicans.[1],[4]

Antifungal agents available for the treatment of systemic and invasive candidiasis are restricted to polyenes, allylamines, azoles, and the recently developed echinocandin class of molecules. Emergence of drug resistance in C. albicans is

reported all over the world.[5] Generally, drug resistance has emerged through the development of acquired resistance and an epidemiological shift towards inherently less susceptible species. High rates of azole resistance in C glabrata and intrinsic azole resistance of Candida krusei are well known.[4]

In view of the above background information, this study was undertaken to study clinical profile of patients with Candida infection, to speciate and characterize the Candida isolates responsible for producing clinical disease, to study the antifungal susceptiblity profile of the Candida isolates.

Materials and Methods

The present study was carried out on patients with Candida infection in both outdoor and hospitalized patients at JNMCH, AMU, Aligarh over the period of 2 years. The study was performed after the approval from Institutional Ethics Committee.

3.1 Selection of Cases

All the patients with laboratory confirmed candida infection irrespective of age and sex were included in the study. Specimen collection & transport: Various clinical specimens including skin swab, nails, oral swab, cervical swab, urine, sputum, BAL, Endotracheal aspirate, CSF, pus and blood culture were collected depending on the suspected site or system involved. The samples were obtained in duplicate and transported to the Mycology Laboratory, Department of Microbiology. The specimens were obtained using standard techniques.

3.2. Specimen Processing:

3.2.1 Direct Microscopy: Specimens like endotracheal aspirate, urine, oral swab, pus, vaginal swab etc., were subjected to direct microscopy by making a KOH mount and a Gram-stained smear.

3.2.2. Urine: Without agitation was used for microscopic examination and culture on cysteine lactose electrolyte deficient agar (CLED).

3.3.3. Blood: Inoculated BACT/ALERT PF PLUS or BACT/ALERT FA PLUS culture bottle was loaded in BacT Alert 3D incubator. When machine beeps for positive, blood culture bottle was unloaded for microscopic examination and culture on blood agar.

3.2.4. Other samples (like Pus, BAL, Cervical swab, sputum): Sample was collected either in sterile syringe or with sterile cotton swabs in sterile vials. For aspirated pus sample, mix the specimen thoroughly and place a drop of the specimen on slide for microscopic examination & culture on blood agar, chocolate agar & MaCconkey agar. For swab specimen, roll the swab over slide for microscopic examination and culture on blood agar, chocolate agar & MaCconkey agar.

3.2.5 Fungal Culture: The positive culture for candida species in different specimen was isolated on two Sabouraud dextrose agar (SDA) tube containing chloramphenicol (0.05 mg/ml) by rolling over the surface. One was incubated at 25˚C and the second was incubated at 37˚C.

The isolates were identified on macroscopic and microscopic morphological characteristics using standard techniques described in Clinical Mycology by [6,7,8].

3.3. Identification of Candida species:

The identification of Candida spp. was in accordance to (i) Colony characteristics, (ii) LCB mount, (iii) Germ-tube test (GTT), (iv) Growth at 42°C, (v) Morphology on CMA, (vi) Hi-Chrome Candida agar, it is a chromogenic medium with which a presumptive identification of Candida species on the basis of colors of the colonies [9]. (vii) Sugar fermentation tests and (viii) KB006 HiCandidaTM Identification kit: standardized colorimetric identification system, based on the principle of pH change and substrate utilization. Organism to be identified was isolated on a common medium like Sabouraud dextrose agar (M063). Inoculum was prepared by picking 2-4 well isolated colonies and a homogenous suspension in 2-3 ml sterile saline was made. The density of the suspension was adjusted to 0.5D at 620nm. Aseptically kit was open and peeled off the sealing foil. Each well was inoculated with 50 µl of the above inoculum by surface inoculation method. Kit was incubated at 25˚C for the duration of 24-48 hours. Results were noted in terms of color change.

3.4. Antifungal Susceptibility Testing: Antifungal susceptibility testing was performed by the disc diffusion and broth microdilution methods. For Disc diffusion method: CLSI M44-A2 method uses Mueller-Hinton agar supplemented with 2% glucose and 0.5 µg methylene blue/ml.[10]

The antifungal tested were Amphotericin B, nystatin, ketoconazole, clotrimazole, fluconazole and itraconazole (HiMedia Laboratories, Mumbai, India). Broth micro dilution method: Broth micro dilution method was adopted in this study as per CLSI (2008) guidelines based on document no. M-27A3.[10] Antifungal agents powders (Amphotericin B, Ketoconazole, and Fluconazole) were purchased from HiMedia Laboratories, Mumbai, India.

