CHARACTERISATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES ISOLATED FROM CLINICAL SAMPLES OF PATIENTS ATTENDING KATIHAR MEDICAL COLLEGE, KATIHAR, BIHAR

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ABSTRACT

BACKGROUND

Of the various candida species, more than 90% of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, *Candida dubliniensis* and *Candida lusitanae*. Clinical manifestations range from mucocutaneous overgrowth to disseminated infections like candidaemia. Virulence factors like tissue adhesion, phenotypic switching and production of extracellular hydrolytic enzymes are responsible for colonisation and invasion of host tissues along with host factors like immunocompromised states.

The aim of this study is to characterise candida strains isolated from patients and to study their anti-fungal susceptibility pattern.

MATERIALS AND METHODS

Samples such as pus, sputum, blood and urine were collected from patients attending OPD's and IPD's of Medicine, Surgery, Obstetrics and Gynaecology, Paediatrics and Casualty. In this study, primary isolation was done on blood and Sabouraud's dextrose agar from clinical samples. Species were identified based on production of coloured colonies on HiCrome agar and other phenotypic tests like germ tube and chlamydospores formation, sugar fermentation and assimilation tests. Statistical Analysis- Statistical analysis was carried out using online statistical software at http://www.physics.csbsju.edu/stats/contingency_NROW_NCOLUMN_form.html. Chi-square test was used to determine the probability.

Study Design- This descriptive study was conducted in the Department of Microbiology, Katihar Medical College, Katihar, Bihar.

RESULTS

Candida species isolated were *Candida albicans* 38.5% followed by *Candida famata* 22.2%, *Candida guilliermondii* 17%, *Candida tropicalis* 6.7%, *Candida kefyr* 5.2%, *Candida dubliniensis* 3%, *Candida glabrata* and *Candida parapsilosis* 2.2% each and *Candida lusitanae* and *Candida catenulata* 1.5% each. Modified Kirby Bauer's Disc Diffusion method was used for performing anti-fungal susceptibility tests. *Candida albicans* and non-*albicans* candida were most sensitive to voriconazole (73.1% and 84.3%) and most resistant to miconazole (51.9% and 66.3% respectively).

CONCLUSION

Candida albicans was the most frequently isolated species in the present study. *Candida* species was isolated most frequently from urine samples followed by vaginal swab and sputum. Highest number of candidiasis patients were from Departments of Obstetrics and Gynaecology 57.0% followed by Paediatrics 17.8% and Medicine 14.1%. *Candida albicans* was the most commonly isolated candida species, 38.5%. Maximum number of *Candida albicans* strains showed dose-dependent sensitivity with nystatin 76.9%. NAC species showed resistance to miconazole 66.3% followed by ketoconazole 47% and fluconazole 25.03%.

KEYWORDS

Candida Albicans, Non-*Albicans Candida*, Anti-Fungal Susceptibility.

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BACKGROUND

Candida is one of the frequently encountered opportunistic fungal pathogen, which is associated with vast spectrum of human infections. *Candida* colonizes healthy intact skin and in recent times there is an increase in diseases caused by non-

albicans candida (NAC).¹ NAC species are currently more frequently recovered from adults and children in tertiary care medical centres and there are numerous reports on increase of *Candida* infection from India as well.² At present there are 163 acknowledged anamorphic species of the genus *Candida*, which are found in different habitats. The increase in the incidence of *Candida* species over the past two decades is significant and NAC species continue to replace *Candida albicans* at most of the clinical sites like the blood stream. Now, *Candida* species constitutes the third to fourth most common causes of nosocomial bloodstream infections.³

Candida species can cause a wide range of infections including blood stream infections (BSIs) and disseminated candidiasis. In spite of advances in the diagnosis and

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treatment of candidiasis, among the pathogens involved in BSI candida ranks fourth in the United States and seventh in Europe.⁴⁻⁷ Few studies from India have reported candidaemia rates of 6% - 18%⁸⁻¹⁰ and increase in isolation of NAC from BSIs.^{11,12} In a recent study, the incidence rate of candidaemia has been reported to be 6.9 per 1000 in intensive care unit (ICU) patients, and 7.5% of ICU patients receiving antifungal therapy.^{13,14} Candidaemia increases mortality rate by 20% - 49%^{15,16} and nosocomial candidiasis are associated with crude mortality rate of over 60%, while the attributable mortality rate may be as high as 49%.^{17,18}

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. Fluconazole is an antifungal agent most commonly used for prophylaxis, as it can be administered orally and is comparatively cheaper than other antifungal agents. Nonetheless, selection of appropriate empiric therapy is complicated considering the increasing prevalence of NAC species.¹⁹

The present study was undertaken to determine the rate of isolation of candida species from different clinical samples, to characterise the isolates and determine their antifungal susceptibility pattern. The study was conducted from December 2015 to May 2017.

