1. INTRODUCTION
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Over the past decades there has been a significant increase in the number of reports of mucosal and systemic infections caused by Candida species. This is mainly attributed to a dramatic rise in, the number of immunocompromised individuals, especially those infected with the human immuno deficiency virus (HIV), and patients receiving immunosuppressive therapy for malignancy and those undergoing transplantation. The wide spread use of broad spectrum antibiotics and the increased use of indwelling catheters and other mechanical devices also are the important contributory factors.¹

*Candida albicans* is the most pathogenic species among of the genus Candida and is consistently most frequently isolated causative agent of Candidal infection in humans. However, in recent years other species of candida have been isolated with increasing frequency from suspected cases of infection. These include *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei* and *Candida lusitaniae*. The other species, including *Candida guilliermondii*, *Candida kefyr*, *Candida famata*, *Candida haemulonii*, *Candida holmii*, *Candida incospicua*, *Candida norvegensis*, *Candida rugosa*, *Candida utilis*, *Candida vishwanathii* and *Candida zylanoides* have been isolated occasionally. The clinical significance of *Candida dubliniensis*, a recently described species of candida associated with recurrent oral candidosis in HIV-infected individuals is still under investigation.²

The improved methods of species detection and identification have significantly contributed to increased frequency of recovery of non- albicans Candida species. It is suggested that the inherent decreased susceptibility of many of these species particularly *C. krusei* and *C. glabrata* to commonly used antifungal agents such as fluconazole may be an important factor in their emergence as opportunistic pathogens.²

Irrespective of the reason for the increased frequency of recovery of candida species (either *C. albicans* or non-albicans species) from cases of infection, their clinical importance at the moment has warranted detail investigation in several aspects related to historical review, taxonomical changes, determinents of pathogenicity, hosts defence mechanism, factors
predisposing to infection, ecology and epidemiological patterns, various clinical
forms and nosocomial importance etc.

The modern mycology laboratory therefore has to perform the important
role in several aspects namely detection, isolation, identification, epidemiological analysis and antifungal susceptibility testing of Candida.

1.1. Historical Studies:

Candida infections have been recognized since antiquity as "thrush" or "moniliasis" and the first description of candidal infection were found in "Hippocrates' Epidemics" from the fourth century. Gruby noted the exact association between thrush and mycotic etiology in 1842 who classified the agent as a "Sporotrichum" species. In the following decades various pathological conditions such as vaginitis, onychomycosis, chronic mucocutanauos candidiasis, cystitis, endocarditis etc. were shown to be associated with the agent causing thrush.3,4,5

The nomenclature of yeast isolated from thrush patients has often undergone changes from "Oidium albicans" (Robin 1853) to "Syringospora robinii" (Quinquad 1868) to "Sacchromyces albicans" (Reiss 1875). Berkhout in 1923, after recognising the differences between Monilia species (isolated from rotting plants and fruits) and yeasts (isolated from patients) has established the genus "Candida".3,4,5

The two major events have revived the interest in fungal infection caused by species Candida. First, was the introduction of broad-spectrum antibiotics, which frequently predisposes patients to candidal infections by causing imbalance of commensal flora in favour of fungi. The second, was the increase in the number immunocompromised patients during last decade, as a result of increasing use of life saving modern therapeutic modalities or infection due to human immunodeficiency virus.3,4,5

Though, about more than 150 different Candida species representing twenty genera have been isolated from human sources, about eight species are reported as potential pathogens, and encountered in deep-seated infections in severely debilitated patients, these are Candida albicans, Candida kefyr, Candida parapsilosis, Candida tropicalis, Candida vishwanathi, Candida
The species rarely encountered are *Candida catenulata*, *Candida famata*, *Candida inconspicua*, *Candida intermedia* species, *Candida catenulata*, *Candida lusitaniae* *Candida norvengensis*, and *Candida zylanoides*. Among industrially important yeasts only *C. tropicalis* is a significant pathogen, other species such as *C. lipolytica* and *C. utilis* are unable to exert pathological effects even when given intravenously to corticosteroid treated animals.³

**1.2. Taxonomical Changes In Candida Species:**

Until recently the classification of fungi was based on the comparison of phenotypic properties viz. structures involved in sexual reproduction. This has caused major anomolies in fungal phylogenies. However, the application of improved technology based on molecular biological techniques, has led to the resolution of some of these problems.⁵,⁶,⁷

