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Original Research Article

A Descriptive Microbiological Study of Oral Candidiasis in a Tertiary Care Hospital

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Abstract

Background: Oral candidiasis is one of the common opportunistic fungal infections which affects the oral mucosa. There have been significant changes in the management of candidiasis in the last few years. Candida albicans and non albicans species are closely related but different from each other with respect to epidemiology, virulence characteristics and antifungal susceptibility. Early diagnosis and management is essential to combat the dreaded complications of multisystemic diseases. So, the present study was undertaken with the aim to speciate the candida isolates and to study the antifungal susceptibility pattern of various candida species isolated from oral swabs collected from patients with oral candidiasis attending a tertiary care hospital.

Methods: The study was conducted in the Department of Microbiology, Tertiary Care Hospital during the period of Jan 2019 to Dec 2019. We assessed the characterization of Candida species by using CHROM differential agar and conventional methods like Corn meal agar and Sugar assimilation test. We also have performed the antifungal susceptibility testing as per CLSI guidelines.

Results: Out of 130 samples, 112 isolates grown on SDA culture of which 50 (44.6%) were *Candida albicans* while 62 (55.3%) were candida non albicans. *C. albicans* (44.6%) was the most predominant species identified, followed by *C. tropicalis* (25%) and *C. Krusei* (14.2%). The overall sensitivity of CHROM agar was 95.88% and specificity was 99.15%. All isolated Candida species were sensitive to Amphotericin B. 82% of *C. albicans*, 89.3% of *C. tropicalis* and 91.7% of *C. parapsilosis* were sensitive to fluconazole.

Conclusion: Though Candida albicans to be the most prevalent species, emergence of non-albicans Candida species necessitates the species identification. The conventional techniques require minimum 3-4 days for species identification. The newer technique CHROM agar is equally useful method for speciation of Candida. There has been significant rise in the resistance in fungal isolates. Antifungal susceptibility testing should be done as a part of routine laboratory practices to know the resistant pattern of the isolated fungi in the local area and to prevent the irrational use of antifungal agents. This will guide the clinician to start antifungal therapy.

Keywords: *Candida albicans, Candida non albicans,* CHROM agar, Antifungal susceptibility.

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Introduction

Oral candidiasis is the most common and opportunistic fungal disease.[1] Normal oral flora comprises of various organisms which includes eubacteria, archaea, fungi, mycoplasmas and protozoa.[2] Among these, fungi are classified as eukaryotes, and the most important to dentistry belong to the genus Candida.[2] Commonest and most virulent pathogenic species of this genus is Candida albicans.[1,3,4] Other than Candida albicans, there are many types of Candida species, which are seen in the oral cavity. Species of oral candidiasis are: C. albicans, *C. glabrata, C. guillermondii, C. Krusei, C. parapsilosis, C. pseudotropicalis, C. stellatoidea, C. tropicalis.*[2,5] Candida albicans has been recognized as an increasingly important human pathogen particularly in immunocompromised hosts because of advanced age, infection or immunosuppressive therapy.[6]

Human infections caused by Candida albicans and other related species range from the more common oral thrush to fatal, systemic superinfections in patients who are afflicted with other diseases.[1] Gradual emergence of non-albicans candida species as a cause of refractory mucosal and invasive candidiasis, particularly in patients with advanced immunosuppression and problem of resistance to azoles and other antifungal agents in the candida species is a point of concern.[7]

Although Candida albicans is the most common causative organism of causing both superficial and deep fungal infections, an increasing incidence of less common species of candida has also been noted in last few years.[1,3,7] A definitive identification of non albicans candida species is important to detect emerging pathogenic strains particularly those that have acquired resistance to antifungal drugs.[3] It is of vital importance that patients should be evaluated clinically and microbiologically for the presence of candida species in the oral cavity.[8] Species level identification with in vitro antifungal susceptibility pattern is essential to choose the appropriate drug.[8] If Candida strains are not susceptible to fluconazole, in those cases other drugs like itraconazole or voriconazole can be used.[7,8] Also reserving antifungal drug prophylaxis for only those with severe and frequent recurrences of candidiasis in immunocompromised patients can go a long way in avoiding antifungal resistance.9

Material and Methods

The study was conducted in the Department of Microbiology, Tertiary Care Hospital during the period of Jan 2019 to Dec 2019. A total of 130 patients presenting with symptoms of Oral Candidiasis to the OPD of our Tertiary Care Hospital, were included in the study. Clinical details of the patients were obtained and recorded.

