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Isolation, Speciation and Characterization of *Candida* Species in Clinical Isolates

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A B S T R A C T

One hundred clinical isolates of *Candida* were studied during a period of one year. Most common clinical sample was urine (38%) followed by sputum (17%) and blood (10%). Most common species isolated was *Candida albicans* (42%) followed by *Candida tropicalis* (34%), *C. parapsilosis* (11%), *C. glabrata* (6%), *C. krusei* (5%) and *C. dubliniensis* (2%). Non albicans candida were isolated at a higher rate (58%) than *Candida albicans*. To conclude the study it showed that prevalence of non albicans candida was higher than *C. albicans* from various clinical specimens.

Introduction

Over the last few years, the incidence of mycotic infections has progressively increased. Fungi once considered as non-pathogenic or less virulent are now recognized as a primary cause of morbidity and mortality in immune-compromised and severely ill patients (Mokaddas *et al.,* 2007).

Candida species are among the most common fungal pathogens. They are ubiquitous yeasts found on plants and form the normal flora of alimentary tract of mammals and the mucocutaneous membranes in humans (Adhikary *et al.,* 2011).

They are commonly found on the skin, mucosa, nails, throughout gastrointestinal tract and female genital tract particularly higher in vagina. The overall carriage rate in healthy individuals has been estimated to

reach 80 %. The most commonly isolated *Candida* species from the gastrointestinal tract of humans is *Candida albicans*, followed by *Candida tropicalis* and *Candida parapsilosis* (Agarwal *et al.,* 2011). *Candida glabrata* is most often isolated from the mouth.

Candidiasis is the commonest fungal disease found in humans. The infection may be acute or chronic, superficial or deep and its clinical spectrum is wide. It is found mainly as secondary infection in individuals with some underlying immune compromised condition and very rarely as primary disease.

Non albicans species are emerging pathogens and can also colonize human mucocutaneous surfaces and invades tissues; leading to life threatening diseases in patients whose cell

mediated immunity is decreased by disease or iatrogenic intervention (Ajello, 1997; Verma, 2003; Akpan, 2002 and Al- abeid *et al.,* 2004). Now, *Candida* species constitute the third to fourth most common causes of nosocomial blood stream infections. In recent years HIV has been identified as one of the most important predisposing conditions for candidiasis. Other predisposing factors for *Candida* infections are prolonged use of antimicrobial drugs, diabetes, chemotherapy and catheterization (Ali Zarei, 2013; Anil K Paswan, 2012; Anil, 1997 and Baradkar *et al.,* 1996). Although *Candida* albicans remains the most common causative agent of both superficial and deep fungal infections, an increasing incidence of Non-albicans species of *Candida* that cause disease has also been documented in the last few years. These include *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida dubliniensis,* *Candida glabrata*, *Candida kefyr* and *Candida lusitaniae*.

Accurate species identification is therefore important for the treatment of the *Candida* infection as the non albicans species of *Candida* continue to be increasingly documented and as not all the species respond to the same treatment.

Hence the present study was undertaken at a Tertiary Care teaching hospital of Jaipur to isolate and speciate genus *Candida* from the various clinical samples and to analyse the predisposing conditions for candidiasis.

Materials and Methods

100 Clinical samples were obtained in a period of one year in the Microbiology Lab of Mahatma Gandhi Medical College and Hospital, Jaipur. A detailed history was taken with a particular emphasis on the natural receptive states like infancy, old age, pregnancy, prolonged administration of antibiotics, past history of chronic diseases such as Diabetes mellitus, tuberculosis, hospital stay duration, AIDS or immunosuppression, cancers, endocrine diseases such as hypothyroidism and post-surgical infections. Presence of signs of other opportunistic infection was looked for and associated diseases if any were recorded. Written informed consent was obtained from each patient. Relevant Clinical samples were collected with complete universal precautions. The samples collected were transported to the mycology lab as soon as possible. Samples were subjected to Gram's stain to look for presence of Gram positive yeast like budding cells with pseudohyphae and KOH mount.

Media used for culture were Sabouraud Dextrose Agar with Chloramphenicol (5mg/ml), Brain Heart Infusion Broth and 5% Sheep Blood Agar prepared as per the standard procedures. The Lactophenol Cotton Blue (LCB) mount was used to study morphological features of fungal isolates. They were further speciated by the germ tube test, chlamydospore formation on Corn Meal Agar and carbohydrate utilization patterns by Sugar Assimilation Tests.

