6. DISCUSSION
# DISCUSSION CONTENTS

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6. DISCUSSION

The past three decades have witnessed major changes in hospital population and health care systems. Along with advances in medical and surgical management, an increase in opportunistic fungal infections among immunocompromised and critically ill patients is often noted. Among the various opportunistic fungal infections, the infection by ubiquitously found Candida species have assumed special importance.

Though over 100 Candida species have been recognized, only few are reported to be pathogenic. The *Candida albicans* is responsible for nearly 80% of infections. Now, the emergence of non-albicans Candida species also has been documented and is attributed to certain factors viz. the route of entry, mode of spread and the selection pressure of antifungal agents particularly fluconazole and itraconazole.

Thus the rising incidence and changing picture of Candida infections has demanded the identification of causative species, the spectrum of infections caused by them, the source of infection, the epidemiological behaviour and analysis of risk factors. The resulting knowledge is sought to help while planning the strategies in prevention of life threatening infections among immunocompromised and the critically ill patients.

6.1. The Study Group

Various general factors, which are likely to predispose susceptible host to acquire exogenous or endogenous candidosis, have been recorded. They are, presence of chronic ailment, prolonged hospitalization necessitating catheterisation (for example patient of head injury with coma, patient of cerebrovascular accidents with deranged cerebral function), patients of uncontrolled diabetes, patients of cancer on radio/chemotherapy, patients of burns (more than 70%), patients on prolonged broad spectrum antibiotic therapy and patients clinically suspected of HIV infection (syndromic patients with evidence of HIV infection viz. ARC, lymphadenopathy, oral candidiasis, diarrhea, chronic
weight loss, CNS infections). These patients are prone to diminished immune status resulting in higher opportunity for ubiquitously present Candida to get colonized either exogenously or endogenously producing situations conducive for establishment of Candida infections.

The present study has therefore been planned to attempt isolation, and identification of medically important Candida from immunocompromised patients attending "Shri Chatrapati Shivaji Maharaj Sarvopchar Rugnalya", Solapur,"Shri Sidheshwar Cancer Hospital", Solapur and "Ashwini Sahakari Rugnalya", Solapur, either on OPD or IPD basis, with definitive signs and symptoms of clinical syndrome locally or systemically manifesting distinct clinical possibility of Candida infection.

A total of 643 patients were selected after thorough clinical history and physical examination along with relevant investigations and were categorized under different clinical groups viz. pulmonary tuberculosis (128), chronically hospitalized patients with catheterisation (128), diabetes mellitus patients with hyperglycemic and glucosuric state (122), patients of cancer with chemo/radiotherapy (104), syndromic patients with clinical suspicion of HIV infections (83), second and third degree patients of burns with evidence of burn wound infection (78). (Table 5.1)

For matter of convenience the results of the study have been presented in tabular format, the same shall be referred for drawing meaningful observations, and interpretations, highlighting specific scientific revelations of the present study during the course of discussion.

Six hundred and forty three clinical cases in the present study are divisible according to their sex and it was observed that, males show higher incidence of suspected Candida infection. The clinical cases could also be grouped in accordance to age into seven groups. The incidence of infection was more commonly noted between the age 31-60 years of age, the incidence being highest in both the sexes between the age group of 30-40 years. (Table 5.2)

Different clinical groups selected for the study could also be analyzed in accordance to age and sex. It was observed that incidence of Candida infection
was higher among male population in patients of pulmonary tuberculosis, patients of cancer, and patients chronically hospitalized needing catheterisation. Earlier workers recorded similar findings.\textsuperscript{1,3,4,8} Significantly high incidence was observed among female burn patients, but generally the incidence of burn injuries are more common among the females than males. Therefore, female predisposition for Candida infection among burn patients may not have any particular relevance.

The pattern of incidence among different clinical groups is observed to vary according to age, dependent on the overall incidence of a particular disease in community as is reported by various workers,\textsuperscript{22,261-268} for example, the, incidence of tuberculosis is seen to be common in the age group 21-50, whereas incidence of cancer, diabetes, chronic hospitalization is seen in the higher age groups, as can be logically analyzed, higher incidence of suspected HIV infection in the younger age group is well known and the same is reflected in the present study. (Table 5.3)

Aging predisposes an individual to dampening of immunological apparatus further complicated by the occurrence of diseases like pulmonary tuberculosis, uncontrolled diabetes, cancer, HIV infection, resulting in situations where ubiquitously present Candida colonizes in higher density enhancing the prospect of clinical infection by Candida in them.

The findings of present study are in accordance to the observations, cited in the literature that, a higher age group individual, when afflicted by disease there is clinical predisposition of Candida infection, the same is represented in tabular form.
Table 6.1. Shows clinical predisposition of Candida infection in higher age individuals as reported by various workers.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Clinical type</th>
<th>Predisposing age in years</th>
<th>Incidence Of Candida infection</th>
<th>Authors and Reference Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pulm. Tuberculosis</td>
<td>31-60</td>
<td>84.03%</td>
<td>Shivananda et.al (1981)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-60</td>
<td>73.43%</td>
<td>Present study (1998)</td>
</tr>
<tr>
<td>2</td>
<td>Cancer</td>
<td>40-80</td>
<td>77.64%</td>
<td>Chen et al (1974)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40-80</td>
<td>70.11%</td>
<td>Cho et al. (1979)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-60</td>
<td>84.61%</td>
<td>Present study (1998)</td>
</tr>
<tr>
<td>3</td>
<td>Diabetes</td>
<td>40-60</td>
<td>43.50%</td>
<td>Odds et.al. (1989)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-60</td>
<td>70.49%</td>
<td>Present study. (1998)</td>
</tr>
<tr>
<td>4</td>
<td>Urinary tract infection in (Chronic hospitalized &amp; catheterized pts.)</td>
<td>40-60  30-60  51-70</td>
<td>32%  64%  44.53%</td>
<td>Odds et al (1988)  Laxmi et al (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Present study (1998)</td>
</tr>
<tr>
<td>5</td>
<td>Burns wound infection</td>
<td>30-40</td>
<td>53%</td>
<td>Kathryan et al (1972)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31-40</td>
<td>47.43%</td>
<td>Present study (1998)</td>
</tr>
<tr>
<td>6</td>
<td>Suspected HIV</td>
<td>20-40</td>
<td>63%</td>
<td>Kate et al. (1993)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21-50</td>
<td>85.54%</td>
<td>Present study (1998)</td>
</tr>
</tbody>
</table>

6.2. Specimen Collection

In the present study from 643 clinical cases, clinical specimens for attempted isolation of Candida have been collected from logical and relevant clinical sites where from, it was thought that if detectable number of Candida were likely to be present, they would be obtained from the clinical samples collected.

Table 5.4 shows distribution of 1701 clinical specimen comprising of urine (835), oral gargle (354), sputum (301), wound swab (125) body fluid and aspirates (42), stool (23) and blood (21) obtained from 643 cases.

For deriving significance of postulated presence of Candida in the clinical specimen an attempt has been made to collect the relevant clinical specimen on more than one occasion as far as possible with reasonable time period gap in the days of collection.
Samples of oral gargles were preferred to throat swabs since the colonization of Candida could be at various sites in the oropharynx and the clinical sample collected should comprehensively represent likely presence of Candida in the oropharynx. When literature was reviewed similar recommendation has been cited, for Candida detection in oropharynx.\textsuperscript{181}

Table-5.5 shows distribution of clinical specimen according to the clinical groups of study and the number of occasions of collection.

6.3. Laboratory Processing

All the clinical specimen were subjected to standard recommended laboratory procedures viz. microscopic demonstration of Candida forms in clinical specimen, cultivation of clinical specimen in Sabourauds dextrose agar with and without antibiotic containing media, germ tube testing, fermentation and assimilation of sugars or carbohydrates. Therefore, the results of the present study are presentable on the basis of smear positivity after microscopic examination of clinical specimen, the culture positivity when detectable number of CFU were seen during the course of incubation of culture media following inoculation and on the basis of different species of Candida isolated from different clinical specimen and different group of patients.

In literature, as it has been cited that presence of Candida is more likely at mucosal sites, an attempt was made to evaluate whether Candida demonstrated, isolated and identified were in significant density by observing either microscopic fields and the number of colony forming units detected from each specimen on cultivation.\textsuperscript{4,22} However, specimen such as blood and body fluids when show presence of Candida even in few number were considered clinically significant after ruling out theoretical possibility of contamination either at the time of collection of sample or during the laboratory procedures.\textsuperscript{29} This was essential since patients of cancer and patients of suspected HIV infection were the clinical groups from whom such samples were collected viz. blood, CSF, pleural fluids and ascitic fluid.
6.4. Direct Smear Examination

It is well documented that mycelial forms of Candida if demonstrated persistently indicate an established colonization therefore, when clinically correlated, direct smear examination results are sensitive and useful adjunct to the culture results. The Gram staining method is quite satisfactory for microscopic demonstration of Candida in direct smears prepared from clinical specimen, our observations are consistent with other workers.

Out of 1701 clinical specimen obtained 21 were samples of blood, which were processed for Candida cultivation without smear examination. In result, out of 1680 clinical specimen, 272 (16.19%) showed presence of Candida either in budding yeast form, pseudohyphae or true hyphae forms.

Table 5.6 shows the distribution of smear positivity in accordance to the type of clinical specimen. The smear positivity ranged from 4% to 26%. Wound swab from patients of burn showing the lowest smear positivity (4%), where as stool from suspected HIV infection cases showing higher smear positivity (26.08%). The occurrence of Candida in stool specimen from this group of patients is an important finding since Candida are known to cause opportunistic infection in these patients, and reflect an altered intestinal content as reported in the literature.

Table 5.7 shows the distribution of smear positivity and culture positivity observed in clinical specimen relevant to the clinical group of study.

Presence of Candida in smear from sputum of patients of pulmonary tuberculosis also has a higher significance and clinical importance since, Candida are known to cause super infections in patients of pulmonary tuberculosis compounding the problems of therapeutic management of such cases. In the present study Candida could be demonstrated in direct smear of 26.81% sputum specimen from chronic PTB cases.

128 PTB cases are divisible on the basis of clinical chronicity and on the basis of whether the lesion is cavity forming or non-cavity forming. 58 cases showed cavity formation of which 21 were fresh cavitatory type while 37 were...
In both the categories sputum examination revealed comparable smear positivity. (Table 5.7A)

Smear positivity was marginally more (27.02%) in chronic cavitatory type than fresh cavitatory type (23.80%). In all these cases sputum examination was done on two occasions.

In 70 cases of non-cavitatory type 39 cases were fresh and 31 were chronic the smear positivity was only marginally more in fresh non-cavitatory type (17.94%) than chronic non-cavitatory type (16.12%), in all these cases sputum examination was made on more than one occasion (twice in fresh, thrice in chronic cases) and smear positivity was seen in all these specimens.

Khanna et al (1977)118 and Jain et al (1982)117 attributed acquisition of fungal infection to treatment with anti-tubercular drugs and chronicity of the disease. Chakravarty et al (1964)272 has explained how fungal infection is acquired among chronic cavitatory type of cases having undergone anti-tubercular treatment since, the acid fast bacilli are eliminated from cavities the left over cavities promote fungal growth by virtue of presence of necrotic material and available oxygen.

In our study however, neither the chronicity of the disease, nor the cavitatory or non-cavitatory form of disease has shown any particular reflection on the smear positivity of the sputum for presence of Candida.

However, in our study when urine samples of these cases were examined for possible colonization of Candida 27 specimen (14.59%) collected from 12 cases showed presence of Candida in smear. In all these cases Candida were seen when the specimen was collected twice or thrice. Persistent presence of Candida confirmed the carrier state.

In the clinical group of chronically hospitalized patients needing catheterisation presence of Candida in the urine deposits has distinct clinical significance.22,261273 Debilitated patients with indwelling catheters for a long duration have higher predisposition for Candida colonisation by ascending route.22 These Candida may vitiate the clinical course due to urinary infection refractory to conventional antibiotic therapy.
In all 128 cases represented the clinical groups of chronically hospitalised patients, needing catheterisation there were seven different kinds of illnesses, as shown in Table 5.7.B. From these cases urine specimens were obtained and were submitted for microbiological examination. Demonstration of Candida in urine smears of these patients (21.83%) in the present study is of significance. Catheterisation for period less than 10 days revealed no smear positivity for Candida, whereas, urine deposit smears beyond 10 days showed smear positivity in 13 cases, smear positivity is in higher proportion according to the chronicity of hospitalisation as is notified in Table 5.7.C.

122 cases of patients of diabetes mellitus on the basis of the duration of diabetes could be classified in four categories and specimen of oral gargles (190) and urine (180) were obtained. Table 5.7.D shows smear positivity among them. 30 samples (15.78%) of oral gargle collected from 15 patients, twice from each patient showed smear positivity, whereas, six cases revealed urine smear positivity in 13 (7.22%) samples.

The smear positivity was more common in patients with diabetes for long duration of 11-12 years.

