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STUDY OF PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA.

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ABSTRACT

Background and Objectives: The yeast like fungus candida is the most common fungal pathogen causing disease in the human beings. As the disease is usually chronic and treatment failure cases were commonly noted , so the study was conducted to know the prevalence and antifungal susceptibility pattern of various candida species isolated from clinical samples.
Materials and Results: A total of 1702 subjects were selected, among which 60.2%were male and 39.8% were female. The identification of various candida species was done by saline wet mount, 10% KOH mount, Gram stain, germ tube test, culture on SDA, Chromagar, cornmeal agar, sugar fermentation tests and sugar assimilation tests. Antifungal susceptibility test was done by disc diffusion method.103 candida species were isolated from 1702 samples.
Conclusion: Candida albicans is the most common species isolated from clinical specimen and the infection is almost same in both the sexes and predisposing factors like diabetes mellitus, prolonged contact it water were noted. They were advised correct treatment for diabetes mellitus, to limit the contact with water and maintenance of good personal hygiene.

KEYWORDS: **Candida, Candidiasis, Antifungal susceptibility.**

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INTRODUCTION

The genus *Candida* comprises about 200 species, of which close to 20 have been associated with pathology in human. *Candida* species are the most common cause of fungal infections worldwide. They can cause a great variety of infections, including simple mucocutaneous to severe invasive infections that can involve virtually any organ. Blood stream infections by *Candida* are increasingly common, and often are associated with high mortality rates[1, 2]. Recently, there has been an increment in the frequency of non-*albicans* species of *Candida*, such as *C.glabrata, C.krusei, C.tropicalis,* and *C.parapsilosis*, as causes of fungemia[3,4]. Candidiasis is a primary or secondary infection involving a member of the genus *Candida*. The clinical manifestations of disease are extremely varied, ranging from acute, subacute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis, and meningitis. The pathologic processes evoked are also diverse and vary from irritation and inflammation to chronic and acute suppuration or granulomatous diseases. The disease represents an opportunistic infection[5].Candidiasis, which accounts for 66-80% of fungal infections, is a superficial or a deep-seated infection caused by opportunistic yeasts belong to the genus *Candida*. Among all *Candida* species, *Candida albicans* is by far the most common in all clinical forms of candidiasis, representing 70-80% of all yeast isolate[6].*Candida*-associated denture stomatitis is a very common inflammatory process affecting about 60 % of the subjects carrier of a prosthesis. Oral carriage of *Candida* species was significantly higher in diabetic patients compared with healthy individual.[7, 8].The frequency of vaginal candidiasis during pregnancy was found to be 38%, in which 27% were symptomatic and 11% were asymptomatic group. Increased ratio of infection was observed in multigravida and diabetic women [9].The presence of *Candida* in urine is referred to as candiduria. The majority of patients with candiduria suffer a completely benign process[10]. However, candiduria is sometimes a marker of disseminated candidiasis[11]. The prevalence of urinary *Candida* infection has been reported in some part of Ghana. A study conducted in Accra, Ghana reported that the prevalence of urinary *Candida* infection increased in both males and females from 3.5% in 2001 to 5.1% in 2003.[12]

In hospitals, *Candida* species represent 8-9% of all nosocomial blood stream infection, and the risk is higher among patients in the intensive care units (ICU) and cancer patients[13].Recent studies indicate that *Candida* may be isolated from the hands of 15-54% of health care workers in the intensive care unit setting and that the strain of *Candida* carried on the hands may be shared by infected patients[14].Twenty years ago, *Candida* spp were commonly regarded as little more than culture contaminants; however, in less than 2 decades these organisms have become major human pathogens[15]. Nowadays, *Candida* spp. is known as the 4th most frequently isolated pathogen from the blood stream, among hospitalized patients in North American hospitals .The reasons for this increase in fungal infections are multifactorial: better clinical evaluation, and diagnosis, greater survival of patients with malignancies, chronic diseases, increasing number of transplants, complex surgical procedures, catheters, implants and use of wide spectrum antibiotics[2,16].With the remarkable modern advances in medicine, there has been an increase in the number of immunocompromised individuals who need extensive care in hospitals. This has resulted in a rise in the incidence of fungal infections, especially those due to *Candida* species. *C.albicans* is amongst the most common fungal causative agent in superficial and deep seated candidiasis. However, non-*albicans Candida* species have also been implicated in recent years[17].The overall increase in candidemia in

recent years is complicated by the emergence of non-*albicans Candida* (NAC) species as both colonizers and pathogens causing nosocomial fungal blood stream infection (BSI). Wingard in a comprehensive review of all published reports during 1952-1992 found that 12 reports showed proportionally higher (>50%) isolation of NAC species. The NAC species isolated were *C.glabrata, C.krusei, C.tropicalis* and *C.parapsilosis*. Other species like *C.guilliermondii, C.lusitaniae, C.dubliniensis, C.kefyr, C.lipolytica*, and *C.pelliculosa* were occasionally isolated[18].The importance of patient age in determining rank order of *Candia* species causing BSI has also been noted. The predominance of *C. albicans* and *C. parapsilosis* and the lack of *C. glabrata* and other NAC species have been observed in neonatal age groups. In contrast, *C. glabrata* becomes an increasingly important pathogen with increase in age. The contribution of individual NAC species also varies with patients and diagnostic groups. Candidemia due to *C. parapsilosis* is generally associated with catheters, hyperalimentation, or prosthetic devices. Also, *C. parapsilosis* is the most common species of *Candida* to be isolated from the hands of health care workers in ICU, especially those who wear gloves. Thus, it is likely that the contamination of prosthetic devices with the organism occurs via the hand of health care workers[18].Similar to the western world, the rise in frequency of NAC species has been observed in tertiary care centers in India as well with isolation rates ranging from 52 to 96%. However, the predominant isolation of *C. tropicalis* instead of *C. glabrata* or *C. parapsilosis* in all age groups in the Indian scenario is unique in this context. It was proposed that these shifts may result from selective pressures imposed by increased utilization of antifungal agents such as azoles[18].