Ketoconazole, amphotericin B was dissolved in dimethyl sulfoxide, and fluconazole was dissolved in sterile distilled water. Stock solutions were diluted with RPMI 1640 medium (with l-glutamine but without bicarbonate) (Sigma Chemical Co., St. Louis, Mo.), supplemented with glucose (2%), and

buffered to pH 7.0 with 0.165 M morpholino-propane-sulfonic acid (MOPS; Sigma).

Quality control: ATCC 24433 Candida albicans, C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were included as the control organisms each time with each drugs.

3.5 Statistical Methods: Microsoft Excel was used for data capturing and Descriptive & Analytical Statistical analysis (when applicable) was performed using SPSS® 25.0 for Windows. Chi-square test was used to compare differences between the groups. A p value of ≤0.05 was considered significant.

Observations and Results

A total of 126 patients with candida infection were included in this study.

It was observed that Candida infection was more common in females (57.1%) as compared to males (42.9%) though statistically the difference is not significant (p>.05). Overall, the female to male ratio was 1.3:1.

It was observed that the maximum number of patients with candidiasis 73 (57.9%) were in the age group of adults (17-60 years), as shown in table 1.

Table 1: Age and sex distribution of patients with candida infection (n=126)

Age group	Male	Female	Total
Infants (<1 Year)	24(19.0)	5(4.0)	29(23.0)
Pediatrics (2-16 Years)	6(4.8)	6(4.8)	12(9.5)
Adults (17-60 Years)	19(15)	54(42.9)	73(57.9)
Elderly (>60 Years)	5(4.0)	7(5.6)	12(9.5)
Total	54(42.9)	72(57.1)	126(100)

Figures in parenthesis indicate percentage

4.3 Associated risk factors in the study group

The most frequent risk factors in this study population included were peripheral catheter in 52 (41.3%) cases followed by pregnancy in 38 (30.2%) cases. Other important risk factors found were neonatal age group in 29 (23.0%), antibiotic intake in 26 (20.6%) cases and steroid intake in 25(19.8%) cases, low birth weight 24 (19.0%) cases, indwelling urinary catheter 23 (18.3%) cases, diabetes mellitus in 18 (14.3%) cases, prematurity 17 (13.5%) cases and HIV infection in 5 (4.0%) cases.

Remark: Different combination of risk factors were found

4.4: Clinical presentation of patients with candidiasis

Fever was seen in majority of the patients 74(58.7%) cases, followed by abdominal pain in 34(27%) cases, cough in 24(19%) cases, low birth weight in 24 (19%), asthenia and malaise in 22(17.5%) cases, cutaneous lesion in 18(14.3%), headache in 17(13.5%), failure to thrive 16(12.7%), vomiting in 15(11.9%) and Itching & vaginal discharge in 10(7.9%) cases. Many patients had a combination of more than one symptom.

Table 2: Clinical presentation of patients with candidiasis

Presenting symptoms	No. of patients	Percentage
Fever	74	58.7%
Cough	24	19.0%
Asthenia and malaise	22	17.5%
Diarrhea	12	9.5%
Vomiting	15	11.9%
Abdominal pain	34	27.0%
Itching & vaginal discharge	10	7.9%
Headache	17	13.5%
Altered sensorium	8	6.3%
Difficulty in swallowing	9	7.1%
Low birth weight	24	19.0%
Failure to thrive	16	12.7%
Difficulty in breathing	12	9.5%
Cutaneous lesions	18	14.3%

Remark: Different combinations of symptoms were found.