Uncontrolled diabetes may promote Candida colonization in oropharynx and the urinary tract due to hyperglycemia and persistent glucosuria, colonisation of Candida at these areas is of significance and may indicate immunocompromisation.274

Detection of Candida in patients of cancer in specimen of oral gargle and urine also is a matter of concern, since the patients are under radiotherapy and chemotherapy, both the types of therapies are known to cause devitalisation of tissues and if Candida are found at vulnerable sites, their presence has profound clinical significance.275 In the present study 286 specimen were obtained from 104 patients of cancer, of which 164 oral gargles collected on more than one occasions whenever possible, yielded 41 (25%) smear positive results, whereas, 101 samples were urine samples collected on multiple occasions, yielded 18 (17.81%) specimen yielded smear positive results.
Twenty one samples of blood were obtained from patients showing indications of candidemia in form of febrile illness or toxicity.

Table 5.7.E. shows distribution of cases according to clinical type of cancer and the number of cases showing smear positivity. Oral gargle specimen deposit was nearly twice more smear positive (25%) than urinary sediment smear positive (17.82%). In general colonization of Candida was more common a feature in the oro-pharyngal cavity.

From 83 patients suspected of HIV infection, 45 cases revealed HIV antibody test positive and showed the clinical symptoms of weight loss, lymphadenopathy, fever and diarrhea in number of cases as shown in Table 5.7.F. Fifteen cases had clinical meningitis, 35 cases had clinical oral candidiasis and eight cases had other complications.

Clinical specimen such as sputum, oral gargles, body fluids such as gastric aspirates, ascitic fluid, pleural fluid, CSF, urine and stool were obtained from them as shown in Table 5.7.G.

Smear positivity was seen maximum in body fluids (10 cases), followed by sputum (6 cases), stool (4 cases), urine deposits (3 cases). Twenty-three cases out of 83 (27.71%) showed smear positivity for presence of Candida blastospores or pseudohyphae.

78 patients of burns could be categorised into four types of burn injuries and each category could be divided into II<sup>nd</sup> and III<sup>rd</sup> degree burn. (Table 5.7.H). Specimen of wound swabs and urine were examined for presence of Candida. Smear positivity was noted in five patients (4%) from wound swabs, urine smear positivity was more common (10 cases 6.84%). Smear positivity was more common among III degree burns, and patients with thermal burn injuries (Table 5.7.I)

6.5. Culture Results

Detection of Candida on cultivation is more scientific method of studying the presence of Candida at vulnerable clinical sites for any meaningful correlation. In the present study out of 1701 clinical specimen 681 (40.03%) specimen showed culture positivity.

Discussion
Table 5.8 shows distribution of culture positivity according to the nature of clinical specimen.

The culture positivity was found to vary according to the nature of specimen, showing the lowest culture positivity of 10.40% in wound swab of burn patients to the highest in sputum specimen (53.48%). (Table 5.8)

It appears that certain clinical samples are more relevant for the clinical group of study than another, for example sputum of pulmonary tuberculosis (culture positivity 80.45%) are more relevant than urine samples (culture positivity 28.64%) from the same patients, similarly oral gargle is more appropriate sample (culture positivity 60.36%) than urine (culture positivity 46.53%) in patients of diabetes. Whereas in patients of cancer both oral gargle and urine revealed higher percentage of culture positivity (60.36 % and 46.53% respectively). High percentage of culture positivity was also seen in sputum and body fluids of suspected HIV patients (69.13% and 50% respectively) (Table 5.7).

Table 5.9 shows the distribution of culture positivity in various clinical groups of patients.

Out of 681 Candida strains isolated, the bulk of Candida was found in patients of PTB (230 ,56.79%%), cancer (152, 53.14%), and uncontrolled diabetes (112,30.27%) and suspected HIV (100,44.05%) (Table 5.9).

In all these clinical groups there is a disease related immunocompromise, which is as long durable as the prevailing disease, giving higher susceptibility to host and increased colonising opportunity to Candida, when compared to situational immunocompromise observed in patients of burns or patient clinically hospitalised and catheterised. In these patients the incidence is found slightly low, possibly because the immunocompromise may not have constitutional relatedness to the host, or possibly, is only situation related persisting till the presence of burn injury or catheterisation.

6.6. **C. albicans and non-albicans Candida species on basis of Germ tube test result.**

Over the years germ tube forming ability was given importance in deciding pathogenicity or invasive nature genetically endowed by Candida, accordingly germ
tube forming Candida were considered pathogenic and since most of these produced porcelain white colonial appearance on cultivation, even the lesions clinically observed as oral thrush, vaginal thrush were characterised by curdly white patches of exudate naming the responsible fungus as C. albicans was considered appropriate, the other Candida were thus designated as non-albicans Candida. 22

In the present study based on germ tube positivity 419 (61.52%) Candida strains were grouped as C. albicans and 262 (38.47%) were grouped as non-albicans Candida. Majority of the C. albicans were found in clinical group PTB (110), diabetes (105) and cancer (103), where as non-albicans Candida were also seen in highest number among patients of PTB (120) followed by suspected HIV (57) and cancer (49). (Table 5.10)

When the culture positivity results are considered according to the clinical group for clinical correlation, certain observations emerged.

In clinical group of PTB, out of 128 cases sputum specimen from 86 patients were culture positive. Sputum culture positivity was distinctly more in patients showing cavitatory lesions and was particularly more when the cavitatory lesions were chronic. 53 sputum samples from cavitatory PTB cases yielded culture positivity of which 32 were chronic cavitatory type, delivering 64 strains of Candida when sputa were cultured on two occasions. From the same patients, isolation of non-albicans Candida was marginally more (34 strains) than C.albicans (30 strains). Out of 42 sputum samples from the fresh cavitatory cases 28 strains isolated were non-albicans and 14 were C.albicans, in both the situations sputa were culture positive on two consecutive occasions. Isolation of non-albicans Candida (62 strains) was more than C.albicans (44 strains). Table 5.10 A.

Seventy one strains of Candida strains were isolated from sputum of non-cavitatory type of PTB cases, the isolation of Candida was marginally more in chronic cases (37 strains) than in the fresh cases (34 strains) and C.albicans (50 strains) were more frequently isolated than non-albicans Candida (21 strains).
Urine samples of PTB cases revealed isolation of 53 strains of Candida of which non-albicans were more commonly isolated (n=40 strains) than *C. albicans* (n=13 strains). Isolation of non-albicans strains was most frequent in fresh non-cavitatory type of cases.

Analysis of Candida culture among PTB cases reveals Candida isolation from both the type of clinical presentation i.e. cavitatory and non-cavitatory, as well as, from both type of clinical specimen i.e. sputum and urine. 123 non-albicans Candida were isolated as against 107 *C. albicans*.

Patients of PTB require hospitalisation for the morbidity that they suffer from, in hospital set-up Candida are ubiquitously present, and *C. albicans* is an established pathogen known to cause opportunistic infection, however, the matter of great concern recently is the isolation of large number of non-albicans Candida.

Recent reports suggest a definite shift in the distribution of infection caused by species of Candida other than *C. albicans*. Use of fluconazole as known anti-fungal agent has been reported to prompt fluconazole insensitive strains of non-albicans Candida to establish in susceptible immunocompromised patients thereby, yielding non-albicans Candida isolates as the likely pathogens in clinical situations.

The present study supports this view with distinct higher isolates of non-albicans Candida species in most common susceptible group of immunocompromised patients i.e. in cases of PTB.

Interestingly it is seen that in 58 number of cases of cavitatory type of tuberculosis isolation of Candida was distinctly more (106 strains) than the non-cavitatory cases (71 strains) and isolation of non-albicans Candida was distinctly more (62 strains) than the *C. albicans* (44 strains). Our study is in accordance to the postulations of Chakravarty et al (1964) that patients of cavitatory tuberculosis under treatment, after bactericidal action on *Mycobacterium tuberculosis*, leave necrotic material and higher concentration of oxygen for Candida to flourish.

From 128 chronically hospitalized patients for different clinical conditions with catheterisation, 32 Candida isolates were found of which 28 (87.50%) were
C. albicans, colonization and isolation of Candida was not found within 10 days of catheterisation but it increased with duration of catheterisation. Table 5.10.B.


Table 5.10.C shows distribution of culture positive results among patients of diabetes mellitus from oral gargles (75 strains 92.59%) and urine (28 strains 90.32%), according to the duration of illness. Isolation of C. albicans from oral gargles and urine was more common than non-albicans Candida and was found to increase with duration of illness. In patients with diabetes more than six years up to 10 years, 44 strains of C. albicans were isolated and from patients with diabetes mellitus beyond 10 years 35 C. albicans and 7 non-albicans strains were isolated.

Uncontrolled diabetes and diabetes refractory to anti-hyperglycemic treatment coupled with certain degree of immunocompromisation prevailing for a duration, enhances prospects of Candida infection and colonization. Presence of higher concentration of glucose allows ubiquitously present Candida to establish opportunistically at the mucosal sites viz. oropharynx, and genito-urinary mucous membrane.97,276,277

Detection of Candida and appropriate treatment in diabetes mellitus becomes highly important clinical decision, since such patients are known to undergo clinical complications due to Candida- polymicrobial infections, the related morbidity and even fatal infections.

Our findings of significant Candida isolation especially C. albicans is consistent with observations of other workers. 22,91,92,261 The reason for lesser frequency of non-albicans species of Candida is not clear.

When data of culture positivity in clinical group of cancer patient was analyzed, from 104 cases, 99 isolates of Candida (C. albicans 72 and non-albicans 27) were found in oral gargle and 47 (28 C. albicans and 19 non-albicans) were from urine samples. Isolation of C. albicans was more dominant feature, whereas Candida were more frequently isolated from cases of oral cancer.( Table 5.10.D)
Oral Cancer is a more frequent type of cancer seen when compared to malignancies at other sites, possibly because of malignant pathology in the oropharynx and due to substantial devitalization of cancer lesion surrounding apparently healthy tissue there appears to be higher colonization of Candida, these Candida may cause opportunistic infection as cancer patients are under either radiotherapy or chemotherapy, either of the therapy resulting in distinct possibility of immunocompromisation.

Detection of Candida in such patients may prove very vital in modifying the clinical therapeutic management. Our study of higher C.albicans isolation compares well with earlier reports. When culture positivity of Candida was analyzed among 83 suspected HIV patients it was found that 100 strains of Candida were isolated, of which 43 C.albicans and 57 were non-albicans. Isolation of non-albicans Candida among HIV patients is well documented.

Isolation of Candida species was more from sputum / oral gargle samples than other sites. Table 5.10 E1&E2.

Opportunistic infections among patients with HIV infection are well known and detection of Candida provides an opportunity of specific anti-candidal treatment in such cases, which may help to enhance quality life expectancy, among these patients.

Patients of burns when examined for presence of Candida from their burn wound sites and from urine samples, it was found that isolation of Candida was more from urine 42 strains (28.62%) than wound sites 13 strains (10.14%). Isolation of C.albicans was marginally more than non-albicans Candida from either of the sources, however, more number of isolates were found in patients receiving thermal burn injuries, when compared with burn injuries due to electric or chemical sources. Table 5.10.F.

Isolation of Candida from patients of burn and their role in morbidity is in accordance to the findings of other workers but most of these workers have reported presence of Candida in form of burn wound infection, whereas, in the present study urinary isolation of Candida is an important
feature. We feel that this is an interesting aspect worth of clinical cognizance in view of the fact that patients of high percentage burns are moribund to their beds and if colonization of Candida occurs in their urinary passage, causes opportunistic infections, which may cause complications in the clinical course. Detection of Candida provides an opportunity of appropriate anti-fungal drug, which may be vital in such cases.

6.7. Candida Species Identification

In view of ubiquitous occurrence of Candida in nature and as commensal in human being, it is possible that under influence of various factors present in the ecosystem and the predisposing factors favoring situational or opportunistic colonisation, certain phenotypic or genotypic variations produce an inevitable overlap of Candida species. Thus, it is always important to identify the concerned Candida species in a given clinical situation for proper clinico-epidemiological correlation.

In the present study every culture isolate has been thoroughly studied for biological and biochemical properties and identification based on the battery of tests has been attempted. 4,23,29,135,136,220

Table 5.11 shows analysis of 681 Candida isolates and their differentiation according to different morphological biological and biochemical characteristics.

Out of 419 germ tube test positive Candida, 412 (60.49%) were identified as C. *albicans*, where as seven of these *C.albicans* demonstrated germ tube test positivity were biochemically identified as *C.tropicalis*. *C.tropicalis* is recently noted as second most important Candida species than *C.albicans* in causing opportunistic infections. Other non -albicans Candida species noted in our study are 101(14.83 %) *C.tropicalis*, *C.parapsilosis* 61(8.95%) *C.krusei* 43 (6.31%), *C.kefy* 33(4.84%) and *C.guilliermondii* 31(4.55%). Association of these Candida with clinical disease is also on record. Our isolation of six different species of Candida having pathogenicity potential is consistent with observation of other workers. 2,4

Discussion

Many workers have highlighted this interesting biological property of Candida, often associated with conclusion of their pathogenicity.\(^{39,148,252,253}\) There are theories explaining germ tube forming ability of Candida, such as effect of yeast cell concentration,\(^{149,150}\) starvation of carbon moiety in the milieu,\(^{255}\) presence of nitrogen containing nutrients,\(^{255}\) concentrations of amino acids in the milieu,\(^{253}\) however, no distinct mechanism was confirmed as responsible for this biological behavior.

