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Prevalence of *Candida* In Doaba Region of Punjab (India)

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ABSTRACT

Background: The most often encountered opportunistic fungi, *Candida* cause several diseases in humans. Prevalence of Non-albicans *Candida* spp. is arising worldwide due to increase in number of immunocompromised patients. Hence, identifying and distinguishing *Candida* is important for successful management of patient.

Aim & Objectives: Isolation, identification and distinction of *Candida* spp. from several clinical samples and determining its prevalence.

Material & Methods: During this 4 months study, a total 39 *Candida* spp. were isolated and were further distinguished using conventional method of microbial identification (Culture on SDA, Gram stain, chlamydospore production, Germ tube test) and HiChrom Candida differential agar method.

Results: Out of 1663 samples, 39 *Candida* spp. were isolated which indicates 2.35% prevalence of *Candida albicans* among the collected samples. Out of these 39 isolates, 19 were *Candida albicans* (49%) and rest 20 Non-albicans *Candida* (51%). Most of the *Candida* spp. was isolated from female patients (58.97%) within the age group of ≥30 to ≤70 (17.95%). Among various clinical samples, maximum *Candida* isolates were obtained from urine sample (56.42%) followed by sputum (28.21%), stool (7.69%), HVS, pus, body fluid (2.56%). Patients admitted to ICU were more affected (25.64%) than the others, admitted to various clinical wards.

Conclusion: In this study apart from *Candida albicans,* there was increased isolation of Non-albicans *Candida* spp. like *Candida tropicalis, Candida krusei and Candida glabrata*. Therefore early

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identification and distinction of *Candida* are important for successful patient management as Non-albicans *Candida* spp. are developing more resistance towards antifungal drugs.

KEYWORDS: *Candida albicans, Non-albicans Candida*, HiChrom Candida differential agar, Chlamydospore formation.

INTRODUCTION

Candida spp. being a human commensal inhabit skin, mucous membranes, oral cavity, gastrointestinal and genitourinary tracts. *Candida albicans* is most frequently encountered fungal opportunistic pathogen [1]. Facilitation of active conversion of commensal *Candida* to potent pathogen is done by several virulent factors which includes extracellular hydrolytic enzymes secretion, biofilm formation and adherence to medical devices & host tissues [2]. It causes infection when the host becomes immunocompromised or when the balance between *Candida* spp. and the host is altered like in extremes of age, pregnancy, diabetes, prolonged administration of antibiotics, secondary bacterial infections, steroid therapy and AIDS [3].
Incidence of candidiasis is increasing worldwide. In developed countries among total isolated yeasts 40-60% is *Candida albicans*, but the Indian studies show increased predominance of Non-albicans *Candida* spp. which includes *C. krusei, C. glabrata, C. parapsilosis* and *C. tropicalis*. Non albicans *Candida* cause more than 90% of spreading infections, however prevalence of the *Candida* spp. depends on the clinical settings, patient population and , geographical location [1, 4].

Diseases ranging from superficial candidiasis to invasive candidiasis are caused by all *Candida* spp., yet they differ in acuteness and antifungal susceptibility [1]. *C. krusei* is naturally resistant and *C. glabrata* is less susceptible to fluconazole. However, *C. tropicalis* is more resistant to antifungal agents, is involved in biofilm formation and has very high arte of adherence to inanimate objects such as vascular and urinary catheters [5-6]. Therefore early identification and distinction of *Candida* are important for successful patient management as more resistance against antifungal drugs has been developed among Non-albicans *Candida* spp.

MATERIALS AND METHODS

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In a 4 months study from January 2018 to April 2018, samples were collected from Out Patient Department (OPD) and In Patient Department (IPD) which included gynecology, surgery, medicine, emergency, special, pediatrics, ICU, and NICU wards at tertiary care hospital, Jalandhar. Several clinical specimens like urine, stool, sputum, high vaginal swab, pus and body fluids were included in our study. From direct clinical sample wet mount was performed and then inoculated on Sabouraud dextrose agar for 24h at 37^{o}C. Differentiation of isolates obtained from clinical samples was performed by Germ tube test, HiChrom Candida differential agar and chlamydospore production.

Direct examination: On a glass slide vaginal swabs and pus samples, mixed with one drop of 10% potassium hydroxide were kept and covered with coverslip. Preparations were examined for budding yeast cell under the microscope.