The taxonomy of genus Candida is confused with the absence of a known sexual phases of reproduction in many species, forcing taxonomists to classify individual species on the basis of physico chemical properties namely, presence or absence of xylose, septal pore complexes, and on carbohydrate assimilation profiles etc. However, application of molecular technique has enabled to establish precise relationship among many candida speices. For example using DNA finger printing analysis, the species *Candida langergonii*, *Candida clausenii* and *Candida stellatoidea* have been confirmed as synonyms of *C. albicans*. Similarly, the analysis of small ribosomal subunit sequences has shown that *C. albicans*, *C. tropicalis*, *C. vishwanathi* form one group with more distant connection to *C. guilliermondii*, *C. kefyr* and *C. glabrata* these findings are in aggrement with co-enzyme Q analysis results.⁵,⁶,⁷

The ambiguities in Candida taxonomy will possibly persist with ongoing improvement in methods of identification, differentiation and classification of the candida, and may result into identification of new pathogenic species, an important example is the identification of *Candida dubliensis*. Recently this species has been isolated from patients of oral candidiasis in HIV-infected and AIDS. Earlier this species was identified as *C. albicans*, however, on the basis
of their ability to produce abundant chlamydospores (in contiguous pairs and clusters), and DNA finger printing results, these two species are separated.\textsuperscript{2,6,8}

Though range of molecular techniques namely partial DNA sequencing (mainly of SS and LS ribosomal genes, their spacers and other genes), PCR – ribotyping and mitochondrial (mt) DNA restriction analysis are introduced and have been proved to be powerful tools for establishing exact taxonomic position of the genera, the application of new techniques has resulted only moderate changes in taxonomy of medically important Candida species.\textsuperscript{6,8}

1.3. Morphogenesis:

In anticipation that hyphal form represents the pathogenic form of Candida and on account of frequent occurrence of hyphal forms of Candida in infected tissue sections, the phenomenon of morphogenesis i.e. change from yeast to mycelial form is extensively studied in the genus Candida.\textsuperscript{9}

Shepherd et al (1941), observed range of intermediate forms namely spherical budding yeast, short to long pseudohyphal form, true hyphae and chlamydospores; and reported the occurrence of phenomenon of pleomorphism in Candida species.\textsuperscript{10}

Sherwood and Gow et al (1941) observed helicle hyphae produced by Candida on cellophane surface and reported the existence of contact – sensing phenomenon i.e. "thigmotrophism" helping the \textit{C.albicans} in penetrating the host strata.\textsuperscript{11}

Several environmental factors such as temperature, pH, carbohydrate type and concentration etc. are reported to influence the development of either of the morphological form.\textsuperscript{12} However, genetic studies has revealed the existence of different morphology specific genes variously described as "extent of cell elongation gene (ECE)", "hyphae specific gene", the " pH responsive gene" etc.\textsuperscript{9,13}

1.4. Virulence Attributes Of Candida:

Candida species are opportunistic pathogens, causing invasive infections only in hosts with locally or systemically impaired antimicrobial defences. It is reported that both cellular and molecular factors determine variations in the inherent virulence properties of different Candida species. Molecular studies on defining the virulence factors of \textit{C.albicans} has revealed
 genomic variation in response to changing environment, and expression of finite number of "variant traits" essential for commensal existence of Candida.\textsuperscript{14,15,16} 

The environmental change stimulates expression of "virulence traits", transducing the signals to phase-specific genes affecting phenotypic switching leading to dimorphic transition (yeast to mycelium transition), these changes leads to evolution of a clonal population containing variants with increased adhesiveness, ability to produce extra cellular enzymes such as secretory aspartyl proteinases (SAP), phospholipases, and cells with altered surface antigenic molecules. These variants evade and / or modulate host immune response and cause invasive infections. Despite of intense research no single dominant "the virulence factor" of \textit{C. albicans} has been identified and therefore, it is reported that Candida possesses multiple virulence attributes each with low propensity for enhancing virulence.\textsuperscript{12} 

1.5. Host Defences: 

Members of genus Candida are opportunistic fungal pathogens, commonly found on skin and mucocutaneous areas. As a result of life long commensalism initiated at birth, the underlying immunity in the form of cell mediated immunity (CMI) is usually present in an immunocompetent host. CMI plays a major role in the defence against mucocutaneous candidiasis, as it is noted in AIDS and chronic mucocutaneous candidiasis (CMC) patients. This immune response include involvement of CD\textsubscript{4}T lymphocytes, various cytokines, such as IFN-\(\gamma\), interleukins (IL), granulocyte macrophage-colony stimulating factor (GM-CSF) and the macrophage. Macrophages upon activation by IFN-\(\gamma\) produced by CD\textsubscript{4}+Th\textsubscript{1} subset releases oxygen radicals and the anticandidal effector molecules.\textsuperscript{17} 