MATERIALS AND METHODS

The present study was carried out in the department of Microbiology, Navodaya Medical College Hospital and Research Centre, Raichur from November 2009 to October 2010

Sample size

All the clinical samples (1702) that came to the Department of Microbiology laboratory during the study period from November 2009 to October 2010 constituted the material for the study.

Specimen Collection: [1,5,19]

The following guidelines followed during specimen collection:

1. Specimen collected from an active lesion containing viable organisms.
2. Specimen collected under aseptic precautions.
3. Sufficient specimen was collected.
4. Sterile collection devices and containers were used.
5. The specimen was labeled appropriately.

The specimens for laboratory investigation were collected from the clinically suspected cases of candidiasis under strict aseptic precautions. Sterile swabs and bottles were used to collect the appropriate clinical materials. The various clinical specimens collected were oral swabs, ear swabs, vaginal swabs, stool, CSF, sputum, blood, pus, nail scrapings etc. The specimen was subjected to preliminary tests like wet mount, Gram stain, culture on SDA and urease test.

The following methods were followed while collecting specimens from various sites.

1. Skin: The site was cleansed with 70% ethyl alcohol and the alcohol was allowed to evaporate. Skin material was then scraped with the flat edge of a sterile scalpel blade.

2. Nail and nail bed: Nails were cleansed with 70% ethyl alcohol and a portion of the infected nail was clipped and excess keratin scraped from the nail bed. In case of paronychia, the exudates were expressed from under the nail folds and the sample was collected on a moist sterile swab.

3. Vagina: Speculum examination was done and the vaginal discharge was collected using a sterile swab from the posterior fornix.

4. Mouth: The lesions were visualized using a tongue depressor and the specimen was collected using a sterile swab.
5. Blood: Blood was placed directly in a culture system like brain heart infusion agar and later transferred onto Sabourauds Dextrose Agar medium.
6. Cerebrospinal fluid and other sterile body fluids: 3-5 ml of the fluid was collected, centrifuged and the sediment was used for mycological investigations.
7. Urine: Fresh, midstream, clean catch urine (50-200 ml) was collected in a sterile, screw capped container.
8. Catheter tip: Sample from catheter tip is inoculated immediately and also after incubating it in glucose broth for two hours.
9. Stool: Stool sample is collected in a clean wide mouthed container
10. Respiratory tract specimens: Deep coughed out early morning sputum was collected in a sterile wide mouthed container.
11. Pus or discharge from any other lesions: The specimens were collected using sterile swabs or aspirator tubes after the area has been cleansed properly.

Two swabs/specimens were taken from each case. The specimens were transported immediately to the laboratory taking care not to dry out the specimens. One of the specimens was subjected for direct examination and the other for the culture. All the above samples were subjected to various mycological tests as detailed in the following section. Besides these above specimens for microbiological tests; routine investigations were conducted such as hemogram, urine for albumin and sugar, fasting and post prandial blood sugar, screening the chest, VDRL, and tests for HIV.

Mycological examinations for 103 Candida species were done:[19,20]

1) Direct examination:
   a) Wet Mount
   b) Gram's stain

a) *Wet Mount*
For direct microscopic examination the specimen was placed on the glass slide then 10% KOH (Potassium Hydroxide) was added and coverslip was placed on the specimen. The slide was slightly warmed and gentle pressure was applied over the coverslip to remove the trapped air. Microscopic examination was done, first under the low power then under high power. In case of nail specimen 40% KOH is added to a small test tube to which nail clipping was added and incubated overnight for dissolving nail keratin. The specimen was then examined under microscope.[19] Attempt was made to identify the pseudohyphae and yeast cells.

b) *Gram's stain*
Smears were made from the clinical sample on a clean and grease free glass slide, then heat fixed by just passing the glass slide over the flame. Then the smear was stained by Gram's method and observed under oil immersion objective and looked for the presence gram-positive oval yeast like budding cells (2-4μm) and / or pseudohyphae.[20]

2) Culture:[19.21]
For Culture Sabouraud's Dextrose Agar (SDA) with Chloramphenicol was used. The sample was inoculated on SDA slopes and incubated at 25°C. The slopes were observed regularly daily for 2 days to 3 weeks. Colonies were identified by the colony characters and by gram's stain. Once the colonies were confirmed speciation was done by the following methods.

a. Germ tube
b. Corn meal agar inoculation
c. Sugar Fermentation
d. Sugar Assimilation
e. CHROMagar Candida

a) *Germ Tube Test: (Reynolds Braude Phenomenon)*
Test tubes with size of 12 × 75 mm were labeled with culture number, date and time of test. Using a Pasteur pipette, dispensed 3 drops of fresh pooled human serum into the tubes which was obtained from our serology lab.

Lightly touch a single colony with a loop and emulsified the colony in 0.5ml of human serum, incubated at 35^0 C and examined every half an hour for 2 to 3 hours by taking a loopful of suspension on a glass slide and placing a coverslip over the suspension and examined under low power objective then confirmed by using high power objective. Germ tubes were seen as long tube like projections extending from the yeast cells. There was no constriction at the point of attachment to the cell (Drumstick appearance). The germ tube formation was seen within 3 hours in *C.albicans*.[19,21]

b) *Cornmeal agar inoculation*

Corn meal agar with tween-80 was used for demonstration of chlamydospores, blastospores and pseudohyphae. Using a marker pen, divided a petri plate into two halves, and labeled each half on the bottom of the plate with the culture number, date and time of inoculation. With a sterile inoculating straight wire, lightly touch the colony, and then made one streak cutting the agar. 3 to 4 streaks were made across the first to dilute the inoculum and then covered with a sterile 22 by 22mm coverslip. The plate was incubated at 22-26^0 C in the dark for up to 3 days. After the incubation, the plate was examined by placing the plate without its lid on the microscope stage and using a low and high power magnification for chlamydospores, blastospores and pseudohyphae.[19,21]

c) *Sugar Fermentation*[19]

Six carbohydrate broths were used, each containing 2% Glucose, Sucrose, Lactose, Maltose, Galactose and Trehalose separately with 1% peptone and 0.5% sodium chloride with Andrade's indicator (0.005%). Durham's tube was immersed for gas detection. Inoculated yeast colonies into each carbohydrate broth and incubated at 25°C for one week and examined at 48-72 hours intervals for acid (pink colour) and gas (in Durham's tubes) production.

