Discussion

The *Candida* species known as human pathogens were ubiquitous colonizers of human and other warm blooded animals. In this study different isolates of *Candida* have been isolated from different samples and were found to be the sole cause of infection. This study has been done to investigate the *Candida* profile and the sensitivity pattern in Barak valley health care centers. The predominance of non-*albicans* *Candida* species over *C. albicans* was found to be a notable feature. No such study has been conducted previously in Barak Valley.

The study has been designed for epidemiological investigation of different *Candida* species for the reason that, although *C. albicans* remained the sole cause of infection but infection with non-*albicans* *Candida* species are now emerging due to the variable susceptibility pattern of non-*albicans* *Candida*, selection of appropriate empirical therapy has become complicated. The changing epidemiology of *Candida* infection has shown a significant shift towards the non-*albicans* *Candida*. This was also associated with the cost of the treatment. The cost of management of candidiasis with non-*albicans* *Candida* was more than *Candida albicans*. The infection with non-*albicans* *Candida* species created an important impact in selecting antifungal therapy. Therefore, species level identification became compulsory for proper selection of drugs. In the present study confirmation
of species has been done by the conventional method from different clinical samples.

The study shows that there has been shift from *Candida albicans* to *non-albicans Candida species* of *Candida* as dominant causative agent (Table.5). 500 different samples were taken from suspected cases of candidiasis and were investigated for *Candida species*. All samples were collected aseptically in a sterile leak proof container. All samples were then cultured on blood agar and SDA. 94 cultures were found positive for bacterial growth but not *Candida species* and in 39 samples yeast like cells seen in microscopy but no growths were found on SDA. 348 samples were both microscopy and culture negative. Microscopically 113 samples showed yeast like cells and were culture positive for *Candida species*, these samples were considered for further identification (Table. 1). Repeat samples were also taken whenever needed.

Among total *Candida sp. C. albicans* exhibited 27.43% positivity and rest were *non-albicans Candida* (Fig.1). The predominant *non-albicans Candida* was *C. glabrata* 31.70% (26/82) followed by *C. tropicalis* 30.48% (25/82) and are found to be associated with hospital acquired infection (Pfaller et al., 1996). There is single isolate difference between *C. glabrata* and *C. tropicalis*. Other *non-albicans Candida species* were *C. guilliermondii* 20% (17/82) and *C. parapsilosis* 17% (Table.6). In all the samples in this study *non-albicans candida* is found in maximum number.

Blood samples were collected in BacT/Alert bottles which had brain heart infusion broth (BHI) and incubated. Subcultures were done in blood agar and SDA.
with antibiotics. Seven blood samples were collected from neonatal intensive care unit (NICU) and one blood was collected from a patient of 9 years old male who had presumptive diagnosis of fever of unknown origin (FUO) and was taking long term antibiotics therapy, as soon as *Candida* was detected, antifungal drug was given. The patient responded well. Second blood culture has been done from the same patient whenever possible for confirmation of the response of antifungal drug, which found no *Candida* in blood. Seven blood positive for *Candida* were isolated from surgical intensive care unit (SICU), with risk factor of long stay in hospital and prolonged antibiotics therapy. Two were from the patients who were receiving chemotherapy. Two blood *Candida* were found to be associated with central venous catheter. For suspected central line related blood stream infection (CRBSI) samples were collected as follows-one blood sample from peripheral and one from central line of the same patient were collected and cultured. In both the samples *Candida species* were isolated, which then correlated with central line tip culture, when it was removed. Twelve *Candida* species were isolated from males of different age group and seven from females (Table.10). Out of 19 positive for blood samples the species distribution is found as follows *C. glabrata* 37% (7/19), 32% *C. albicans* (6/19) and *C. tropicalis* 32% (Table.13). Total 68% (13/19) non-albicans *Candida* and 32% *C. albicans* were isolated from the suspected case of candidemia (Table.14). One *C. glabrata* and one *C. tropicalis* are found to be associated with central line related blood stream infection (CRBSI). Biomaterial infection is increasingly alarming problem. Modern health care technology has allowed the use of wider and newer variety of medical devices. Biomedical engineering investigations have attempted to minimize these material traits to
reduce device susceptibility to these infections (Nett and Andes, 2006). Early detection and removal of infected device need to establish cure of Candida infection. Although the reasons for Candida infections are multifactorial, better clinical assessment and diagnosis is needed. In Barak Valley there is no previous epidemiological information regarding the prevalence of Candida sp. However, the present study also emphasizes the immergence of C. glabrata, C. tropicalis as non-albicans Candida and C. albicans in association with blood stream infections.

