CHAPTER I
1.1 Introduction

"Candida albicans has an identity crisis; it thinks it’s a part of the human body"

-Carl Kummato.

In this world with such a large population, people are suffering from various diseases caused by different microorganisms. When a patient is infected with HIV, tuberculosis, cancer or when an organ transplant has been done, the immune system weakens or is suppressed. This leads to the opportunistic activity of the commensal population of microbes living inside our body. Candidiasis is one such opportunistic infection predominantly seen in tuberculosis infected patients (Rippon, 1988; Smith, et al., 1989; Musial, et al., 1998 and Loeffler, et al., 2000). Tuberculosis (TB) is a respiratory disease caused by a bacterium Mycobacterium tuberculosis. It remains the single largest infectious disease causing high mortality in humans, leading to 3 million deaths annually, about five deaths every minute. Approximately 8-10 million people are infected with this pathogen every year (WHO, 2009). Out of the total number of cases, 40 per cent of cases are accommodated in South East Asia alone. In India, there are about 500,000 deaths occurring annually due to tuberculosis, with the incidence and prevalence being 1.5 to 3.5 million per year, the proportion being higher for men (3.5%) than for women (2.0%) (WHO, 2009). With the spread of AIDS, tuberculosis continues to be ignored by large populations. The emergence of
drug-resistant organisms threatens to make this disease once again incurable. Tuberculosis continues to be a major health problem worldwide. In 2008, the World Health Organization (WHO) estimated that one-third of the global population was infected with TB bacteria (George Schiffman, 2009). The lungs are the major site of infection.

There are two forms of TB:

1) **TB infection**
2) **TB disease (active TB).**

Most affected people come under TB infected category. People with TB infection have no symptoms and cannot spread TB to others. People with TB disease have symptoms and can spread TB to others. People with TB infection can take medicine to keep them away from getting TB disease. People with TB disease can usually be cured with anti-TB drugs. Most persons infected with TB bacteria never develop TB disease. If TB disease does develop, it can occur 2 to 3 months after infection or years later. The chances of TB infection developing into TB disease lessen as time passes (Raja, 2004).

The symptoms of TB disease depend on wherein the body the TB bacteria are multiplying. Tuberculosis bacteria usually multiply in the lungs and cause a bad cough that lasts longer than 2 weeks, chest pain and coughing up blood or sputum (phlegm from deep inside the lungs). Other symptoms are: weakness or tiredness, weight loss, chillness, fever, and night sweats. (Long, et al., 2002).
1.2 Immunology of tuberculosis

The tubercle bacilli enter the body via the respiratory route. The bacilli spread from the site of initial infection in the lung through the lymphatics or blood to other parts of the body, the apex of the lung and the regional lymph node being favoured sites. The phagocytosis of *M. tuberculosis* by alveolar macrophages is the first event in the host-pathogen relationship that decides outcome of infection. Within 2 to 6 weeks of infection, cell-mediated immunity (CMI) develops, and there is an influx of lymphocytes and activated macrophages into the lesion, resulting in granuloma formation (Spector and Lykke, 1966). The exponential growth of the bacilli is checked and dead macrophages form a caseum. The bacilli are contained in the caseous centers of the granuloma. The bacilli may remain forever within the granuloma, get re-activated later or may get discharged into the airways after enormous increase in number, necrosis of bronchi and cavitation. Fibrosis represents the last-ditch defense mechanism of the host, where it occurs surrounding a central area of necrosis to wall off the infection when all other mechanisms failed (Jayasankar and Ramanathan, 1999).