Sugars	*C. albicans*	*C. tropicalis*	*C. krusei*	*C. dubliniensis*	*C. Para-psilosis*	*C. guillier-mondii*	*C. glabrata*
Glucose	AG	AG	AG	AG	AG	AG	AG
Lactose	-	-	-	-	-	-	-
Sucrose	-	AG	-	-	-	+	-
Maltose	AG	AG	-	AG	-	-	-
Galactose	V	AG	-	V	V	AG	-
Trihalose	V	AG	-	V	-	AG	AG

A-Acid G –Gas V- Variable

d) *Sugar Assimilation*[22]

Yeast nitrogen base agar was used for the sugar assimilation test. Yeast suspension from a 24-48 hrs old culture was prepared in 6 ml of distilled water and then turbidity was adjusted to McFarland No. 5 standard. 30ml of yeast nitrogen base medium required for each strain. Two petridishes were labeled with isolate number and name of the sugars used. Than yeast suspension was poured equally into both the plates. Than 15 ml of cooled (50^0C) yeast nitrogen agar base was poured into the yeast suspension and mixed well and allowed to set at room temperature. Discs were prepared from Watman No 1 filter paper are soaked in 20% carbohydrate solution using a 2mm loop and placed in the designated area and incubated for 24 hours at room temperature. Those isolate which assimilate a particular sugar, grow well around the discs. The pattern of assimilation is noted.

Sugars	C. albicans	C. tropicalis	C. krusei	C. dubliniensis	C. parapsilosis	C. guillier mondii	C. glabrata
Glucose	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-
Sucrose	+	+	-	-	+	+	-
Maltose	+	+	-	+	+	+	-
Galactose	+	+	-	+	+	+	-
Mellabiose	-	-	-	-	-	+	-
Cellabiose	-	+	-	-	-	+	-
Xylose	+	+	-	-	+	+	-
Raffinose	-	-	-	-	-	+	-
Trihalose	+	+	-	-	+	+	+

CHROMagar Candida

CHROMagar prepared according to manufacturer's instructions (Hi Media, Mumbai). 21.02 gm of dehydrated media was suspended into 500 ml of distilled water. Then the suspension was heated to boil gently (not to be autoclaved) to dissolve the medium completely. After cooling to 50°C, the media were poured into petri plates and allowed to set. The growth of SDA sub-cultured on chromogenic medium and incubated at 35^{0}C. All yeast isolates grew well and developed distinctive colored colonies after overnight incubation. The plates were further incubated for a total of 48 h to get better developed colonies.

Antifungal Susceptibility Testing

Antifungal susceptibility testing of the yeast isolates was assessed using the agar diffusion method according to CLSI guidelines. The discs were supplied by Hi-Media, Mumbai. Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue was used for the sensitivity testing. The addition of the glucose speeds up growth of the yeasts and the addition of the methylene blue enhances edge-definition of zones. Disc diffusion method was used in the present study. The antifungal agents used for disc diffusion method are:

1. Amphotericin B (100 IU)
2. Fluconazole (25µg)
3. Nystatin(50 µg)
4. Ketoconazole (50µg)
5. Clotrimazole (10 µg)

Preparation of inoculum for the agar diffusion test

Using a sterile inoculating loop, distinct colonies of yeast from the SDA plates were transferred into 2ml of normal saline in a test tube. The colonies were emulsified in the saline solution to form a suspension of turbidity equivalent to 0.5 McFarland.

Inoculation of the agar plates

A sterile cotton swab was dipped into the inoculum already prepared. The soaked swab was rotated firmly several times against the upper inside wall of the test tube to remove excess fluid. Using the cotton swab, the dried

surface of the Mueller-Hinton agar (supplemented with glucose and methylene blue) was inoculated with the test organism. The plates were left for 5 minutes to allow for any surface moisture to be absorbed before the drug impregnated discs were applied. Using sterile forceps, the discs were removed from the container and then placed on the Mueller-Hinton agar. The sterile forceps was used to press down the discs to make contact with the surface of the Mueller-Hinton agar. The plates were incubated at 37ºC.

Reading of the plates and the measurement of diameter of zone of inhibitions

The plates were examined after 20 to 24 hours of incubation. The diameters of zones of complete inhibition were measured to the nearest whole numbers in millimeters using meter rule and recorded.

To determine whether the isolates tested against Amphotericin B, Nystatin, Fluconazole, Ketoconazole, Clotrimazole were susceptible, intermediate or resistant; the diameters of the zones of inhibition obtained were compared with the standard zones interpretive breakpoints published by CLSI M44-A2 guidelines[23], Dota KFD et al[24], Rosco Diagnostica[25].