Culture on SDA: Sabouraud Dextrose Agar (SDA) were inoculated with clinical samples obtained from different wards. Plates were incubated at 37^{o}C for 24-48h. Yeast cell growth as creamy to white color, pasty colonies was checked.

Gram staining: A smear was prepared from clinical isolates on a glass slide. After heat fixing the slide, smear was stained with Gram stain reagents. After drying, slide was observed under microscope and checked for gram positive budding yeast cell.

Germ tube formation test: Small amount of inoculum from SDA was mixed with 5 drops of human or sheep serum in a tube that was obtained when blood centrifugation done at 15000 rpm for 15 minutes. Inoculated serum tubes were incubated for 24-48h at 37^{o}C. One drop of inoculated serum sample was kept on a grease free glass slide after incubation, a cover-slip placed on it and then examined for the production of germ tube under the microscope.

Differentiation on Hichrom agar: All clinical isolates obtained were inoculated on HiChrom Candida differential agar. The plates were incubated at 25^{o}C for 48h.

Chlamydospore formation Assay: All clinical isolates obtained were inoculated on Corn meal agar by making a deep horizontal cut and placing cover slip on this inoculated area. Inoculated plates were incubated at 25^{o}C for 24-48h.

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RESULT

Out of 1663 clinical samples, total of 39 *Candida* spp. were isolated. Hence, prevalence is 2.35%. Out of these 39 isolated spp. *Candida albicans* isolates were 19 and Non-albicans *Candida* isolates were 20.

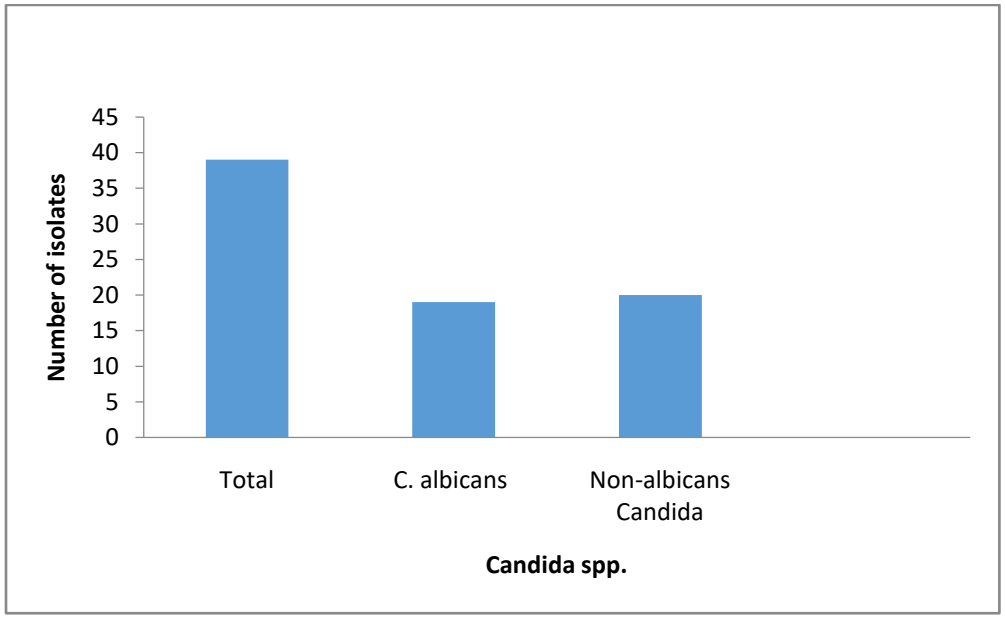

Fig. 1 Prevalence of *C. albicans* and Non alboicans *Candida*

From total Non albicans *Candida* isolates (20), rate of occurrence of *Candida tropicalis* (45%) was higher followed by *C. krusei* (30%) and *C. glabrata* (25%).