Macrophages are the important cell types, which combat the pathogen. Various aspects of macrophage-*Mycobacterium* interactions and the role of macrophage in host response such as binding of *M. tuberculosis* to macrophages via surface receptors, phagosome - lysosome fusion, mycobacterial growth inhibition / killing through free radical based mechanisms such as reactive oxygen and nitrogen intermediates; cytokine-mediated mechanisms, recruitment of accessory immune cells for local inflammatory response and presentation of antigens to T cells for development of acquired immunity macrophage apoptosis in containing the growth
of the bacilli. This is also a mechanism to kill the bacilli (Goren, et al., 1976 and Selvaraj, et al., 1988). The role of other components of innate immune response such as natural resistance associated macrophage protein (Nramp), neutrophils and natural killer cells were involved to suppress the growth of the *M. tuberculosis*. (Fleischmann, et al., 1986; Bermudez and Young, 1991). The specific acquired immune response through CD4 T cells, are mainly responsible for protective Th1 cytokines and through CD8 cells bringing about cytotoxicity. The role of CD-1 restricted CD8+ T cells and non-MHC restricted γ/δ T cells are involved, although it is incompletely understood at the present time. (Raja, 2004). Suppression of immune response leads to reactivation of tuberculosis, whereas severe tuberculosis may itself cause immunosuppression (Menon, 1997).

### 1.3 Opportunistic fungal infection

The opportunistic fungi are potential pathogens in the immunocompromised patients, patients with some pre-existing disease and patients with a long history of antibiotics (Schell, 1995 and Khan and Chugh, 2000). The rate of opportunistic infections in tuberculosis patients is also very high. The reasons for increased prevalence are: lowering of immunity due to tuberculosis, the use of antituberculosis drugs of non-specific action which promote the growth and reproduction of fungus flora which in turn aggravate the course of underlying process in lung tissues (Sain, et al., 1991 and Solov’eva, et al., 1991).

Among the fungal pathogens, *Candida albicans* is common yeast isolated from tuberculosis patients and it is responsible for causing severe secondary infections in such patients (Pukhlik, et al., 1990). Besides a syntropic relationship between *Candida albicans* and *Mycobacterium tuberculosis*, it has also been reported in a number of studies where tubercle bacilli were found to enable *C.*

Moreover C. albicans also stimulated the growth of Mycobacterium tuberculosis of reduced viability (Mankiewicz, 1954). Another study confirmed the effect of polysaccharide fraction of C. albicans for enhancement of growth as well as reduction of the generation time of tubercle bacilli (Ghafoor, 1967). Candida was first isolated in 1844 from the tuberculosis patient (Mandel, et al., 1994). Candida is a component of the normal micro flora of the alimentary tract and the mucocutaneous membrane of a healthy host. However, slight alteration in the physiological state can turn normally harmless commensal yeast into aggressive pathogen causing mucosal, superficial or even life threatening systemic infection (Candidemia) in the immunocompromised host pointing to the pathogenic potential of Candida species (Newman and Holly, 2001). The role of C. albicans in causing severe secondary infection in tuberculosis patients has also been reported in a study, where, inspite of successful completion of antituberculosis chemotherapy, patients suffered from chronic cough, sputum or occasional hemoptysis (Kim, et al., 1988).

1.4 CLASSIFICATION OF CANDIDA ALBICANS

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<th>Kingdom</th>
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<tr>
<td>Phylum</td>
<td>Ascomycota</td>
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<tr>
<td>Sub phylum</td>
<td>Saccharomycotina</td>
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<tr>
<td>Class</td>
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<td>Family</td>
<td>Saccharomyceteaceae</td>
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<td>Order</td>
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<tr>
<td>Genus</td>
<td>Candida</td>
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<td>Species</td>
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(Guarro, et al., 1999)
1.5 Clinical manifestations of Candidiasis

Clinical manifestations of candidiasis are extremely varied, ranging from acute, sub acute, chronic to episodic involvement which may be localized to the mouth, throat, skin, scalp, vagina, fingers, toes, nails, bronchi, lungs or gastrointestinal tract. It may also be systemic as in septicemia (circulating in blood and causing damage to blood vessels and sometimes blood cells), endocarditis and meningitis. Pathologic processes evoked are diverse and vary from irritation and inflammation to chronic and acute suppuration or glaucomatous response.