A. Fluconazole (25µg):

Sensitive : ≥ 19mm zone clearance
Intermediate : 14-19mm
Resistance : ≤14mm

B. Ketoconazole (50 µg)

Sensitive : ≥ 20mm zone clearance
Intermediate : 10-20mm
Resistance : ≤10mm

C. Amphotericin B (100 IU)

Sensitive : ≥ 15mm
Intermediate : 10-14mm zone clearance
Resistance : ≤10mm

D. Nystatin (50 µg)

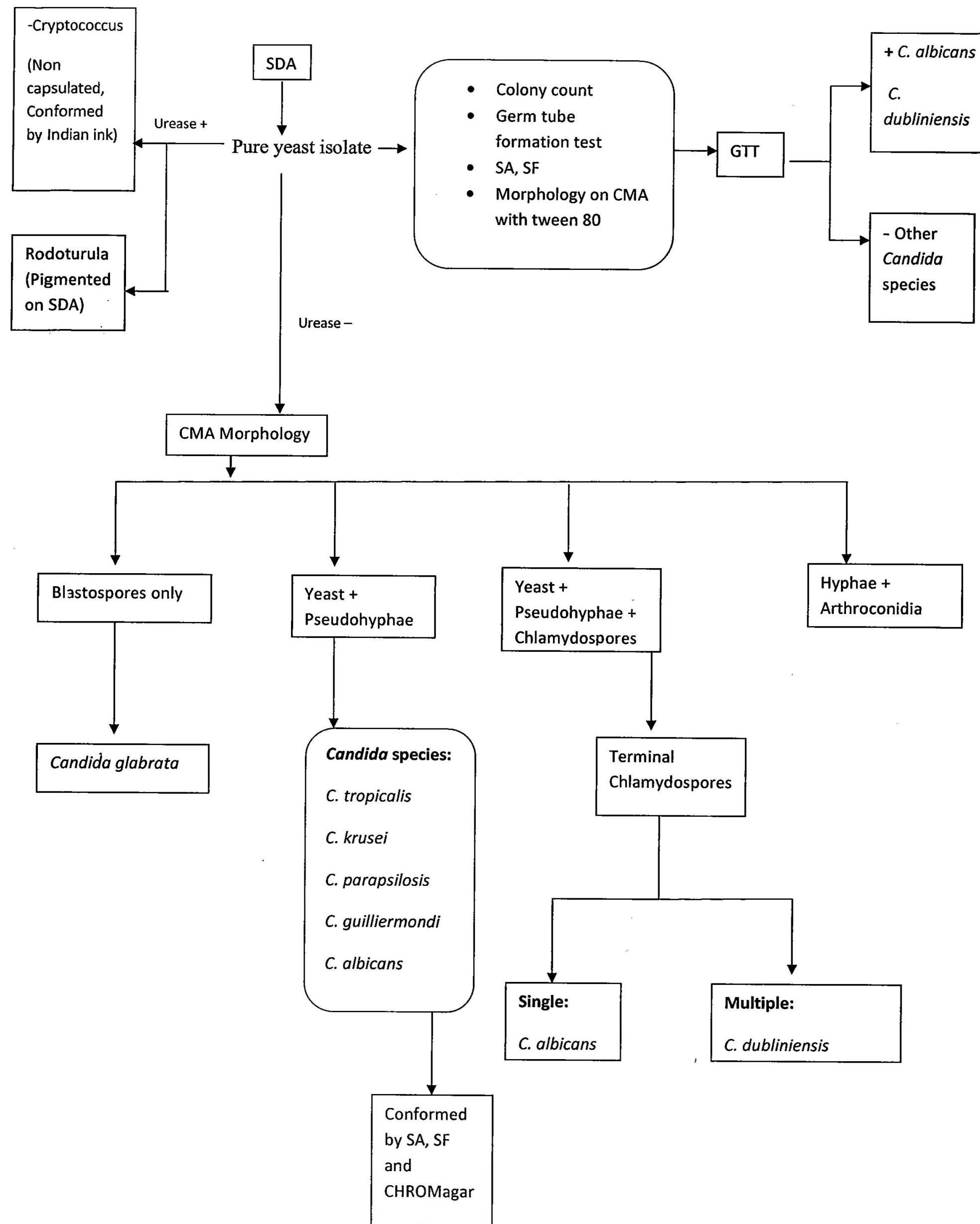
Sensitive : > 15mm zone clearance
Intermediate : 10-14mm
Resistance : ≤10mm

E .Clotrimazole (10 µg)

Sensitive : ≥ 20mm zone clearance
Intermediate : 12-19mm
Resistance : ≤11mm

Controls used are **:** *Candida parapsilosis* ATCC 22019
: *Candida albicans* ATCC 90028

Yeast Identification Scheme



SF – Sugar Fermentation Test
SA – Sugar Assimillation Test
GTT – Germ Tube Test

RESULTS

Statistical analysis

Descriptive statistics such as mean, standard deviation (SD), proportions and graphs were used to describe the data. Differences between proportions were analyzed using χ^2 tests, or by Fisher's exact test, if the sample sizes were small or unbalanced. A two-tailed P-value <0.05 was considered statistically significant. During the study period of November, 2009 to October, 2010, a total of 1702 clinical samples were screened. The prevalence of *Candida* species are studied in relation to age, sex, site of isolation, underlying conditions and predisposing factors.

Table-1
Distribution of patients according to age

Age	Sex		Total
	Female	Male	
0-9	5	7	12
10 - 19	2	0	2
20-29	18	1	19
30-39	15	2	17
40-49	12	11	23
50-59	5	10	15
60-69	3	7	10
70-79	2	3	5
Total	62	41	103

Mean age 37.3 and SD 18.74

The age of the patients showed an asymmetric distribution with more patients included in the age group of 20-49 years comprising 57.2% with a mean age of 37.3 ± 18.74. Age distribution of patients was from 1 month to 79 years. The highest incidence was seen in the age group 40-49 years.