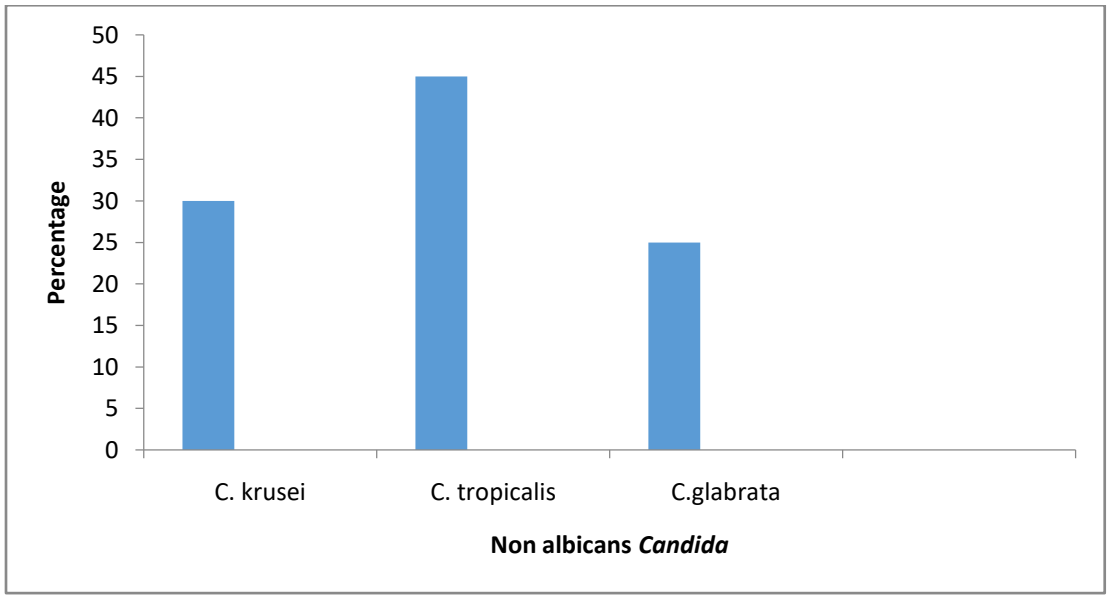

Fig. 2 Prevalence of Non-albicans *Candida*

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Vol-22-Issue-16-August-2019

Candida spp. isolates obtained from females were more (58.97%) as compared to males (41.03%)

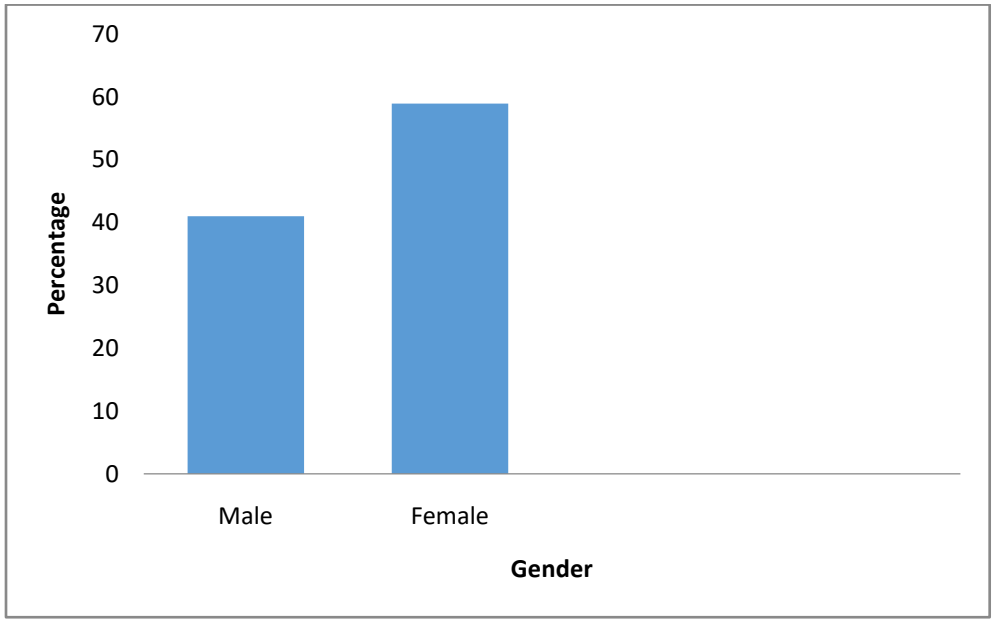

Fig. 3 Distribution of *Candida* spp. among different gender

C. albicans were mostly isolated from female samples (57.89%) than from male samples (42.11%). Also maximum of Non-albicans *Candida* was isolated from female samples (60%) and minimum was *isolated from male samples (40%).*

