

Original Research Article

A STUDY OF SPECIES IDENTIFICATION, DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA

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ABSTRACT

Background: Candidiasis is the most commonly encountered opportunistic infection caused by candida species. Although candida albicans is the major species contributing to the various invasive and non-invasive diseases, the non-albicans candida (NAC) has been noted in recent years and during the COVID-19 pandemic. The organisms have also shown resistance to multiple antifungals mainly the azoles. So, the present study is conducted to identify the various species of candida and their antifungal susceptibility pattern.

Materials and Methods: speciation was done by conventional and automated methods. Antifungal susceptibility testing was conducted by both disc diffusion and vitek 2 automation using fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine.

Results: Isolation of both candida albicans and non-candida albicans was seen. Although candida albicans is more commonly encountered, the non-candida albicans C.guilliermondii, C. tropicalis, C. parapsilosis, C. utilis etc. were also noticed. Antifungal resistance to fluconazole and amphotericin-B were noted. The most common specimens encountered were cervical swabs, blood, urine.

Conclusion: In the present study candida albicans was the major isolate and other species like C.guilliermondii, C.parapsilosis, C.tropicalis, C..utilis, were also noted. The major species isolated were from cervical swabs, blood, and urine. The antifungal resistance was also seen in non-candida albicans. Therefore, speciation and antifungal susceptibility testing should be done to avoid treatment failure.

Keywords: Speciation, non-candida albicans, antifungal susceptibility testing. Disc diffusion, Vitek2.

INTRODUCTION

Candidiasis is an infection caused by the overgrowth of pathogenic yeast from the genus candida. Its prevalence accounts for the most common type of opportunistic fungal infection affecting human health globally, with more than a billion cases every year. Typically, yeast can live harmlessly on the host's mucosal tissues, such as in the oral cavity, gastrointestinal mucosa, and vaginal mucosa, unless the balance is disrupted. The immunosuppressed, elderly population and palliative patients are highly susceptible to Candida infections.^[1]

The risk factors for candidemia and candidiasis have been discussed in several studies, highlighting the role of age (>60 years), as well as underlying conditions, such as diabetes mellitus, acute renal failure (ARF), being on hemodialysis (HD), burns, and having malignancies. Additional risk factors include previous Candida colonization, complicated abdominal surgeries, central venous lines, total parenteral nutrition, corticosteroids and broad-spectrum antibiotics, pregnancy, prematurity, and immunosuppressive therapy. The coronavirus disease 2019 (COVID-19) is a recently identified risk factor for candidemia.^[2]

Infections resulting from *Candida albicans* and non-*albicans* species have increased significantly in the last decade and are among the leading causes of nosocomial infections, which result in serious public health problems.^[3]

Although *Candida albicans* is the most common causative agent of both superficial and deep fungal infections, an increased 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*.^[4]

The threat due to MDR *C. auris* further increases in the COVID-19 pandemic as the ratio of hospitalized patients increases, providing a vulnerable environment for nosocomial infections. A recent study reported a mortality rate of 67.85% for coronavirus-associated *C. auris* infections. Timely diagnosis and proper antifungal treatment are required to improve clinical outcomes and manage candidiasis.^[5]

Thus, the present study was carried out to determine the species distribution and antifungal susceptibility profile by conventional as well as automated methods.

MATERIALS AND METHODS

This is an observational study conducted in the department of microbiology Mahadevappa Rampure Medical College Karnataka. The present study was carried out from 1st August 2022 to 31st January 2024. Samples were collected from clinically suspected cases of candidiasis attending the Hospital. Informed consent was taken from each patient after briefing them about the study.

A detailed history was taken regarding name, age, sex, address, OP/IP no., and occupation. The presence of predisposing factors like diabetes, history of long-term antibiotic intake, steroid use, HIV, catheterization, pregnancy, prematurity, etc. was considered.

A thorough physical examination was carried out in every case and was noted for varied manifestations of candidiasis.

Specimen collection: The specimens for laboratory investigation were collected from clinically suspected cases of candidiasis under strict aseptic precautions. Sterile swabs and bottles were used to collect the appropriate clinical material. The various clinical sample collected were oral swab, blood, urine, cervical swab, vaginal swab, nail scrapping, ear swab, wound swab, sputum etc. The specimen was labeled properly.