Hazen and Cutler (1979)\(^{148}\) have proposed an interesting mechanism designated as "morphogenic autoregulatory substance" production. In their opinion this is a major influencing factor in determining Yeast-Mycelial (Y-M) conversion in \textit{C. albicans}. They further add that, the other factors like starvation of carbon, presence of nitrogen, relative concentration of amino acids has only supportive or coincidental influence on germ tube formation. However, metabolic pathways governing respiration, glycolysis, anaerobic fermentation, amino acid degradability through amino peptidase could be responsible for state of the flux observed in vivo as well as in vitro, between yeast and mycelial forms of Candida.\(^{155,252,253,254,255}\)

In the present study, germ tube formation of sizable number (n=50 each) of \textit{C. albicans} and \textit{C. topicalis} using different support media was carried out to study the factors governing germ tube formation. Pooled normal human serum was found to be best medium in comparison to other media, similar observations are reported by other workers.\(^{149,252,253,254,281}\) (Table 5.12).

In our study starvation period of 45 minutes yielded highest results of germ tube test positivity than 15 and 30 minutes starvation. Starvation results into change in glucose metabolism and acquisition of pluripotency i.e. to resume growth in either morphological form.\(^{39,255,282}\) Various have workers reported starvation period in the range from 20 minutes (Shepherd et.al 1985)\(^{39}\), to 60 minutes, (Cho et.al 1992)\(^{252}\) and to 180 minutes (Holmes et.al 1988)\(^{255}\) (Table 5.13).

The present study also revealed that inoculum prepared from 48 to 72 hours old Candida cultures produce highest germ tube positivity than cultures
incubated upto 24 hours (Table 14). Similar studies are reported by Cho et.al (1992)\textsuperscript{252} and (1994).\textsuperscript{254} However, the present study provides the comparison among both the germ tube forming species of Candida, i.e. \textit{C.albicans} and \textit{C.tropicalis}, which is an interesting aspect of our study.

In order to study effects of amino acids on germ tube formation in different species of Candida, glycine, histidine and cysteine containing 0.5% glucose medium as supportive media were used. Interestingly, glycine was found to be promotive for both \textit{C.albicans} (50% germ tube positivity) and \textit{C.tropicalis} (14% germ tube positivity) while histidine (50% germ tube positivity) and cysteine (14% germ tube positivity) were found to be promotive amino acids for \textit{C.albicans} and \textit{C.tropicalis} respectively. The germ tube positivity in different amino acids containing media indicated involvement of different metabolic pathway in Y-M transition in both the species, as reported by Shepherd et.al (1985)\textsuperscript{39} and Joshi et.al (1979)\textsuperscript{253}

On the basis of ability to form germ tube or otherwise, \textit{C.tropicalis} has shown scope for differentiation, Martin et.al (1981)\textsuperscript{263} have demonstrated subgroups of \textit{C.tropicalis} based on electron microscopic studies of germ tube formation by \textit{C.tropicalis}. They have identified different populations of \textit{C.tropicalis}, some showing abortive filamentation, where as some showing continued germ tube formation. The formation of germ tube was appreciably reducible by subculturing as reported by Joshi et.al (1983).\textsuperscript{153}

Germ tube formation by \textit{C.tropicalis} also has been demonstrated by Tierno et.al (1977)\textsuperscript{155} and Joshi et.al (1983)\textsuperscript{153} wherein changes in respiration and glycolysis, so also abrupt change from aerobic to fermentative metabolism of glucose have been suggested as factors responsible for filamentation i.e. germ tube formation, generating a speculation whether \textit{C.tropicalis} are metabolically subverted forms of \textit{C.albicans}.\textsuperscript{252} This is an interesting possibility that in absence of other scheme of identification, germ tube forming Candida may be erroneously concluded as \textit{Candida albicans} less knowing that some of them could be \textit{C.tropicalis}. 

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Our observation of *C. tropicalis* showing germ tube formation in 6.93% is consistent to the findings of other workers who have reported germ tube test positivity up to 26% in *C. tropicalis*.\(^{153}\)

### 6.9. Morphological Identification of Candida Species.

Identification of Yeast or Yeast like fungi into different species using standard recommended criteria such as microscopic appearance, colony morphology and biochemical reactions like carbohydrate fermentation and assimilation are technically time consuming, expensive and demand technical skill, therefore, reliable and alternative methods were under consideration of many scientists.

Different support media have been used to study various morphological growth phases of Candida species. Study of production of germ tubes and chlamydosporos was considered important for morphological identification of Candida. Dolan et.al (1971)\(^{162}\) and Joshi et.al (1993)\(^{165}\) have described elaborately typical mycelial morphological forms of Candida species which are helpful in microscopic identification of different species of Candida presumptively within 24 to 72 hours of culture, in specially defined media like glucose agar (GA), rice extract agar (REA) and corn meal agar (CMA). Similar reports are available in literature of attempted morphological identification of yeast like organism.\(^5,143,270\)

In our study of 681 strains of Candida cultivated on GA with Tween 80 and on CMA, and it was observed that GA with Tween 80 was better medium in view of higher growth potential as well as better morphological discrimination of different growth phases of Candida namely yeast forms, chlamydosporos, mycelial forms with blastospores, giant forms etc.(Table 5.15)

Our study suggests that Candida isolated from clinical specimen on culture medium could be fruitfully identified into different Candida species, when subcultured on GA with Tween 80 within 72 hrs, providing even much desired prospects of early species identification for meaningful and prompt therapeutic management.

### 6.10. Identification of Candida species based on Chemical and Dye sensitivity test.

Based on scientific possibility that microorganism demonstrates differential sensitivity or resistance to certain chemicals and dyes similar to their sensitivity or
resistance to antimicrobial drugs, Sobczak in 1985\textsuperscript{257} suggested a scheme using previously experimented dyes and chemicals for identification of Candida species.

In the present study, the 681 cultivated Candida strains identified on the basis of recommended standard procedures, were also identified on the basis of chemical and dye sensitivity by disk diffusion test as recommended by Sobczak (1985).\textsuperscript{257} It was possible to sub-classify six species of Candida into 12 distinct codes viz. 412 strains of \textit{C.albicans} into three codes, \textbf{120406} being the most common code found in 383 strains, \textit{C.tropicalis} into two codes of which 123456 code being the most common (96 strains), \textit{C.parapsilosis} into three codes of which, 020450 being more common than the other two (57 strains), \textit{C.krusei} into two codes of which 123456 being more common than the other (40 strains), whereas 33 strains of \textit{C.kefyr} and 31 strains of \textit{C.guilliermondii} could not be classified but they were represented by 123456 and 120456 codes respectively (Table 5.16)

This was an interesting aspect of Candida identification especially for non-albicans Candida for epidemiological purpose. For \textit{C.albicans} different methods of typing are available like serotyping, resistotyping and biotyping in addition to well established morphotyping. Until such time similar methods are available for non-albicans Candida, their classification by chemical and dye sensitivity using disk diffusion test could be used fruitfully for clinico-epidemiological correlation after their morphological and biochemical identification into different species.

Series by Sobczak (1985)\textsuperscript{257} consisted of 594 yeast isolates including \textit{Geotrichum, Rhodotorula}, and \textit{Torulopsis} in addition to the strains of Candida. Present study of 681 Candida isolates probably is the largest series, for such type of differentiation using chemical and dye sensitivity discs.

Table no.5.17, 5.18,5.19 shows distribution of different Candida species found in different clinical samples in various clinical groups.

Candida isolation was more in patients of PTB (56.79%), cancer (53.14%), patient suspected of HIV (44.05%) and uncontrolled diabetes (30.27%). \textit{C.albicans} was found nearly in comparable proportions from all the six clinical groups, whereas
other species of Candida namely *C. tropicalis* was found more commonly from PTB, *C. parapsilosis* was found more commonly from PTB, *C. krusei* from PTB and cancer, *C. kefyr* and *C. guilliermondii* from PTB and HIV than the other clinical groups (Table 5.17). Yield of Candida was more in sputum 78.07%, in oral gargle 50.84%, body fluid 50%, than other clinical specimen viz. stool 39.13%, blood 28.57%, urine 25.98% and wound swab 10.4%.(table 5.18)

Table 5.18 also show that *C. albicans* is the predominant isolate from all these specimen (60.49%). The isolation of non-albicans candida species viz. *C. tropicalis* 14.83%, *C. parapsilosis* 8.95%, *C. krusei* 8.31%, *C. kefyr* 4.84% and *C. guilliermondii* 4.55% is an important finding therefore, the general contention that Candida infection is *C. albicans* infection is proved wrong. Our study demonstrates importance of speciation of Candida in all the clinical situations for the fact that this may help in better therapeutic management. The issue is of importance in specific clinical situation viz. non-responding PTB cases, uncontrolled diabetes, patients of cancer on chemo or radiotherapy and patients of suspected HIV infection in search of better quality life.

Fungal super infections among patients of pulmonary tuberculosis are known to cause refractory phase among patients on anti-tubercular drugs, prolonging the period of hospitalization and enhancing rate of morbidity and mortality.\(^{116, 117, 266, 269, 270}\) Candida are the commonest fungi reported by various workers, and *C. albicans*, *C. tropicalis*, *C. krusei* are the common Candida species, associated with super infection.\(^{117, 266, 269}\)

The reported rate of isolation of Candida species by other workers in comparison to the present study is as shown in table.
Table 6.2. Showing isolation rate of different Candida species among cases of PTB as reported by other workers.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Authors</th>
<th>C.alb</th>
<th>C.tropic.</th>
<th>C.krusei</th>
<th>C.pseudo</th>
<th>C.parap</th>
<th>C.guillier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pandalai &amp; Kurup et.al (1962)</td>
<td>Nil</td>
<td>Nil</td>
<td>2.9</td>
<td>Nil</td>
<td>8.7</td>
<td>Nil</td>
</tr>
<tr>
<td>2</td>
<td>Grower &amp; Junnarkar (1965)</td>
<td>Nil</td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
<td>2</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Khanna et.al (1977)</td>
<td>62.5</td>
<td>Nil</td>
<td>1.8</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>Malik (1981)</td>
<td>38.1</td>
<td>11.3</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>Shivananda et.al (1981)</td>
<td>62.5</td>
<td>12.5</td>
<td>15</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>Jain S.K.(1982)</td>
<td>18.57</td>
<td>2.14</td>
<td>1.4</td>
<td>1.4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>7</td>
<td>Jain S.K.(1982)</td>
<td>31.70</td>
<td>3.65</td>
<td>2.43</td>
<td>2.43</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>Present study(1998)</td>
<td>46.52</td>
<td>21.73</td>
<td>6.52</td>
<td>6.08</td>
<td>13.47</td>
<td>5.65</td>
</tr>
</tbody>
</table>

*All figures are in percentage

In the present study out of 230 strains of Candida isolated from cases of PTB, 107 (46.52%) are *C.albicans* whereas 123 (53.47%) strains are non-albicans Candida. In absence of speciation many of these Candida would have been regarded as co-incidental isolation under the belief that *C.albicans* is the only pathogenic species of Candida. Among the non-albicans Candida though *C.tropicalis* strains are in sizable numbers 21.73% (50 strains), presence of *C.parapsilosis* is also of great importance in as many as 13.47% (31 strains) isolated from PTB cases. Whereas *C.krusei, C.kefyr* and *C.guilliermondii* are also isolated with comparable frequency (15, 14, 13 strains respectively). (Table 5.19)

In view of higher prevalence of pulmonary tuberculosis in India, and paucity of medical mycology laboratories most of pulmonary mycotic infections are wrongly diagnosed and treated with antituberculosis drugs, further
worsening the situation. With the advent of oral azoles early recognition of candidal infection has become mandatory in the recent days.

Super infection by opportunistic fungal infection, has received greater importance due to the recent knowledge that, there is dangerous association of tuberculosis and HIV infection, unless all the cases of tuberculosis yielding fungi especially different species of Candida are screened for HIV infection it will not be possible to disassociate likely probability of associated HIV infection among pulmonary tuberculosis cases.\(^{268}\)

More studies are required in this area before assuming marker role of Candida isolation among PTB cases for HIV infection. This possibility may improve therapeutic management of refractory cases of tuberculosis, since C. albicans is suspected of inducing HIV amplification in latently HIV infected cells.\(^{21}\)

The putative predisposing factor causing candiduria are indwelling catheters, chronic debilitation, immunocompromisation due to prolonged antibiotic therapy, use of steroids or cytotoxic drugs as a part of therapeutic management. Colonized Candida are likely to increase in number due to urethral trauma secondary to catheterisation. \textit{C.tropicalis} and \textit{C.albicans} are reported common species of Candida by Chakravarti et.al (1997) \(^{279}\), whereas Laxmi et.al (1993) \(^{261}\) have isolated \textit{C. stellatoidea} almost in the same number as \textit{C.albicans}. Detection of Candida in urine holds a special significance since these are known to invade the mucosal barrier to enter rich capillary bed of the urinary organs leading to bacterimia, to metastatic candidal abscesses and septicemia.\(^{22}\) In the present study also 32 Candida isolates are found dominated by \textit{C.albicans} (n=28).