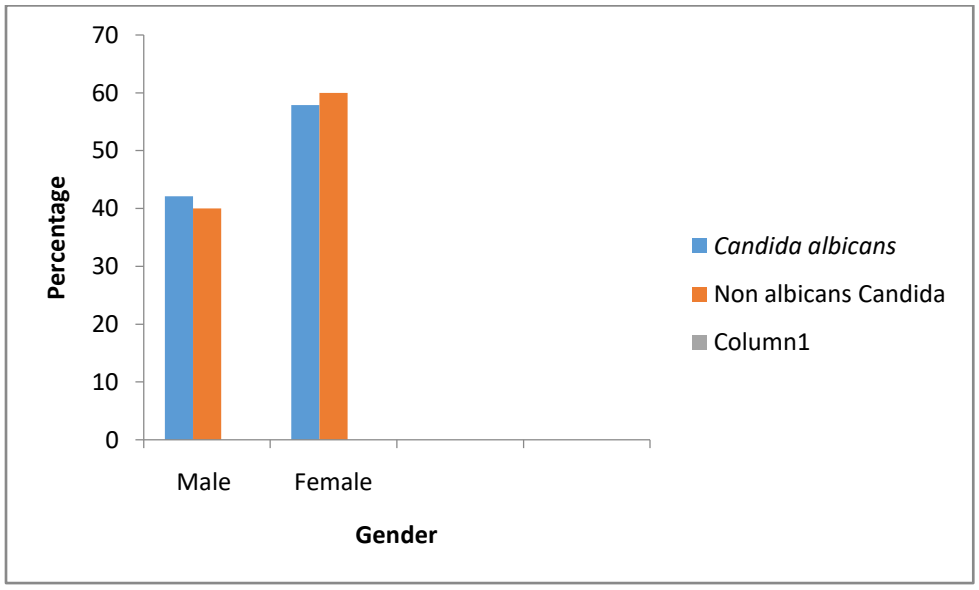

Fig. 4 Distribution of *C. albicans* and Non albicans *Candida* among gender

Maximum isolates of *Candida* spp. were isolated from the patients in age groups 31-40, 51-60 and 71-80 and minimum were isolated from 11-20 and 81-90 age group patients.

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Vol-22-Issue-16-August-2019

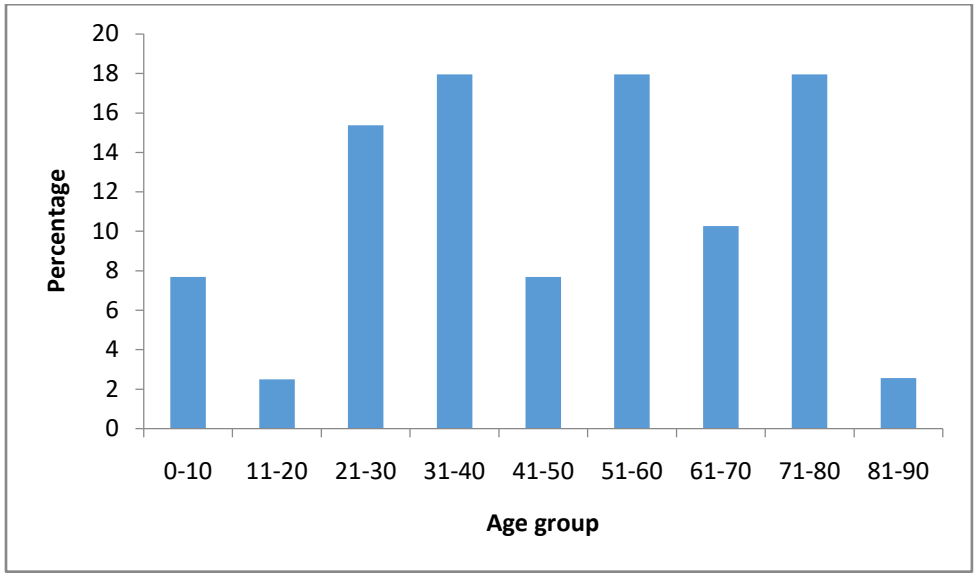

Fig. 5 Age wise distribution of *Candida* spp.