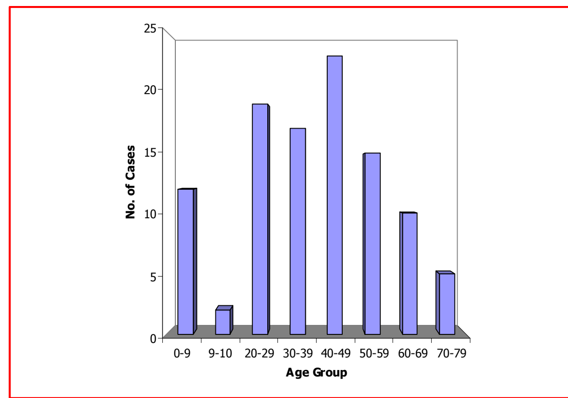


Figure-1
Distribution of patients according to age

Table-2
Distribution of patients according to sex

Sex	Number of patients	Percent
Female	62	60.2
Male	41	39.8
Total	103	100.0

Ratio: Male: Female = 0.66: 1

The *Candida* species isolated more from female (60.2%) than male (39.8%) patients in ratio of (M:F) 0.66: 1

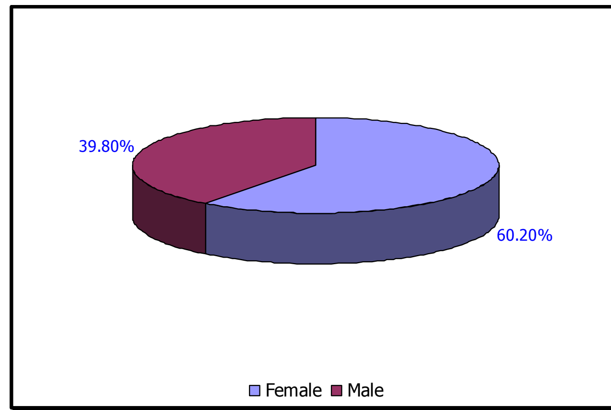


Figure-2
Distribution of patients according to sex

Table-3
Prevalence of Candida species in clinical specimens

Clinical specimens	Total No. screened	Total No. of isolates	Percentage
HVS	360	42	11.7
Sputum	300	21	7.0
Oral Swabs	60	12	20.0
Pus	330	6	1.8
Urine	550	6	1.1
Ear Swabs	15	5	33.3
Nail	10	5	50
Stool	40	4	10.0
Conjunctival swabs	5	1	20.0
Blood	32	1	3.1
Total	**1702**	**103**	**6.1**

χ^2 = 77.43, p<0.0001, Significant

Hundred and three (103) samples out of the 1702 tested positive for *Candida* species giving a prevalence rate of **6.1%** (p<0.0001).

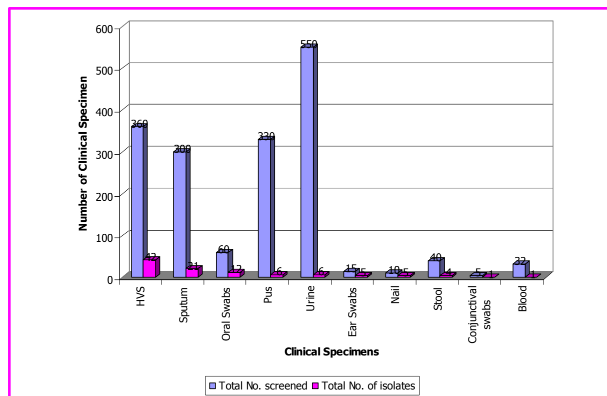


Figure-3
Prevalence of Candida species in clinical specimens

Table-4
Different species of Candida isolated

Organism	Total isolates (103)	Percentage
C. albicans	67	65.0
C.tropicalis	25	24.3
C.krusei	11	10.7
Total	103	100.0

Candida albicans was the major species accounting for 67(65%) of the total isolates. Non *albicans Candida* constituted 25 (24.3%) of *C.tropicalis,* followed by C.*krusei* 11(10.7%).

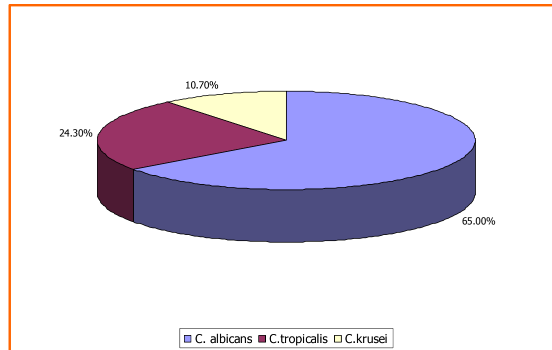


Figure-4
Different species of Candida isolated

Table-5
Identification of species by conventional method and CHROM agar

Species	Conventional method	CHROM agar
C. albicans	67	67
C. tropicalis	25	25
C. krusei	11	11
Total	103	103

All the *Candida* species, isolated from various clinical specimens who were characterized by conventional method, could correctly identified by CHROM agar.

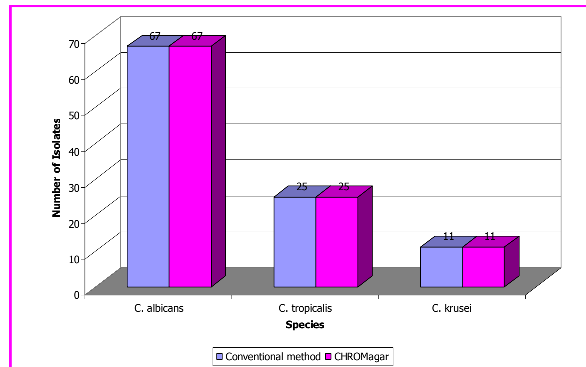


Figure-5
Identification of species by conventional method and CHROM agar

Table-6
Distribution of isolates among various predisposing factors

Predisposing Factor	Male	Female	Total (%)
DM	18	16	34 (33)
Pregnancy	0	23	23 (22.3)
Prolonged drug intake and secondary to other disease (PD and SD)	12	11	23 (22.3)
HIV	4	2	6 (5.8)
Prolonged contact with water (PW)	3	2	5 (4.9)
Others	4	8	12 (11.7)
Total	41	62	103 (100)