4.6: Identification of candida species by various laboratory methods**,** 4.6.1: Direct Microscopy: Gram's Staining and LCB Staining**.** Table summarises the Gram's Staining and LCB Staining findings in specimens positive for Yeast like growth on Sabouraud dextrose agar (SDA)

Table 3: Gram's Staining and LCB Staining direct observation results

Sample	No. of Candida isolates (%)	Gram's Staining		LCB Staining	
		Yeast Like budding cells seen (%)	Yeast Like budding cells not seen (%)	Yeast Like budding cells seen (%)	Yeast Like budding cells not seen (%)
Urine	42(33.3)	26(61.9)	16(38.1)	23(54.8)	19(4.2)
Blood	40(31.7)	NA	NA	NA	NA
Vaginal discharge	19(15.1)	14(73.7)	5(26.3)	16(84.2)	3(15.7)
Pus	9(7.1)	6(66.7)	3(33.3)	5(55.6)	4(44.4)
Sputum	7(5.6)	7(100)	0	7(100)	0
BAL (Broncho-alveolar lavage)	2(1.6)	1(50)	1(50)	1(50)	1(50)
Tracheal aspirate	6(4.8)	2(33.3)	4(66.7)	3(50)	3(50)
Oral swab	1(0.8)	1(100)	0	1(100)	0
Total	126(100)	57(66.3)	29(33.7)	56(65.1)	30(34.9)

Figures in parenthesis indicate percentage

4.6.2: Morphology of Candida isolates on CHROMAgar as shown in table

Table 4: Morphology of Candida isolates on CHROM Agar

Candida species	Colour production	No. of isolates	Percentage
Candida albicans	Bluish green	42	33.3%
Candida tropicalis	Deep blue	38	30.1%
Candida krusei	Pink, pale borders	24	(19.0%)
Candida parapsilosis	white	10	(7.9%)
Candida glabrata	Pink to purple	7	5.6%

4.6.3: Identification of candida species by Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests

Table 5 shows the speciation of 126 Candida isolates included in the study by using germ tube test, morphology on cornmeal agar and sugar fermentation.

In the Candida isolates included in the study, 42 out of 126 isolates shows the formation of germ tube in the germ tube test, pseudohyphae with terminal chlamydospore (A) on cornmeal agar and fermentation of glucose (1) & maltose (2) with variable (v) fermentation of galactose (5).

And 84 out of 126 Candida isolates do not form germ tube in the germ tube test but shows different morphology on cornmeal agar, among 84 isolates 40 shows the formation of blastoconidia anywhere along pseudohyphae (B) on cornmeal agar and fermentation of glucose (1) & maltose (2) with variable (v) fermentation of sucrose (3) & galactose (5).

Among 84 isolates 26 shows the formation of pseudohyphae with cross-matchsticks or treelike blastoconidia (C) on cornmeal agar and fermentation of glucose (1). And 12 out of 84 isolates shows the formation of blastoconidia along curved pseudohyphae (D) and fermentation of glucose (1) with variable (v) fermentation of galactose (5). Among 84 isolates 3 do not shows the formation pseudohyphae but small cells with terminal budding (E) on cornmeal agar and fermentation of glucose (1). And 3 out of 84 isolates shows the formation of fairly short, fine pseudohyphae & clusters of blastoconidia at septa and fermentation of glucose (1), sucrose (3) & galactose (5).

Table 5: Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests results

Methods	**Observations**																																	
GTT	**Positive**						**Negative**																													
Cornmeal Agar	A						B						C						D						E						F					
Sugar Fermentation	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	+	+	0	0	v	0	+	+	v	0	v	0	+	0	0	0	0	0	+	0	0	0	v	0	+	0	0	0	0	0	+	0	+	0	+	0
Species	C. albicans						C. tropicalis						C. krusei						C. parapsilosis						C. glabrata						C. gullermondii					
No. of isolates	42						40						26						12						3						3					

[A-Pseudohyphae with terminal chlamydospores, B-Blastoconidia anywhere along pseudohyphae, C-Pseudohyphae with cross-matchsticks or treelike blastoconidia, D-Blastoconidia along curved pseudohyphae, E-No pseudohyphae; cells small; terminal budding, F-Fairly short, fine pseudohyphae; clusters of blastoconidua at septa]

[1-Glucose, 2-Maltose, 3-Sucrose, 4-Lactose, 5-Galactose, 6-Cellobiose] [(+): positive, (-): Negative, (v): variable]

4.6.4 Identification of Candida species by KB006 HiCandidaTM Identification Kit (n = 70)

Table 6 shows the maximum no. of isolates 47(67.1%) were C. tropicalis, followed by 5(7.1%) isolates were C. pintolopessi and 3(4.2%) isolates of C. albicans, C. kefyr, C. krusei each by using Identification of Candida species by KB006 HiCandidaTM Identification Kit.

