1.0 INTRODUCTION

*Candida* species form part of normal micro biota of human body. These organisms inhabit various parts of body such as skin and the mucosal surfaces viz the gastrointestinal tract and female genito-urinary tract specially vagina. Because of their commensal nature, they cause endogenous infections. There are 163 known species of *Candida* and only 20 of these are significant pathogens implicated in different infections (Chander, 1996). *Candida albicans* is a harmless commensal of humans because the colonization of these organisms does not induce inflammatory responses in the host (Grank et al., 2008, Kuleta et al., 2009). *Candida albicans* is the most studied among the *Candida* species, which causes candidaemia, vulvovaginitis affecting women of all age groups, oropharyngeal infections. The incidence of candidiasis is higher in human immuno deficiency virus (HIV) infected patients. Those individuals whose immunity is lowered or compromised due to prolonged steroid or antibiotic therapy, prolonged stay in the intensive care units, transplant recipients and HIV infected patients constitute high risk group (Enwuru et al., 2008, Odds et al., 2007, Bagtzoglou et al., 2009). *Candida* is yeast with expanded cellular morphology from pseudohyphae to true nonconstricted hyphae. The filamentous form of fungus consists of uninucleated compartments separated by septa. *Candida* is known as dimorphic fungus as it exhibits "dimorphic transition" from a budding yeast cell to a filamentous form. This dimorphic nature may help to invade epithelial cells (Gow, 1997). Although *Candida albicans* is the predominant species which has been implicated in various forms of candidiasis, however, of late, non *albicans* species like *C.glabrata*, *C.tropicalis* and *C.parapsilosis* are also emerging as pathogens in candidiasis. Different *Candida* species can be differentiated from each other on the basis of morphological features of their colonies on different media. *Candida* species are major fungal pathogens in the country (Rani et al., 2002). *Candida* species has been reported as the fourth most common cause of hospital acquired bloodstream infections in United States and Scotland (Chander, 1996). *Candida tropicalis* has been identified as the main organism in hospital settings in Taiwan (Yang et al., 2008).

Chakrabarti and co-workers (1992) studied the incidence of candidaemia and the relation of prolonged hospitalization in Indian hospital over a ten year period. On culturing blood of the patients, 50% strains of *Candida albicans*, 17% *C. guilliermondii*, 15% *C.tropicalis* and 8% *C. parapsilosis* were recovered.

*Candida* species possess certain virulence factors which contribute to pathogenesis of fungal infections which include; host recognition receptors, morphological dimorphism, secretion of
hydrolytic enzymes, biofilm formation, phenotypic switching due to alteration in antigen structure, colony morphology and tissue affinities. The process of phenotypic switching provides flexibility to cells and this may result in the adaptation of the organism to the host environment (Calderone and Fonzi 2001).

Several antifungal agents have been used in treating cases of Candidiasis. The prolonged use of these drugs result in the development of resistance to them which poses a great challenge in treating patients of Candidiasis. Such resistance has been reported against fluconaozle in Candida albicans and non albicans species (Uppuluri et al., 2008). Various studies have been carried out to elucidate the mechanism of drug resistance in Candida species. The fluconazole resistance in Candida albicans has been linked with increased expression of genes encoding multiple drug resistance, ATP binding cassette transporters and target enzymes in the ergosterol pathway and nucleotide substitutions at various position of ERG11 gene (White et al., 2002). The ERG 11 gene encodes for lanosterol 14α-demethylase which is a drug target enzyme. A transcription factor Upc2p has been associated with regulation of ERG11 gene expression in Candida albicans. Overexpression of this factor increases the resistance in Candida albicans leading to accumulation of lanosterol demethylase consequently resulting in emergence of resistance to azoles. The alterations if any in this factor, on the other hand, may result in hyper susceptibility to fluconazole. However, the exact mechanism for this is not well understood (Dunkel et al., 2008).