History, clinical findings, co-morbid conditions, surgical interventions and results of relevant investigations were also recorded. Ethical clearance was obtained.

Inclusion Criteria

All the oral swab samples collected from the patients of all age group with oral candidiasis attending a tertiary care hospital were included in this study.

Exclusion Criteria

Other oral lesions were excluded from the study.

Methodology

Sterile swabs were used for collection of the sample from oral cavity by swabbing over the oral lesions (present overt buccal mucosa, tongue, gingiva and palate).[8] Specimens were transported to the laboratory immediately and processed.

Direct Microscopic Examination of Specimens [10]

Stained Preparation

Gram Staining

The smears were prepared from collected swab samples of oral candidiasis patients. Gram staining was done by using Hucker Modification method and smears were examined under 100X oil immersion field. Gram positive round or oval yeast like budding cells with or without pseudohyphae were visualized in relevant samples (Fig1).

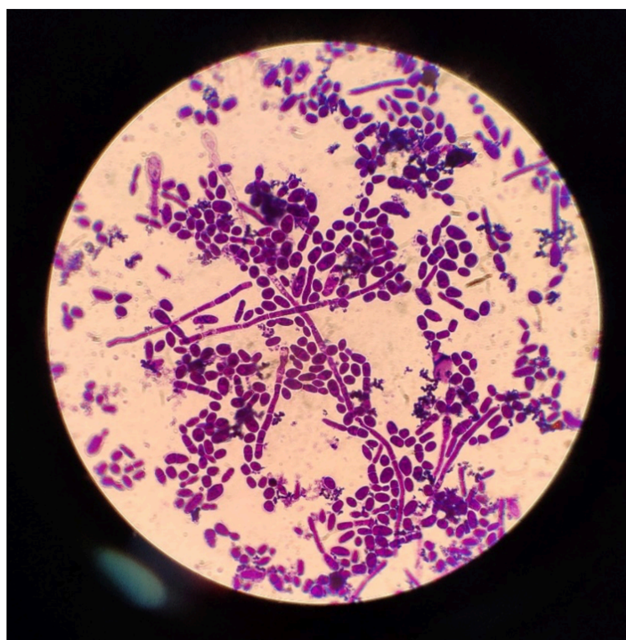

Figure 1: Gram staining showing gram positive budding yeast cells with pseudohyphae

Culture [1,10]

The clinical specimens were inoculated on SDA slopes and incubated at 37°C. The slopes were examined for every 24 hrs for first 3 days and maximum up to 7 days (as culture of yeast to be held for 7days). Colonies which appeared cream colored, pasty and smooth were examined for Gram positive budding yeast cells by Gram's stain to confirm them as Candida species.

Figure 2: SDA slant culture showing creamy white coloured, smooth pasty colonies of candida

Species Identification Methods

Germ tube test (Reynolds Braude Phenomenon) [1,3,10]

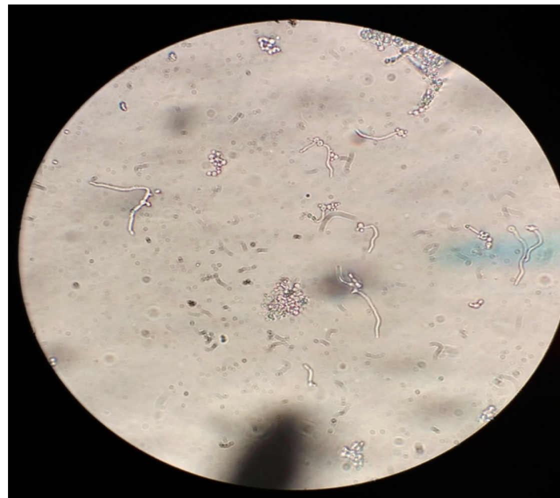

Figure 3: Positive germ tube test showing tube like elongation from the parent cell with no constriction at the point of origin

A small portion of an isolated colony was suspended in a test tube containing 0.5 ml of human serum. The test tube was incubated at 37°C for 2 hours. A drop of yeast suspension was placed on a microscope slide, overlaid with a coverslip and examined microscopically for the presence of germ tubes which are long tube-like projection from yeast cells. Isolates producing germ tubes were presumptively identified as *C.albicans* or C.dubliniensis.