χ^2 = 0.772, p=0.3, Not Significant

In the study, diabetes mellitus was the major predisposing factor constituting 34(33%) followed by pregnancy 23(22.3%), prolonged drug intake / secondary infection 23(22.3%), HIV seropositive 6(5.8%) prolonged contact with water 5(4.9%). Others 12(11.7%) in predisposing factors include, catheterization, stay in ICU, parenteral nutrition, prematurity and low socio-economic status, poor personal hygiene. Even though there is female predominance as compared to male, it is statistically not significant (p>0.05)

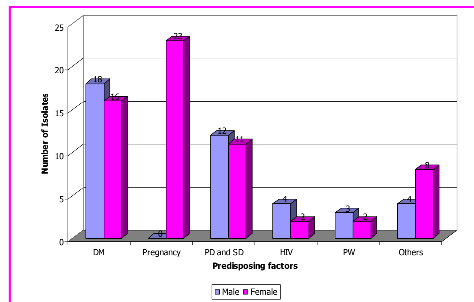


Figure-6
Distribution of isolates among various predisposing factors

Table-7
Distribution of different species of Candida among various clinical specimens

Clinical specimens	*C. albicans (%)*	*C. tropicalis (%)*	*C. krusei (%)*	Total (%)
HVS	34 (81)	6 (14.2)	2 (4.8)	42 (40.8)
Sputum	12 (57.1)	6 (28.6)	3 (14.3)	21 (20.4)
Oral Swabs	5 (41.7)	5 (41.6)	2 (16.7)	12 (11.7)
Pus	4 (66.6)	1 (16.7)	1 (16.7)	6 (5.8)
Urine	2 (33.3)	3 (50)	1 (16.7)	6 (5.8)
Ear Swabs	4 (80)	0	1 (20)	5 (4.9)
Nail	3 (60)	2 (40)	0	5 (4.9)
Stool	2 (50)	1 (25)	1 (25)	4 (3.9)
Conjunctival swabs	0	1 (100)	0	1 (1)
Blood	1(100)	0	0	1(1)
Total	67	25	11	103

X^2 = 8.697, p=0.013, significant

The highest number of isolates was from High vaginal swab isolated from vulvovaginitis constituting 42(40.8%). The other major samples were sputum 21(20.4%), oral swabs 12(11.7%), urine 6(5.8%), pus 6(5.8%), nail scrapings 5(4.9%), ear swabs 5(4.9%), followed by stool 4(3.9%) and blood 1(1.67%). Among the 42 High vaginal swabs, 34(81%) isolates were *C.albicans*, 6(14.2%) were *C.tropicalis,* and 2(4.8%) were *C.krusei.* Among 21 sputum samples, 12 (57.1%) were *C.albicans*, 6(28.6%) were *C.tropicalis,* 3(14.3%) were *C.krusei*. Among 12 oral swabs, 5(41.7%) were *C.albicans*, 5(41.6%) were *C.tropicalis* and 2(16.7%) were *C.krusei.* Among 6 pus samples, 4(66.6%) were *C.albicans*, 1(16.7%) each were *C.tropicalis* and *C.krusei.*

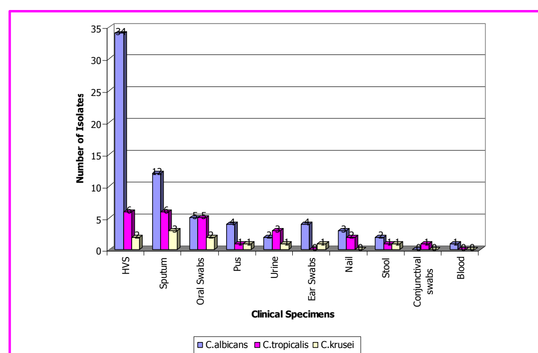


Figure-7
Distribution of different species of Candida among various clinical specimens

Among 6 urine samples, 2(33.3%) were *C.albicans*, 3(50%) were *C.tropicalis* and 1(16.7%) was *Candida krusei.* Among 5 nail scrapings, 3(60%) were *C.albicans* and 2(40%) were *C.tropicalis*. Among 5 ears swabs, 4(80%) were *C.albicans*, 1(18.18%) one was *C.krusei* (20%). Among 4 samples of stool, 2(50 %) species were *C. albicans* and one (25%) each of *C.tropicalis* and *C.krusei.*

Table-8
Distribution of different species of Candida among various predisposing factors

Predisposing Factor	*C.albicans*(%)	*C.tropicalis*(%)	*C.krusei*(%)	Total
DM	20(58.82)	10(29.41)	4(11.76)	34
Pregnancy	18(78.26)	3(13.04%)	2(8.65)	23
Prolonged drug intake and secondary to other disease (PD and SD)	16(69.56)	3(13.04)	4(17.39)	23
HIV	2(33.33)	4(66.66)	0	6
Prolonged contact with water (PW)	3(60)	2(40)	0	5
Others	8	3	1	12
Total	67	25	11	103

χ^2 = 2.149, p=0.342, Not significant

C.albicans 20(58.82%) was the major isolate in diabetes mellitus, followed by *C.tropicalis* 10(29.41%) and *C.krusei* 4(11.76%). Among 23 *Candida* isolates having pregnancy as predisposing factors, 18(78.26%) were *C.albicans*, 3(13.04%) were *C.tropicalis*, 2(8.69%) was *C.krusei.* The 23 *Candida* isolated from patients on prolonged broad spectrum antibacterial agents, corticosteroid and secondary to other infection were *C.albicans* in 16 (69.56%), *C.tropicalis* in 3(13.04%) cases and *C.krusei* (17.39%) in 4 case. HIV infection with *C.tropicalis* 4(66.66%) as the major isolate and *C.albicans* 2(33.33%) Among 5 *Candida* isolates with prolonged contact with water 3(60%) isolates were

C.albicans and 2 (40%) isolates were *C.tropicalis.* Among the 12 isolates of various other predisposing factor were *C.albicans* 8(66.66%), *C.tropicalis* 3(25%) and *C.krusei* 1(8.33%).