The highest isolation of *Candida albicans* was in 71-80 years age group (31.58%) and minimum isolation was in 81-90 years age group (5.25%) whereas maximum isolates of Non-albicans *Candida* were in 51-60 years age group (25%) and minimum in 0-10, 11-20, 41-50 and 71-80 years of age group.

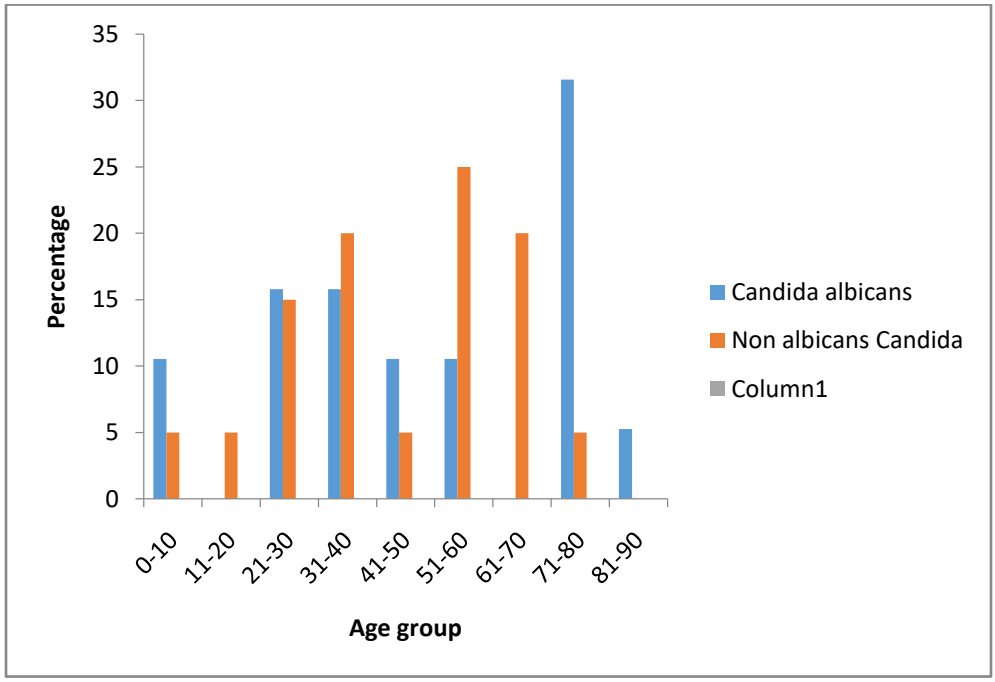

Fig. 6 Distribution of *C. albicans* and Non albicans *Candida* among different age group

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Vol-22-Issue-16-August-2019

Isolates of *Candida* spp. was obtained more from Medicine ward (25.64%) and minimum was isolated from Surgery, Special and Chest & TB ward (2.56%).