In contrast, the immunity to deep seated or systemic candidiasis involves both cellular (CMI) and humoral elements. The polymorphonuclear cells are the ultimate effectors in eliminating candida causing systemic infections. The cells phagocytise candida and kill them via action of myeloperoxidase, various other enzymes and defensins.\textsuperscript{18} Recently, synthesis of an immunoregulatory cytokine by PMN has been documented.\textsuperscript{18,20}

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It is reported that several factors such as route of infection, the tissue infected, the regional immune response, genetic background of the host and the infecting strain influence development of immune response either in form of Th1 or Th2 form. \(^ {19,20,21}\)

1.6. Predisposing Factors:
Various risk factors local and systemic that predispose host to candidiasis can be categorised as. \(^ {22,23,24}\)

1.6.1. Natural Factors:
Which include (a) several microbial infections, (b) idiopathic, congenital or other debilitating diseases or disorders e.g. endocrine dysfunction, lymphocyte defects, phagocytic abnormalities etc. (c) digression from normal physiological status e.g. pregnancy, infancy, old age etc.

1.6.2. Dietary Factors:
Such as excess or deficiency of foodstuffs that may alter the composition of endogenous microbial flora e.g. carbohydrate rich diets and vitamin deficiencies.

1.6.3. Mechanical Factors:
Such as trauma in burns, accidental wounds, local occlusion or maceration of tissues resulting due to use of dentures, thumbsucking habits, wearing of occlusive clothing, etc.

1.6.4 Iatrogenic Factors:
Treatment with drugs viz. antibiotics, corticosteroids and other immuno suppressive agents, that alter the endogenous microbial flora or host defence against infections and also surgical procedures or use of prosthetic materials create favourable nidus for colonisation and multiplication of Candida at these areas. \(^ {19,20}\)

1.7. Ecology And Epidemiology:
Yeasts are distributed ubiquitously in terrestrial and aquatic habitat, and are commonly associated with plants and insects. But the yeasts causing human infections have restricted natural distribution. The spectrum and types of Candidal infections in animals resembles to the infection in humans with superficial, deep and disseminated infections. Although, the yeasts occur widely throughout the animal kingdom, the most important human candidal infections are due to endogenous Candida. \(^ {25}\)
Normally *C.albicans* and few other species are found in the mouth, oropharynx, skin, vagina, gastrointestinal tract and other sites, however, higher carriage is reported in patients receiving medical attention.\(^{25}\)

The intestinal carriage of Candida species in human is common than the carriage at other sites, colonisation of Candida species rises in circumstances indicative of pathological processes and these act as a endogenous source of infection.\(^{21}\) Oral spread of candidosis is an important route of infection in burn patients. Faecal candida form an important source of infection in vaginal candidosis and diaper rash. Extensive gastrointestinal colonisation and persorption of yeast is reported as a major source of disseminated candidiasis. Prolonged use of catheter also predisposes to systemic candidiasis. Candida species are frequently found in the hospital environment viz. in foods, on floors and other surfaces and act as a source of infection.\(^{26}\)

### 1.8. Clinical Candida Infections:

Candida virtually infects every tissue of the body and causes a variety of infections. As number of immunocompromised patients is increasing, infections previously not recognised or were rarely observed have emerged. Broadly clinical Candida infections can be categorised as (A) mucocutaneous infections and (B) invasive candidiasis. Invasive candidiasis is further grouped as candidemia, tissue-proven disseminated candidiasis and single organ candidiasis. Disseminated candidiasis is further classified into acute and chronic type as shown in table.\(^{26,27}\)

<table>
<thead>
<tr>
<th>Table 1.1 Showing clinical types of Candida infections:</th>
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<tbody>
<tr>
<td><strong>A. Mucocutaneous candidiasis:</strong></td>
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<tr>
<td><strong>B. Invasive candidiasis:</strong></td>
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<tr>
<td>I. Acute disseminated candidiasis:</td>
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<tr>
<td>Occur in granulocytopenic or agranulocytopenic patients.</td>
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<tr>
<td>II. Chronic disseminated candidiasis:</td>
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<tr>
<td>Occur in leukemic patients recovering from granulocytopenia.</td>
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1.9. Nosocomial Candidiasis:

The CDC’s National Nosocomial Infection Surveys (NNIS) found increase in the rate of nosocomial candidemia. The *C. albicans* remains the most frequent cause of fungemia and hematogenously disseminated candidiasis.