Our findings of \textit{C.albicans} in chronically hospitalized patients urine is consistent with the findings of Laxmi et.al (1993) \(^{261}\), Goldberg et.al (1979) \(^{273}\) and Chakravarti et.al (1997).\(^{279}\)

Patients of diabetes who are not effectively controlled for their hyperglycemic and glucosuric state are prone to candidal invasion at the site of uroepithelium as well as mucosal surfaces of oropharynx. Candida species are often associated and \textit{C.albicans} is the frequent species found.\(^{89,50,91}\)
In the present study out of total 112 (30.27%) Candida isolates from patients of uncontrolled diabetes, 103 (91.96%) were *C. albicans*. Presence of *C. albicans* is significant in them since they are known for their invasive character. Our findings are in corroboration to the findings reported by other workers. \(^3,8,9\) (Table 5.19)

The virulence of *C. albicans* is reported to be enhanced by higher blood or tissue glucose level, low lactate level, impaired T cell response, low opsonic index, increased adhesive potential of *C. albicans* through accumulated enzymes, directly causing damage at tissue or cellular level. Therefore, presence of Candida in either urine or oral gargle among diabetes is not an health encouraging situation. \(^22,90,91,98\)

Many workers have reported opportunistic fungal infection among cancer patients where they found Candida was more frequent and among Candida, *C. albicans* and *C. tropicalis* were the commonest Candida isolates.\(^{22,262,263}\) Where as Kiehn et.al (1980) \(^{275}\) have reported a large series of yeast isolated (33-40 isolations) in 15 months period and they have found in addition to *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. kefyr* as the other Candida species isolated. Our finding of *C. albicans* 66.44% (101 strains) as dominant Candida species along with *C. tropicalis* 12.5% (19 strains) *C. krusei* 9.21% (14 strains), *C. parapsilosis* 5.92% (9 strains), *C. kefyr* 3.28% (5 strains) and *C. guilliermondii* 2.63% (4 strains) as other species in that order of frequency are consistent with findings of other workers. (Table 5.19)

The reported rate of isolation of Candida species by other workers in cancer patients in comparison to the findings of the present study is as shown in the table.
Table 6.3. Showing rate of isolation of different Candida species among cancer patients as reported by other workers.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Authors</th>
<th>C.alb</th>
<th>C.tropic</th>
<th>C.parap</th>
<th>C.krusei</th>
<th>C.guill</th>
<th>C.pseu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kiehn et al (1980)</td>
<td>275</td>
<td>12.9</td>
<td>3.7</td>
<td>1.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>Wingard et al (1979)</td>
<td>67.41</td>
<td>28.08</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Martin et al (1981)</td>
<td>56.8</td>
<td>16.1</td>
<td>1.1</td>
<td>1.9</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>Present study (1998)</td>
<td>66.44</td>
<td>12.5</td>
<td>5.92</td>
<td>9.21</td>
<td>2.63</td>
<td>3.28</td>
</tr>
</tbody>
</table>

* All figures are in percentage.

On account of devitalisation of mucosal surface in patients of cancer on radio or chemotherapy, lesser prone species of Candida also demonstrate potential of causing the disease. Unless these Candida infections are attended to by specific antifungal drug therapy higher five year survival rates are unlikely.

Presence of Candida in oropharynx as well as in lower end of esophagus has assumed a clinical marker status of the degree of immunodeficiency due to HIV infection. Every HIV suspected clinical patient is thoroughly examined for Candida presence. In the present study apart from C.albicans (43 strains), C.tropicalis (15 strains), C.guilliermondii (12 strains), C.parapsilosis (11 strains), and C.kefyr (11 strains) are also found in sizeable number and the incidence of Candidal infection is 40%, the reported incidence ranges from 12-93%. 268

Presence of Candida at the site of burn wounds is important, since patients are already receiving antimicrobial drugs either to prevent or control common bacterial infections. Infections may become chronic inspite of antibiotic treatment when they are of fungal origin. There are many studies of role of Candida in burn patients, in present study Candida were isolated in 55 (20.29%) cases. Isolation being dominated by C.albicans (30 strains), C.tropicalis (13 strains), and C.parapsilosis (7 strains). Neely et al (1988)102 reported 85% of burn patients colonized or infected by C.albicans, 18% by C.tropicalis and 11% by C.parapsilosis. Other workers also reported higher occurrence of Candida wound infection.98,99,100,101,103,104 The reported incidence
of Candida colonization in burn wounds ranges from 11.4% to 54.7%. Degree of burn injury and site of burn injury plays a very vital role in inducing Candida colonisation and infection.\textsuperscript{101,103}

6.11. Mixed Candida Isolation.

Microbial involvement in causation of disease could be in monomicrobial or poly-microbial form. The monomicrobial isolations assumed importance when are found in close body cavities unlikely to be fostering microbial presence in health, such as when isolated from pericardial, pleural, peritoneal fluids and joint fluids. Monomicrobial isolations from sites showing commensal presence of microorganisms assumed clinical significance when they show high concentration or density than is comprehensible or when the isolation is carried out on repeated occasions.\textsuperscript{286,287}

Polymicrobial isolations are suggestive of mixed microbial infections, however, it is difficult to know which of the microorganism is preceding than the other and whether their combined presence has any relatedness in inflicting pathogenicity on the host.

Microbial synergy is one of the established possibilities based on supportive roles coexisting microorganisms render towards mutual benefit. It has been exemplified more vividly among bacteria viz. coexistence of aerobic/facultatively anaerobic and obligatory anaerobic bacteria in combined infections where anaerobes and facultative anaerobes are responsible for using up oxygen and providing relative anaerobiosis, the same can be said about Eh of the milieu due to presence of the either of the microorganisms.\textsuperscript{287}

Microbial synergy could be nutritional, one providing the essential growth factor for the other.\textsuperscript{287}

The third mechanism stipulated is protection of a susceptible organism from antimicrobial activity due to antimicrobial agent (drug like antibiotic) or the colicin by another micro-organism resulting in an infection opportunity.\textsuperscript{287}

Certain other mechanisms prompting microbial synergy have been postulated by Mackowiac 1978.\textsuperscript{286} He has stated association of Pneumocystis carinii and cytomegalovirus (CMV), CMV being carried by P. carinii at the sites of host tissues.
Association of certain parasites and viruses is also on record where parasites act as a vector of the pathogen for example *Strongyloidis latti* and swine influenza virus. Further stipulating is the suggestion that other nematode vectors may act as vector for viruses keeping the virus protected from eco-adversities and host defenses.\textsuperscript{286}

Peculiar association between Schistosomiasis and Salmonella infection is well known but exact mechanism how Schistosomiasis facilitate salmonella infection is not clear. Association of a nematode with bacteria is exemplified similarly between murine nematodes carrying enteropathogenic *E.coli* as a cause of bacterial gastroenteritis in man.\textsuperscript{286}

"Stuffy nose syndrome" is well known for preceding *Hemophilus influenza* infection followed by Staphylococcal infection of respiratory tract. Association of Chlamydosporium species and Penicillin species from tomato juice with *Clost.botulinum* resulting in a prospect of botulism through the modification of acidic pH is another example sited, of bacterial synergy with a facultative mode.\textsuperscript{286}

Association of *Toxoplasma gondii* and CMV, Malaria and viral hepatitis predisposing host to secondary bacterial infections by theory of nutritional support is also reported. Increased virulence of micro organisms by acquiring factors from other micro organism, is another mechanism of microbial synergy, where as acquiring R factors from commensal microbial flora of the bowel, exemplified by salmonella, obtaining drug resistance in bowel generating MDR Salmonellae causing outbreaks in close communities or prospective nosocomial infection is also on record.\textsuperscript{285}

Table 5.20 shows 372 strains of *C.albicans* isolated in mono microbial form, whereas in 40 strains of *C.albicans* were isolated with other Candida species viz. *C.tropicalis n=12, C.krusei n=12* and *C.parapsilosis,n=10* were the common from the samples of sputum and oral gargle then other clinical specimen.

Poly microbial infections are more frequently observed when the patients are immuno-supprresed. Our findings of co-existence of *C.albicans* with species other than *C.albicans* is suggestive of mixed candidal infection. This is a very

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significant observation in all the clinical groups. Interventional treatment against Candida would be clinically warranted and empirically clinician may prescribe anti-candidal drugs assuming that pathogen would be C. albicans. Co-existing non-albicans Candida species may make these cases refractory to treatment if they are resistant to conventional anti-candidal drugs. Similar observations will enlighten further this postulated concept of mixed candidal infection with a probable candidal synergy enhancing infection opportunity.

An attempt was made to analyze type of other Candida isolations. Out of 101 C. tropicalis strains isolated 81 (80.19%) isolates were isolated as pure isolates, whereas 20 (19.80%) were in combination with other Candida, C. albicans (n=12) C. parapsilosis (n=4), and C. guilliermondii (n=2) were the more common species associated. As in C. albicans sputum and oral gargoyle were the two clinical specimen yielding more frequent combined isolates, PTB, cancer and HIV were the clinical syndromes more common to yield poly microbial candidal isolation. (Table 5.21)

Out of 61 C. parapsilosis, 44 (72.13%) were pure isolates and 17 (27.86%) were combined isolations. C. albicans (n=10), C. tropicalis (n=4), and C. kefyr (n=2) were the other Candida commonly associated. Interestingly urine from patients of Cancer has shown combined isolations than other specimen and clinical group. Urinary isolations in combined form were also associated in PTB, HIV and diabetics, suggesting stronger association of C. parapsilosis with uro-epithelial colonization. It is well known that uro-pathogenic E. coli are so pathogenic due to their increased adherence due to D-mannose resistant surface pili, though still unknown it is likely that, similar virulence mechanism may be responsible for C. parapsilosis association with the urinary epithelium. (Table 5.22)

A total 43 C. krusei isolates were obtained of which as many as 19 (44.18%) were associated with other candidal species, C. albicans (n=12) and C. guilliermondii (n=4), being the common Candida isolation in combination. Association of C. krusei like other Candida with sputum and oral gargoyle in groups of PTB, cancer and HIV is consistent with other finding in our study. (Table 5.23)

C. kefyr were isolated in 33 instances of which only 8 (24.24%) shows combined isolation, with no particular Candida species associated with any
particular specimen or clinical group. But 11(33.33%) of the C.kefyr were isolated from patients of HIV infection and PTB is an interesting observation. (Table 5.24)

Out of 31 C.guilliermondii, 12 (38.70%) were isolated in combination with other Candida, C.kefyr being more commonly found. Isolation C.guilliermondii in HIV and PTB group is significantly high than other states of immunocompromisation. (Table 5.25)

Mixed yeast infections are on record, many workers have reported isolations of more than one variety of Candida from different clinical syndromes. The reported prevalence of multiple species of Candida is less than 10% and the combination of C.albicans with C.glabrata, C.krusei or C.tropicalis or often reported. Brooks et al (1985) reported isolation of C.albicans, C.tropicalis and C.glabrata in combination from patients undergoing transplantation.

Dyess et al (1985) reported mixed isolation of C.albicans, C.parapsilosis, C.tropicalis and C.rugosa from systemic candidiasis patient. In their study Staphylococci have been noted in association with Candida. Similar association was also reported by Burchard et al (1983).

Carlson et al (1982) experimentally demonstrated synergistic virulence between Staphylococcus aureus and Candida albicans. Whereas C.albicans along with T. glabrata were recovered from patients of endophthalmitis (Klein et al. 1979).

In the present study we report isolation of 681 Candida strains comprising of 412 (64.27%) C.albicans, 101(15.75%) C.tropicalis, 61 (9.51%) C.parapsilosis as the major potential pathogens in mono-microbial as well as poly-microbial forms. It will be interesting to know whether any significant microbial synergy existed among these syndromic patients yielding these Candida, especially since all the subjects had definitive evidence of certain degree of immunocompromisation.

In the present study isolation of 269 (39.50%) non-albicans Candida is a significant finding. Secondly, combined isolation of Candida species on 116 occasion is also a matter of great concern. These combined isolations nearly amounting to 17 % of total Candida isolations cannot be disregarded as chance
isolation. There is a strong possibility that co-existence of different species of Candida in immunocompromised patients may have etio-pathogenetic association. The present study reveals that Candida from immunocompromised patients deserve to be discriminated into different species. Therefore, a clinical sample inoculated on Candida supporting culture medium should be looked for any variability in colonial morphological appearances, with an aim to isolate and identify as many Candida species as possible, for they may predict clinical significance.