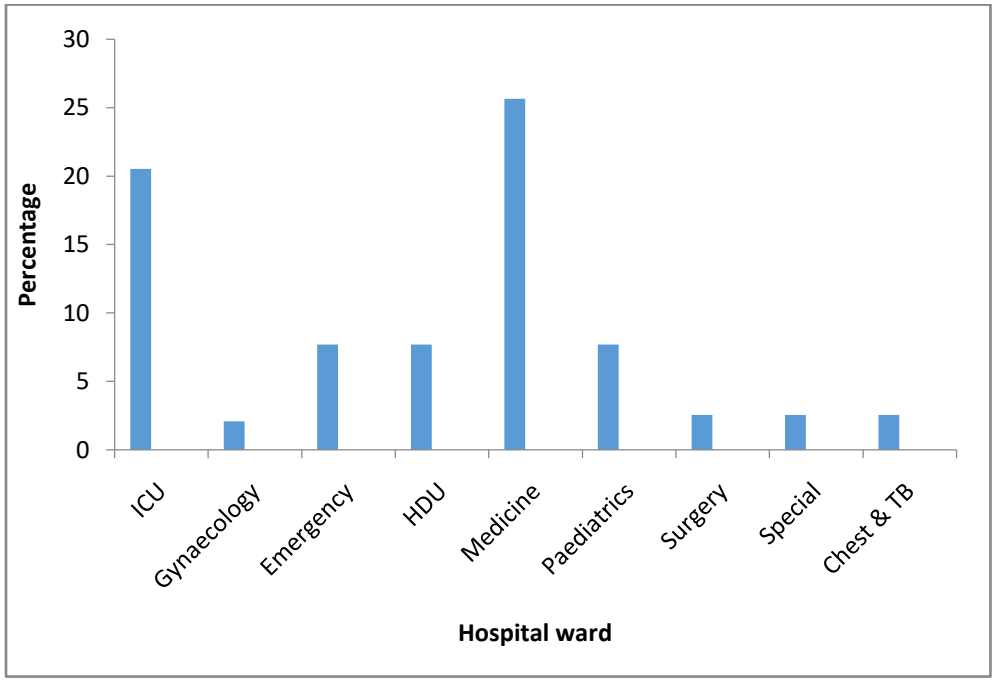

Fig. 7 Ward wise distribution of *Candida* spp.

Maximum number of *C. albicans* is isolated from Gynaecology department (27.78%) and minimum is isolated from Surgery and Chest & TB department (5.56%).

Whereas maximum number of *Non-albicans candida* is isolated from Medicine ward (33.33%) and minimum is isolated from Emergency, Paediatrics and Special ward (4.76%).

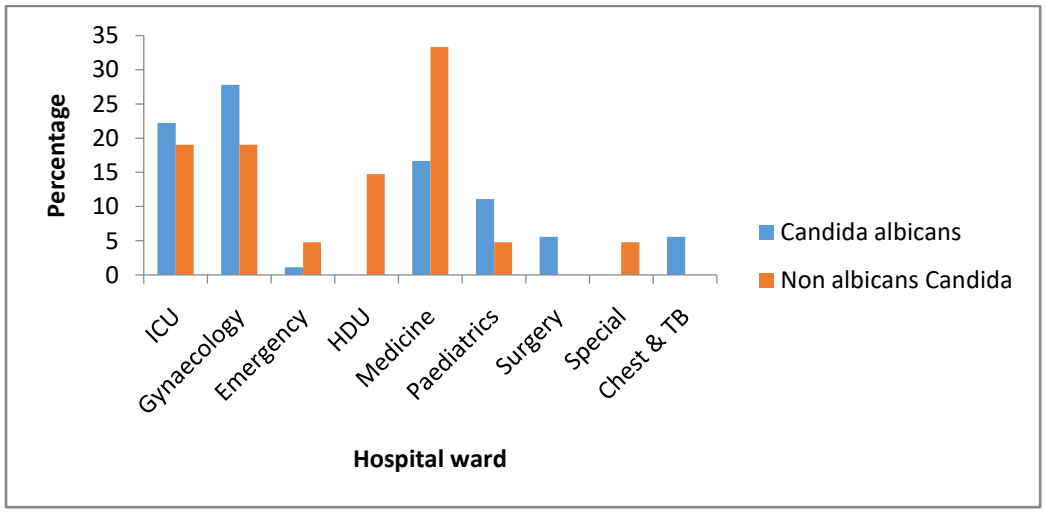

Fig. 8 Prevalence of *C. albicans* and *Non-albicans candida*in various departments.

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Isolation of *Candida* spp. was high from urine sample (56.42%) and minimum from HVS, Pus and Body fluids (2.56%).

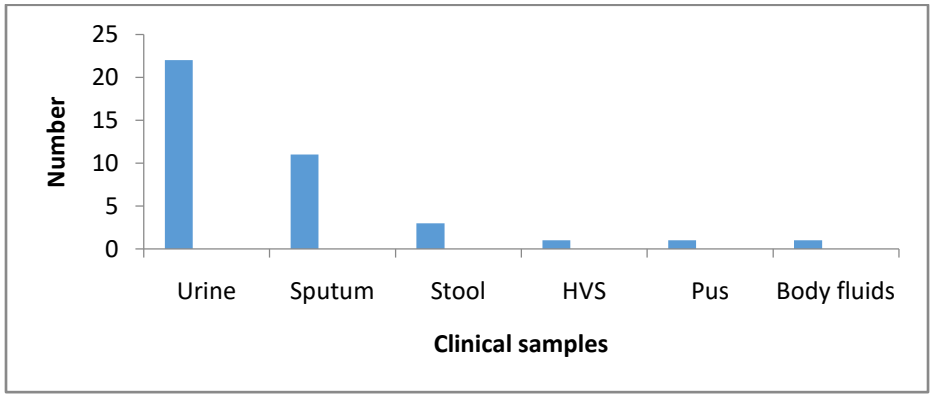

Fig. 9 Sample wise distribution of *Candida spp.*

Maximum number of *C. albicans* is isolated from urine samples (68.42%) and minimum from pus samples (5.26%).