The following methods were used while collecting specimens from various sites: -

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

Nails and nail bed: nails were cleaned with 70% isopropyl alcohol and a portion of the infected nail was clipped. In the case of paronychia, the exudate was expressed from below the nail folds and the sample was collected on a sterile swab.

Blood: blood is collected by minimal asepsis using one-step skin disinfection with alcohol collected in a vacutainer. for automation in BacTec /Alert blood culture bottles are used.

Urine: fresh, mid-stream, clean catch urine (50-200ml) was collected in a sterile screw-capped container.

Cervical swab: A speculum examination was done and the cervical discharge was collected using a sterile swab.

Pus or discharge: the specimens were collected using sterile swabs after the area had been cleansed properly. Two swabs were taken from each case. The specimens were transported immediately to the laboratory taking care not to dry out the specimen. One specimen was subjected to direct examination and the other to culture.

Sputum: sputum is collected in a sterile container in the early morning and spot. They are collected in a well-ventilated area. Gastric aspirate for infants was collected.

Mycological Investigation:

A) Direct examination- each specimen was examined microscopically before culturing by the following methods.

Potassium hydroxide (KOH) wet mount: Placed the specimen on a clean glass slide add 1 to 2 drops of 20% KOH and place the cover slip. The slide was gently heated and after the material softened (after about 15-20 minutes), gentle pressure was applied over the coverslip to force out any trapped air, and specimen examination was conducted first under low (10x) and then under high power objective (40x). In the case of nail clipping, 40% KOH is used about 0.5 to 1ml of 40% KOH was first taken in a small test tube, nail clipping has to be put and kept overnight to dissolve the nail keratin. The specimen was then examined under low and high-power objectives.

Gram stain: Smears were prepared on a clean, dry glass slide from each specimen and heat-fixed. The fixed smear was stained by the Gram staining method and observed under an oil immersion lens (100x) to look for the presence of Gram-positive oval yeast-like budding cells and pseudohyphae.

B) Culture:

Culture Medium: Sabouraud's dextrose agar medium was used for the cultivation of fungi. It had a slightly acidic pH of 5.6, which is favorable for the growth of many fungal species, including *Candida*. The addition of the antibiotic chloramphenicol helps prevent bacterial contamination and overgrowth in the culture, allowing for the selective growth of fungi.

Cycloheximide: The decision to avoid cycloheximide in the medium was based on the sensitivity of certain *Candida* species (*C. krusei*, *C.*

parapsilosis, and *C. tropicalis*) to this antifungal agent. Cycloheximide can inhibit the growth of some *Candida* species, and its omission ensures that these species can grow and be detected.

Inoculation and Incubation: Freshly collected specimens are inoculated onto two Petri dishes of Sabouraud's dextrose agar and incubated at temperatures 37°C.

Colony Characteristics: After 48-72 hours of incubation, the colonies of *Candida* species appeared as cream-colored, smooth, and pasty. Growth was identified by the colony characteristics and the growth of *Candida* was confirmed by gram staining. The SDA Petri dishes with growth were processed further for speciation by physiological tests

C) Physiological tests

Germ tube test (Reynolds-Braude phenomenon):

A small portion of an isolated colony of *Candida* to be tested is suspended in a test tube containing 0.5ml of human serum. The test tube is incubated at 35°C for about 2-4 hours. A drop of suspension was placed on a microscopic slide, overlaid with a coverslip. This was examined under low power objective to locate the group of cells and later the presence of germ tube was confirmed under high power objective of microscope. Over 90% of *C. albicans* produced germ tubes. The germ tube is an elongated projection from the yeast cell, typically about half the width and 3 to 4 times the length of the mother cell. One important characteristic is that there is no constriction at the point of origin of the germ tube.

Corn Meal Agar Inoculation (Dalmau technique):

Prepared corn meal agar and added 1% Tween-80 to the corn meal agar. Mixed thoroughly to ensure even distribution. Sterilized the medium by autoclaving. The medium was poured into Petri dishes to solidify. Using a sterile loop, made 2-3 parallel cuts on the surface of the corn meal-tween-80 agar. Each cut was 3.5-4 cm long and 1.2 cm apart. Placed a flame-sterilized and cooled cover glass over the central part of the streak to create a microaerophilic environment. Incubated the plates at 22°C for 48 hours, after 48 hours of incubation, examine the plates. Placed the plate on the microscope stage and observed for the presence of: Blastoconidia: Yeast cells reproducing by budding. Pseudohyphae: Chains of elongated yeast cells without true septa. Chlamydospores: *C. albicans* formed large thick-walled, terminal chlamydospore.