Typing of micro-organism having potential to cause infections especially in a hospital setting is essential for clinico-epidemiological correlation. Similarly, species of Candida being ubiquitous, since now are not uncommonly associated with various clinical syndromes among hospitalised patients and the isolation of Candida revealing in most of the laboratories Candida albicans or non-albicans level of identification in some laboratories even species level identification based on biological and biochemical characterisation having been adopted already, still discrimination of the strains isolated on different occasions is difficult.

Discrimination of clinical Candida isolates using different methods in addition to the well-documented bio-chemical identification is feasible and has been reported by different workers.34,167,179,180,185,190

Hasenclever and Mitchell (1961)167 have carried out typing of C. albicans isolates using serotyping procedure on the basis of agglutination patterns observed with specific antiserum and designated C. albicans types as serotype A isolates and serotype B isolates.

Hunter et al. (1987)293 have carried out typing of C. albicans on the basis of their morphology appearance and have reported different morphotypes found among the clinical isolates of C. albicans on the basis of linear streak inoculation and the pattern of growth identifiable as without fringe, or with fringe, on the basis of the character of the fringe as discontinuous fringe or continuous fringe. In addition to the streak surface topography of the culture.

McCreight and Warnock et al. (1985)184 have classified C. albicans on the basis of their biological ability to resist certain dyes and chemicals, when
they are grown in presence of these dyes and chemicals incorporated media, and have reported different resistotype represented into alphabetic abbrevations from A to G, when growth of the test strains indicated selective resistance of the strain to that specific chemical or dye.

Whereas, Odds and Abbott (1980)\textsuperscript{189} have used biotyping as method also of great utility in discriminating \textit{C.albicans} strain and have suggested (Odds & Abbott 1983)\textsuperscript{191} numerical codes using three group of parameters and based on the score in each group have proposed a three digit code for each isolate, thereby achieving \textit{C.albicans} strains discrimination.

Use of single method of typing even though provides classification of the strain, clumping of common types by either of the methods described above seems inevitable. Clumping of strains limits the purpose of typing. Even though the four different methods of typing of \textit{C.albicans} after their conventional identification using standard morphological and biochemical parameters provide reasonable scope for epidemiological correlation and has gained popularity among different workers. We feel combination of typing procedures shall provide better opportunity of strain discrimination and more meaningful clinico epidemiological correlation.

Use of molecular biological genotypic methods of typing of \textit{C.albicans} provide the best strain discrimination but, limitations of the facilities of highly sophisticated specialized laboratory equipment and technical expertise being available at selected places causes restricted use of these procedures in general.

Since the facilities of molecular genotyping techniques are not available at our setup, in the present study efforts have been made to generate maximum possible informative typing based on phenotypic methods such as serotyping, morphotyping, resistotyping and biotyping of \textit{C.albicans}.


Different immune responses are obtainable from Candida, which shows immuno- reactivity against corresponding antibody in form of agar gel diffusion, immunoeletrophoresis, cross immuno-electrophoresis and agglutination
reaction. Recently raising of monoclonal antibodies against Candida antigens, is done using immunoblot technique.\textsuperscript{166} Agglutination – adsorption experiments to divide \textit{Candida albicans} into serotype A and serotype B is more practicable method of differentiating clinical isolates of \textit{C.albicans}. Isolation of serotype A is more common and is associated with greater pathogenic potential related to higher colonisation rate. However, detection of serotype B though in low percentage still indicates that serotype B also may have pathogenicity potential.\textsuperscript{48,176}

Since Hasenclever and Mitchell in (1961)\textsuperscript{169} have demonstrated serotype A and B, and since Tsuchiya et.al (1974), \textsuperscript{168} have shown a possibility of 'serotype C' using cross immuno electrophoresis, serotyping of clinical isolates of \textit{C.albicans} has generated interest.

Epidemiological studies reported from Europe, Africa and North America, has revealed higher prevalence of serotype A than serotype B. Auger et.al (1979)\textsuperscript{170} and (1983)\textsuperscript{173} have shown that or expression of serotype A epitopes has resulted into higher reports of serotype A identification. But, Dupont et.al (1995)\textsuperscript{48} and Brawner et.al (1989)\textsuperscript{177} have shown higher percentage of serotype B occurrence among HIV patients who were syndromic and were treated by antifungal agents like 5FC, possibly the sensitivity of serotype A cells to 5FC has eliminated the serotype A cells thereby leaving behind only serotype B variants in higher number.

Chande et.al.(1994)\textsuperscript{258} has reported 4 times more occurrence of serotype A than serotype B among clinical \textit{C.albicans} isolated from patients of PTB.

Using modified serotyping method i.e. double diffusion in place of agglutination, Martin et.al. (1983)\textsuperscript{294} reported occurrence of majority of serotype \textit{A C.albicans} (71.5%) and minor number of serotype B (26.3%) in a study of oral Candida isolate form 10 year old children.

The serotyping has a slight drawback due to sharing of antigenic determinants between \textit{C.albicans} serotype A and \textit{C.tropicalis}, so also between \textit{C.albicans} serotype B and \textit{C.stellatoidea} warranting detailed scheme of species identification and confirmation before an attempt of serotyping.\textsuperscript{169}
Present study reveals more than 80% of the *C. albicans* belonging to serotype A and an average of less than 20% of serotype B among different clinical groups. (Table 5.27) Patients of burns and suspected HIV infection have revealed relatively higher occurrence of serotype B (50% and 48.83%) respectively (Table 5.28)

When source of these Candida was considered, it appears that samples of sputum, oral gargles reveal higher percentage of serotype A. (Table 5.29)

Our findings of higher prevalence of serotype A is in agreement with earlier reports. Our findings of higher prevalence serotype B among patients of HIV infection also corroborates with findings of other workers.

It appears that serotyping of *C. albicans* is a useful adjunct to other typing methods.


Phongpaichit et al. (1987) studied variation in morphology of *C. albicans* among the population of Candida developing on a suitable culture medium and demonstrated that *C. albicans* exist in yeast or mycelial form and the pattern of the colonial growth generally correspond to the morphological character of *C. albicans*. This phenomenon of outgrowth into mycelial form, from the previously existing yeast form, was named as "phenotypic switching" the number of yeast forms of Candida having a property to develop mycelial form was associated with the virulence character and was correlated with clinical condition.

The cells of *C. albicans* in a phase of mycelial forms grow on a culture medium as a continuous streak with fringe where as the cells of *C. albicans* switching between the two morphological forms i.e. yeast form and mycelial form, produced discontinuous fringe at the site of streak inoculation. This population of cells is considered more invading type and is associated with more virulence.

In addition to the continuous or discontinuous nature of the single streak line of the culture other characters such as texture, surface topography, width of fringe, helped in differentiating different strains of *C. albicans*. Table (5.30) The single streak line of the inoculum also shows the other culture characters known typical of *C. albicans*, such as smooth- white colonies, wrinkled surface or fringe borders.
Hunter et al. (1989)\textsuperscript{179} modified the code proposed by Phongpaichit et al. (1987)\textsuperscript{295} and in result could differentiate 446 strains of \textit{C. albicans} into 50 different morphotypes and reported discontinuous fringe types to be frequently associated with fatal deep infections.

Hunter et al. (1990)\textsuperscript{180} used morphotyping in combination with resistotyping for epidemiological typing of \textit{C. albicans} recovered from patients and staff of intensive care unit.

Borromeo et al. (1992)\textsuperscript{181} reported single morphotype in mouth of healthy non-smoker whereas multiple morphotypes in mouths of smokers.

Oliver et al. (1993)\textsuperscript{182} reported different morphotypes of \textit{C. albicans} recovered from patients of HIV infections, the morphotypes differed when isolated in early stage than from advanced stage of infection with ARC & AIDS.

Apparently morphotyping seems to be the only technique invitro which indicates virulence of \textit{C. albicans}.\textsuperscript{34}

In the present study, morphotyping on the pattern of coding suggested by Hunter et al (1989)\textsuperscript{179} has been carried out using features of fringe distribution, width of fringe, texture of fringe along with streak surface topography. (Table 5.31)

It was possible to define 18 different morphotypes from among 412 \textit{C. albicans} isolates, however, bulk of the isolates i.e. 388 could be grouped under nine common morphotypes, of which fringless variant "000/0" was the commonest found, among 254 strains, the second common morphotype is "324/0" showing discontinuous fringe in 51 strains followed by morphotype "323/0" in 37 strains (Table 5.31). Fringless smooth strain was similarly found to be common by Hunter et al. (1989)\textsuperscript{179} they considered this morphotype, to represent wild and more virulent type of \textit{Candida albicans}. Kakru et al. (1999)\textsuperscript{296} demonstrated highest proteinase activity among the smooth and fringless variant of \textit{C. albicans}, the isolates were more from the blood and body fluids.

The cells of \textit{C. albicans} showing ability to spontaneously and reversibly switch from yeast to mycelial form has been considered as more strong character associated with virulence and discontinuous fringe formation. In the
present study 117 strains showed discontinuous fringe and four morphotype codes. The bulk of isolates were from sputum, oral gargle and urine.

Table- 5.31 shows distribution of *C.albicans* according to the type of clinical specimen and the morphotype.

Table- 5.32 shows distribution of morphotypes according to the clinical groups as well as the clinical specimen yielding *C.albicans*.

Discontinuous fringe morphotype "324/0" was seen commonly in sputum of PTB and oral gargle of cancer patients. Whereas, morphotype "323/0" was more common among diabetics in oral gargle specimen. The same morphotype was also found in sputum and body fluid of suspected HIV patients and in the wounds of the burn patients.

It is interesting to observe that sputum of PTB shows variety of morphotypes. Similarly oral gargle of cancer patients, urine of burn patients also showed variety of morphotypes.

The technique of morphotyping has revealed reasonable discrimination to classify the number of *C.albicans* obtained from different clinical groups and clinical specimen.

The *C.albicans* isolation obtained on repeated culture methods revealed in certain situation the same morphotype emphasizing the epidemiological utility of typing the strain. This was considered of significant importance in patients of HIV, PTB, cancer where the problem of opportunistic and super infections is of great clinical value.

Freshly isolated strains are typeable morphologically into different types with an advantage of discrimination. Repeated sub-culturing and storage, for a long period resulted into loss of ability to produce same morphological feature.


Similar to the methods based on resistance to organic or inorganic compounds used to delineate bacteria, Warnnock et al. (1979)\(^{183}\) have suggested resistogram method for differentiation of *C.albicans*. The number of chemicals or dyes used has been variable, some have used five chemicals, and some have used six, whereas some have used even twelve chemical inhibitors.
Generally, if a strain has shown ability to resist particular chemical incorporated in the medium, the resistance is evidenced in the form of visible growth, whereas if the strain is sensitive there is inhibition of growth.

McCreight et al. (1985)\textsuperscript{297} suggested to use alphabetical code to designate resisting ability of the strain thereby denoting a pattern of resistance and sensitivity for each of the isolate of \textit{C. albicans}. He has compared the resistogram of \textit{C. albicans} isolates obtained from different clinical wards against isolates from OPD subjects.

Hunter et al. (1989)\textsuperscript{179} used resistogram studies against 12 chemical inhibitors and classified these strains showing positive or negative or equivocal reactions.

Hunter et al. (1990)\textsuperscript{180} used resistotyping in combination with other typing procedures such as morphotyping and correlated the different strains isolated, source of the isolate and the frequency of the isolations. Applications of numerical index of discriminating power comparing four typing methods based on physicochemical typing were reported by Hunter and Fraser et.al (1989).\textsuperscript{179}

It is apparent from the literature that, use of resistotyping for generating different resistograms can provide scope for discrimination and clinical correlation. But the method of typing is laborious and requires good technical skill.

In the present study six chemicals and dyes designated with alphabetical codes from A to G were used as inhibitors of \textit{C. albicans} and the resistogram patterns were noted (Table 5.33). All the clinical isolates were classifiable into eleven resistogram patterns of which certain patterns were more common than the others. It was possible to distribute 412 \textit{C. albicans} strains into eleven resistogram types with minimum of 13 strains representing pattern of A--D--G as the lowest number obtained, and to A--C D--F G as the highest pattern obtained in 79 strains. But it was interesting to note that each pattern had fairly well distribution among 412 \textit{C. albicans} strains, so also the strains represented different clinical syndromes and different clinical sources, in well distributed manner. Gross clumping was not noticed, this may be viewed as an advantage over serotyping and morphotyping where clustering was inevitable in serotype
A, and morphotype 000/0, morphotype 324/0 and 323/0 showed fair amount of clustering than the other morphotypes. (Table 5.34)

6.12.d Biotyping

Odds and Abbott (1980) developed nine tests scheme to biotype isolates of *C. albicans*, the tests were based on growth and no growth end points. They suggested a three-digit code to the results of nine tests scheme grouped in 'threes', their method has potential to distinguish 512 strains of *C. albicans*.

Mouth and vaginal samples from 85 patients and healthy volunteers were studied for biotyping by this method by them, and 45 biotypes were distinguished of which eleven types were detected more than once. Whereas, single isolates of 32 different biotypes were also found by them. Interestingly, they found some biotypes from oral and vaginal sources in seven out of eight patients, the method was found to be useful for epidemiological investigations when source detection was considered important.