Cornmeal agar (CMA) [1,10]

The media was prepared as per the manufacturer's instruction, autoclaved and poured in Petri dish. A streak was made down to the centre and 3-4 streaks across the first and cover slip was placed over it so as streak line should not project beyond the cover slip. The cover slip was placed to create partial anaerobic environment. Plates were incubated for 48 hours at 25°C. Growth was observed at the interface of the cover slip and line of inoculum under low power and high power subsequently for chlamydospores, blastospores, pseudohyphae and hyphae. Findings were recorded.

CHROM agar [1,10,11]

Figure 4: CHROM agar showing light green-coloured colonies of *C. albicans* and pink coloured colonies of *C. Krusei.*

It is a selective media for the isolation and identification of different species of Candida. Media was prepared as per the manufacturer's instruction and was dispensed in petri dishes after being allowed to cool slightly. 4-6 Isolates were inoculated on each CHROM agar plate from primary culture. These plates were incubated at 30°C for 48 hours. The various species of Candida were identified by their colony colour, size, texture, and presence of colour diffusion into the surrounding agar presumptively in 48hrs. Colours produced by various Candida species were recorded.

Sugar assimilation (Auxanographic techniques) [1,32

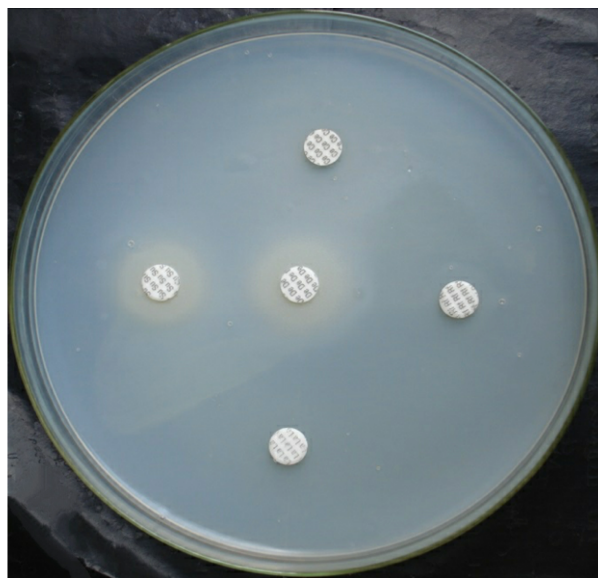

Figure 5: Sugar assimilation test

18ml quantities of agar and distilled water was dispensed in screw capped tubes. Autoclaved at 121°C for 20 minutes and stored at 4°C. A heavy inoculums yeast suspension was prepared from 24 hours old culture in 2ml Yeast Nitrogen Broth. The prepared suspension was poured into 18 ml of molten agar cooled at 45°C, and then poured into a 90mm Petri plate. The Petri plate was set at room temperature until the agar surface hardens. Sugar discs were placed in a circle with sterile forceps, such that they were at least 30mm apart from each other from centre to centre. These plates were incubated at 37°C for 3-4 days. Presence of growth around each disc indicates assimilation of respective sugars.

Antifungal susceptibility testing: [1,12]

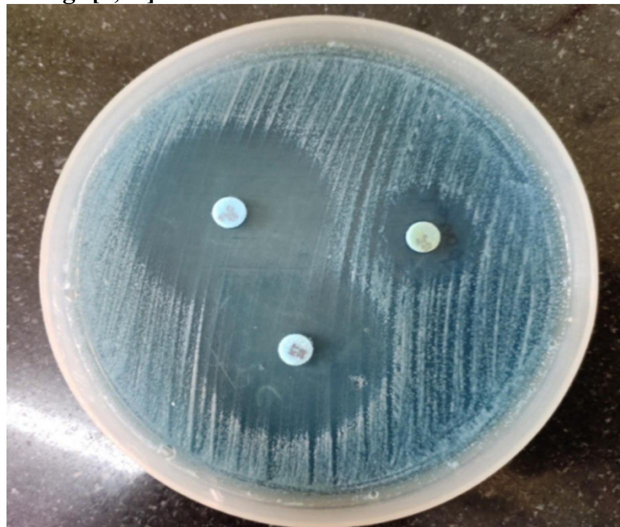

Figure 6: Antifungal susceptibility testing by disc diffusion test

Antifungal Susceptibility Testing for Candida isolates was done by disc diffusion method, as per CLSI Guidelines on Antifungal Susceptibility testing.[12]

Disc diffusion method

Glucose methylene blue (GMB) stock solution was prepared and Autoclaved for 15 minutes at 121°C. The solution was stored at room temperature and were not refrigerated as this may cause precipitation. Mueller-Hinton agar with 2% glucose and 0.5 µg/ml methylene blue Mueller-Hinton agar was prepared according to the manufacturer's instructions. GMB solution with methylene blue was poured into the freshly prepared and cooled Mueller Hinton agar medium and then they were poured into plastic, flat bottomed petri dishes to a depth of approximately 4 mm. Then agar medium was allowed to cool to room temperature and stored at refrigerator temperature (2 to 8°C) The agar medium should have a pH between 7.2 and 7.4 at room temperature.