Urine samples were collected from suspected urinary tract infection (UTI) and catheterized patients. Most nosocomial urinary tract infections are associated with the use of indwelling bladder catheter. Out of 37 Candida isolates from females a cluster of 29 were seen between the age group of 8 years to 40 years (Table.11). These observations represent higher susceptibility of catheterized females to hospital acquired urinary tract infection (Kamath et al., 2009), which could be attributed to shorter length of female urethra and absence of prostatic secretions in females. On the other hand out of 16 urine isolates from males only six were found in this age group. Study also showed in total urine isolates 30% were from male and 70% from female, indicating that its prevalence is more in female (Table.10). In total urine isolates 75% (40/53) were non-albicans Candida and 25% (13/53) C. albicans (Table.14). In present study, among non-albicans Candida C. glabrata 25%(13/53), followed by parapsilosis 21% (11/53), C. tropicalis 19% (10/53), C. guilliermondii 11% (6/53) were found (Table.13). Forty four Candida species were isolated from urine of catheterized patients and it was considered that urinary tract infection is due to catheter association. Fungal biofilm formation on catheter is a complex phenomenon and may be related to the severity
of infection due to drug cannot reach the organism at its optimal concentration.

Antifungal susceptibility test of *Candida sp.* is advantageous in addition to removal of foreign devices to optimize the management (Krcmery and Barnes, 2002)

Vulvovaginal candidiasis is an important cause of morbidity in women of reproductive age. Maximum number of isolates (71%; 15/21) were recovered from 21 years to 40 age group (Table.11) and can be related to the sexually active age group which was well mentioned in a study by Kashid et al. (2011) and Dalal and Kelker (1980). It was also found that 76% non-*albicans Candida* was isolated over *C. albicans* (Table.14). There was significant increase in non-*albicans Candida* (Spinillo, 1997). Significant influence of use of antibiotics also can increase the incidence of vulvovaginal candidiasis, as antibiotics are known to destroy the normal protective vaginal flora and helps in colonization with *Candida* (Jindal et al., 2007). Approximately three-quarters of all women experience at least one episode of vulvovaginal candidiasis during their life time and nearly half of them suffer from multiple episodes (Ferrer, 2000). This study showed *C. guilliermondii* (29%) as predominant *Candida sp.* followed by *C. albicans* (24%), *C. tropicalis* (24%), *C. glabrata* (14%), *C. parapsilosis* (10%) in vaginal secretions of suspected vulvovaginal candidiasis (Table.13). In this study *C. guilliermondii* found to be associated more with vulvovaginal Candidiasis.

If we exclude vulvovaginal candidiasis which was isolated from females only, a cluster of positivity has been noticed in the age of 0-10 years and 51 to more than 60 years of age group (Table.7). The natural immune status of this age group is found to be less and they were more prone to infection.
Sixty percent non-albicans Candida was isolated from cerebrospinal fluid (Table 14). Cerebrospinal fluid was incubated in brain heart infusion broth (BHI) for 24 hours and sub culture was done in blood agar and SDA. Candida meningitis is the most frequent manifestation of invasive candidiasis–related CNS candidiasis (Nesrin et al., 2007).

In this study 67% (10/15) Candida isolates were found in sputum of males followed by 33% (5/15) in females (Table 10). Maximum cases (67%) of sputum positive are found in the patient age group of 51 years to 60 years (Table 11). The species distribution in sputum found as followed C. albicans (5/15), C. guilliermondii (4/15), C. tropicalis (3/15), C. glabrata (2/15), and (1/15) C. parapsilosis (Table 13). The main risk factors which were found in this study were diabetes mellitus, prolonged use of antibiotics, chronic obstructive pulmonary disease (COPD), tuberculosis, malignancy and smokers.

The data was analyzed by SPSS version 17.0. As it was observed from Fig. 2, that the incidence of candidiasis is more among females (72/113) compared to males (41/113), but statistically by chi square test it has been seen that the incidence of Candida species distribution is independent of sex ($\chi^2 = 0.141$ and the critical value $\chi^2 = 3.84$ at five percent level of significance with one degree of freedom). One way ANOVA (Table 21.b) is used in our analysis as we have assumed only one way relationship of equality between means of C. albicans and non-albicans Candida in our analysis and conclude that prevalence of C. albicans and non-albicans Candida are equal ($p>0.05$). Although the prevalence of non-albicans Candida and C. albicans was done by paired t-test ($p<0.10$) and
correlation test (p<0.05), in both the method prevalence of non-albicans Candida is found as significant over C. albicans at ten percent and five percent level of significant respectively (Table.23,Table.24;). The increase prevalence of non-albicans species found to be replacing C. albicans and this finding is correlating with a study by Jha et al. (2006).