MATERIALS AND METHODS

A brief clinical history of patient including age, sex, socio-economic status, religion, history of intake of antibiotics/ antifungal agents and past history were taken. A total of one hundred and thirty five candida species were isolated from 54 male and 81 female patients attending or admitted to the Departments of Medicine, Surgery, Obstetrics and Gynaecology, Paediatrics and Casualty. Wound, vaginal, oral and aural samples were collected with the help of sterile swab sticks. Mid-stream early morning urine samples were collected in sterile containers. Blood samples were collected for culture in blood culture bottles and sputum samples in sterile containers.¹⁹

10% KOH mounts and Gram stained smears were made from samples and examined for presence of yeast like cells with or without pseudohyphae. Specimens were inoculated on routine culture media and Sabouraud's dextrose agar. Blood culture was done by incubation of inoculated blood culture bottles containing brain heart infusion broth at 37°C and open subcultures were done on blood agar and SDA after 24 hrs., 48 hrs. and 7 days of incubation. Bottles showing no growth after 7 days of incubation were discarded as negative.¹⁹

For identification of candida species, HiCrome agar (HiMedia Laboratories, Mumbai) plates were inoculated with the test strain and incubated at 37°C for 24 to 48 hours. The appearance of colonies of various colours was then noted down. Commercially available corn meal agar with 1% Tween 80 was prepared and inoculated with the test strain. Plates were incubated at 25°C for 3 - 5 days following which morphological features like hyphae, pseudohyphae, blastospores and chlamydospores formation of various candida species was observed microscopically for differentiation of *Candida albicans* from non-*albicans* candida species.²⁰ Germ tube Test, Sugar Fermentation Tests (glucose, lactose, maltose and sucrose) and Sugar Assimilation Test

was done as confirmatory tests for characterisation and identification of candida species against panel of sugars.¹⁹ Antifungal Susceptibility Testing by Kirby-Bauer disc diffusion method was done using Mueller-Hinton agar, 2% glucose and (0.5 µg/mL) methylene blue dye. Zone size interpretation of various candida species against amphotericin B (100 µg), voriconazole (1 µg), fluconazole (25 µg), itraconazole (10 µg), ketoconazole (10 µg), miconazole (30 µg) and nystatin (100 units) was done as per standard protocol.²⁰

Statistical analysis was carried out using online statistical software at http://www.physics.csbsju.edu/stats/contingency_NROW_N_COLUMN_form.html. Chi-square test was used to determine the probability.

RESULTS

A total of one hundred and thirty five candida strains were isolated in the Department of Microbiology from December 2015 to May 2017 from various samples, which were taken up for further study. Institutional Ethical Committee clearance and written consent was obtained from each and every patient before conducting the study.

Out of the 135 patients from whom candida species was isolated, 60.0% were females and 40.0% were males. In age group of 21 - 30 years the occurrence of candida infections was more in females, a finding that was found to be statistically highly significant ($p=0.000$). As for male patients, these infections were most frequently encountered in the age group of 1 - 10 years, which was also found to be highly significant ($p=0.003$). Overall, in the present study the female-to-male ratio was 1.5: 1 (Table 1).

Candida species were isolated from urine 36.3%, vaginal swab 18.5%, sputum 17.0%, wound swab 11.9%, blood 8.9%, oral swab 4.4% and aural swab 3.0% (Table 2).

Highest number of candidiasis patients were from Department of Obstetrics and Gynaecology 57.0% followed by Paediatrics 17.8% and Medicine 14.1%. *Candida albicans* was the most commonly isolated candida species 38.5%. Amongst the non-*albicans* candida sp. that was isolated, *Candida famata* 22.2% was the commonest followed by *Candida guilliermondii* 17%, *Candida tropicalis* 6.7%, *Candida kefyr* 5.2%, *Candida dubliniensis* 3.0%, *Candida glabrata* and *Candida parapsilosis* 2.2% each and *Candida lusitanae* and *catenulata* 1.5% each (Fig. 1).