Inoculum preparation

Inoculum was prepared by picking five distinct colonies from a 24-hours-old culture of Candida species and the colonies were suspended in 5 ml of sterile normal saline. The turbidity was adjusted equivalent to 0.5 McFarland standards (1x 106 to 5 x 106 cells/ml) resulting in semi-confluent growth.

Inoculation of the agar plate: A sterile cotton swab was dipped into the suspension. The excess fluid was removed from the swab by pressing firmly against the inside wall above the fluid level. Then a lawn culture was made on the dried surface of the agar by streaking the cotton swab according to the standard three directional method. The plate was left open for 3-5 minutes, allowing excess moisture to be absorbed, and then the antifungal discs were dispensed onto the plate. Application of disks to inoculated plates: Antimicrobial discs were dispensed onto the surface of an inoculated agar plate by means of a sterile forceps and pressed down. The discs were evenly distributed on the plate with a distance of 2.5cms from centre to centre of the discs.

Incubation

Plates were inverted and incubated at 35°C ±2°C within 15 minutes after placing the discs. The plates were observed for formation of zone of inhibition around the disc after 24 hrs of incubation. If no visible growth with particular strains, the plates were reincubated for 48 hours and then read. Zone of inhibition was measured at the point where there was prominent reduction in growth. The findings were recorded and tabulated.

Statistical analysis: Qualitative data is represented in form of frequency and percentage. Nominal data among qualitative variables include age, sex, risk factors of the subject. Association between the quantitative variables were found using Chi-square tests at 95% confidence interval. The results were graphically represented where deemed necessary. Appropriate statistical software, including but not restricted to MS Excel, PSPP was used for statistical analysis. Graphical representation was done in MS Excel 365.

Results

It is observed that majority of the study participants were from the age group of 31-40 years (20.8%) and 41-50 years (20.8%) followed by age group of 51-60 years. The mean age of the study participants was 34 +/- 19 years. Least number of study participants were from the age group of 11-20 years. Majority of the study participants were females (61.5%) and only 38.4% were males. It is observed from the above table that majority of the study participants had risk factors among which HIV (36.92%) was most common followed by Infant age group (10.0%) and

then followed by carcinoma (9.23%) and Diabetes (7.69%). While 18.46% had no risk factors.

It is observed that 116 (89.23%) samples showed positive findings on gram staining. 112 (86.15%) samples showed growth on SDA agar while 18 (13.85%) showed no growth. It is observed from the above table that 50 (44.6%) samples were positive test while 62 (55.3%) were negative for germ tube test. Corn meal agar detected 50 (44.6%) *C. albicans*as predominant species, followed by 28 (25%) *C. tropicalis* and 16 (14.2%) *C. Krusei.*

Table 1: Distribution of sugar assimilation properties among candida species

Species	GLU	MAL	SUC	LACT	GALCT	MEL
C. albicans	50	50	50	0	50	50
C. glabrata	6	0	0	0	0	6
C. Krusei	16	0	0	0	0	16
C. parapsilosis	12	12	12	0	12	12
C.tropicalis	28	28	28	0	28	28
Total	112	90	90	0	90	112

It is observed that all species of *Candida albicans* showed assimilating properties in Glucose, Lactose and Maltose while *Candida albicans, Candida parapsilosis, Candida tropicalis* showed assimilation in Maltose and Sucrose. The difference in assimilating properties of candida species was found to be significant at 95% CI as proved Chi-square test.

Table 2: Distribution of sugar assimilation properties among candida species

Species	CEL	INO	XYL	RAF	TREH	DUL
C. albicans	0	0	50	0	50	0
C. glabrata	0	0	0	0	6	6
C. Krusei	0	0	0	0	0	0
C. parapsilosis	0	0	12	0	12	0
C. tropicalis	0	0	28	0	28	0
Total	0	0	90	0	96	6

It is observed that all species of *Candida albicans* showed assimilating properties in Cellobiose, Inositol and Raffinose while *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis* showed assimilation in xylose. The difference in assimilating properties of candida species was found to be significant at 95% CI as proved Chi-square test.