It is worth noting that the results of KB006 HiCandidaTM Identification Kit showed major discordance as compared to the identification of candida species by Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests. C. famata, C. kefyr, C. pseudotropicalis and C. pintolopessi were exclusively identified by KB006 HiCandidaTM Identification Kit, also, C. albicans was identified only in 4.2 % of isolates by KB006 HiCandidaTM Identification Kit while in 33 % by Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests.

Table 6: Results and Interpretation Chart of KB006 HiCandidaTM Identification Kit (n = 70)

	Well number of KB006 HiCandidaTM Identification Kit									
Sugar	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Urease	-	-	-	-	-	-	-	-	-	+
Melibiose	-	-	+	-	-	-	-	-	+	-
Lactose	-	-	-	+	+	-	-	-	-	-
Maltose	+	+	-	-	-	-	-	-	-	-
Sucrose	-	+	+	+	+	-	-	-	+	-
Galactose	+	+	-	+	+	-	-	-	+	-
Cellobiose	-	+	+	+	+	-	-	-	+	-
Inositol	-	-	-	-	-	-	-	-	-	-
Xylol	+	+	+	-	+	-	-	+	+	-
Dulcitol	-	-	+	-	-	-	-	-	+	-
Raffinose	-	-	+	+	+	-	-	-	+	-
Trehalose	+	+	+	-	-	-	+	-	+	-

Candida species	C. albicans	C. tropicalis	C. famata	C. kefyr	C. pseudo-tropicalis	C. pintolo-pessi	C. glabrata	C. parapsilo-sis	C. guiller-mondii	C. krusei
No. of isolates	3 (4.2)	47 (67.1)	1 (1.4)	3 (4.2)	2 (2.8)	5 (7.1)	2 (2.8)	2 (2.8)	2 (2.8)	3 (4.2)

[(+) = positive reaction, (-) = negative reaction] Figures in parenthesis indicate percentage

4.7: Distribution of Candida albicans isolates in relation to clinical diagnosis (n=126)

Various clinical presentations observed in patients with candidiasis. The maximum number of patients 42 (33.3%), presented with UTI followed by septicemia in 40 (31.7%) and vaginitis in 19 (15.1%) patients. Other varied presentations included pneumonia 15 (11.9%), skin infection 9(7.1%) and oral thrush 1 (0.8%).

Table 7: Distribution of Candida species isolates in relation to clinical diagnosis

Clinical diagnosis	No. of Candida albicans isolates	Percentage (%)
UTI	42	33.3%
Septicaemia	40	31.7%
Vaginitis	19	15.1%
Pneumonia	15	11.9%
Skin infection	9	7.1%
Oral Thrush	1	0.8%
Total	126	100%

4.9: Distribution of various species of Candida species isolated from various specimens

In urine samples out of 42 candida species 26 were Candida albicans, 13 were Candida tropicalis, 5 were Can-dida krusei, Candida parapsilosis and in blood samples 19 out of 40 samples were Candida tropicalis, 11 were Candida krusei, 7 were Candida albicans and vaginal discharge out of 19 samples 7 were Candida krusei, 6 were Candida albicans, 4 were Candida parapsilosis.

Sputum of 7 patients was positive for yeast cells and abundant pseudohyphae, in which 6 were also positive for Candida albicans and 1 for Candida tropicalis growth.

Table 8: Distribution of various species of Candida isolated from various specimens

Candida species	Clinical specimens						Total
	URINE	BLOOD	VAGINAL DIS-CHARGE	PUS	SPUTUM	OTHERS (BAL, TA, ORAL SWAB)	
C. albicans	19	7	6	1	6	3	42(33.3)
C. tropicalis	13	19	1	5	1	1	40(31.7)
C. krusei	5	11	7	1	0	2	26(20.6)
C. parapsilosis	3	1	4	2	0	2	12(9.5)
C. glabrata	1	0	1	0	0	1	3(2.4)
C. guilliermondii	2	1	0	0	0	0	3(2.4)
Total	42	40	19	9	7	9	126

Figures in parenthesis indicate percentage

4.10: Susceptibility pattern of Candida isolates to various antifungal agents

Table 9 shows the susceptibility pattern of Candida albicans isolates in the study group. Resistance was observed in 38.9% isolates to fluconazole, 26.2% isolates to itraconazole 8.7% isolates to ketoconazole,12.7% isolates to clotrimazole, , 3.2% isolates to amphotericin B, 4.8% isolates to nystatin.