CHROM agar inoculation: CHROM agar (Hi-Media) is a selective and differential medium commonly used for the isolation and differentiation of various *Candida* species. CHROM agar was prepared and the subculture was done in brain heart infusion broth by suspending colonies from a fresh blood agar plate. The BHI broth was kept for 45 minutes in the incubator, taken out, and then the yeast suspension was streaked onto the CHROM agar plate with the help of a sterile loop to form single colonies. Incubated the CHROM agar plate at 37°C for 48-72 hours and observed different *Candida* species

producing colonies with distinct colors on the CHROM agar plate.

Sugar Fermentation Test: prepared fermentation test media with peptone, Andrade's indicator, a suitable range of sugars, and water. The test organism was subcultured onto a sugar-free medium to deplete the presence of fermentable carbohydrates, and inoculum preparation was done. Inoculated the test organism by adding one drop of inoculum suspension in each tube containing 1% sugar concentration and incubated for 48-72 hours at 30°C. The ability to ferment sugar is shown by acid (indicator turns pink) and gas trapped in the Durham tube. The different species of *Candida* were identified by fermentation patterns which were specific to each species of *Candida*.

Sugar assimilation test (auxanographic plate method):-

prepared the basal media using yeast nitrogen base, agar, and water, dispensed in a universal container, and autoclaved for 115°C for 15min. The test organisms were grown on a basal sugar-free medium and the carbohydrate-depleted *Candida* colonies were made into suspension in sterile saline to a density equivalent to match Mc Farland no.4/5 density. The YNB agar plate is poured with inoculum and with the help of sterile forceps selected carbohydrate discs were placed on the surface of the YNB agar plate, 30mm apart. The plate was incubated at 30°C for 24-48 hours. The 2% sugars used were glucose, sucrose, lactose, maltose, trehalose, and xylose. The growth around individual sugar disc, indicating assimilation of that particular carbohydrate, and the absence of growth is interpreted as no assimilation. Different species of *Candida* utilized specific carbohydrate substrates and standard carbohydrate profiles were used to identify the species.

Antifungal susceptibility testing by conventional method:

The antifungal susceptibility testing was done by the Kirby-Bauer disc diffusion method.

A) antifungal susceptibility testing by disc diffusion method.

Muller-Hinton agar with 2% glucose and 0.5µg/ml of methylene blue dye is used.

Inoculum preparation-distinct colonies of *Candida* from an SDA plate were transferred using a sterile inoculating loop into 2ml normal saline in a test tube and the cell density was adjusted to 0.5 Mc Farland standard, the resulting suspension equals $1-5 \times 10^6$ cell/ml. The working solution is prepared by diluting the 1ml above solution with 19ml of normal saline resulting in a suspension containing $0.5-2.5 \times 10^3$ cell/ml.

The following antifungal discs were used fluconazole (10mcg/disc), amphotericin B (100units/disc), voriconazole (1mcg/disc) caspofungin, micafungin, and flucytosine.

Testing procedure: Pour the inoculum suspension into the entire Petri plate. The plates were allowed to dry and with the help of sterile forceps the antifungal discs were placed 30mm apart from each other on the

surface of the plate. The plates were incubated at 37^oc for 48 hours and the diameter of the clear zone of inhibition around the discs was noted. An interpretation was done according to standard CSLI guidelines.

Mycological examination by automated method VITEK-2 System (YST card) procedure for candida species identification and Yeast Susceptibility Testing

- 2 plastic tubes (no. 1 and no.2) were taken for isolation and placed on a cassette
- 3ml of suspension solution in each tube from the dispensator was taken
- tube1 as ID tube (for identification), and tube2 as YST tube (for sensitivity)
- picked similar isolated colonies from quarant4 from the blood agar plate, and made a suspension in tube1.
- fixed the turbidity of tube 1 up to 2.2-2.4 McFarland with the help of densi-chek plus.
- transferred 145µl of suspension from tube1 to tube 2
- placed ID card in tube1 and YST card in tube 2
- Place the cassette in the vitek2 compact machine and readings are taken by using software.