Odds and Abbott et.al.(1983) in the study of genital candidosis demonstrated same biotype from different anatomic sites such as mouth, rectum, or urethra among 25 women studied for candidosis. In11 women the biotype was same, supporting the contention that *C. albicans* was from an endogenous reservoir, whereas Odds et al (1983) tried to examine *C. albicans* strains from genitalia of symptomatic and asymtomatic patients by examining 50 males and 181 females but failed to draw any conclusion in comparing virulence among different biotypes, in their study of 282 isolates.

Odds and Abbott et al (1983) also tried three code biotyping combined with serotyping and 5-FC resistance of isolates from U.K. in comparison to isolates from five different geographic locations of U.S. The number of isolates studied were 247 from U.K. and 330 from U.S. These isolates yielded 160 biotypes with ten major clusters of related types more prevalent in both the populations. No specific association could be found between the biotypes in association to anatomic sites or infected versus colonized individuals.

Odds and Abbott (1983) in due course suggested modification of their original nine tests scheme by adding certain tests, by substituting certain tests
in an attempt to study biotyping of not only *C. albicans*, but also other medically important Candida species, retaining the three digit pattern. They demonstrated feasible differentiation of species and strains, among *C. glabrata*, *C. guillermondii*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis* and *C. tropicalis* in addition to the main *Candida albicans*. They however, stressed the role of biotyping primarily for epidemiological correlation of presumptively identified Candida species since the reproducibility of the system was good. In their study they have shown the same yeast type even in mixture of Candida species or *C. albicans* were examined on frequent occasions, suggesting tendency on the part of the host, of carriage of phenotypically consistent types of Candida providing a scope for clinico-epidemiological correlation as and when desired.

In the present study also, all the 412 *C. albicans* isolates were subjected to three group, nine tests scheme on the lines of Odds and Abbott (1983)\(^1\) scheme with slight modifications. Proteinase production, 5-FC resistance, sorbose assimilation, glycine assimilation, tetrazolium salts resistance was not feasible, in our laboratory setup. Group I represented pH 1.4 tolerance, pH 1.55 tolerance and urea assimilation. Group II comprised of salt tolerance (NaCl), cetrimide resistance and sodium periodate resistance, whereas in-Group III boric acid resistance, citrate assimilation and saffranine resistance. All these tests are included in the 14 biochemical test parameters recommended by Odds and Abbott (1983)\(^1\), coding was done on the same line as suggested by Odds and Abbott (1980)\(^1\) as 1,2,4 for serial number 1,2,3 in each group, depending upon the results of the test, in form of growth or no-growth end points, three digit code was designated to each strain of *C. albicans* tested. (Table 5.35)

Twenty-four biotypes could be recognized, of which biotype \(356\) (n=52), \(322\) (n=51), \(355\) (n=43), \(336\) (n=29), \(545\) (n=28), \(252\) (n=27) and \(325\) (n=24) showed clustering, whereas single biotype was represented by three biotypes \(143\), \(156\), and \(345\). Certain biotypes were found in more than one clinical source for example biotypes \(322\) was seen in sputum, oral gargle, urine as well as in bodyfluids, similarly biotypes \(356\) and \(355\) was seen in sputum, oral gargle
and urine. Biotype 532 was found in sputum, urine and body fluids, and biotype 336 was found in sputum, oral gargle and stool.

412 C. albicans when biotyped showed 24 biotypes viz 356 (n=52), 322 (n=51), 355 (n=43), 336 (n=29), 545 (n=28), 252 (n=27) and 325 (n=24) of which seven biotypes could be classified into 257 isolates, where each biotype showed more than 20 isolates in each group, representing more than 9 to 19 strains, whereas, 13 isolates showed eight different biotypes. (Table 5.36)

Biotyping based on commercial 'carbon' assimilation pattern and enzyme profiles is feasible using API systems, but the kits are expensive and have to be imported. API system suggests combination of different kits to get better discrimination.34

Typing based on protein variability on gel electrophoresis and immunoblotting techniques permit strain delineation but the procedure is time consuming requiring special equipment.34

Typing by sensitivity to killer toxins provides scope for typing of C. albicans but panels of killer toxins required are not easily available. (Polonelli et.al.1983) 196

Strain delineation based on, genotypic methods namely electrophoretic, karyotyping, RFLP and PCR technology are modern methods of typing, and these are beyond the scope of moderate sized laboratory like ours. However, simultaneous typing of strains of C. albicans by multiple phenotypic typing methods viz. serotyping, morphotyping, resistotyping and biotyping could be practicable method of differentiating C. albicans strains.

6.13. Combination Phenotyping:

Table 5.37.A shows the frequency distribution of different combination phenotype patterns obtained from sputum of PTB cases. The 94 C. albicans strains isolated from sputum specimen on repeat occasions are typed into 31 different combination phenotypes. Similarly, Table 5.37.B shows combination phenotype of C. albicans isolated from urine of PTB cases according to their frequency of distribution. 13 C. albicans strains isolated from urine specimen are typed into 8 different combination phenotypes.

Table 5.38 shows the frequency distribution of different combination phenotype patterns of C. albicans isolated from urine of chronically hospitalised
and catheterized patients according to their frequency of distribution. Out of 28 \textit{C. albicans} strains isolated 12 combination phenotypes patterns were obtained.

Table 5.39A shows the frequency distribution of different combination phenotype patterns of \textit{C. albicans} obtained from oral gargle of diabetes mellitus cases. The 75 strains of \textit{C. albicans} can be separated into 18 combined phenotype patterns. Similarly, table 5.39 B shows combination phenotype of \textit{C. albicans} isolated from urine of these cases according to their frequency of distribution. The 28 strains of \textit{C. albicans} can be separated into 10 combined phenotype patterns.

Table 5.40 A show the frequency distribution of different combination phenotype patterns obtained from oral gargle and blood from patients of cancer. 21 combined phenotype pattern from among 73 \textit{C. albicans} strains were obtained. Similarly, table 5.40 B shows combination phenotype patterns (n=14) of \textit{C. albicans} strains (n=28) isolated from urine from patients of cancer according to their frequency of distribution.

Table 5.41A show the frequency distribution of different combination phenotype patterns (n=12) obtained from sputum from suspected HIV cases. Similarly, table 5.41 B shows combination phenotype patterns (n=14) of \textit{C. albicans} strains (n=16) isolated from urine, stool and body fluids from suspected HIV cases according to their frequency of distribution.

Table 5.42 shows the frequency distribution of different combination phenotype patterns (n=14) of \textit{C. albicans} strains (n=30) obtained from wound swabs and urine specimen collected from burn cases.


All the 412 \textit{C. albicans} strains obtained from different clinical groups have been subjected to typing procedures viz. serotyping, morphotyping, resistotyping and biotyping. Each typing method, provided scope for classifying \textit{C. albicans} into certain number of patterns, however, clustering was inevitable. Combination of typing methods has been effectively used in finger printing of microbial strains. In the laboratories, where, genetic methods involving molecular biological techniques like nucleic acid probes, RFLP, DNA finger printing, PCR are not feasible, combination typing method remains the only
logical choice of phenotypic typing procedures suitable for meaningful clinico-epidemiological correlation.

In the present study also an attempt has been made to type *C. albicans* strains from different clinical groups and present some inferences of the study by stating the frequency distribution of the similar strains found in clinical groups.

When 107 *C. albicans* obtained from pulmonary tuberculosis cases, were analyzed according to the clinical type of pulmonary tuberculosis 39 patterns of combined phenotype were found and are presented in tabular form, in Table5.37A &B.

Cavitatory form of PTB was found either as fresh cavitatory type or as chronic cavitatory type. Number of *C. albicans* strains obtained were more from chronic cavitatory type (n=64) than fresh cavitatory type (n=42).

In most of the cases isolation was obtained when clinical sample was repeated and repeat cultures were undertaken. It is an interesting observation that *C. albicans* strain obtained on repeat isolation has shown the same pattern more frequently for example from 3 patients of chronic cavitatory type of PTB same combined phenotype i.e. A/0000/0/A- D - - -/322 was obtained when sputum specimen was collected on 2 occasions indicating etiopathogenic relatedness of a particular *C. albicans* strain. Similar findings were also observed in 18 chronic cavitatory type of PTB cases.

It is remarkable observation in this study that, different patients have yielded a different pattern of combined phenotype for example in patients of chronic non cavitatory type of PTB 11 patterns were obtained from sputum specimen collected on one occasion, which provides a scope to exclude a clinical possibility of cross infection, this will have a significant clinical bearing that each clinical case will need an independent clinical consideration for management. It further suggests that, since Candida are opportunistic pathogens and are ubiquitously present, there may be inherent diversity among the strains isolated from even one particular clinical condition. In the present study, diverse combined phenotypes are seen in cavitatory form of PTB and similar different diverse types are found in non-cavitatory type of PTB cases.

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Discussion
No particular combined phenotype was found to be predominant in any particular clinical group i.e. cavitatory or non-cavitatory, fresh or chronic. (Table 5.43.A).

Ninety-four *C. albicans* strains obtained from sputum samples could be typed and correlated with the type of clinical tuberculosis. We found combination typing very useful in differentiating *C. albicans* strains.

When 13 *C. albicans* isolates obtained from urine samples of the PTB cases were analyzed, it was found that a serotype B was more prevalent than the serotype A and in 3 fresh cavitatory type of cases, when a single random urine collection was made, three *C. albicans* isolates were obtained and all 3 had the same phenotype B/324/0/A- -D--/356. Similarly, two fresh cases of non-cavitatory type also showed the same combination phenotype B/000/0/A-CD-FG/152, whereas, from two chronic cavitatory type of PTB cases, when urine was collected on 3 occasions, one case yielded the same combination phenotype on two earlier occasions, whereas, on third time urine showed a different phenotype. Second case, revealed a different phenotype on each time, urine was collected. These observations, are interesting and suggest that combination phenotyping provides scope for analysis and clinical inference (Table 5. 43.B).

In some cases of PTB, we found that the same combination phenotype in sputum as well as in urine. This also provides scope of correlation between source and site of colonisation.

Many workers 177, 261, 268, 269 have reported *C. albicans* from PTB cases, and the same has been reported in our study, isolation of other species of Candida, but typing of *C. albicans* or other species and correlation has not been reported.

In the present study isolation of *C. albicans* has been most frequent in PTB cases than other clinical groups and it has been possible to differentiate the cases into four clinical subgroups, two major subgroups cavitatory and non-cavitatory each group, divisible as fresh and chronic. *C. albicans* found in the sputum and urine could be typed by all four methods independently and could be correlated in combination, providing good scope for analysis of super infections among PTB cases. We suggest, that every PTB case, also be investigated for further isolation, especially Candida, more so *C. albicans*,

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elaborate phenotyping be attempted and if feasible, HIV testing should be
carried out to investigate whether any association exist between PTB, HIV and
fungal infection, and to consider a possibility whether *C. albicans* detection
emerges as a laboratory marker of HIV association of these PTB cases.

From the clinical group of **chronically hospitalized patients** needing
catheterisation only urine samples were available, 32 Candida isolates were
found of which only four were non-albicans Candida, whereas 28 strains of
*C. albicans* were found. The isolation was more frequent when catheterisation
lasted for two weeks or more. When 28 *c. albicans* were analysed 12 combined
phenotype patterns were obtained.

Same combination phenotypes were obtained from four cases beyond
fifteen days duration of catheterisation, when the urine sample was collected on
two consecutive occasions, similarly in three separate cases when
catheterisation was extended for 13 to 15 days, three consecutive urine
samples yielded the same combination phenotype and in one case the different
combination phenotype was found. In a same group of patients with duration of
catheterisation for more than 15 days, one case presented independent
*C. albicans* combination phenotype, **B/000/0/- CDE - G/545**. Similar finding
was also seen in one case with 10-12 days catheterisation. Table 5.44.

No one particular combination phenotype was seen more commonly than
the other.

Laxmi.et.al (1993) 261 and Chakravarti et.al. (1997) 279 also found higher
*C. albicans* isolation among chronically hospitalised patients, however, they have not
differentiated their *C. albicans* strains as has been done in the present study.

In patients of **diabetes mellitus**, 112 Candida species were found of
which 103 were *C. albicans* and 9 were non-albicans species form clinical
specimens of oral gargles. On the basis of duration of the diabetes disease, it
was possible to make four groups. Isolation of Candida was more commonly
observed in patients with longer duration of disease. All strains of *C. albicans*
were subjected for different typing methods and the combination phenotypes
were analysed. No particular combination phenotype was predominant,
however, serotype A and morphotype 000/0 was more common, but on the basis of resistotyping and biotyping, it was possible to discriminate them into different phenotypes.

