CHAPTER 1 - INTRODUCTION

1.1 GENERAL INTRODUCTION

Candidiasis is an important opportunistic fungal infection of immunocompromised hosts with changing epidemiological trends. A relatively small number of Candida species are pathogenic for humans among the normal flora and it need some predisposing factors to act as a pathogen in man. According to the data provided by the Centre for Diseases Control and Prevention and the National Healthcare Safety Network, Candida species are ranked fifth among hospital acquired pathogens and fourth among blood stream infection pathogens, resulting in mortality rates as high as 50% (1).

The increased rate of immunosuppressive conditions during the last few decades, as a result of chemotherapy or disease like Acquired Immune Deficiency Syndrome (HIV/AIDS), diabetes mellitus, transplantations, frequent use of antibiotics, long stays in the intensive care units, pregnancy, have led to a parallel increase in the incidence of Candida albicans species and non- Candida albicans species causing mucosal and systemic infections (2, 3). Resistance to antifungal agents has also represented a major challenge for the treatment of candidiasis.

Studies from India also showed a trend towards an increasing prevalence of non-Candida albicans candidiasis, and its frequency is influenced by the long term treatment regimens, and other supportive care measures. The emergence of antifungal resistant non- Candida albicans and the irrational use of antibiotics are also complicated the antifungal therapy of candidiasis. So the change in epidemiology and antifungal resistance has created the need of its speciation and susceptibility testing in selecting the antifungal agent for the treatment of Candida infections (4, 5).

1.1.1 Candida as a nosocomial agent

An increase in nosocomial infection due to Candida over the last decade has been linked to iatrogenic factors like invasive medical procedures, use of more potent long term antimicrobial agents; increased life expectancy and duration of hospital stay (6).
This situation points towards the need of continuous surveillance for preventing the nosocomial infections due to *Candida species*.

### 1.1.2 Virulence factors of *Candida*

The virulence factors of *Candida* species includes more than 200 proteins that aids in host tissue penetration, nutrient acquisition, tissue destruction, formation of the extracellular matrix, cell separation, cell wall remodeling and biofilm formation. Proteases and phospholipases facilitate the adherence of the fungus to host cells, cause dysfunction or even rupture of host cell membranes. Biofilm may lead to life threatening conditions in patients with candidemia. Candidial biofilms are established in intravascular catheters and other implanted devices serve as reservoirs of infectious particles, which subsequently break off from the initial structure and causing metastatic infections (7).

### 1.1.3 Treatment and drug resistance

Appropriate antifungal therapy depends upon a good knowledge of the agents causing the infections and their antifungal spectrum. Antifungal susceptibility testing *in vitro* ensures that the drug that chosen will be active against the infecting organism and therefore provide beneficial therapeutic effect to the patient under treatment. The emergences of resistance to antifungal agents and broad-spectrum azole agents to which some *non-Candida albicans* species are inherently resistant have complicated the treatment for candidiasis (8). Thus, the knowledge of susceptibility profile of *Candida* isolates is important in deciding treatment option. The adverse toxic effect during long term therapy coupled with antifungal resistance of *Candida species* have lead to life threatening conditions in patients. So the need for new treatment strategies with minimal or no side effects is the need of the hour.

### 1.1.4 Role of Yeast killer toxin as antimycotic agent

Natural antifungal proteins are produced by a diverse group of organisms including bacteria, fungi, insects, vertebrates, invertebrates as well as plants. Yeast killer toxins are considered in this group and have been shown to have a broad spectrum of killing activity against various plant and human pathogens.
It has been reported that killer phenomenon is widely distributed and almost 100 yeast species belonging to more than 20 genera are killer toxin producers (9). Among the killer yeasts *Wickerhamomyces anomalus* has been found to have a potential killing effect on pathogenic and non pathogenic fungus.

The antifungal spectrum of purified killer toxins is giving a promise for the upcoming infection in humans. *Candida* infections have increased during the past few years in immunocompromised patients and the isolates have found to have gained antifungal resistance. Thus researches on yeast killer toxins focused on the use of these agents as potential antifungal is going on. The strong therapeutic activity exerted against different experimental mucosal and systemic mycoses by monoclonal and recombinant microbicidal killer toxin antibodies as well as by a synthetic killer peptide (KP- an antibody fragment engineered from the sequence of a recombinant killer toxin antibody) suggested new potential strategies for transdisease anti-infective prevention and therapy (9-11).

1.1.5. The present scenario

In clinical practice antifungal drugs are sometimes prescribed for the treatment of yeast infections without confirming the etiology by culture. There is lack of data on speciation and susceptibility of yeasts species to antifungal agents in present diagnosis settings. Moreover, continued surveillance of candidemia is important to document changes in its epidemiological features and its antifungal resistance pattern. The minimum inhibitory concentration is an important indicator of emerging resistance. So the need of a novel antifungal agent with less toxicity and wide spectrum of action from a natural source also pointing towards the use of killer toxins from yeasts. India studies on yeast killer toxin activity against pathogenic *Candida* species are scanty. Hence the present study was chosen to evaluate the anticandidial effect of yeast killer toxin produced by *Wickerhamomyces anomalus* from environmental isolates.
1.2. AIM OF THE STUDY

- To determine the *Candida* species responsible for infections from clinical samples, study their virulence characters and antifungal susceptibility including its susceptibility to killer toxin produced by environmental yeast *Wickherhamomyces anomalus*.

1.3. OBJECTIVES OF STUDY

- Isolate and identify *Candida* species from clinical specimens.

- Determine the production of virulence factors like biofilm, protease and phospholipases produced by the isolated *Candida* species.

- Study the antifungal susceptibility of *Candida* species by broth dilution method.

- Elucidate the mycotoxicological properties of purified *Wickerhamomyces anomalus* killer toxin against pathogenic *Candida* isolates.

- Perform experimental modulation of different parameters like temperature and pH to determine their effects on killer toxin production.
1.4. SOCIAL RELEVANCE OF THE STUDY

1. Candidial nosocomial infections with changing epidemiology due to HIV and increasing antifungal resistance can be tracked by routine culture and susceptibility testing.

2. The wide antimicrobial spectrum of activity of yeast killer toxin and yeast killer toxin anti-idiotypic antibodies suggest new potential strategies for transdisease anti-infective prevention and therapy.

3. The yeast killer phenomenon can be used in epidemiological studies and biotyping of opportunistic pathogenic yeasts.

4. Yeast killer toxins have a role in industrial processes like fermentation, food preservation.

5. Yeast killer toxin and anti-idiotypic antibodies can be used in recombinant DNA technology, as secretory vectors in genetics and vaccine preparation.