Candida isolates were done using the VITEK® 2 System (AST-YS08) (BioMérieux, France) and analyzed according to the 2017 Clinical and Laboratory Standards Institute guideline (CLSI M60). The panel of antifungal agents tested included amphotericin B, caspofungin, fluconazole, flucytosine, micafungin, and voriconazole. C. albicans ATCC 10231 and C.parapsilosis ATCC 22019 were used as controls during the laboratory procedure.

RESULTS

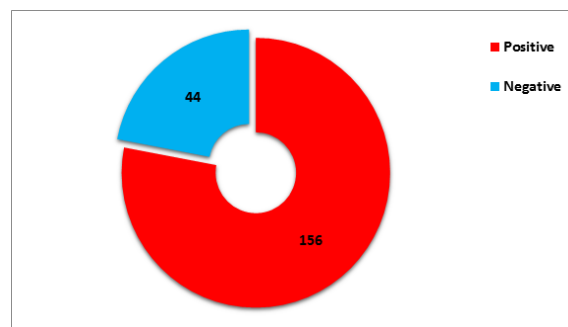


Figure 1: Pie chart presents culture positivity in clinically suspected cases of candidiasis

The [Table 1 and Figure 1] depict: A total of 200 cases were examined for the mycological investigation to confirm the presence of candidiasis, among them 156 cases were culture positive accounting for 78% and 44 (22%) were culture negative. The 156 culture-positive isolates were further evaluated for a series of tests for speciation.

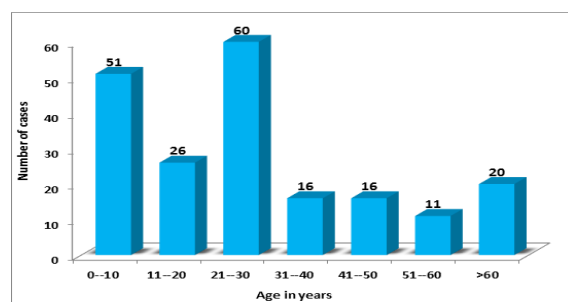


Figure 2: The bar diagram represents the age-wise distribution of cases

Table 1: Culture positivity in clinical suspected cases of candidiasis

Culture report	No. of cases	Percentage
Positive	156	78.0
Negative	44	22.0
Total	200	100.0

Table 2: Age Distribution

Age in years	No. of cases	Percentage
0-10	51	25.5
11-20	26	13.0
21-30	60	30.0
31-40	16	8.0
41-50	16	8.0
51- 60	11	5.5
>60	20	10.0
Total	200	100.0

The [Table 2 and Figure 2] show the age incidence of candidiasis in the present study. The highest incidence is seen in the age group of 21-30 years

comprising 30%, followed by 25.5% in the age group of 0- 10 years. The lowest incidence was in the age group of 51-60 (5.5%) years.

Table: 3 Sex Distribution of Cases

SEX	NO. OF CASES	PERCENTAGE
FEMALE	128	64.0
MALE	72	36.0
TOTAL	200	100.0

Among 200 patients, females were 128(64%) and males were 72(36%). The incidence was higher

among females than males. The male-to-female ratio is 1:1.7

Table 4: number of culture positives among various clinical specimens

Clinical specimen	No. Of cases	Percentage
CERVICAL SWAB	75	37.5
BLOOD	42	21.0
URINE	54	27.0
SPUTUM	9	4.5
PUS	7	3.5
NAIL SCRAPING	3	1.5
SUCTION TIP	2	1.0
EAR SWAB	2	1.0
SKIN SCRAPING	2	1.0
CORNEAL SCRAPING	2	1.0
SKIN BIOPSY	1	0.5
ASCITIC FLUID	1	0.5
TOTAL	200	100.0

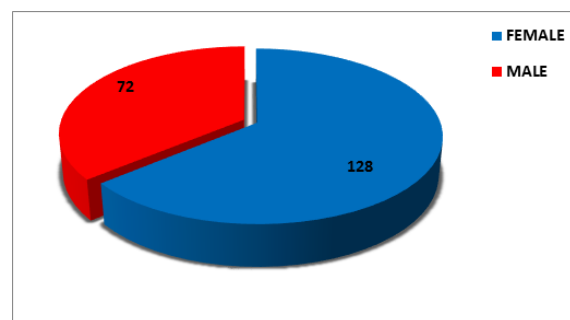


Figure 3: The pie chart represents the sex-wise distribution of cases.