Table 3: Distribution of species characteristics observed on CHROM agar

Species	Colour in CHROM agar					
	Blue purple	Light green	Mauve	Pink	Purple	Total
C.albicans	1 (1.9%)	48 (94.12%)	1 (1.9%)	1 (1.9%)	0	51
C.glabrata	0	0	0	1 (16.6%)	5 (83.3%)	6
C.krusei	0	0	0	15 (100%)	0	15
C.parapsilosis	0	0	12 (100%)	0	0	12
C.tropicalis	28 (100%)	0	0	0	0	28

X^2 Value	df	P Value	Significance
245.763	16	0.01	Significant

Light green colour appearance was seen in 94.12% subcultures of Candida albicans in CHROM agar, 83.3% *Candida*galbrata showed purple colour, 100% candida krusei showed pink colour. While *Candida parapsilosis* showed mauve colour and candida tropicalis showed blue purple colour. The difference in appearance in CHROM agar was found to be significant at 95% CI as proved Chi-square test.

Table 4: Antifungal Susceptibility Testing

Species	Fluconazole	Ketoconazole	Amphotericin B
C.albicans(50)	41 (82%)	29 (58%)	50 (100%)
C.glabrata(6)	0 (0.00%)	2 (33.33%)	6 (100%)
C.krusei(16)	1 (6.25%)	1 (6.25%)	16 (100%)
C.parapsilosis(12)	11 (91.7%)	9 (75%)	12 (100%)
C.tropicalis(28)	25 (89.30%)	12 (42.9%)	28 (100%)

It is observed from table on antifungal susceptibility that all strains were 100% susceptible to Amphotericin B. 82% of *C. albicans* was, 89.3% *C. tropicalis*, 91.7% of *C. parapsilosis* were sensitive to fluconazole while 6.25%

of *C. Krusei* were sensitive to fluconazole. All strains of *C. glabrata* were resistant to fluconazole. To ketoconazole 58% of *Candida albicans* were sensitive while 33.3% of *Candida glabrata*, 6.25% of *Candida krusei*, 75% of *Candida parapsilosis* and 42.9% of *Candida tropicalis* were sensitive.

Table 5: Association between risk factors and candida infection

Risk factors	Candida albicans	Percentage	Candida Non albicans	Percentage
CARCINOMA(12)	7	14	5	8.1
DM(10)	4	8	6	9.7
HIV(48)	23	46	25	40.3
HTN(8)	2	4	6	9.7
ICU Pt(8)	2	4	6	9.7
INFANTS(13)	7	14	6	9.7
NIL(24)	2	4	4	6.5
PREGNANCY(7)	3	6	4	6.5
Total (130)	50	100	62	100

We have compared the association between risk factors and infection of various species of candida. 46% of albicans species and 40.3% of non albicans species had HIV as risk factor. Thus, *Candida albicans* was more commonly associated with HIV than candida non albicans. The association between risk factor and candida infection is significantly associated as proved by Chi-square test at 95% CI ($p<0.01$).

Table 5: Predictive ability of CHROM agar

Species	True Positive (TP)	True Negative (TN)	False Positive (FP)	False negative (FN)	Sensitivity*	Specificity+	PPV#	NPV @
C.albicans	49	61	0	2	96.08	100.00	100.00	96.83
C.krusei	15	95	2	0	100.00	97.94	88.24	100.00
C.glabrata	5	106	0	1	83.33	100.00	100.00	99.07
C.parapsilosis	12	99	1	0	100.00	99.00	92.31	100.00
C.tropicalis	28	83	1	0	100.00	98.81	96.55	100.00

*Sensitivity = TP/TP+FN +Specificity= TN/TN+FP #PPV=TP/TP+FP @NPV=TN/FN+TN

The above table shows sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CHROM agar in diagnosis of candida species. The overall sensitivity of CHROM agar was 95.88 and specificity is 99.15%. The positive predictive value is 95.42 while negative predictive value is 99.18.