Though *C. albicans* is most commonly isolated species many recent reports have documented emergence of non- *albicans* species of Candida as nosocomial pathogens, viz. *C. tropicalis, C. glabrata, C. parapsilosis, C. krusei* and *C. lusitaniae*. Many of these infections arise from an endogenous source and the patient population, the various treatment regimens, and the antibiotics influence their frequency or other supportive care measures employed at specific institutions. Infections may be acquired by exogenous route via the hands of health care workers, contaminated infusates and biomaterials and the inanimate environment.²⁸

1.10. Role Of Clinical Mycology Laboratory In Diagnosis Of Candidiasis:

With availability of newer and advanced techniques in recent years, clinical mycology laboratory has experienced technical expansion than diminution. The number of useful roles it can play in clinical diagnosis and evaluation include, the isolation and characterization of species, testing in vitro susceptibility to antifungal agents, and the detection of strains involved in hospital outbreaks, detection of antigens and specific immune response to these antigens.²²⁹

The diagnosis of mucocutaneous candidiasis by direct smear examination and isolation of Candida from the clinical specimen is quite satisfactory. However, the diagnosis of invasive Candidiasis, an important cause of morbidity and mortality among severely immunocompromised patients is difficult. Clinically there are no specific signs indicative of invasive candidiasis and the diagnosis depends on combination of microbiological (microscopy and culture), histopathological and serological test results.²²⁹ The results of microbiological investigation depends on nature of clinical specimen being examined as well as on stage of infection. The positive results are only available in advanced stage of infection and the negative results do not rule out the diagnosis of invasive candidal infection.
Histological technique, though confirm the tissue invasion but require invasive procedures, which are sometimes not practicable in serious patients. The serological tests provide rapid means of establishing diagnosis of fungal infection, enabling prompt therapy to be initiated, and the tests can be used to monitor the disease course by serial detection of antigen and or antibody titer. Most of the serological tests which are currently used are based on detection of antibodies to Candida, but the test for detection of Candida antigen are also used with increased frequency now a days.

The antibody tests are widely used but these have their own demerits e.g. false positive results are often obtained in healthy individuals because of exposure to environmental fungi and the test are dependent on hosts immune response. However, ELISA and immunoblot assays offer better qualitative analysis of antigens and antibody specifically associated with invasive infection.\(^{30}\)

However, there have been developments over recent years in production of specific antigens from different growth phases of Candida and the antigens likely to be associated with infection process such as acidic carboxyl proteinase, aggressins and enolase.\(^{31}\)

Nevertheless, the intrinsic advantage of culture based diagnostic techniques is that, the isolate can be further subjected to antifungal susceptibility testing and epidemiological studies. On account of shortcomings of serological tests, these tests are used as adjunct to the culture techniques. However, in view of development of highly sensitive molecular technique such as PCR etc, rapid and reliable diagnosis of invasive candidal infections in very near future is anticipated.\(^{31,32,33}\)

1.11. Epidemiological Candida Typing:

With the increase in incidence of Candida infections interest in gaining a better view of the pathogenesis and epidemiology of *Candida albicans* infections has risen in the recent years. Number of methods are introduced to differentiate species further into strains and types. Broadly these methods are categorized as those based on phenotypic characters such as morphotyping, resistotyping, serotyping and killer typing those on genetic character such as
restriction fragment length polymorphism (RFLP), electrophoretic karyotyping etc. $^{34,35}$

Phenotypic typing methods have permitted differentiation of many classes but the reproducibility is poor because of difficulty in standardization of technique, unstability of karyotype and end point reading. The methods based on genetic markers typing has provided a better and reliable means of discrimination among Candida species, however, it is reported that neither of these tests alone provide an ideal answer to an epidemiological question because the strains with identical karyotype may not express their genes in the same way, the unstable karyotype may result into varying phenotypes. Thus the optimal approach for epidemiological studies with Candida demand use of combination of genotypic and phenotypic methods. In view of poor discriminatory ability of the existing typing methods and that the strains of C. albicans isolated from clinical material are highly homogenous, use of single method with high discrimination ability or use of more than one typing method either in parallel or in a hierarchical system is recommended to obtain better discrimination. $^{34,35,36}$

In recent times the incidence of Candida isolation and their possible association with clinical disease is frequently noticed in our hospital. In the present situation whether there is simultaneous increase of immunocompromising states among the hospital attending patient population due to diseases like severe degree of clinical tuberculosis, spectra of clinical suspected HIV infection associated clinical conditions, patients of diabetes mellitus, patients with severe burns and patients of cancer undergoing chemotherapy or not, more meaningful clinico-epidemiological co-relation of Candida infection is essential.

The present study therefore attempts isolation and speciation of medically important Candida species, epidemiological typing of the clinical Candida isolates based on morphological, biochemical and biological properties, and their susceptibility to antifungal agents. Proposed biotyping and serotyping of these clinical isolates along with relevant virulence studies will hopefully enrich a speculation of their probable role in a given clinical setting.