1.6 Types of Candidiasis

<table>
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<th>TYPES OF CANDIDIASIS</th>
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<tr>
<td>INFECTIOUS CANDIDIASIS</td>
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<td>ALLERGIC CANDIDIASIS</td>
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<td>Candidiasis</td>
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<td>Eczema, Asthma, Gastritis</td>
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Mucocutaneous involvement
- Oral thrush
- Stomatitis
- Glossitis
- Cheilitis

Alimentary
- Esophagitis
- Gastritis

Genital
- Vulvovaginitis
- Balanitis
- Balanoposthitis

Chronic
- Mucocutaneous Candidiasis
- Ocular Candidiasis

Cutaneous involvement
- Intertriginous
- Paronychia & Oncomycosis
- Diaper disease

Systemic involvement
- Urinary Tract Candidiasis
- Pulmonary Candidiasis
- Endocarditis
- Meningitis
- Candidemia
- Arthritis
- Osteomyelitis
- Endophthalmitis
1.7 Oral *Candida* infection in TB patients

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an over growth of *Candida* species, the commonest being *C. albicans* (Akpan and Morgan, 2002).

Immunocompromised patients like those with tuberculosis are vulnerable to the opportunistic *Candida* infection leading to an increase in the mortality and morbidity rate of the patients (Ingrott, et al., 1987; Rippon, 1988 and Smith, et al., 1989). In the immunocompromised cases due to administration of prolonged antibiotic therapy the oral flora are disturbed and the salivary secretions are also impaired, promoting the growth of *C. albicans* (Odds, 1988; Epstein, 1990; and Peterson, 1992). The attachment of yeast cells in mucosal cell of buccal cavity is found to be 88% in immunocompromised patients, 30 % of *Candida* cells are attached even in the healthy host (Sandin, et al., 1982).

The oral hygiene involves cleaning the teeth and washing the oral cavity. Cleaning the teeth and washing the buccal cavity is important because individuals sleeps a minimum of six hours, during which their oral cavity favours and promotes the growth of *Candida* spp. both aerobically and anaerobically by utilizing the food remains in the dentures (Lehmann, 1998).

Due to immunodeficiency or suppression of immunity in tuberculosis cases, the opportunistic fungal infections are high (Ellner, 1982 and Nash and Douglos, 1988). It has been reported that patients with tuberculosis have several dysfunctions in macrophages, monocytes and T cells as well as chemotaxis that predispose them to opportunistic fungal infections. (Gronye, 1984 and Sehmite, 1993). Drugs that
reduce the salivary gland secretions can lead to an increased risk of oral candidiasis (Abu-Elteen and Abu-Alteen, 1998 and Perterson, 1992).

The last two decades have seen an increase of candidiasis, which is attributed to the widespread use of antibiotics and immunosuppressive drugs. *C. albicans* was reported as opportunistic human pathogen (Datta, 1992). Drugs such as broad spectrum antibiotics alter the local oral flora creating a suitable environment for *Candida* to proliferate (Epstein, 1984). The normal oral flora is restored, once the antibiotics are discontinued. Immunosuppressive drugs such as the antineoplastic agents have been shown in several studies to predispose the oral candidiasis by altering the oral flora, disrupting the mucosal surface and altering the character of the saliva (Bergman, 1991 and Francis and Wash, 1992).

Fungal infections are one of the major diseases of public health importance in India, but studies with regard to opportunistic fungal infections are a few. Keeping in view the role of *Candida* existence in tuberculosis patients, the study aims at the co-infection of *C. albicans* in tuberculosis patients in relevance to their gender and age and to evaluate the significance of the finding at the clinical level.
1.8 OBJECTIVES OF THE PRESENT STUDY

1. Isolation and identification of *Candida* species co-existing with hospitalized tuberculosis patients in India.

2. Analysing socio-biological factors associated with *Candida* infected patients.

3. Antibiotic sensitivity study on the *Candida* isolates.

4. Sequencing the drug resistant genes of Fluconazole resistant gene (MDR1) and Ergosterol synthesis gene (ERG2) taken from *Candida* spp that were isolated from tuberculosis patients. (This will help to develop new drugs for candidiasis).

5. Submission of sequenced genes to NCBI and BLAST analysis of sequenced gene.