Results and Discussion

In this population-based study we have shown the potential clinical importance of species-level identification as *Candida* species. Our results show, that in an unselected hospital population, as many as one-third of all cases of *Candida* infection may be attributable to nosocomial clusters, and the risk is highest in wards providing intensive care. Non Albicans *Candida* species are on the rise due to increasing immune compromised states. In this study of total 100 cases from various clinical isolates, 51 patients (51%) were male and 49 were female patients (49%). Majority of the male patients were in the age group of 51-60 yrs (29%), and majority of female patients were of the age group 31-40 yrs

(39%). Out of 10 HIV positive patients the male: female ratio was 2.3:1. Higher male:female ratio shows that males are at increased risk in comparison to females because their jobs and habits entail them to be more migratory. A similar age group was reflected in studies of Maiken Arendrup (2013). Male predominance was noted in our study (51%) with *Candida* infection more common in 51-60 years age group males consistent with study of Deorukhkar *et al.,* (2012). However in the study by Kandhari *et al.,* (1969) the incidence was found to be higher in females (61.2%) than in males (38.8%) and Rizwi *et al.,* (2011) reported female preponderance in their study group with a ratio of 0.85 : 1 (M:F).

In all the 100 patients included in this study the most common clinical sample was urine in 38 (38%) patients followed by sputum 17 (17%) and blood samples 10 (10%). High vaginal swab was seen in total of 6 (6%) patients followed by 4(4 %) each of pus/wound, ascitic fluid, stool, suction tip and oral swab, 3 (3%) samples were of ear swab followed by 2 (2%) samples each of catheter tip and endotracheal tube tip. Central line tip and Broncho alveolar lavage were 1 % respectively.

Our observation is similar with the studies of Deorukhkar *et al.,* (2014) where urine samples were in majority (34.6%) and Patel *et al.,* (2012) where urine showed the highest number of isolates (34.5%) followed by sputum (28.9%) and blood (26%). Similar findings were seen in study by Marcia Cristina Furlaneto *et al.,* (2011). Studies which were done earlier by Pfaller *et al.,* (1996), have reported *Candida* species as the seventh most common nosocomial pathogen hospital wide and as that which caused 25% of all urinary tract infections. Most common species isolated was *Candida albicans* (42%) followed by *Candida tropicalis* (34%),

Candida parapsilosis (11%), *Candida glabrata* (6%), *Candida kruseii* (5%) and *Candida dublinensis* (2%) in clinical samples. The present study is in agreement with the studies conducted by Dastider (72.8%), Gupta (64%) and Mokaddas *et al.,* (2007) (39.5%) which all found *Candida albicans* to be the commonest isolate. In the present study Non albicans *Candida* were isolated at a higher rate (58%) than *Candida albicans* (42%) which was in agreement with the findings of the studies by Mokaddas *et al.,* (2007) which also showed the Non albicans *Candida* incidence (60.5%) to be higher than that of *Candida albicans* (39.5%). A study by Chakrabati *et al.,* (1996) also showed Non albicans *Candida* to have a higher incidence than *Candida albicans* (75%) and (25%) respectively. Similar findings were observed in study by Saroj Golia *et al.,* (2013). Comparative studies on different *Candida* species by Manchanda showed that *C. tropicalis* (55.03%) was higher while it was 34% in our study.

These findings seem to suggest that the Non albicans *Candida* are emerging as important pathogens. In the urine sample isolates *Candida tropicalis* (47%) was the most common species followed by *Candida albicans* (34%), *Candida parapsilosis* (8%), *Candida glabrata* (5%), *Candida kruseii* and *Candida dublinensis* in 3% each. Studies conducted in Brazilian medical centres have also shown increased rates of isolation of *C. tropicalis* from urine.

The most common species isolated from sputum was *Candida albicans* (53%) followed by *Candida tropicalis* (24%). Blood samples showed the predominance of *Candida tropicalis* (30%) and *Candida parapsilosis* (30%) being the most common species followed by *Candida albicans* (20%) and *Candida krusei* (20%). The incidence of blood stream infection (BSI) caused by Non

albicans *Candida* species (80%) was higher than *Candida albicans* (20%). Among the NAC species *C. tropicalis* followed by *C. parapsilosis*, and *C. krusei* predominantly caused BSI.