Whereas maximum number of *Non-albicans candida* isolates is obtained from urine samples (45%) and minimum from stool, HVS and Body fluids (5%).

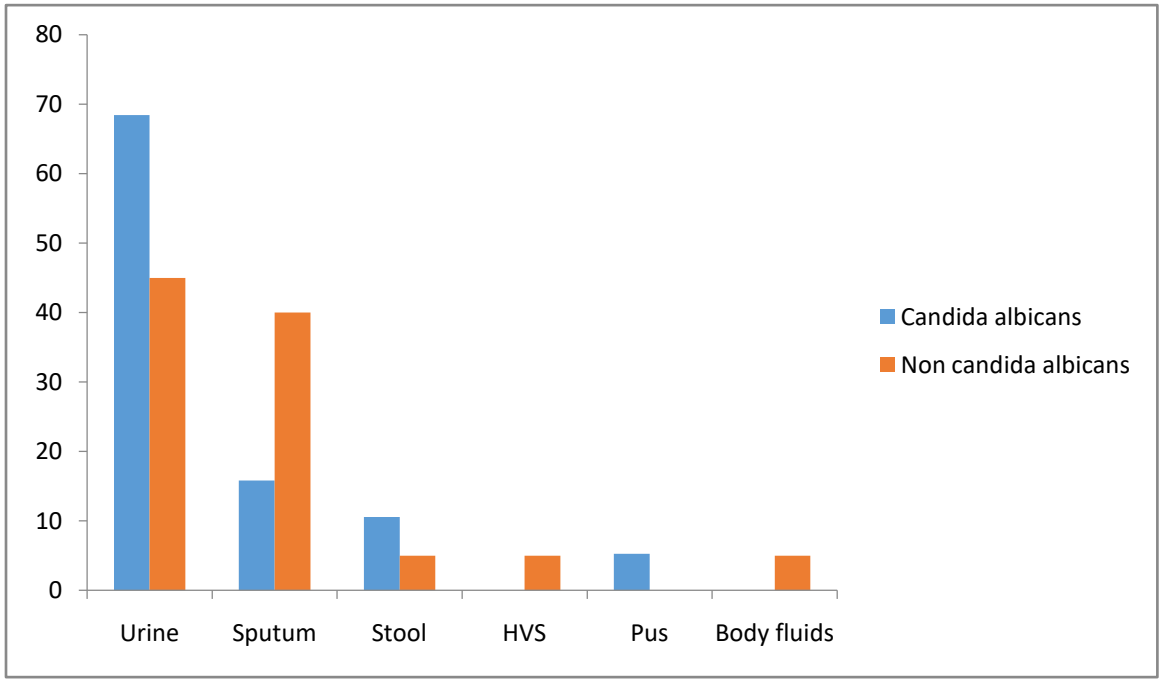

Fig. 10 Prevalence of *C. albicans* and Non albicans *Candida* among different samples

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DISCUSSION

C. albicans and Non albicans *Candida* both are causing invasive candidiasis, and the antifungal resistance of invasive *Candida* amplifies the situation. Due to increased in number of immunocompromised and recurrently ill patients the frequency and prevalence of *Candida* infections is increasing. So it is important to identify *Candida* spp. as they differ by their antifungal susceptibility and virulence factors. In this study, 39 *Candida* spp. were isolated in a period of 4 months (1st January to 30th April) from different clinical samples. Samples were collected from OPD and IPD section. From all clinical samples, *C. albicans* isolates were 19 and of Non-albicans *Candida* were 20.