The [Table 4 and Figure 4] depict the various clinical samples taken from the patients, among them cervical swabs were the highest(n=75) followed by

urine(n=54), blood(n=42), sputum(n=9), pus(n=7), nail scraping(n=3). Suction tip, ear swab, skin scraping, and corneal scraping were two each and both skin biopsy and ascitic fluid were one respectively.

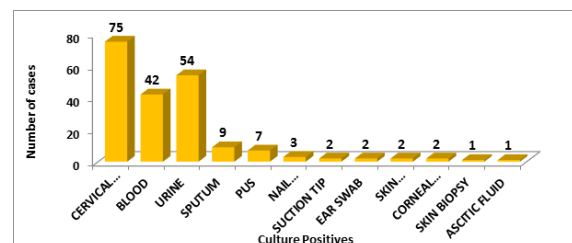


Figure 4: The bar diagram presents culture positives among various clinical specimens.

Table 5: different species of candida isolated from culture-positive cases

Species	No. Of cases	Percentage
Candida albicans	60	38.5
Candida guilliermondii	33	21.2
Candida utilis	22	14.1
Candida parapsilosis	16	10.3
Candida tropicalis	12	7.7
Candida glabrata	10	6.4
Candida inconspicua	1	0.6
Candida dubeliensis	1	0.6
Candida famata	1	0.6
Total	156	100.0

The [Table 5 and Figure 5] data shows the different species of candida isolated from 156 culture-positive cases. Among 156 isolates C.albicans was the predominant species, accounting for 38.5%,

C.guilliermondii 21.2%, C.utilis14.1%, C.parapsilosis 10.3%, C.tropicalis 7.7%, C.glabrata 6.4%, and C.inconspicua, C.dubeliensis, C. famata 0.6% each.

Table 6: Distributions of different candida species among various clinical samples

Source	Total	Candida albicans	Candida guilliermondii	Candida utilis	Candida parapsilosis	Candida tropicalis	Candida glabrata	Candida inconspicua	Candida dubeliensis	Candida famata
Cxswab	64	18	-	20	15	-	9	1	1	-
Blood	42	3	33	2	1	2	-	-	-	1
Urine	34	25	-	-	-	8	1	-	-	-
sputum	6	6	-	-	-	-	-	-	-	-
pus	3	3	-	-	-	-	-	-	-	-
Suction tip	2	2	-	-	-	-	-	-	-	-

Ear swab	1	1	-	-	-	-	-	-	-	-
Nail scrapin g	2	-	-	-	-	2	-	-	-	-
Skin scrapin g	2	2	-	-	-	-	-	-	-	-
Total	156	60	33	22	16	12	10	1	1	1
Fisher exact test P-value	P = 0.0173, Significant									

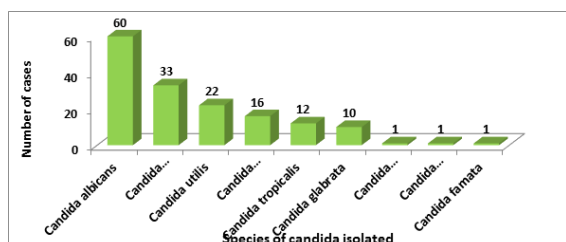


Figure 5 The bar diagram presents species of candida isolated from culture-positive cases

There was statistically significant difference of distribution of candida species among various clinical samples ($P < 0.05$)

The above table depicts the distribution of various species among clinical samples in cervical swab candida utilis was the highest $n=20$, in blood *C. guilliermondii* was the highest $n=33$, and in urine candida albicans was highest $n=34$.