In three situations, an interesting observation was that serotype, morphotype and resistotype is same, and it was possible to discriminate these phenotypes into independent biotypes by biotyping procedure, whereas in one instance, same morphotype, resistotype and biotype was found, oral gargle specimen revealed the same combination phenotype on two consecutive occasions, but on the third occasion the C. albicans showed different serotype i.e. serotype B. It has been well documented that, the strains of C. albicans display difference in vivo epitope expression, therefore, yielding different serotype on different occasions.34,35

Combination phenotyping of C. albicans, strains obtained from oral gargles of the patients of diabetes mellitus therefore has revealed interesting observations, from 6 patients with duration of illness for 11 to 15 years same combined phenotype A/000/0A –C D E F - / 356 and A/000/0A –C D E F - / 256 was obtained when oral gargle specimen was collected on 2 occasion from each patient, highlighting the clinical correlative importance of C. albicans type, the effort has provided distinct scope to differentiate similar looking C. albicans strains, only when all the four typing methods were considered together. This therefore, provides definite scope of using this procedure for any epidemiological study whenever the situation demands. (Table 5.45.A.)

When urine specimen from patients of diabetes mellitus were analysed, again, some interesting observations were made, same combination phenotype A /000/0/ABC-- - /545 were found in four patients when the urine was twice cultured for Candida isolation. While in same group, in 3 patient same combined phenotype B/000/0/A- -D- - /532 was obtained when urine was collected on single occasion from each patient. In two cases with duration diabetes from 6-10 years the A/000/0/A-CD-FG/152 type was obtained. Table 5.45.B

Various workers have demonstrated isolation of Candida species in patients of uncontrolled diabetes and C. albicans has been reported as the
frequent Candida species found. Even though virulence of *C. albicans* is reported to be enhanced by blood/tissue glucose level, low lactate level, impaired T-cell response, low opportunistic index, indicating sedimentation adhesive potential of *C. albicans* through accumulated enzymes, ability to cause direct damage at tissue cell level is well established. It would be interesting to study among different patients yielding the same combination phenotypes, whether there is any variability in the virulence and whether it gets correlated with the clinical presentation of cases of diabetes mellitus for example *C. albicans* isolated from oral gargle from a case of uncontrolled diabetes mellitus, clinical variability may exist in form that in one case *C. albicans* may have caused oral thrush, whereas, in another, it may have got only colonized without any clinical evidence. In both these cases oral gargle will yield *C. albicans*. Similarly isolation of *C. albicans* from urine could be an innocent colonization or could be urinary tract infection, in either of the situations, combination phenotype could be same. The present study reveals, scope for such correlation in specific situations. We therefore suggest that, in every case of diabetes mellitus an attempt be made, to isolate *C. albicans* from oropharynx or urogenital site and study by combination phenotyping, whether more meaning could be attributed to *C. albicans* isolation.

In patients of cancer, 152 Candida strains were isolated of which, 101 were *C. albicans*, 72 strains of *C. albicans* were from oral gargle specimen, whereas, 28 strains were from urine specimen, only one *C. albicans* was isolated from blood culture. Out of 72 *C. albicans* from oral gargles as many as 52 were from single group of patients of oral cancer. When 101 *C. albicans* strains were analysed using combination phenotyping procedure 35 combined phenotype were obtained and certain interesting observations were found. From two oral cancer patients, when oral gargle was collected on three consecutive occasions, the same combination phenotype A/0000/A-CD-FG/355, but for the difference in biotype i.e. 252 instead of 355 was found. Similarly in patients of ovarian cancer, when the samples of oral gargle were collected on three occasions from two patients the same combination phenotype
A/000/0/AB - E - /355 was found. Isolation of same combination phenotype in the instances cited is less likely to be co-incidental, there seems to be a strong possibility, that *C. albicans* strain has got firmly established in these cases. Many workers have shown that *C. albicans* is a predominant Candida species, found in patients of cancer and oral cancer is a more frequent type of cancer seen. Table 5.46. A.

Another interesting observation was, in one case of oral cancer, when oral gargle was collected on two different occasions, different combination phenotype was found, when further analysed, both these combination phenotypes had the same pattern of serotype, morphotype and resistotype (A/000/0/A -CD-FG), but the difference was only in the biotype i.e. 323 and 355. Similarly from 3 patients of CML oral gargle collected on 2 occasions same combined phenotype A/00/0/A B C - - - G/322 was found. From one case of multiple myeloma a single strain of *C. albicans* was isolated which revealed distinct pattern i.e. A/323/0/A -D - -/315.

Similarly in two different cases of oral cancer, when oral gargle was collected on two different occasions, same combination phenotype A/000/0/A C D–F G was found, on further analysis it was found that, *C. albicans* found in them, had the same serotype, morphotype and resistotype but the variation was seen only in the biotype i.e. 355 and 252, here importance of biotyping in combination with other phenotypic typing procedure is high lighted.

Whereas, when a case of multiple myeloma was examined for presence of *C. albicans* by collecting oral gargle specimen on two occasions, on both of these occasions the *C. albicans* isolated showed the same serotype, resistotypes, and biotype, but the difference was seen in the morphotype of the strains, here the importance of morphotyping is highlighted.

When 28 *C. albicans* isolates obtained from urine specimen were examined, it was seen that five cases of multiple myeloma yielded the same combined phenotype on two different occasions, however, each of the combined phenotype was different than the other. Similarly, one case of CML and one case of oral cancer...
showed identical combination phenotype, each time urine was collected. Similar observations was made in patients of ovarian cancer.

Colonisation of C. albicans in patients of cancer at vulnerable mucosal sites due to effects of radiotherapy or chemotherapy causing devitalisation of healthy tissue were established fact. Detection of C. albicans with clinical evidence of candidal infection necessitates addition of antifungal agents in the management regime of such cases. Combination phenotyping of C. albicans provides scope for clinico-epidemiological correlation. The present study has shown various interesting aspects. In a modest laboratory setup, if isolation of C. albicans is supplemented with different typing procedures, it will provide, an interesting approach, since patients of cancer are hospitalized for a longer duration, planned prospective studies could provide excellent monitoring tool to the clinician, in the management of these disease.

Even though, many workers have demonstrated detection of Candida in patients of cancer, such data of well discriminated C. albicans is not reported.

From 83 patient suspected of HIV infection 100 strains of Candida were isolated of which 43 were C. albicans and 57 were non-albicans.

Presence of Candida in the oropharynx and upper GIT has been assumed as a clinical marker of degree of HIV infection. In the present study different clinical samples viz. sputum, oral gargle, urine, body fluids and stool have yielded Candida isolations. When 43 C. albicans were analysed 26 combined phenotype pattern were obtained

Sputum or oral gargle has revealed 27 isolates of C. albicans and 31 non-albicans, higher non-albicans Candida isolation was also seen in urine samples, but body fluids like ascetic fluid, plural fluid, CSF and specimen like stool have shown higher C. albicans isolation than non-albicans Candida species. When 43 C. albicans were analysed on the basis of combination phenotyping, according to different cases some interesting observations are noted, like in other clinical groups, isolation of same combination phenotype on multiple occasions from the same patient.

Discussion
Same combination phenotype A/323/0/-CDEF- was found in two different patients but for the difference in the biotype 532 and 322 in a sample from body fluid. In two different oral gargle/ sputum sample obtained on different occasions, the same combination phenotype A/A--D--/363 was found but for the difference in morphotype viz. 754/0, 732/0 and 733/0 only and in stool samples collected on two consecutive occasion from patients showing HIV antibody positive and oral candidiasis clinically detected same combination phenotype A/ABC---/336 was obtained but for the difference in morphotype 654/0, 722/0 and 734/0 whereas, in one case two stool samples showed same combination phenotype A/A-CD-FG/336 except for difference in morphotype 323/0 and 000/0. Similar findings of same combination phenotype except a different morphotype was also seen in one patient clinical suspicion of HIV infection without HIV antibody presence, in the same group one patient revealed the same combination phenotype B/000/0/A-CD-FG/552 from urine specimen obtained on two different occasions. Detection of C.albicans as well as non-albicans Candida in HIV infected group is clinically very important as opportunistic fungal infection are well known to occur among these patients. (Table 5.47.A,B)

The patients of burn included in the present study could be classified into different categories depending on the nature of burns viz. patients with thermal burns, electric burns, chemical burns and other miscellaneous burns.

Patients were also classified into third degree and second degree burns. The clinical material obtained was in the form of wound swabs and urine. The samples were obtained more frequently whenever possible. 30 C.albicans were obtained of which eight were from wound swabs and 22 from urine samples. C.albicans from wound swabs as well as urine were more common from patients of thermal burn injury. When the combination phenotype of C. albicans (n=30) were analysed 14 combined phenotype patterns were obtained. The wound swabs in one case of thermal burn injury showed distinctly different combination phenotype in the second sample.
Urine samples obtained from two different patients revealed the same combination phenotype \textit{A/000/0/A-CD-FG/154} on both occasions the urine was collected, suggesting the possibility of cross infection. Whereas, in one case, urine samples collected on two different occasions, revealed the same serotype, morphotype, and biotype but different resistotype.

One case of electrical burn revealed the same combination phenotype \textit{A/324/0/AB - - E- -/322} when urine was collected on two consecutive occasions. These are some interesting observations supporting importance of multiple typing methods, simultaneously adopted to achieve better discrimination of \textit{C.albicans} strains. (Table 5.48)

Different workers have isolated Candida strains from wound swabs of burn patients, \textit{C.albicans} being the dominant Candida species, than non-albicans species however, in present study we have isolated more Candida strains from urine samples than wound swabs. In our opinion, when patients of high percentage of burns are admitted, they are immobilized to their beds, this provides opportunistic colonisation of their urinary passage by Candida and due to certain degree of immunocompromisation, these patients are always at risk of acquiring Candida infection. Candida infections in these patients can lead to increased morbidity and complications like candidemia, multiple foci of abscess, following candidemia.

Nosocomial infections are well known among patients of burn, cross infection due to Candida could also be a source of worry, if Candida are colonized in these burn patients. Early detection of Candida among these patients with clinico-epidemiological correlation through combination typing procedures may help the clinician to restrict the presence of Candida among burn patients.

\textbf{6.15. Antifungal Sensitivity Testing}

Prevalence of fungal infections especially those caused by Candida have become more frequent. The studies on epidemiological correlation of opportunistic and nosocomial candidal infections, though few in number have revealed importance of non-albicans Candida species along with well established importance of infections due to \textit{C.albicans}. Since, some of such infections are life-threatening infections and occasionally are compounded as they exhibit decreased susceptibility
to antifungal drugs. When opportunistic fungal infections are envisaged in patients with immunocompromisation, prophylactic antifungal therapy is indicated, however, there are problems in the management of such patients either due to intrinsic resistance of the Candida envisaged or due to the problems of many known adverse side reactions of the drugs used, along with systemic toxicity of the antifungal agents, proposed to be used.

Antifungal drug sensitivity testing is comparatively difficult laboratory procedure when compared with invitro anti-microbial drug sensitivity testing against bacteria. Different methods such as agar dilution, broth dilution, disk diffusion are available. The results of anti-fungal drug susceptibility testing have recently been accredited and now NCCLS proposes certain methods for testing of yeast fungi.\textsuperscript{202}

Reports of studies of anti-fungal drug susceptibility testing performed invitro, supported by MIC in broth dilution method & agar diffusion method (E-test) are available. However, the methods described are highly technical requiring rich resources and therefore, are practiced in select reference laboratories.\textsuperscript{211}

But the problems of Candida infections is universal and a need for appropriate laboratory support for effective clinical management is essential at many health care centres. In view of observable resistance found, such susceptibility testing of isolates from clinical samples is recommended in all the mycological laboratories of the country.

The agar disk diffusion method can emerge as a practicable method of anti-fungal drug susceptibility testing, when properly monitored by using the standard recommendations, and known control standard strains.

In the present study 681 number of Candida strains comprising of 412 \textit{C.albicans}, 101 \textit{C.tropicalis}, 61 \textit{C.parapsilosis}, 43 \textit{C.krusei}, 33 \textit{C.kefyr}, and 31 \textit{C.guilliermondii}, were isolated, from different clinical groups, where immunocompromise and likely fungal infections was strongly suspected. From these isolates 200 strains comprising of 70 \textit{C.albicans}, 40 \textit{C.tropicalis}, 30 \textit{C.kefyr}, 20 strains each of \textit{C.parapsilosis}, \textit{C.krusei} and \textit{C.guilliermondii} represent the total strains subjected for anti-fungal susceptibility testing against

\begin{center}
\textbf{Discussion}
\end{center}
AmB (amphotericine, the polyene), miconazole (Mcz), and fluconazole (Fcz, the azoles), by using agar disc diffusion method. The test strains results were compared with control strains (Y/16) and reported as sensitive, intermediate, or resistant, based on diameters in mm, of zones of inhibition observed around 10 \( \mu g \) concentration discs of AmB, Mcz and Fcz.

Out of 200 strains tested 180 (90%) showed sensitivity to AmB, 173 (86.5%) each to Mcz and Fcz. Seventeen strains (8.5%) were intermediate to AmB and Fcz, whereas, 18 (9%) were intermediate to Mcz. Only three (1.5%) were resistant AmB, whereas, 10 (5%) and 9 (4.5%) were resistant to Mcz and Fcz respectively. Interestingly, none of the strains of \textit{C. albicans} revealed resistance to drugs used in the test, either they were sensitive or intermediate. From the table (5.49) it is distinctly clear that most of the clinical Candida isolates of the present study are susceptible to the commonly used anti-fungal agent and only a few non-albicans Candida are showing resistance, the resistance to azoles is more common than to polyenes.