In high vaginal swab samples *Candida albicans* was most common (50%) followed by *Candida tropicalis* (33%) and *Candida glabrata* (2%).

In study done by Gross T Norma et al., (2007), *C. albicans* was the predominant species (75%) followed by *C. parapsilosis* (12%), *C. tropicalis* (5.3%) and *C. glabrata* and *C. famata* 3.5% each. Similar findings were seen in studies done by Jindal *et al.,* (2006).

In stool samples *Candida tropicalis* (75%) was the commonest species followed by *Candida parapsilosis* (25%). *Candida albicans* was the only species isolated from ascitic fluid, central line tip and bronchoalveolar lavage (BAL) samples.

Most number of IPD cases from the ICU/CCU (37%), followed by casualty/burn wards (12%), medicine (16%), paediatrics (5%), gynae (3%), surgical ward (8%) and remaining from other wards. In this study most frequently associated risk factor is ICU/CCU prolonged stay, being 40% of the total number of cases.

Candida albicans (16) and *C. tropicalis* (16) were the predominant species isolated; 40% of each, followed by *C. krusei* (10%), *C. parapsilosis* (7.5%) and *C. dubliniensis* (2.5%). Similar findings were seen in a study done by Paswan *et al.,* (2012). In a study done by Giri *et al.,* (2013) *C. tropicalis* was the most common isolate (74.35%).

In a study done my Chun Fang Ma *et al.,* (2013) *C. tropicalis* was isolated in 30.8% of the cases followed by *C. parapsilosis* and *C.*

albicans in percentage of 20% and 17.9% respectively. Incidence of candiduria was high among the patients admitted in ICU/CCU followed by patients who were diabetic and those on prolonged antibiotic intake in the present study. Similar findings were seen in a study done by Deorukhkar *et al.,* (2014). Second most common risk factor in the present study is the immunocompromised status that is 36% of the cases. In the present study most predominant *Candida* species in such patients is *C. albicans* followed by *C. tropicalis*; 42% and 31% respectively. Other species were *C. glabrata* (11%), *C. parapsilosis* (8%), *C. krusei* (6%) and *C. dubliniensis* (3%).

The third most common risk factor in the present study is the long duration of antibiotic intake accounting for 20% of the total cases.

Candida species isolated from such patients in our study were *C. albicans* 60%, followed by *C. tropicalis* 30% and *C. parapsilosis* 10%. These findings were similar with the study done by Sajjan *et al.,* (2014) where *C. albicans*, *C. krusei* and *C. tropicalis* were 69.7%, 17%, and 13% respectively.

Candida species are emerging as a potentially pathogenic fungus in patients with bronchopulmonary diseases.

In this study *Candida* co-infection in patients with pulmonary tuberculosis was observed in 16 patients (16%); out of which *C. albicans* was the most common isolate observed in 50% (8) of the cases, followed by C. *tropicalis* 31%(5) and *C. parapsilosis* 19%(3). Similar findings were observed in a study done by Arunava Kali *et al.,* (2013), where *C. albicans* (50%) has been reported to be the most predominant isolate from TB patients followed by *C. tropicalis* and *C. glabrata* (20%) of each, *C. parapsilosis* (6.7%) and *C. krusei* (3.3%). The result is in keeping with other similar studies.
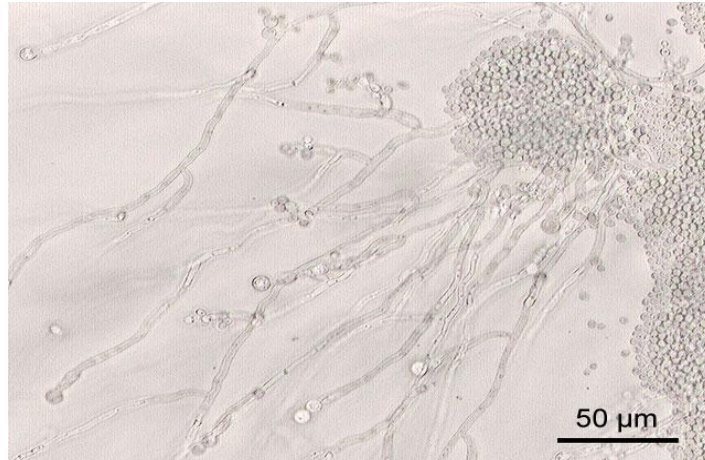
Image.1 *Candida albicans* on Corn Meal Agar