Table 7: Antifungal Susceptibility Patterns Of Various Species

SPECIES	FLU			VORI			CASPO			MICA			Amph-B			FLY		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>C.albicans</i>	48	-	12	60	-	-	60	-	-	60	-	-	48	-	12	60	-	-
<i>C.guilliermondii</i>	-	-	33	33	-	-	33	-	-	33	-	-	33	-	-	33	-	-
<i>C.utilis</i>	22	-	-	22	-	-	22	-	-	22	-	-	22	-	-	22	-	-
<i>C.parapsilosis</i>	16	-	-	16	-	-	16	-	-	16	-	-	16	-	-	16	-	-
<i>C.tropicalis</i>	12	-	-	12	-	-	12	-	-	12	-	-	12	-	-	12	-	-
<i>C.glabrata</i>	10	-	-	5	5	-	2	-	8	10	-	-	10	-	-	10	-	-
<i>C.inconspicua</i>	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	-	1	-
<i>C.dubeliensis</i>	-	-	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
<i>C.famata</i>	-	-	1	1	-	-	1	-	-	1	-	-	1	-	-	1	-	-
TOTAL	108		48	151	5	-	148	-	8	156	-	-	143	-	13	155	1	
PERCENTAGE	69.23		30.77	96.79	6.41		94.89		5.11	100			91.61		8.39	99.35	0.65	
Fisher exact test P-value	P = 0.4873, Not significant																	

Flu=fluconazole. Vori=voriconazole, caspo=caspofungin, mica=micafungin, fly=flucytosine, amph-b=amphotericin-b.

There was statistically not significant difference of distribution of antifungal susceptibility patterns of various species ($P > 0.05$)

The above table shows the antifungal susceptibility patterns of the different species of candida by disc diffusion and vitek2 automated method. the fluconazole showed 69.23% sensitivity and 30.7% resistance, voriconazole 96.79% and 6.41%. and others showed almost 100% sensitivity to caspofungin, micafungin, flucytosine, amphotericin-B showed 8.3% resistance.

DISCUSSION

Candidiasis is the most common fungal disease in humans affecting mucosa, skin, nails, and internal organs. The clinical manifestations of candidiasis vary, ranging from acute, subacute, chronic, and episodic. It is found mainly as a secondary infection in individuals with some underlying

immunocompromised condition and very rarely as the primary disease.

Although candida albicans remains the most common causative agent, an increasing incidence of less species of candida has been documented in the last few years. These species include *C.tropicalis*, *C.guilliermondii*, *C.parapsilosis*, and *C.glabrata* and tend to be less susceptible to azole, particularly fluconazole.^[5]

The study conducted by Gulcan Sahal et.al. the frequencies of different Candida species isolated in this study shows that most of the Candida species were *C. parapsilosis* (31.3%; $n = 31$) and *C. tropicalis* (30.3%; $n = 30$), followed by *C. krusei* (15.2%; $n = 15$), *C. albicans* (13.1%; $n = 13$), *C. glabrata* (8.1%; $n = 8$), *C. kefyr* (1%; $n = 1$), and *C. orthopsilosis* (1%; $n = 1$). Furthermore, the majority of Candida isolates were observed to be isolated from vaginal swabs (49.5%; $n = 49$), followed by specimens of tracheal aspirate (10.1%; $n = 10$), blood (9.1%; $n = 9$), sputum (9.1%; $n = 9$), urine (8.1%; $n = 8$) and catheter (1%; $n = 1$). In our study majority of species were identified in cervical swabs, blood, and urine samples.^[6]

In the study done by Sarkate PR et.al. The sugar fermentation test and sugar assimilation test done for speciation of *Candida* showed that 40% of cases (n = 16) were *C. albicans* and 60% of cases (n = 24) were non-*C. albicans*. The non-*C. albicans* included 45.8% cases (n = 11) of *C. glabrata*, 25% cases (n = 6) of *C. tropicalis* and *C. krusei*, and 4.1% cases (n = 1) of *Candida guilliermondii*.

In the above study, the majority of the *C. albicans* were resistant to commonly used antifungal drugs such as fluconazole (42%), Flucytosine (28%), and amphotericin B (14%). Among the non-albicans *Candida* sp., *C. tropicalis* showed 49% resistance to fluconazole, 42% resistance to flucytosine, and 10% resistance to amphotericin B. *C. krusei* and *C. parapsilosis* was more resistant to azoles, particularly fluconazole, than *C. albicans*. All the isolates were uniformly sensitive to micafungin, voriconazole, and caspofungin. In our study. Resistant to fluconazole and amphotericin B is noted for *C. albicans* and non-candida albicans. 100% resistance was noted for fluconazole by *C. guilliermondii* species.^[7]