Discussion

Oral candidiasis is the most common human fungal infection, especially in the immunocompromised patients. Oral candidiasis if untreated can lead to systemic candida infection, sepsis and candida oesophagitis. Although close to 200 Candida species have been described, only 20 Candida species are significant pathogens. Out of these, seven are well-known opportunistic pathogens for humans. The diversity of Candida associated with human infections, provides new challenges in the diagnosis and treatment of candidiasis and in the study of their virulence. Though Candida albicans remains the most common agent of both superficial and deep infections, an increasing incidence of non albicans candida species have also been documented in the recent years. The current study was conducted in a tertiary care hospital in the Department of Microbiology. A total of 130 clinically diagnosed oral candidiasis cases were included in whom oral swabs were collected. The characterization of Candida species in patients with oral candidiasis were assessed in the study. The characterization and antifungal susceptibility testing will help clinician to initiate specific antifungal therapy and management to avoid systemic complication. This study was an attempt to understand the epidemiological distribution of various species of candida in patients with oral candidiasis and their antifungal resistance.

In the present study it was observed that majority of the study participants were from the economically productive age group of 31-50 years with a mean age of 34 +/- 19 years. Similarly, Hu et al. conducted a study on candidiasis in mucosal disease and reported the mean age to be 51.1+/-27.2 years.13 Leandro et al. also reported in their study on oral candidiasis, majority of the study participants to be in the age group of 51-70 years.[14] Common involvement of this age group may be explained by the fact that risk factors like infection by HIV and underlying diseases mainly affect the economically productive age group. Majority of the study participants in our study were females (61.5%), similar to gender distribution seen in number of other studies. Gauch et al. in their study on association between diabetes and oral candidiasis reported females to be 60% of their study sample.[15] Pakfetrat A, et al in their study of oral manifestations in HIV patients showed majority of patients were male.[16] It seemed that

cultural and economic conditions affect the gender distribution. Candida species are component of normal flora in human beings commonly found on skin, mucosa, throughout gastrointestinal tract, female genital tract particularly higher in vagina during pregnancy. There is crucial role of differentiating between colonisation and invasion at mucosal surfaces. Number of various factors are known to predispose superficial and deep candidiasis. The T-cell mediated immunity is important in protection from high-level mucocutaneous candida colonisation. Whenever immunity is suppressed due Infections or therapeutic drugs or due to physiological condition, there is alteration in the balance of normal microbial flora of the body and causing the candidal invasion to mucosa and infection.[1]

HIV (36.9%) was the most common risk factor observed in our study. It was followed by infant age group (10%), then by carcinoma (9.2%) and diabetes (7.69%). The risk of oral candidiasis is enhanced in patients of HIV infection because of development and progression of cellular immunodeficiency. Similarly, Menzes et al. reported in their study on risk factors of oral candidiasis as HIV being the second most common associated risk factor.[17] Candida species colonisation of infants occurs early in 113 life and this may be affected by variety of common practices in neonatal intensive care units.[18] Kawashito et al. also reported a high incidence of oral candidiasis in patients of carcinoma.[19] This explains the compromised cell mediated immunity in cancer patients on chemotherapy or radiotherapy predisposing them to opportunistic oral candidiasis. Also, Sampath et al. reported a significant association of oral candida carriage amongst diabetics.[20] We found no risk factors in 24 (18.4%) study participants. Though these were clinically diagnosed cases of oral candidiasis, only 8 Candida species were isolated from clinical specimens. This may be due to poor oral hygiene, nutritional deficiencies. Similar finding were reported by Dan et al. who reported Candida nonalbicans species was 44.5% among women which denotes infection in the absence of risk factors.[21] Table no.4 shows association between risk factors and infection of various species of candida. Candida albicans was found to be the main cause of oral candidiasis in patients with HIV infection and AIDS.20 46% of albicans species and 40.3% of non albicans species isolated were associated with HIV as a risk factor.

Our study reported candida non albicans species (61.54%) were more than the albicans species (38.4%). Study by Novikova et al. showed 87% Candida albicans, 9% Candida krusei, 4% Candida glabrata and one case Candida parapsilosis and one case of mixed infection with Candida albicans and Candida krusei.[22] But the recent studies have shown increased incidence of non albicans species. Reports from kumari et al have documented similar results and has shown that non albicans has increased in prevalence with 32.39% Candida albicans, 22.5% Candida glabrata, 45% Candida parapsilosis and 39% Candida tropicalis.[23] Vijaya et al have also reported increased number of non albicans species. They observed C. glabrata was most common followed by *C. tropicalis* and C. parapisilosis.[24] In Jawed ahmed et al. reported their results as 41.7% Candida albicans, 16.7% Candida tropicalis, 16.7% Candida krusei and 14.8% Candida glabrata.[25]

In our study, *C. tropicalis* were 25%, *C. Krusei* were 14.2%, *C. parapsilosis* were 10.7%, C. glabrata were 5.3%. This emphasizes nonalbicans candida speciation is very essential. In our study we observed 116 (89.23%) samples showed positive findings on gram staining Direct smear gram stain microscopy is always helpful for presumptive identification of fungal infection. The microscopic findings should be confirmed by culture. We observed 112 (86.1%) samples showed growth on SDA medium while 18 samples had not shown growth on SDA. Among these 18 culture negative samples, only 4 samples showed positive findings on microscopy which is statistically not significant. This may be because of previous improper treatment, less quantity of live organism. Germ tube test is the simple method used to differentiate between candida albicans and non albicans.