Hospitalized patients with different underlying illness and immunocompromised patients were found with Candida infection. Disease usually originates from gastrointestinal tract or skin. Most organisms that inhabit endogenous reservoirs were acquired exogenously. Studies have showed common risk factors for fungal infections (Narain, 2003; Hachem et al., 2007) and most of the risk factors were very common in all hospitalized patients and therefore it is very crucial to find out which patients were at greater risk for developing nosocomial fungal infection. A broad group of patients can be defined as who were immunocompromised (e.g., malignancy, corticosteroids, chemotherapy, malnutrition, HIV) are exposed to Candida infection, other distinct group who are exposed to infection with Candida were the hospitalized patients in which the many other factors primarily provided route of infection (long term catheter use, burn), and the use of broad spectrum antibiotics. As with the widespread use of antibiotics, the selective pressures exerted by the use of azole antifungals encourage the proliferation of resistant species, which create difficulty in management of critical patients.

C. albicans remained the most common species responsible for oral candidiasis in HIV positive patients. The increasing trend of infection with non
*albicans Candida* was also seen in patients with HIV infection. In the present study distribution of species in HIV positive patients has shown identical distribution with the non HIV positive populations, suggesting that infection with *Candida sp.* in HIV positive may be because of the defects due to the host defenses. The study found 12 *Candida sp.* out of 20 samples of oral swab of HIV positive patients (Fig.3). Out of 12 *Candida sp.* 10 were non-*albicans Candida* (Table.15) and *C. tropicalis* was found as maximum number followed by *C. parapsilosis* (Table.16).

In this study ten *Candida sp.* from males and two from females were isolated (Fig.4) The reason for the increased candidiasis in HIV positive individuals was due to the decrease CD4+ T lymphocytes, which cause major damage to the cell mediated immunity and also humoral immunity affected (Kashid et al.,2011).

Antifungal susceptibility testing was still an unexploited method in many Indian routine clinical microbiology laboratoris. This study has also provided a focus on the use of antifungal disc in routine clinical laboratory. The simplicity and flexibility of disc diffusion is very appealing method to introduce in the work flow of routine microbiological laboratories; on the other hand which can contribute a useful aid in treatment.

The susceptibility pattern of all *Candida* isolates showed that 91% were sensitive to amphotericin B, 65% voriconazole followed by 49% itraconazole35% fluconazole. Interestingly 94% *C. albicans* were susceptible to amphotericin B, followed by 92% *C. tropicalis*, 88% *C. glabrata*, 94% *C. guillermondii*, 86% *C. parapsilosis*. *C. tropicalis* showing 28% fluconazole, 40% itriconazole, 48% voriconazole sensitivity. *C. albicans* showing only 26% sensitivity against
fluconazole (Table.18). It has been noticed from this study the second most
effective drug is voriconazole after amphotericin B. The graphical representation of
sensitivity pattern of Candida species can be seen in Figure (Fig.5,6,7,8,9). The
isolates which recovered from HIV positive individual were shown a prevalence of
non-albicans Candida and found more resistant to fluconazole. Non-albicans
Candida species played an important role as causative agents in oropharyngeal
candidiasis and have been associated with severe symptoms. Medicine such as
antibacterial, antiretroviral and antifungal agents can interfere in changes in species
distribution (Nweze, 2011). A variety of antifungal agents are now available for the
treatment, in this study Candida sp. isolated from HIV infected patients were
highly sensitive to amphotericin B, except one C. tropicalis and one C. parapsilosis
which were resistant (Table.17).

Rapid species identification from different clinical specimens and standard
drug susceptibility testing would be an effective approach for controlling outbreak
by non-albicans Candida species in different clinical settings. When patients
doesn’t respond to antibiotics, it is necessary to test the patients for Candida and
also necessary to analyze the antifungal susceptibility pattern in order to timely
management of life threatening fungal infection.

From the study it was concluded that despite significant development in
Health care, the organism which is normal flora in healthy human, play a dominant
role in causing infection in hospitalized patients and the patients fall in extra
burden of cost as well as the adverse side effects of drugs. A variety of local and
host factors contribute to the prevalence of Candida infection, therefore yeast
infection with even host colonizer may not be neglected in critical care patients and clinical diagnosis must be correlated with laboratory investigation. The prevalence of *Candida species* involvement in candidiasis of HIV positive patients didn’t show any variation indicating that the infection was due to the decrease in immunity. This study therefore, emphasized the need for species level identification, which can be achieved by implementation of routine germ tube test, biochemical test for yeast and for effective treatment; antifungal susceptibility testing should be implemented in microbiology laboratories. Identification by molecular technique although gives an accurate detection but it is in experimental stage in most of the Indian routine clinical microbiology laboratories. The present study also supported the need of periodic surveillance of the antifungal susceptibility pattern of the prevalent *Candida species*, as it provided implication of selection of appropriate antifungal drug for the critical care patients.