Table 9: Susceptibility pattern of Candida isolates to various antifungal agents

Antifungal agent	Sensitive	Resistant
Clotrimazole	110(87.3)	16(12.7)
Fluconazole	77(61.1)	49(38.9)
Amphotericin B	122(96.8)	4(3.2)
Nystatin	120(95.8)	6(4.8)
Ketoconazole	115(91.3)	11(8.7)

Itraconazole	93(73.8)	33(26.2)

Figures in parenthesis indicate percentage

4.11: Susceptibility pattern of various species of Candida isolates to antifungal agents

The Antifungal Susceptibility profile of various species of Candida isolates was performed by using disk diffusion test, Determination of MIC ranges for fluconazole, Ketoconazole and Amphotericin B was also performed. 4.11.1: Antifungal susceptibility profile of Candida species by disk diffusion

a) Table 9 shows the susceptibility pattern by disk diffusion of different species of Candida isolates included in the study.

Maximum resistance in the drug Clotrimazole and Itraconazole was shown by C. parapsilosis 5 out of 12 (41.7%) each, in the drug Fluconazole maximum resistance was shown by C. krusei 24 out of 26 (92.3%), in the drug Nystatin and Amphotericin-B maximum resistance was shown by C. albicans 2 out of 42 (4.7%) and 3 out of 42 (7.1%) respectively and in the drug Ketoconazole maximum resistance was shown by C. parapsilosis 4 out of 12 (33.3%).

Table 10: Antifungal susceptibility profile of Candida species by disk diffusion

Antifungals	**Clotrimazole**		**Fluconazole**		**Nystatin**		**Amphotericin B**		**Ketoconazole**		**Itraconazole**	
	S	R	S	R	S	R	S	R	S	R	S	R
C. albicans	36	6	35	7	40	2	39	3	40	2	37	5
C. tropicalis	38	2	29	11	39	1	38	2	37	3	29	11
C. krusei	24	2	2	24	25	1	25	1	25	1	15	11
C. parapsilosis	7	5	9	3	12	0	12	0	8	4	7	5
C. glabrata	2	1	0	3	3	0	3	0	2	1	1	2
C. guillermondii	3	0	2	1	3	0	3	0	3	0	3	0
Percentage	87.3 %	12.7 %	61.1 %	38.9 %	96.8 %	3.2 %	95.2 %	4.8 %	91.3 %	8.7 %	73.0 1 %	26.9 %

S=sensitive R=resistant

b) Comparison of Antifungal susceptibility profile of Candida albicans versus Candida Non-albicans species by disk diffusion. A Chi-Square test was done to compare the difference in the antifungal susceptibility profile of C. albicans and C. Non-albicans isolates to various antifungal drugs. A significant difference in the susceptibility profile was found against Fluconazole and Itraconazole, which suggest that the C. Non-albicans are more often resistant to Fluconazole and Itraconazole. While Clotrimazole, Nystatin, Amphotericin B, and Ketoconazole do not have a significantly different susceptibility profile against Candida Non-albicans when compared to Candida albicans.

Table 11: Comparison of Antifungal susceptibility profile of Candida albicans versus Candida Non-albicans species by disk diffusion

Antifungals	**C. albicans**		**C. Non-albicans**		**P-value**
	Sensitive	Resistant	Sensitive	Resistant	
Clotrimazole	36	6	74	10	0.70
Fluconazole	35	7	42	42	<0.05
Nystatin	40	2	82	2	0.47
Amphotericin B	39	3	81	3	0.37
Ketoconazole	40	2	75	9	0.26
Itraconazole	37	5	55	29	<0.05

4.11.2: Determination of MIC ranges for fluconazole of various Candida species isolated from patients in the study group

Table 12 shows the MIC values for fluconazole of various candida species isolated from patients in the study group. It was observed that 2/42 (4.7%) isolates of Candida albicans and 1/3 (33.3%) isolates of Candida glabrata and 1/3 (33.3%) isolates of Candida guillermondii had MIC value of 32 µg/ ml. And 9/42 (21.4%) isolates of Candida albicans, 3/40 (7.5%) of Candida tropicalis, 3/26 (11.5%) isolates of Candida krusei, 2/3 (66.6%) of Candida glabrata had a MIC value of 64 µg/ ml.