We observed that all germ tube positive candida species were *C.albicans* (38.46%) only and none of the non albicans species were positive for Germ tube test. Meis et al. and Terlecka et al. in their study reported similar findings that only Candida albicans species show positive germ tube test.[26,27] Also Kim et al. reported in their study that a unique way to differentiate Candida albicans and non albicans is their ability to produce unique germ tube formation at 39oC.[28] Thus our study findings were in concordance with published literature. Among these isolated candida species, further characterisation was done by inoculation on corn meal agar. Growth on corn meal agar showed various patterns of growth. On cornmeal agar (Dalmau plate method) at 25°C for 72 hrs, pseudohyphae (and some true hyphae) are formed with clusters of round blastoconidia at the septa. Large, thick - walled, usually single terminal chlamydospores are characteristically formed. Also *C.tropicalis* forms blastoconidia singly or in very small groups all along graceful, long pseudohyphae.

We observed that pseudo hyphae were present in Candida albicans and Candida Krusei. Branching pseudohyphae were present in Candida tropicalis. While thin pseudo hyphae were present in Candida parapsilosis. Blastoconidia in cluster were present in Candida albicans. Single blastoconidia were found

in Candida tropicalis. While Tree like blastoconidia were present in Candida krusei. Terminal clamydospore was seen in 98.4% isolates of Candida albicans. Similarly, several studies by Citiulu et al. Staib et al. and Babin et al. reported similar findings.[29,30,31]

Table no. 1 shows sugar assimilating properties of each species isolated. We observed that Candida albicans, Candida tropicalis and Candida parapsilosis assimilate all sugars except dulcitol. Candida glabrata assimilated only three sugars [glucose, xylose and dulcitol]. Candida krusei is biochemically inert which assimilates only xylose. Lactose was not assimilated by any of the candida species. Glucose was assimilated by Candida glabrata, Candida parapsilosis and Candida krusei. Glucose and maltose were assimilated by Candida albicans, apart from these two sugars, sucrose was also assimilated by Candida tropicalis. Simillar findings were reported by Devadas et al who reported the auxanographic carbohydrate assimilation profiles for; *C.albicans*, *C.tropicalis*, *C.krusei*, *C.parapsilosis*, *C.glabrata*, Out of all, C.kefyr assimilated all sugars, Candida glabrata assimilated glucose and trehalose; *C.krusei* assimilated glucose only. Lactose was assimilated only by Candida kefyr not by other candida species. Cellobiose assimilation was positive for *C. tropicalis* which differentiates it from *C. albicans* and *C. parapsilosis*. Dulcitol was not assimilated by these Candida species.[32]

Table no.2 shows findings on CHROM differential agar. Light green colour appearance was seen in Candida albicans species on CHROM differential agar, Candida galbrata showed purple colour while Candida krusei showed pink colour. Candida parapsilosis showed mauve colour and Candida tropicalis showed blue purple colour. Similar findings were reported by Sharma et al.[33] Novikova et al. reported that Use of chromogenic agar is a convenient and reliable means to detect colonization by Candida and can differentiate between *C. albicans* and non-albicans species.[22] Guisiano et al. reported in their study on species identification of candida on CHROM agar. He noticed that the colour and texture of Candida albicans, C. tropicalis, *C. Krusei* was always constant and particularly distinctive for a reliable identification. C. glabrata and C. parapsilopsis colonies also showed constant colour.[34] Nurat et al. in their study on characterisation of Candida species observed that, the CHROM agar test showed the highest occurrence of Candida albicans 54.3%, followed by C. glabrata 25.7%, *C. tropicalis* 5.7% and C. dublinensis 14.3%.[35]

Table no.5 shows the predictive ability of CHROM agar. We have applied statistics and compared conventional method and CHROM agar for speciation of Candida. We observed the overall sensitivity of CHROM agar was 95.88% and specificity was 99.15%. Nurat et al reported in their study CHROM agar sensitivity and specificity for C. glabrata were 91.6%% and 96.2% respectively. The CHROM agar had 89.4% sensitivity and 90.4%.[36] Merlino et al. also concluded used CHROM agar technique, incorporating the use of a concentrated inoculum from preliminary growth. It offered another alternative cost-effective use of chromogenic medium for the phenotypic distinction of *C. albicans* from other commonly encountered yeast species after 8 to 24 h of incubation.[37] Thus, CHROM agar is equally useful method for speciation of Candida as compared to conventional techniques. This method requires less incubation time and do not need expertise for interpretation.