It could have been interesting to follow the individual cases of non-albicans Candida strains, whether they receive anti-fungal drug prior to the specimen collection, or whether they had become refractory to certain anti-fungal drugs because of drug usage and development of resistance. Unfortunately such clinical follow-up was not feasible and shall remain the shortcoming in the present study. However, that non-albicans Candida strains isolated in the present study, have shown demonstrable resistance to azoles and polyenes, thus clinical groups from where these strains were isolated shall require close monitoring during therapeutic management. This finding is significantly important contribution of the present study. This finding shall generate much desired awareness among clinicians for referring the patients for routine fungal culture and anti-fungal drug susceptibility testing.

Chakrabarti et al (1995)\textsuperscript{206} also have reported 2-5% resistance to AmB in different species of Candida and in their study also 10-20% strains revealed intermediate sensitivity. Our results of the observed resistance of 4.5-5% and intermediate resistance from 8.5 to 9% are nearly comparable.
Polyene resistant Candida have been reported by Drutz and Lehrer (1978) among patients with very low host resistant, similarly polyene resistance among non-albicans Candida species especially *C.tropicalis* and *C.lusitniae* have also been reported by Dick et al (1980) and Odds et al (1988). Lynch et al (1994) also showed increased resistance to azoles among non-albicans species isolated from vaginal source, most of the patients were refractory to treatment, and azole exposure may attribute the development of resistance among *C.albicans*.

In laboratory, the occurrence of resistance to azoles has been attributed to the tendency of these agents to the trailing effect, making the end point result interpretation highly subjective.

6.16. Study of Healthy Controls:

Candida are known to survive and grow ubiquitously and are also found in carrier state of human as well as animal sources. There are also reports of isolations of different Candida species from inanimate sites. When found on different anatomical sites of human beings, the Candida do not necessarily bear any clinical relevance unless the predisposing factor generating infections are operative, or the host is having immunocompromisation due to some reasons.

In the present study the purpose of obtaining strains from healthy controls was (1) To determine whether any particular phenotypic factor such as personal habits of an individual, viz. tobacco chewing, tobacco and pan chewing, smoking, smoking and pan chewing predisposes the oropharyngeal mucous membrane for an increased density of colonization by Candida, or for any mechanical reason such as ill-fitting dentures, abrasive dental prosthesis, leading to trauma, favoring higher colonization in the healthy controls. (2) To compare the strain isolation pattern with the pattern of Candida isolation from clinical groups. Another important reason for studying the healthy controls was, (3) to find frequency distribution of different Candida species at carrier sites and further study the type of *C.albicans* isolated from healthy controls, viz. serotype, morphotype, resistotype and the biotype. (4) To compare the type of *C.albicans* from healthy controls with types of *C.albicans* found in the clinical
groups included in the present study. (5) To discover whether different types of *C. albicans* inhabit the immunocompromised host than the types found in healthy controls. Another important purpose of this study among the healthy controls was, (6) to find out whether carrier stage could promote a prospect of nosocomial infection in hospital wards.

There are methods described for obtaining Candida from carrier sites, from the sites of mucous membrane higher culture positivity is found using imprint culture method, lavage with buffered saline provides the next best culture results, and the least results are obtained with swab method. (Odds et.al 1988).

The reported common sites to yield the presence of Candida are mouth, skin and different anatomical sites, faeces, and urine.

In the present study 26 females and 24 males belonging to different age groups form the subjects for the carrier study. Table 5.50.1 and 5.50.2 show the distribution of healthy controls according to habits, age, and sex.

Oral gargle and mid-stream urine sample formed the nature of clinical sample obtained from 50 healthy controls.

Out of 50 specimen comprising of oral gargle and urine, 19 (38%) oral gargle and nine (18%) urine specimen revealed microscopic evidence of presence of blastospores. (Table 5.50.3).

The reports indicate fairly high number of colony forming units (c.f.u.) obtainable from carrier sites. In the present study any sample seldom has revealed c.f.u exceeding beyond 15 c.f.u indicating very low density of Candida in healthy controls. (Table 5.50.4)

In the present study 24 oral gargle and 13 urine samples yielded positive culture for growth of Candida. The 24 isolates from oral gargles comprises 13 *C. albicans*, three strains each of *C. tropicalis* and *C. parapsilosis*, two strains each of *C. krusei*, and *C. psuedotropicalis* and only one strain of *C. guilliermondii*. Urine showed isolation of Candida in 13 cases comprising of 7 *C. albicans* to each of *C. psuedotropicalis* and *C. guilliermondii*, and one each of *C. tropicalis* and *C. parapsilosis*. (Table 5.50.5)

Discussion
Out of 37 Candida isolates 20 strains were *C. albicans* which are comparable to the higher isolation reports of these species among the clinical patients, than the reports of non-albicans Candida species.

Four isolations each of *C. tropicalis* and *C. parapsilosis*, *C. krusei* and *C. psuedotropicalis* show that no particular non-albicans Candida is more prevalent among the healthy controls. Whereas, in clinical patients included in the present study, *C. tropicalis* (n=101 strains) was the main non-albicans Candida species found followed by *C. parapsilosis* (n= 61 strains) being the next predominant species.

Table-5.50.6 show distribution of *C. albicans* from healthy controls according to their personal habits, it is clear that there is no bearing of personal habits on the frequency of Candida carriage.

13 *C. albicans* obtained from oral gargle showed nine serotype A and four serotype B, whereas, seven of the 13 *C. albicans* strains belong to 000/0 morphotype, five to 342/0 and one belong to 254/0 morphotype. (Table 5.50.7)

High prevalence of Serotype A and morphotype 000/0 found among healthy controls is comparable to the higher prevalence of these types found in clinical groups, included in our study.

Biotyping of *C. albicans* from healthy controls revealed 355 as the common biotype, 322 and 363 as the next common biotype. Both these types are also found in the clinical groups included in our study (Table 5.50.8)

Table-5.50.9 shows that frequency distribution of resistotypes of *C. albicans* among the healthy controls. No particular resistotype was found to be common. Combined typing incorporating serotyping, morphotyping, resistotyping and biotyping of *C. albicans* from oral gargle and urine of healthy controls revealed the distribution of the types evenly. Table 5.50.10

The data of the healthy control study reveals that *C. albicans* is found at the carrier site, and shows comparable frequency of the isolation and the type, when compared with the *Candida albicans* type isolated from clinical groups in our study.
6.17. Animal Pathogenicity:

In the present study of 643 cases involving collection of 1701 number of clinical specimen 681 Candida isolates were obtained. 412 strains were identified as *C. albicans*, whereas, as many as 269 strains of non-albicans Candida species comprising of 101 *C. tropicalis*, 61 *C. parapsilosis*, 43 *C. krusei*, 33 *C. kefyr* and 31 *C. guilliermondii* were identified.

*C. albicans* has well established pathogenicity potential and is almost always associated with causation of disease, only rarely *C. albicans* has been found to be non-pathogenic since it could be differentiated into high, moderate and low virulence category on basis of their lethality observed in mice.\textsuperscript{174,228,243,248} Certain biotypes of *C. albicans* have been found to lack virulence determinants like surface adherence, ability to produce toxins and enzymes, so also, genetic attributes of Y-M dimorphic changing potential.\textsuperscript{88,174,173}

Non-albicans species of Candida though assumed to be either less pathogenic or non-pathogenic recently have been found to be associated with clinical situations. In fact, in patients who are immunocompromised they may have nearly same potential as *C. albicans* has.\textsuperscript{2}

Among the non-albicans species *C. tropicalis* is the most common pathogenic species. Whereas, *C. parapsilosis* is associated with low grade pathogenicity, other species which are rarely pathogenic are *C. krusei*, *C. kefyr* and *C. guilliermondii* etc.\textsuperscript{241,242,243,245}

Opportunistic pathogenicity of Candida species, depends mostly on determinants of virulence. Some of such determinants are adherence to host cells or tissue surface, ability to demonstrate dimorphism, ability to produce certain toxins causing erythema, pyrogenicity, intraluminal intestinal fluid flux, chemotaxis, endotoxins like substances and candidotoxin.\textsuperscript{89} Production of certain enzymes like coagulases, protienases, and phospholipases enhance their virulence potential.\textsuperscript{89}

Though Candida has been used to demonstrate pathogenicity potential in variety of animals, more commonly used animals are mouse, rabbit, rat and guinea pig. However, use of mouse is most popular among the experimental
studies of Candida strains. In mice intra-peritoneal inoculation commonly leads to disseminated infection, however, it requires ten times higher dose than the dose used by intravenous route of inoculation.69

Disseminated infection involving parenchymal organs like kidney, lungs and liver with sub-surface micro-abscesses showing focal collection of Candida in yeast pseudohyphae and hyphal forms, some times this foci are massively infiltrated with PMN and macrophages and when the virulence is slightly less granuloma formation can be demonstrated. These are the common histopathological changes seen after experimental animal inoculations.

Since, in the present study sizable number of non-albicans Candida are isolated after their biochemical identification certain prototype strains belonging to each species viz. C.tropicalis, C.parapsilosis, C.kefyr, C.krusei and C.guilliermondii were inoculated intra-peritoneally in mice. Three prototype strains of each species were inoculated intraperitoneally in different mice.

Infection at the site of inoculation was found in C.albicans and C.tropicalis, the size of the abscess was larger in C.albicans than C.tropicalis, whereas, white patches of micro-abscesses in parenchymal organs of peritoneal cavity were seen in two animals out of three in C.albicans, in one animal out of three in C.tropicalis. C.parapsilosis, C.krusei, C.kefyr and C.guilliermondii neither showed abscesses at the sight of inoculation nor showed white patches of micro-abscess in the peritoneal cavity. (Table 5.51.1)

Smear and culture examination was attempted from the inoculated animals from the site of inoculation peritoneal fluid and from the tissue homogenates of the visceral organs like lungs, liver, heart, kidney and brain.

Table 5.51.2 shows the results of smear and culture examination. C.albicans was demonstrated smear and culture at the site of inoculation in all the three animals, from the peritoneal fluid of 2/3 animals inoculated, whereas, lung and liver homogenate of one animal showed C.albicans from smear and culture.

C.tropicalis was demonstrated at inoculation site in one animal, in peritoneal fluid of 2/3 animals and lung homogenate of 1/3 animal.
C.parapsilosis, C.krusei, C.kefyr and C.guilliermondii could not be recovered from the inoculated animals.

Table 5.51.3 shows comparison of histopathological changes observed in C.albicans and C.tropicalis inoculated animals in different visceral organs. C.albicans shows disseminated lesions in lung, liver, kidney, heart, spleen, pancreas and meninges. Whereas, C.tropicalis showed lesions in lung and spleen.

The observations of the present study are consistent with the findings of other workers,\textsuperscript{241,242,243,245,246} that C.albicans and C.tropicalis are the two major species of Candida, which display pathogenicity potential, other species of Candida viz. C.parapsilosis, C.krusei, C.kefyr, and C.guilliermondii have no pathogenic potential. However, due to local constraints experimental study in animals could not be conducted more elaborately therefore the observations found that C.parapsilosis, C.krusei, C.kefyr, and C.guilliermondii are not showing the evidence of pathogenicity require more elaborate study.

The present study had limited purpose of proving, whether the Candida isolates obtained could show demonstrable animal pathogenecity.

The present study has been highly rewarding in aspect of Candida isolation from clinical group of patients with immunocompromisation, regarding the important aspect of species identification.

The present study has also revealed the important aspect of invitro germ tube studies which is a very useful laboratory procedure for determining C.albicans and C.tropicalis.

The scheme of biochemical identification based on carbohydrate assimilation has been very satisfying.

Very interesting aspect of the present study is different typing procedures adopted for classifying C. albicans strains, the success of use of combined-phenotyping procedures in discriminating C. albicans strains.

Reasonable degree of correlation was achieved. The present study has provided scope for analysis of common frequency of certain phenotype in different clinical groups.
Study of antifungal drug sensitivity of the Candida isolates also has provided useful information.

However, since the study has involved very elaborate laboratory procedures, has considered multiple facets of Candida infections involving different clinical groups of patients where immunocompromisation was likely, the study has become very vast and broad based.

We feel, more elaborate clinical follow-up in a specifically identified clinical group for a considerable duration involving series of sample collection over a period of time from the same patient with details of laboratory procedures adopted, revealing species identification and specific combination phenotype shall be more epidemiological relevant data and would be the future prospect of the study.

The present study has highlighted the importance of combination phenotyping to reduce the clustering effect of different typing procedures. In absence of laboratory facilities for more accurate genotypic typing procedures to reach maximally informative distinct phenotype seems to be the only logical alternative for a modest set-up.

The present study has clearly shown, the importance of setting up of Mycological laboratory, for investigation of clinical infections among the immunocompromised patients which are so in plenty especially because of feared HIV pandemic.