In another study done by Nazir A et.al. A total of 424 samples from clinically diagnosed septicaemic neonates were received in the Department of Microbiology during the 1 - year study period. A total of 246/424 (58.01%) cases were blood culture positive. Out of these, 80/246 samples tested positive for candidemia (32.5%). Among the positive samples for candidemia, non-*albicans Candida* was responsible for 82.85% of cases, whereas 17.5% of cases were due to *Candida albicans*. *C. tropicalis* (13.8%) was the predominant species isolated among the nonalbicans *Candida* followed by *Candida krusei* (4.8%), *C. parapsilosis* (3.2%), *Candida guilliermondii* (2.8%), and *Candida dubliniensis* (2.0%).⁶⁰ In our study *C. guilliermondii* was the major isolate in neonates and blood samples. Around 33(21.15%) cases of *C. guilliermondii* were identified in blood samples.^[8]

The study done by Bitew A et.al. showed the following findings Species distribution of *Candida* isolates from 210 patients with vulvovaginal candidiasis

Species of candida	Number of isolates	% of the total isolates
<i>C. albicans</i>	51	58.6
<i>C. krusei</i>	15	17.2
<i>C. dubliniensis</i>	8	9.2
<i>C. glabrata</i>	3	3.4
<i>C. inconspicua</i>	1	1.2
<i>C. tropicalis</i>	2	2.3
<i>C. kefyr</i>	2	2.3
<i>C. guilliermondii</i>	2	2.3
<i>C. lusitanae</i>	1	1.2
<i>C. parapsilosis</i>	2	2.3
Total isolates	87	10

In the above study, they assessed yeast carriage associated with vaginal candidiasis symptoms. The overall prevalence of vulvovaginal candidiasis was 41.4%. Younger women, between 15 and 24 years had a somewhat lower prevalence (35.1%) of vulvovaginal candidiasis, while in the 25 years and older group, the prevalence was between 40.0 and 44.8%. drug susceptibility pattern of *Candida* species against the five antifungal drugs tested. The highest overall resistance rate of *Candida* species was observed against fluconazole (17.2%), followed by flucytosine (5.7%). All *Candida* isolates were 100% susceptible to voriconazole, caspofungin, and micafungin.^[9] In our study, *C. albicans* and non-candida albicans were identified in cervical swabs. Among females were 128(64%) and cervical swab samples were 75(37.5%) and *C. utilis* was the major(n=20) species identified in cervical swabs. Antifungal susceptibility pattern was fluconazole, voriconazole, caspofungin, micafungin, amphotericin-B, and flucytosine was 100% sensitive for *Candida utilis*.

The study done by kothalawala et.al. the VITEK2 YST® identified 110 (91.6%) samples as candidemia. Out of 110 detected, Candidemia was detected in 9.1% (110 out of 1200) of overall bloodstream infections. When considering a single

species *C. albicans* was the most common etiological agent responsible for candidemia and it was isolated in 34 (31%) cases. Overall non-albicans spp. responsible for causing 76 (69%) cases of candidemia. *C. parapsilosis* 22 (20%), *C. tropicalis* 18 (16.4%), *C. glabrata* 12 (11%), *C. krusei* 12 (11%), and other non-albicans were isolated in cases following candidemia. In our study out of 42 blood samples *C. guilliermondii* was thirty-three (n=33) accounting for 21.15%. of candidemia.^[10]

In the present study *Candida guilliermondii*, *Candida utilis*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata* and *Candida inconspicua*, *Candida dubliniensis*, and *Candida famata* are the various species identified. All showed almost 100% sensitivity to caspofungin, micafungin, flucytosine, and voriconazole. Resistance was seen among fluconazole, amphotericin-B, and *Candida glabrata* showed resistance to caspofungin. It is noted resistance was seen in both *Candida albicans* and non-albicans *Candida* mainly for fluconazole.

CONCLUSION

In this study *C. albicans* was the major isolate identified and the non-candida albicans were also

identified. Significant resistance was seen with fluconazole among both *C. albicans* and non-candida *albicans*, possibly due to inadequate antifungal use. Although *C. albicans* was the major species identified, the non-*albicans* candida species were also noted in significant proportion and showed resistance to various antifungals, thus it is necessary to do speciation and antifungal susceptibility testing for the proper treatment of candidiasis.

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