This shows that 9 (21.4%) isolate of Candida albicans, 3(7.5%) of Candida tropicalis, 3(11.5%) isolates of Can-dida krusei, 2(66.6%) of Candida

glabrata were resistant to fluconazole and 2 (4.7%) isolates of Candida albi-cans and 1 (33.3%) isolates of Candida glabrata and 1 (33.3%) isolates of Candida guillermondii 2 (4.7%) iso-lates was dose dependent sensitive, Overall 19(15.1%) of Candida species were resistant to fluconazole.

Table 12: Determination of MIC ranges for fluconazole of various Candida species isolated from patients

Candida species	MIC of fluconazole (µg/ ml)								Total
	0.5	1	2	4	8	16	32	≥64	
C. albicans	6	14	4	7	-	-	2 (4.7)	921.4)	42
C. tropicalis	34	2	1	-	-	-	-	3 (7.5)	40
C. krusei	20	2	-	1	-	-	-	3 (11.5)	26
C. parapsilosis	10	-	-	-	-	-	-	2 (16.7)	12
C. glabrata	-	-	-	-	-	-	1 (33.3)	2 (66.7)	3
C. guillermondii	2	-	-	-	-	-	1 (33.3)	-	3
Total	72	18	5	8	-	-	4	19	126

Figures in parenthesis indicate percentage

(S, <8 µg/ ml; SDD, >8 µg/ ml and ≤32 µg/ ml; R, >32µg/ ml)

4.11.3: Determination of MIC ranges for Ketoconazole of various Candida species isolated from patients

Table 13 depicts the MIC values for ketoconazole of various Candida species isolated from patients in the study. It was observed that 15 (35.7%) isolates of Candida albicans , 13(32.5%) isolates of C. tropicalis, 7(26.9%) isolates of C. krusei,7(58.3%) isolates of C. parapsilosis, 2(66.7%) isolates of C. glabrata and 1(33.3%) isolates of C. guillermondii had MIC value of >0.125 µg/ ml which showed that they were resistant to ketoconazole.

Overall 45(35.7%) isolates of Candida species were resistant to ketoconazole.

Table 13: MIC ranges for Ketoconazole of various Candida species

Candida species	MIC of Ketoconazole (µg/ ml)									Total
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	
C. albicans	25	2	6 (14.2)	4 (9.5)	5 (11.9)	-	-	-	-	42
C. tropicalis	22	5	10 (25)	3 (12)	-	-	-	-	-	40
C. krusei	17	2	3 (11.5)	1 (3.8)	3 (11.6)	-	-	-	-	26
C. parapsilosis	3	2	3 (25)	4 (33.3)	-	-	-	-	-	12
C. glabrata	1	-	-	1 (33.3)	1 (33.3)	-	-	-	-	3
C. guillermondii	2	-	-	-	1 (33.3)	-	-	-	-	3
Total	70	11	22	13	10	-	-	-	-	126

Figures in the parenthesis indicates percentage

(MIC of >0.125 µg/ ml are less likely to respond to ketoconazole)

4.11.4 Determination of MIC ranges for Amphotericin B of various Candida species isolated from patients in the study group. Table 14 depicts the MIC values for Amphotericin-B of various Candida species isolated from patients in the study. It was observed that 3 (7.1%) isolates of Candia albicans, 3(7.5%) isolates of Candida tropicalis and 2(66.7%) isolates of Candida glabrata had MIC values of >1 µg/ ml which showed that they were resistant to Amphotericin-B. Overall, 8(6.3%) isolates of Candida species were resistant to Amphotericin-B.

Table 14: Determination of MIC ranges for Amphotericin B of various Candida species

Candida species	MIC of Amphotericin B (µg/ ml)									Total
	≤0.06	0.125	0.25	0.5	1	2	4	8	16	
C. albicans	29	2	4	4	-	3 (7.1)	-	-	-	42
C. tropicalis	27	5	3	2	-	3 (7.5)	-	-	-	40
C. krusei	19	2	4	1	-	-	-	-	-	26
C. parapsilosis	6	1	3	2	-	-	-	-	-	12
C. glabrata	-	-	-	1	-	2 (66.7)	-	-	-	3
C. guillermondii	3	-	-	-	-	-	-	-	-	3
Total	84	10	14	10	-	8 (6.3)	-	-	-	126

Parenthesis indicate percentage (MIC of >1 µg/ ml are probably resistant)

Discussion

Prompt and rapid diagnosis of candidiasis is essential. Past few years have witnessed a rising number of reports of Candida infections from the Indian subcontinent. In patients who had presence of multiple risk factors, the incidence of Candida isolation was seen to be higher than in those with a single risk factor.