Species wise distribution

So present study reports 49% of *Candida albicans* and 51% of Non-albicans *Candida*, which is in concordance with result of Sachin et al. [14] where *Candida albicans* were 44.4% and *NAC spp.* were 55.5%. Another study by Mokaddas et al. [15], showed that prevalence of Non-albicans *Candida* (60.5%) is higher than *Candida albicans* (39.5%). According to the study of Chakrabati A et al. [6], the prevalence of Non-albicans *Candida* is higher (75%) than *Candida albicans* (25%). Therefore by these findings it is suggested that Non-albicans *Candida* are emerging as potent pathogens.

But a study in Kenya by Kangogo et al. [16], reported that out of 150 species, 130(86.7%) were *C. albicans,* and rest 13.3% were Non-albicans *Candida.* According to a study by Zaini et al. [17], out of 313 isolation, 199(63.5%) were reported as *C. albicans* and 114(36.5%) as *non-albicans Candida.* So their results are dissimilar with our result.

In our study among all *NAC spp. Candida tropicalis*is reported to be the most predominant species whose prevalence is 45%, which is in concordance with the result of Golia S et al.(2013) [18], Patel LR et al. [19] and Adhikary R et al.[7]. Another study conducted by Basu et al. (2011) also reported high prevalence of C. tropicalis followed by C. krusei and C. glabrata.

Sample wise distribution

In our study most of the *Candida* spp. isolates were from urine (56.42%), followed by sputum (28.21%), stool (7.69%) and HVS, Pus & Body fluids (2.56%), which is in concordance with the result of T Singh et al. [20], where reported that most of the *Candida* spp. were isolated from urine (74.7%). Another study of Capoor MR et al. [9] at Safdarjung Hospital, New Delhi also

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Vol-22-Issue-16-August-2019

reported that most of the species were isolated from Urine sample (62.7%) which is in concordance with our result.

But in a study of Agarwal S et al. [21] at a Pediatric tertiary care hospital isolated 169 species from Blood (58.5%) followed by Urine (26%), tracheal aspirate (10%) and CSF (0.6%) etc. which is dissimilar with our result.

Age wise distribution

In our study patients that belong to age group of ≥ 30 years up to ≤ 70 years has shown the highest prevalence of candidiasis which is in concordance with the studies of Dalal PJ and Kelkar SS [11] at Mumbai reporting highest prevalence of candidiasis in the age group of 21-40 years. Another study by R A Kashid et al. [13] reported that the highest prevalence of candidiasis occurs in the age group of above 60 years (24.48%).

Gender wise distribution

In our study most of the species were isolated from females (58.97%) than males (41.03%) which is in concordance with the study of Kandhari KC et al. [22], where prevalence in females was reported higher (61.2%) than in males (38.8%). Stimultaneously, candiduria in females (80%) was more common than in males. Another study by Amar C S et al. [23], reported that isolation of *Candida* spp. in females (60.2%) was more than males (39.8%) which is in concordance with our study.

But a study which is dissimilar with our study, conducted by Patel et al. [19], recorded that males were more predominant than females, with an overall male: female ratio of 2:1. And according to the study of R A Kashid et al. [13], *Candida* spp. isolates were obtained more from males (55.10%) than females (44.8%) which is dissimilar with our result.

Ward wise distribution

In our study most of the *Candida species* were isolated from ICU department (25.64%) followed by Gynaecology ward (23.09%), Medicine ward (20.52%), Emergency and Paediatric (7.69%).

The above result is in concordance with the studies conducted by Capoor MR et al [9], Shivprakasha S et al [24], Sahni V et al [10] and Nur Yapar et al. [25] where reported that the maximum number of isolates were obtained from ICUs. Another study of Trick et al. [26] reported that a substantial increase in prevalence of bloodstream infections occurred by C. glabrata were mostly found among ICU patients in the United States during 1989-1999.

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Vol-22-Issue-16-August-2019

But the study by Dr. A. Arasi et al. [12] reported isolates of the *Candida* spp. were more from Medicine ward (20%) followed by ICU (10.4%), Paediatrics (6.4%) etc. which is dissimilar with our result.

CONCLUSION

In this study not only *Candida* albicans, but NAC spp. also have appeared as potent pathogens causing diseases from superficial mycosis to invasive mycosis. Hence, this study highlights the condition of fast and exact identification of Candida isolates by individual species distinction, as prevalence of Non-albicans *Candida* are on rise which are more resistant to antifungal drugs .

CONFLICT OF INTEREST

The author declare no conflict of interest

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