Table no.3 shows antifungal susceptibility pattern of each species isolated. 82% of C. albicans, 89.3% C. tropicalis, 91.7% of *C. parapsilosis* were sensitive to fluconazole while all strains of C. glabrata were resistant to fluconazole. Pfaller et al. reported very little variation in fluconazole susceptibility among isolates of *C. albicans* (89–100% S, 0–9% R), *C. tropicalis* (91–100% S, 0–9% R) and *C. parapsilosis* (93–100% S, 0–2% R). These species accounted for 78% of all BSI and remained highly susceptible (91-100% susceptible) to fluconazole from 1992 to 2001 irrespective of geographic origin.[38] Later on, change in resistance pattern of this antifungal agent were seen in several studies. Goswami et al. reported overall 67.1% of patients with diabetes and 47.3% of controls continued to show persistence of candida growth on high vaginal swab culture following fluconazole treatment.[39] Similarly Xion et al. reported susceptibility rates to azoles among *C. parapsilosis* species were ≥97.5%. However, 11.6% and 9.5% of *C. tropicalis* isolates were resistant to fluconazole and respectively (7.1% were resistant to both). Approximately 14.3% of C. glabrata were fluconazole resistant, and 11.6% of C. glabrata isolates were cross-resistant to fluconazole.[40] Lei et al. reported that in vitro susceptibility of Candida spp. to fluconazole, itraconazole and voriconazole exhibits a moderate correlation.[41] Thus our study findings were in concordance with the published literature.

In our study, we observed that 58% of Candida albicans, 75% of Candida parapsilosis, 42.9% of Candida tropicalis and 33.3% of Candida glabrata, were sensitive to ketoconazole. Highest sensitivity to ketoconazole was seen among Candida parapsilosis. Chokoeva et al. reported in their study on antifungal susceptibility of candida species that *C.krusei* was 100% resistant to ketoconazole, and sensitivity of *C. albicans* varied between 60-80%.[42] The variation in the rates of azole resistance between our study and published literature may be due to previous medication. We observed in our study all species of Candida were 100% sensitive to Amphotericin B.

Similarly Pakshir et al. reported in their study Candida species isolated from oral lesions of patients were identified as Candida albicans Candida glabrata and Candida dubliniensis and were sensitive to amphotericin B.[43]Prazynska et al, in their study on susceptibility of Amphotericin B to candidal isolates reported all strains were susceptible to Amphotericin B detected by widely accepted broth microdilution method.[44] Though the Candida albicans to be the most prevalent species, emergence of nonalbicans Candida species necessitates the species identification and antifungal susceptibility to be done as a part of laboratory evaluation of Oral Candidiasis. Fluconazole showed 82% sensitivity to Candida albicans. Amphotericin B was found to be the more sensitive drug followed by azoles. As C. glabrata is intrinsically resistant to fluconazole, it should not be used empirically. Earlier microbiological diagnosis for oral candidiasis is warranted to prevent the complications and to direct the clinicians in appropriate way of management. According to this study results, the identification of causative fungal species and treatment according to the drug sensitivity pattern will reduce the complication of azoles and the development of emerging resistance in antifungals drugs.

Conclusion

Oral Candidiasis is an opportunistic infection. Though Candida albicans to be the most prevalent species, emergence of non-albicans Candida species necessitates the species identification. The conventional techniques like sugar assimilation and corn meal agar method require minimum 3-4 days for species identification. The newer technique CHROM agar is equally useful method for speciation of Candida. Now a days there is significant rise in the resistance in fungal isolates. Antifungal susceptibility testing should be done as a part of routine laboratory practices to know the resistant pattern of the isolated fungi in the local area and to prevent the irrational use of antifungal agents. This will guide the clinician to start antifungal therapy. Further it will be helpful for public health measures and to reduce the patient’s sufferings.

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