In our study, Non- albicans Candida were responsible for 66.7% of all candidiasis and the incidence of C. albicans candidiais was 33.3%. Similar observation were shown in the study of Weinstein et al. [11] Among non-albicans Candida, most common isolated species was C. tropicalis (31.7%), followed by C. krusei (20.6%), C. parapsilosis (9.5%), C. gullermondii (2.4%), C. glabrata (2.4%). Rani et al, Kothavade et al, Deorukhkar et al, they also showed C. tropicalis as the most frequently isolated species. This species variation may be due to the differences in empiric or prophylactic practices. [12,13,14].

The results of KB006 HiCandidaTM Identification Kit showed major discordance as compared to the identification of candida species by Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests. C. famata, C. kefyr, C. pseudotropicalis and C. pintolopessi were exclusively identified by KB006 HiCandidaTM Identification Kit, also, C. albicans was identified only in 4.2 % of isolates by KB006 HiCandidaTM Identification Kit while in 33 % by Germ tube test (GTT), Chlamydospore morphology on Cornmeal Agar, and Sugar Fermentation tests.

A possible explanation for this distribution of candidiasis among the patients, having the most common group was patients with UTI 42(33.3%), followed by septicemia 40(31.7%), vaginal candidiasis 19(15.1 %), pneumonia 15(11.9%), skin infection 9(7.1%) and oral thrush 1 (0.8%), could be the presence of known risk factors like pregnancy, broad spectrum antibiotic intake and presence of indwelling urinary and intravenous catheter in these patients.

In this study, most common species isolated in patients with UTI (42 out of 126) was C. albicans (45.2%), followed by C. tropicalis (30.9%), C. krusei (11.9%), C. parapsilosis (7.1%), C. guilliermondii (4.7%), C. glabrata (2.3%). Similar results also seen in the study of, Ozhak-Baysan et al in 2012. [15]

And in patients with septicaemia (40 out of 126) most common species isolated was C. tropicalis (47.5%) followed by C. krusei (27.5%), C. albicans (17.5%), C.parapsilosis and C.guilliermondii (2.5%) each. Sim-ilar results was also observed by Goel et al. [16]

The susceptibility pattern of Candida isolates shows that 61.1% isolates were susceptible to Fluconazole, 87.3% isolates were susceptible to Clotrimazole and 91.3% isolates were susceptible to Ketoconazole, 73.8% isolates were susceptible to Itraconazole, 96.8% to Amphotericin B, 95.8% to Nystatin. Resistance was observed in 38.9% isolates to Fluconazole, 8.7% isolates to Ketoconazole and 12.7% isolates were susceptible to Clotrimazole, 26.2% isolates to Itraconazole and 3.2% isolates to Amphotericin B. 4.8% isolates to Nystatin.

These findings are in agreement with the study conducted by Narang et al and Kotwal et al who found a lower rate of fluconazole resistance 24% and 26% respectively [17,18]. And in contrast to the study by Xess et al 2007 who reported 11.7% resistance to fluconazole and Belet et al 2011(8.5%) and Rizvi et al, 2011 (10.3%) [19, 20,21].

In India, there is a lack of multicentric studies regarding antifungal susceptibility pattern. However, there are few studies from different parts of the country which give some idea regarding the epidemiology of antifungal resistance among candidemia isolates.

In this study we found more resistance to azole group of antifungal agents as compared to amphotericin B in Candida isolates similar to the study by Changdeo et al. [22]

In our study the results of susceptibility by disc diffusion method and with broth microdilution method were found to be different. Overall 15.1% Candida spp. were resistant to fluconazole by broth microdilution method as compare to disc diffusion method which was (38.9%), and some isolates of C. albicans were dose dependent sensitive. In ketoconazole also results were different, 35.7% isolates of Candida species were resistant by broth microdilution as compare to 8.7% resistant by disk diffusion. And in Amphotericin-B, 6.3% of Candida species isolates were resistant by broth microdilution as compare to 3.2% resistant by disk diffusion method.