Microscopic examination of Gram stained smears and 10% KOH mount showed yeast like cells with or without pseudohyphae in 59.3% and 63% of cases respectively. Germ tube formation was seen in 96.2% of *Candida albicans*, 33.3% of *Candida famata* and 17.4% of *Candida guilliermondii*. All the strains of *Candida dubliniensis* were positive in the germ tube test. Chlamydospore formation was seen in only 38.5% of *Candida albicans* and 10% and 8.6% of *Candida famata* and *Candida guilliermondii* respectively.

Typical reactions in the Sugar Assimilation Test were seen in only 96.2% of *Candida albicans*, 90% of *Candida famata*, 88.9% of *Candida tropicalis* and 87% of *Candida guilliermondii*. All the other species of candida gave typical reaction in 100% of cases.

All candida species were confirmed on the basis of sugar fermentation test and sugar assimilation test. The sugar assimilation test was found to be the best test for speciation

of candida. However, as far as *Candida albicans* is concerned, the germ tube test was found to be as sensitive as the sugar assimilation test.

Candida albicans strains were most sensitive to voriconazole 73.1% followed by fluconazole 63.5% and amphotericin B 61.5%. Maximum number of strains showed dose dependent sensitivity with nystatin 76.9%. Maximum resistance was seen with miconazole 51.9% followed by ketoconazole 40.4% and fluconazole 34.6%. Dose dependent susceptibility was most commonly encountered with nystatin 71.1% followed by itraconazole 43.4% and ketoconazole 31.3% (Table 3). NAC species too showed maximum sensitivity to voriconazole was 84.3% followed by amphotericin B 71.1% and fluconazole 65.1%. Majority of the strains NAC showed resistance to miconazole 66.3% followed by ketoconazole 47% and fluconazole 25.03% (Table 4).

Age Group (Years)	Male (%)	Female (%)	Total
1-10 *	21 (38.9)	9 (11.1)	30 (22.2)
11-20 **	2 (03.7)	15 (18.5)	17 (12.6)
21-30 ***	5 (09.3)	39 (48.1)	44 (32.6)
31-40	3 (05.6)	13 (16.0)	16 (11.9)
41-50	5 (09.3)	1 (01.2)	06 (04.4)
51-60	8 (14.8)	0	08 (05.9)
61-70	8 (14.8)	2 (02.5)	10 (13.5)
71-80	2 (03.7)	0	02 (01.5)
81-90	0	2 (02.5)	02 (01.5)
Total	54 (100.0)	81 (100.0)	135

Table 1. Age and Sex-Wise distribution of Patients with Candidiasis

*P= 0.003, **P= 0.023, ***P= 0.000

Sample	Number of Candida Species Isolated	Percentage
Urine	49	36.3
Vaginal Swab	25	18.5
Sputum	23	17.0
Wound Swab	16	11.9
Blood	12	08.9
Oral Swab	06	04.4
Aural Swab	04	03.0
Total	135	100.0

Table 2. Recovery of Candida Species from different Clinical Samples

Figure 1. Candida Species Isolated from different Clinical Samples

Antibiotic	Sensitive (%)	Resistant (%)	Dose Dependent (%)
Voriconazole	38 (73.1)	14 (26.9)	0
Fluconazole	33 (63.5)	18 (34.6)	01 (5.2)
Amphotericin B	32 (61.5)	17 (32.7)	03 (5.7)
Itraconazole	23 (44.2)	05 (9.6)	24 (46.1)
Ketoconazole	13 (25)	21 (40.4)	18 (34.61)
Miconazole	13 (25)	27 (51.9)	12 (23.1)
Nystatin	04 (7.7)	08 (15.4)	40 (76.9)

Table 3. Antifungal Susceptibility Pattern of Candida albicans

Antibiotic	Sensitive (%)	Resistant (%)	Dose Dependent (%)
Voriconazole	70 (84.3)	13 (15.7)	0
Amphotericin B	59 (71.1)	5 (6.0)	19 (22.9)
Fluconazole	54 (65.1)	21 (25.3)	8 (9.6)
Itraconazole	35 (42.2)	12 (14.4)	36 (43.4)
Ketoconazole	18 (21.7)	39 (47.0)	26 (31.3)
Miconazole	12 (14.4)	55 (66.3)	16 (19.3)
Nystatin	12 (14.4)	12 (14.4)	59 (71.0)