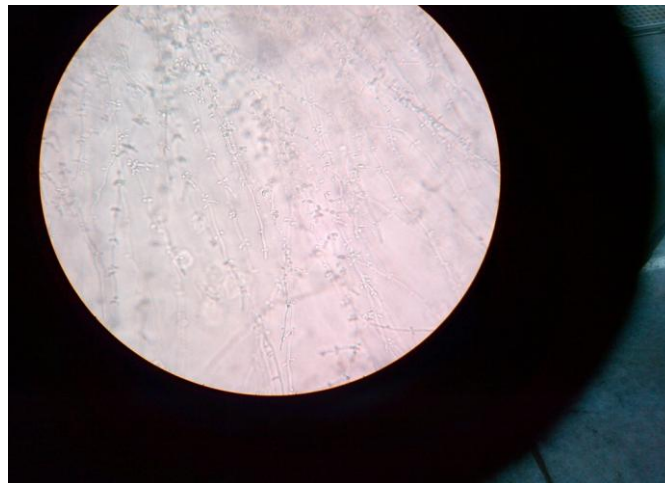**Image.2** *Candida tropicalis* on Corn Meal Agar

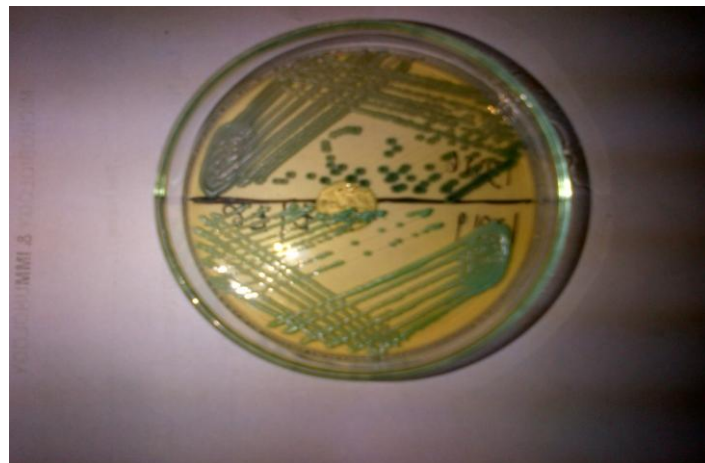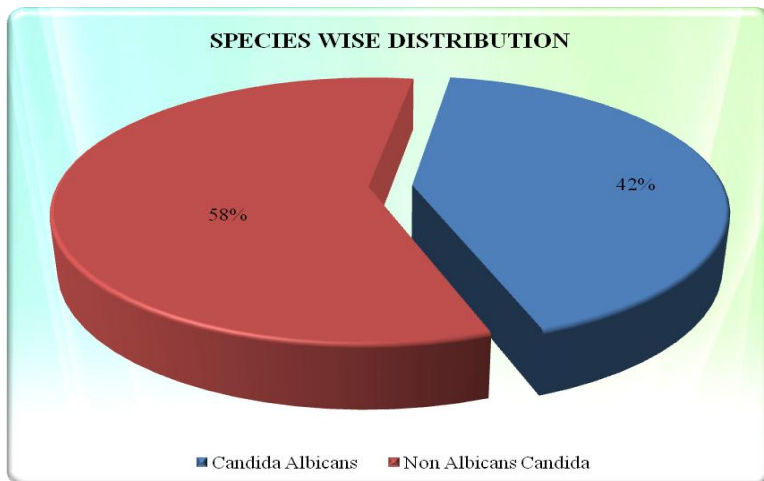**Image.3** *Candida albicans* (Apple Green) *Candida dubliniensis* (Dark Green) on chrome agar
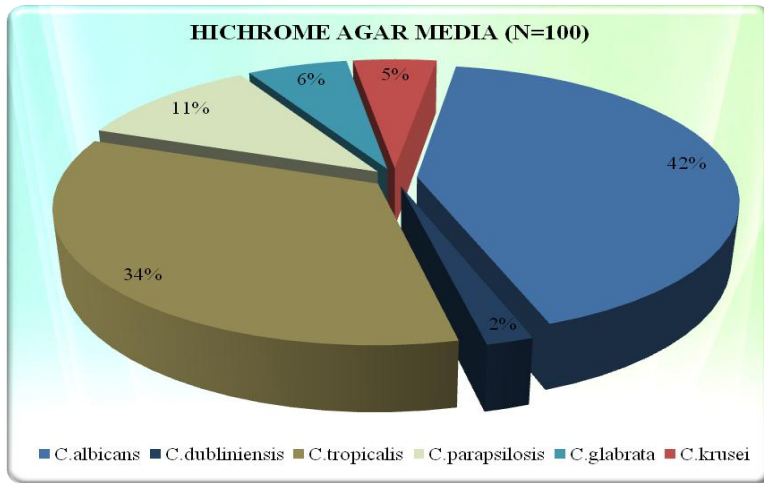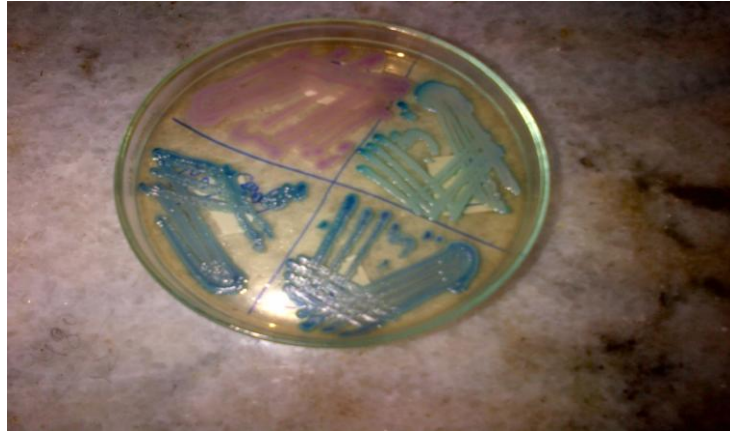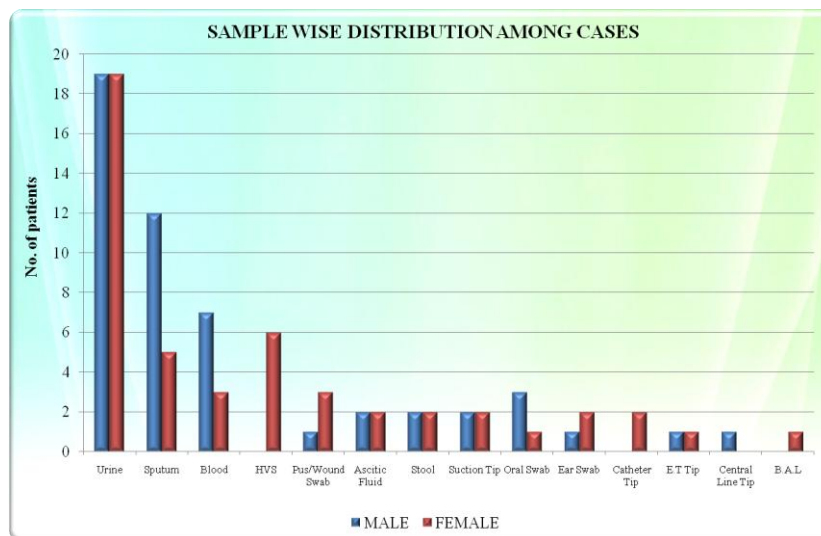
Image.4 *Candida krusei* (Pink Fuzzy) *Candida albicans* (Apple Green) *Candida tropicalis* (Blue)

Out of 100 patients 94% of the cases were admitted in the hospital (IPD) and remaining 6% were outdoor patients.

Table.1 Age wise distribution

AGE	MALE	FEMALE	TOTAL
≤ 1 year	2	1	3
< 10 year	2	2	4
10-20 year	-	1	1
21-30 year	4	9	13
31-40 year	5	19	24
41-50 year	7	7	14
51-60 year	15	4	19
61-70 year	8	4	12
71-80 year	7	2	9
>80 year	1	-	1
Total	**51**	**49**	**100**

Table.2 Clinical profile of the patients

n=100

Presenting complaints	No. of cases	%age
Fever with/without chills	61	61%
Weight loss	22	22%
Sepsis	16	16%
Cough	14	14%
Burning micturition	14	14%
Pain abdomen	11	11%
Chest pain	9	9%
White discharge/pruritis	6	6%
Diarrhoea	5	5%
Oral lesions	4	4%