Antifungal drug resistance is a rapidly changing problem; especially in the immunocompromised patients. At least two mechanisms of resistance are evident. In one, the azole drug fails to cross the cell envelope. In the other, azole-induced blockade of the C-14 sterol demethylation step in the formation of ergosterol is circumvented [23]. Treatment failure, attributable to the development of azole resistant C. albicans strains, appears to become common nowadays, but still seems to be confined to patients receiving long-term treat-ment.

A recent case control study from Johns Hopkins indicated that fluconazole resistance is most likely to develop in patients with advanced HIV disease

(i.e. CD4 cell counts below 50 cells/mm3) who have had significant prior exposure to the drug and many prior episodes of candidiasis [24].

Non- albicans Candida are more resistant to antifungal drugs, especially azoles as compared to Candida albicans. In our study, resistant to Fluconazole was 50% in Non-albicans Candida as compared to 16.7% in Candida albicans, similarly resistant to Ketoconazole was 10.7% in Non-albicans Candida as compared to 4.8% in Candida albicans and resistant to Itraconazole was 34.5% in Non-albicans Candida as compared to 11.9% in Candida albicans, similar findings were also observed in a recent study. [25] Similarly Caliskan et al in 2011 were also observed that overall resistance rates of C. albicans and non-albicans Candida spp. against fluconazole, as 15% and 54.2% respectively, while those rates were 24.2% and 68.7% respectively against itraconazole. [26] And Panizo et al in 2009 observed C. albicans remains the most susceptible of the yeasts studied to fluconazole and itraconazole when compared with non albicans candida. Candida spp., C. krusei showed the greater cross-resistance to azoles, followed by C. glabrata, C. tropicalis and C. parapsilosis, while C. albicans isolates did not demonstrate this characteristic. [27]

Conclusion

Today, Candida has become common nosocomial pathogen and serious systemic Candida infections frequently lead to death. Intensive use of antifungal drugs has led to an incessant increase in the number of resistant fungal strains retaining viability due to their resistance mechanisms. Keeping all this in mind, the present study was conducted to determine the profile of Candida infections with respect to the predominant species and antifungal susceptibility analysis of the isolates.

- Among the patients with candidiasis, the most common age group was of adults (17 to 60 years) with female preponderance.
- Most common clinical presentation of the patients in this study was urinary tract infections.
- Female preponderance was found in this study with a female: male ratio of approximately 1.3:1.
- Pregnancy, neonatal age group, peripheral catheters, indwelling urinary catheter, steroid intake, antibiotic intake and diabetes mellitus were the common risk factors found in the patients belonging to this study. And most common among them was peripheral catheters.
- Non albicans candida constitute the 66.67% of Candida species isolates included in the study but individually C. albicans (33.3%) was the most common species followed by C. topicalis (31.7%) isolated from Candida species isolates included in the study.
- In patients with UTI, most common species isolated was C. albicans (45.2%), followed by C. trop-icalis (30.9%), C. krusei (11.9%), C. parapsilosis (7.1%), C. guilliermondii (4.7%), C. glabrata (2.3%).
- And in patients with septicemia most common species isolated was C. tropicalis (47.5%) followed by C. krusei (27.5%) C. albicans (17.5%) C. parapsilosis and C. guilliermondii (2.5%) each.
- In disk diffusion method resistance was observed in 38.9% isolates to fluconazole, 26.2% isolates to itraconazole, 12.7% isolates to clotrimazole, 8.7% isolates to ketoconazole, 4.8% isolates to nystatin and 3.2% isolates to amphotericin B.
- In our study, resistant to fluconazole was 50% in Non-albicans Candida as compared to 16.7% in Candida albicans, similarly resistant to ketoconazole was 10.7% in Non-albicans Candida as compared to 4.8% in Candida albicans and resistant to itraconazole was 34.5% in Non-albicans Candida as compared to 11.9% in Candida albicans.
- In broth microdilution method resistance was observed in 15.1% isolates to fluconazole, 35.7% isolates to ketoconazole, 6.3% isolates to amphotericin B.

The incidence of candidiasis is significant as per our study in Aligarh region especially in high risk pa-tients. So, clinicians should keep this in mind and such patients should be worked up accordingly with proper investigations of culture and sensitivity. And as we have also observed here, the high percentage of resistance with azoles and other common antifungals, judicious prescription of antifungals should be done.

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