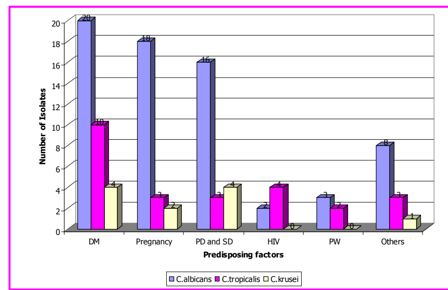


Figure-8
Distribution of different species of Candida among various predisposing factors

Table-9
Antifungal susceptibility pattern

Species	Amphotericin B			Nystatin			Fluconazole			Ketoconazole			Clotrimazole		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C.albicans	66	0	1	66	1	0	62	4	1	66	0	1	62	0	5
C.tropicalis	25	0	0	24	1	0	24	0	1	25	0	0	23	0	2
C.krusei	11	0	0	9	2	0	0	0	11	11	0	0	10	0	1
Total	102	0	1	99	4	0	86	4	13	102	0	1	95	0	8
χ^2 value	0.526			3.13			11.37			0.526			0.02		
p-value	0.99			0.056			0.001			0.99			0.87		

In the present study, among 103 *Candida* isolates tested, Ketoconazole and Amphotericin B showed resistance in 0.97% cases. For Nystatin, no strains showed resistance and 3.88% strains showed intermediate sensitive. For Clotrimazole, 92.23% strains showed sensitive, 7.76% strains were resistant and among them 4.85% were *C.albicans*, 1.97% for *C.tropicalis*, and 0.97% for *C.krusei*. For fluconazole, 83.49% strains showed sensitive, 12.6% showed resistance and 3.88% showed intermediate sensitivity (or dose dependent susceptibility) (p= 0.001) Amphotericin B and Ketoconazole are more sensitive followed by Nystatin, Clotrimazole and Fluconazole. Resistance is seen significantly among non-*albicans Candida* for Fluconazole (p=0.001) and Nystatin showed intermediate sensitive (p=0.056).

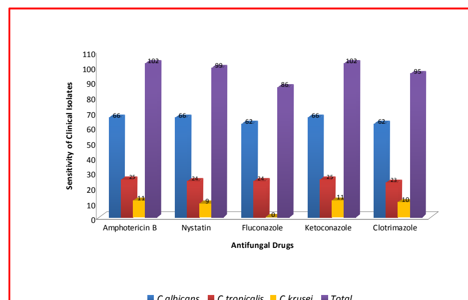


Figure-9
Antifungal susceptibility pattern

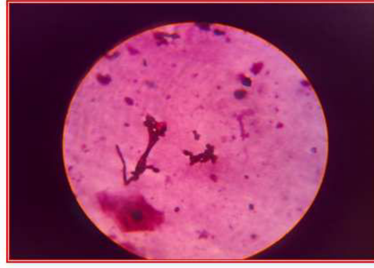


Figure-10
Direct gram stain of sputum showing budding yeast cells with pseudohyphe

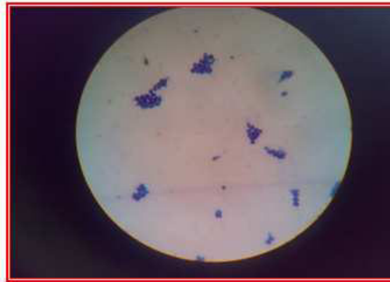


Figure-11
Gram stain showing gram positive yeast like budding cells

Figure-12
Candida growth on SDA

Figure-13
***Candida albicans* showing Germ tube formation**

Figure-14
Candida albicans showing chlamydospores on CMA

Figure-15
Sugar assimilation test

Figure-16
Sugar fermentation test

Figure-17
Hi Crome Candida Differential agar base modified (supplied by Hi Media Laboratories)