Most common presenting complaints in the present study were fever (61%)

The prevalence of NAC spp. is increasing and maybe associated with the inadequate response to anti tubercular drugs. 14% of the total cases in the present study were diabetic. Out of these 14 cases; *C. tropicalis* was the predominant species [7] (50%), followed by *C. albicans* [3] (21%), *C. krusei* [2] (14%) and *C. glabrata* and *C. dubliniensis* [1] (7%) of each. NAC spp. are not only well adapted to the urinary tract but also are difficult to eradicate than *C. albicans*. Increase in concentration of glucose in the tissue, blood, and urine promotes growth of *Candida*. The present study is in keeping with studies done by Kandhari *et al.,* with an incidence of 20.4% and Shroff with an incidence of 14.66%. The predominant species isolated in the present study in 6% cases of VVC is *C. albicans* (50%) followed by *C. tropicalis* (33%) and *C. glabrata* (17%).

Present study is similar to the studies done by Rylander *et al.,* where *C. albicans* species were isolated in 90 out of 219 samples and *C. glabrata* in three and by Neeraja Jindal *et al.,* (2006) on 400 patients, 92 cases were cultures positive for *Candida* of these 64 (69.57%) were *C. albicans* 8 (8.7%), *C. glabrata,* 6 (6.55%) each *C. tropicalis* and *C. krusei*, and 4(4.3%) each *C. parapsilosis* and *C. guillirmondi.* In the present study occurrence of oral candidiasis was 40% out of 10 HIV positive cases. The occurrence of oral thrush ranged from 12% in Tanzania to around 94% in Zaire. The most common type of Candidiasis was the Pseudomembranous type ranging from 1% in Kenya to 70% in Peru followed by Erythematous candidiasis, the frequency of which ranged from 3% in India to 35% in Peru. In a study done by Luis Octino *et al.,* (2005) and Diz Dias *et al.,* (2001), the most common lesion found in HIV positive patients was Pseudomembranous which were in percentage of 97.5% and 69% respectively. *Candida* albicans was the most common species

isolated from oral lesions/swabs of HIV positive patients accounting for 50% (2) of total 4 oral swabs. In previous studies done by Dunic *et al.,* (2004), Mrudula Patel *et al.,* (2006), Omar *et al.,* (2008) *C. albicans* was the most common species isolated in oral swabs of HIV positive patients in the percentage of 77.7%, 83.5%, 78.6% and 84.5% respectively. Percentage of NAC spp. isolated from oral swabs was *C. tropicalis* and *C. parapsilosis* in the percentage of 25% respectively. In the present study 5% of the total cases came with the complaint of Diarrhoea. *C. tropicalis* was the most common species isolated from the stool samples accounting for 60%, followed by *C. parapsilosis* and *C. krusei* in the percentage of 20% respectively. This study was similar to the findings in a study by Banerjee *et al.,* (2012) where *C. tropicalis* (43.8%), *C. krusei* and *C. albicans* (15.6%) of each and *C. parapsilosis* was (6.3%).

Observations

Total 100 isolates of *Candida* were taken from various clinical samples. The age of the patients were ranging from minimum of <1 year to maximum of >80 years (Table 1). Maximum no. of patients belonged to 31-40 years of age group, Followed by 51-60 years. Out of 100 patients 51 were males and 49 were females. The male to female ratio was M:F 1.04:1

Sample wise distribution among cases

Most common form of candidiasis seen was candiduria type (38). Respiratory system was involved in 17 cases, while in 10 cases blood cultures were positive for *Candida* species.

Identification of *Candida* isolates

Out of total 100 samples 44% *Candida* isolates were found to be germ tube positive while 56% were negative.

42% species produced light green coloured colonies on HiChrome Agar while blue coloured colonies were produced by 34% of the species. 2% species produced dark green colonies. Cream to pale pink, pink to purple, and pink with white edges (matt surface) colonies were produced by 11%, 6% and 5% respectively (Image 3 and 4).

Out of 100 samples 42%were *Candida albicans* and remaining 58% were NAC spp.

To conclude, the study showed that prevalence of Non *Candida albicans* were higher from various clinical specimens. It is essential that an early and accurate diagnosis be made of infecting species of *Candida*. This will aid the clinician in timely institution of the appropriate and accurate antifungal drug to be used and will restrict the empirical use of antifungal agents as being commonly done today.

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