Table 4. Antifungal Susceptibility Pattern of Non-Albicans Candida Species

DISCUSSION

Candida species was isolated in 62.6% of males and 37.3% of females in a study conducted in Andhra Pradesh.²⁰ Also, another study from North-East India reported greater number of isolation of candida from males 62% in a study group that included patients from ICU.²¹ In the present study, however, 60% of strains were isolated from females.

Although, a fairly good number of *Candida famata* (33.3%) and *Candida guilliermondii* (17.4%), isolates produced germ tubes, maximum number of strains producing germ tubes was seen with *Candida dubliniensis* (100%) and *Candida albicans* (96.2%). Chlamydospore formation was seen mostly in *Candida albicans* and in few strains of *Candida famata* and *Candida guilliermondii*.

Different media for germ tube production of *C. albicans* and *C. dubliniensis* was ascertained by some workers who found that 90% of *C. dubliniensis* isolates produced germ tube in trypticase soy broth and pooled human serum, while in horse serum only 87.7% of isolates showed germ tube formation. As for *C. albicans*, germ tube production was seen in 98.5% of isolates in trypticase soy broth and 93.9% in pooled human serum and horse serum.²²

Voriconazole was found to be the most useful antifungal drug against *Candida albicans* and non-albicans candida species as determined by the disc-diffusion method. Dose dependent sensitivity was highest with nystatin, a polyene antifungal agent. Maximum resistance was seen against triazoles such as miconazole and ketoconazole.

Other workers have reported maximum sensitivity to amphotericin B (91%) followed by voriconazole (65%) and itraconazole (49%).² A few workers also reported that 78% of *C. albicans* were sensitive to fluconazole sensitivity to nystatin and amphotericin B was 100%, a finding quite different from the present study.²³

Candida species was isolated most frequently from urine samples followed by vaginal swab and sputum. Amongst the candida species, *Candida albicans* was the most frequently

isolated species in contrast to findings in other places in India where *Candida tropicalis* is the commonest.

Candida famata, a rare candida species was isolated as the most common non-albicans candida species. *Candida famata* (previously known as *Debaryomyces hansenii*) is a commensal yeast found in cheese, dairy products and the environment. It has been described in human infections including catheter-related infections, bloodstream infections, peritonitis, acute zonal occult retinopathy and mediastinitis. Though reported from other parts of India, the number of isolations of *Candida famata* was very few as compared to the present study. Direct microscopy of samples gave better results with 10% KOH preparation than Gram staining. Sugar assimilation test was found to be the best method for speciation of candida isolates. However, for *Candida albicans* the germ tube test was found to be as efficacious as sugar assimilation test.

CONCLUSION

The incidence of the various fungal pathogens has increased dramatically over the past few decades. *Candida* species is the commonest of these fungal pathogens. These infections are often severe, rapidly progressive, difficult to diagnose and refractory to therapy.

C. albicans was previously responsible for nearly 80% of candidaemia in many hospitals. However, in recent years there has been a shift in the distribution of infections with non-albicans candida species being increasingly detected. In the present study, candida species were isolated more in young adult females who were in age group of 21 - 30 years.

Majority of males were young children in the age group of 1 - 10 years. *Candida albicans*, the germ tube test was found to be as efficacious as sugar assimilation test. The high occurrence of *Candida famata* in this region, however, needs to be investigated further with a larger patient population. *Candida* species was isolated most frequently from urine samples followed by vaginal swab and sputum. In present study, *Candida albicans* was the most frequently isolated species in contrast to findings in other places in India where *Candida tropicalis* is the commonest.

Candida famata, a rare candida species was isolated as the most common non-albicans candida species. *Candida famata* (previously known as *Debaryomyces hansenii*) is a commensal yeast found in cheese, dairy products and the environment. It has been described in human infections including catheter-related infections, bloodstream infections, peritonitis, acute zonal occult retinopathy and mediastinitis. Though reported from other parts of India, the number of isolations of *Candida famata* was very few as compared to our study.

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