This article can be downloaded from www.ijpbs.net

Figure-18
Candida albicans on CHROMagar candida

Figure-19
Candida tropicalis on CHROMagar candida

Figure-20
Candida krusei on CHROMagar candida

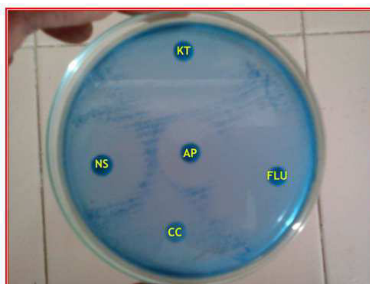


Figure-21
Antifungal susceptibility testing by Disc diffusion method

DISCUSSION

The study identified the specific species of *Candida* that causes infections among patients who attended Navodaya Medical College Hospital and Research Center. The study also determined the in vitro susceptibility of *Candida* species isolated from the various clinical samples. The antifungal agents tested include Amphotericin B, Nystatin, Fluconazole, Ketoconazole and Clotrimazole. The antifungal agents used in this study were chosen on the basis of being commonly available in the local market and were among the drugs that are prescribed at our hospital. *Candida albicans* is by far the most common species causing infection in humans. *Candida albicans* and non-*albicans* species are closely related but differ from each other with respect to epidemiology, virulence characteristic and antifungal susceptibility. All *Candida* species have been known to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet differences in disease severity and susceptibility to different antifungal agents have been reported.[26,27] Distinction between species facilitates the understanding of epidemiology of *Candida* species particularly regarding the reservoir and mode of transmission which is requirement for the development of effective measures to prevent and control transmission of resistant pathogens.[28] In the present study it was found that candidiasis can occur at all ages and in both sexes. The youngest in our study was one month old baby while the oldest was 79 years and the mean age was 37.3 years. The majority of the patients were in the age group of 21-49 years and this might be related to the fact that, this is the age group with higher hormonal variation, sexual activity and rate of pregnancy. This is supported by the percentage of vaginal sample included in our study and predisposing factors like diabetes, prolonged contact with water was seen in higher age group. According to study conducted by Dalal et al[29], Akorth et al[30], Puri KJ et al[88], Jayalaxmi et al[32], maximum cases were in the age group of 21-40 years. Clayton et al[33] in a study on observations on the epidemiology of Candida species, found majority of patients belonging to the age group of 20-60 years. Sahni et al[34] in their study, the mean age was 43.4 years. This study is in accordance with above study. Sex distribution in present study showed that females are more commonly affected than males with an incidence of 62 (60.2%) and 41(39.8%) respectively in a ratio of 0.66:1(M:F). In a similar study conducted by Kandhari KC et al[35] at AIIMS, New Delhi, the incidence in females was about 61.2% while in males it was only 38.8% with a ratio of 1:1.57(M:F) and Rizvi MW et al[36], also reported female preponderance in their study group with a ratio of 0.85:1(M:F).

In the present study, *Candida* species isolated from various clinical samples and the highest was from high vaginal swab (40.8%), followed by sputum (20.4%), oral thrush (11.7%), urine (5.8%), pus (5.8%), nail scrapings (4.9%), ear swabs (4.9%), stool (3.9 %), blood (1%) and conjunctival swab (1%). Basu et al[37] isolated *Candida* from HVS (7.6%) which was lower than Dastidar et al[38] who isolated *Candida* from HVS (26.4%) which is similar to this study. Basu et al[37] in their study, respiratory tract secretion (34%) was the most common source for *Candida* isolation Dastidar et al[38], in their study found oral thrush in 12% of patients while in our study it was 11.6% which is similar to the above study. The present study identified *Candida* species by conventional method and CHROMagar.

Table-10
Studies done by various research workers

Author	Place	*C.albicans*	*C.tropicalis*	*C.krusei*
Yang et al[95]	Taiwan	69%	12%	0.6%
Basu et al[93]	Delhi	45.8%	24.7%	7%
Vijaya et al[99]	Bangaluru	45.9%	35.29%	10.78%
Present study	Raichur	65%	24.3%	10.7%

The above table compares the Candida species isolated by different workers.

The predominant species isolated in our study was *Candida albicans* constituting 65%. The non-*albicans Candida* isolated are *Candida tropicalis* (24.3%) followed by *Candida krusei* (10.7%). In a study by Basu et al[37] in Delhi, the *C.albicans* (45.8%) was the predominant isolate followed by *C.tropicalis* (24.7%), *C.krusei* (7%). This study is similar to above studies. In the present study, the results of CHROM agar were exactly paralleled to that of conventional method. The higher incidence of candidiasis in diabetics may be due to the fact that hyperglycemia limits neutrophil function by inability to phagocytose and kill *Candida* especially among person with type 1 diabetes. So diabetics may not be able to clear pathogens as well as by non-diabetics.107 It has been established that the prevalence of genital candidiasis increases in pregnancy. During pregnancy, levels of both progesterone and estrogen hormones are elevated. Progesterone has suppressive effects on the anti-*Candida* activity of neutrophils, while estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida* species and also decrease immunoglobulins in vaginal secretion resulting in increased vulnerability of pregnant women to vaginal candidiasis. In the present study, *Candida* species was isolated from 22.3% cases pregnancy. Among the *Candida* isolates, 78% were *C.albicans*, 13% were *C.tropicalis* and *C.krusei* in 8.7% cases. Praveen et al[9] found that frequency of vaginal candidiasis during pregnancy was 38%. In the present study, *Candida* species were isolated from 54.8% of the pregnant and 45.2% of the non-pregnant and menopausal women. The above studies reported by different authors indicate that, morbidity in pregnancy due to vulvovaginal candidiasis is more when compared to non-pregnant women. In our study, *Candida* species were isolated from patients on prolonged corticosteroids, broad-spectrum antibiotic use and secondary to other diseases accounted for 22.3% of cases. Among the *Candida* species isolated, 69.7% were *C. albicans*, 17% were *C.krusei*, and 13% were *C.tropicalis.* Broad spectrum antibiotics, corticosteroids and cytotoxic agents are commonly cited risk factor for candidemia. Drugs such as broad spectrum antibiotic alter the local oral flora creating a suitable environment for *Candida* to proliferate. The normal oral flora is restored once the antibiotics are discontinued. Arora D et al[2] reported that, 35% of candidiasis was due to prolonged use of antibiotics. In this study, second most samples were *Candida* species isolated was from sputum in which the presence of primary infection accompanied by malnutrition and debilitation favored *Candida* infection. Studies done by the above authors indicate that use of broad spectrum antibiotics and corticosteroids are important predisposing factors which can cause *Candida* infection upto 66% and also showed that it can cause secondary infection. In the present study 6 cases are attributed of HIV infection, *C.tropicalis* was isolated from 4 patients and *C.albicans* isolated from 2 cases. In a study by Jaylaxmi et al[32] found that vulvovaginitis incidence was 26.5% in HIV positive patients. In the present study, *Candida* isolated from 4.8% cases of onychomycosis. All of them had history of prolonged contact with water. Three of them were agriculturist and two were working in laundry and they had frequent history of contact with water. Other comprised of 11.7% in predisposing factors which include, catheterization, stay in ICU, parenteral nutrition,

prematurity, poor socio-economic status, poor personal hygiene and oral contraceptive. In a study by Mendiratta DK et al[19], it was shown that the colonization of *Candida* in preterm (33.5%) was more than that in the healthy term babies (10%).

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