Low level resistance during early stage of treatment has been correlated to nondisjunction of the chromosomes due to frequent alterations in the chromosomes because of prolonged exposure to this drug (Perepnikhatka et al., 1999). The overexpression of MDR1 gene is a major cause of drug resistance to fluconazole in Candida albicans. This gene encodes a multidrug efflux pump (Dunkel et al., 2008). Due to the development of drug resistance in Candida species, alterations in the physiological and biochemical activities have been reported which may enhance virulence of the resistant strains. This increase in pathogenicity has been demonstrated in systemic mouse infection model (Angiolella et al., 2008). Several approaches have been followed to overcome resistance against fluconazole in Candida species, including multidrug chemotheraphy. Further, since the conventionally used antifungal agents such as amphotericin B have toxic effects on the patients, the search for novel compounds such as use of inhibitors of ergosterol biosynthetic pathway alone or in combination with fluconazole continues. Simvastatin is one of such inhibitor. This drug acts on HMG-CoA (3 hydroxy-3-methylglutaryl-C0A) reductase which is an essential enzyme in ergosterol
pathway of *Candida albicans*. Reduced levels of ergosterol in this organism have been correlated with decreased biofilm production (Liu *et al.*, 2009, Wikhe *et al.*, 2007). Recent studies have demonstrated that these inhibitors exhibit *in vitro* antifungal activity against yeasts and other pathogenic filamentous fungi. These compounds thus, have potential for effective antifungal activity, when used alone or in combination with other drugs. The synergistic combinations of these agents with other antifungals may establish a basis for a newer and safe applicable therapy (Galgoczy *et al.*, 2011). Statins are other category of therapeutic agents which also interfere with the enzymes involved in cholesterol biosynthetic pathway in human leading to lowering of cholesterol. As these compounds cannot be clinically used because of associated side effects and contraindications in humans subjects (Liu *et al.*, 2009, Wikhe *et al.*, 2007). Therefore, interest of medical personnel has focused on safely applicable and clinically introduced other drugs with significant antifungal activity to treat fungal infections.

Certain other categories of therapeutic agents such as antibiotics, antineoplastic and novel immunosuppressive agents used in cancer treatment may directly affect the growth and virulence of *Candida* species. These compounds also possess anti-candidal activity, and may act synergistically with antifungal drugs. One such compound, calcineurin affects morphology of *Candida* and other fungi and reduces azole tolerance. However, conflicting results have been reported in *in vivo* studies. Quinolone and some other agents may augment activity of azole and polyenes. The correlation of *in vitro* and *in vivo* effects might prove more important and valuable (Chen *et al.*, 2011).

The availability of new antifungal agents with novel mechanisms of action has enhanced the use of combination antifungal therapy. The high mortality rate and limited efficacy associated with currently used antifungal agents have stimulated the use of polyene, azoles, and echinocandin-based combinations in the treatment of Candidiasis. However, it may be assumed that the use of two or more drugs with different mechanisms of action might give better results in combination than when used alone. The effect of such combinational antifungal therapy needs to be evaluated and reviewed before their clinical application (Johnson *et al.*, 2004).

Keeping all the above mentioned facts in mind, the present study has been designed to study the phenotypic and virulence traits of different *Candida* species such as *C. albicans* *C. glabrata*, *C. parapsilosis*, *C. guillermondii* and *C. tropicalis* which were recovered from blood stream infections. Also, the susceptibility of these strains to fluconazole and inhibitors of ergosterol pathway alone and in combination with fluconazole to correlate the fluconazole resistance with variability among
ERG11 genes of different *Candida* species has been planned. The present study has, therefore, been planned with the following objectives.

**1.1 OBJECTIVES**

1. Phenotypic characterization and confirmation of clinical isolates of *Candida* species.
2. To determine *in vitro* susceptibility of the fungal strains to fluconazole.
3. To study virulence traits of the fluconazole resistant and selective sensitive strains of *Candida* species.
4. Evaluation of *in vitro* efficacy of ergosterol biosynthetic pathway inhibitors alone and in combination with fluconazole.
5. Amplification of ERG 11 gene of different *Candida* species and the comparative analysis of nucleotide sequences of their amplicons.