5.0 DISCUSSION

Candidal infections due to *Candida* species are gaining importance world over including India because of their progressively increasing incidence. The *Candida* species are normal inhabitants of skin and mucosal surfaces of human and animal hosts. But these organisms have been implicated in a number of clinical conditions such as oral candidiasis particularly in HIV infected patients, vulvovaginal infections, urinary tract infections, less commonly in endocarditis and osteomyelitis, nosocomial non-postneurosurgery spondylodiscitis and candidaemia-associated sepsis syndrome. *Candida* is the leading agent associated with blood stream infections. *Candida* has been emerged as 7th most common pathogen associated with the nosocomial infections (Chander, 1996).

A marked increase in the incidence of patients of candidaemia has been reported in India (Rani et al., 2002). The incidence of invasive mycosis associated with *Candida* species has increased rapidly in immuno-compromised patients (Torres et al., 2009). Novel species of non albicans *Candida* species are also emerging as blood stream infections (Oberoi et al., 2012). In India, infections due to *Candida albicans* are most commonly observed infections, however, other non albicans *Candida* species like *C. glabrata*, *C. parapsilosis* and *C. tropicalis* are also frequently being isolated now a days (Rani et al., 2002, Chakrabarti et al., 1996, Oberoi et al., 2012, Chakrabarti et al., 2008).

The management of candidal infections becomes difficult as it requires an accurate early diagnosis of the strains involved in these infections and selection of appropriate therapy keeping in view the rising trend of drug resistance among strains of *Candida* species (Playford et al., 2010). It is therefore, essential to use effective diagnostic tools which could rapidly identify the etiologic agent as novel species of *Candida* are emerging particularly in blood stream infections. Conventionally these agents can be identified and differentiated on the bases of colony morphology on different selective and differential media, other phenotypic traits such as production of chlamydosporic and pseudohyphae, germ tube production, biochemical tests and virulence factors such as biofilm production and production of hydrolytic enzymes, hemolysin and other factors.

In the present study we characterized 72 isolates of different *Candida* species recovered from blood stream infections from patients at PGIMER Chandigarh. We could clearly differentiate among various Candida species on the basis of morphological and microscopic features, biochemical tests and other factors as mentioned earlier. Our results were fully in concordance with those of NCCPF (PGIMER Chandigarh).
Several workers have used the parameters as mentioned for the characterization of Candida species recovered from different origins. Shaheen and Taha (2006) conducted the comparative study on *Candida* isolates from hospitalized and non hospitalized patients. These workers identified them on the basis of colony morphology on SDA, CHROM agar, pseudohyphae production on Corn meal agar and biochemical tests. The consistency in phenotypic traits was observed in different species of *Candida* by these workers. Resende and co-workers (2004) studied phenotypic traits of 242 yeasts isolated from 200 patients from different clinics and identified them as *C. albicans* (105), *C. tropicalis* (62), *C. parapsilosis*(28), *C. glabrata* (19), isolates of *C. krusei* (8) and *C. guilliermondii* (5) (Resende et al., 2004). The isolates exhibited the variation in the color of colonies produced on CHROM agar (Odds and Bernaerts 1994).

We have observed a shifting trend of occurrence of candidal infections towards non albicans *Candida* species which demonstrates the implication of this group in blood stream infections from patients at PGIMER Chandigarh. Although it is difficult to conclusively comment on the prevalence of different *Candida* species for want of adequate data in this regard, however, it does certainly pinpoint the prevalence of non albican *Candida* species in blood stream infections at this center which covers patients from different parts of northern India. Among non albicans group, we observed *C. tropicalis* as a prominent non albicans *Candida* species as reported by other workers in respect of other countries (Kothavade et al., 2010).

Although the work has been carried out on identified isolates but in our laboratory, we have utilized the experience to differentiate various fungal agents including yeasts and dermatophytes in superficial mycoses. We also plan to apply the expertise to directly identify from the blood stream infections in near future. The PCR based methods can act as adjunct to the phenotypic identification which we plan to develop so as to achieve quick diagnosis. Other methods such as sequencing of rRNA gene (18S covering ITS1 and ITS2) can be undertaken for identification of *Candida* species as has been done by others (Alhussaini et al., 2013).

The rising trend of drug resistance among strains of *Candida* species is another challenge for the clinicians besides early diagnosis. The management of candidal infections becomes difficult as it requires an accurate early diagnosis of the strains involved in these infections and selection of appropriate therapy keeping in view (Playford et al., 2010). We conducted *in vitro* susceptibility testing of 72 isolates in order to identify the strains resistant to fluconazole. The azoles, fluconazole in particular have been most commonly used antifungal agents in treating candidiasis. These compounds inhibit ergosterol pathway at 14-α-demethylation step leading to accumulation of methylated sterols, consequently resulting in disruption of cell membrane integrity (Song et
Use of fluconazole over a period of time has led to increasing frequency of resistance to this drug (Torres et al., 2009). We recorded resistance in 15/72 (25.86%) strains (Table-4.5), of which Candida albicans constituted only one third i.e. 5/15. The distribution of rest 10 resistant non albicans Candida species was as follows; Candida tropicalis (2), C. guillermontii (2), C.parapsilosis (5) and C. glabrata (1). The development of resistance among Candida albicans and non albicans Candida species reflects that such resistance might pose serious challenge in treating candidal infections. Several workers have demonstrated the development of resistance to this drug. Mohanty and co-workers (2007) have reported different prevalence rates among isolates of different origins and found 30% isolates from vulvovaginitis cases as sensitive dose dependent while 70% were susceptible. Enwuru and co-workers (2008) reported similar observations as small percentage of resistant strains (9.5%) among 74 isolates from oral specimens of HIV/AIDS patients. In their study, 78.4% strains were susceptible and 12.1% sensitive dose dependent (SDD).

The drug resistance may be attributed to various virulence factors of the Candida species. Studies are therefore, required on these aspects. There has been sequential increase in fluconazole resistance among Candida species (Ruhnke et al., 2000). In the present study, only 15 of 72 isolates tested (20.83%) were found resistant to fluconazole by both the disc diffusion and broth dilution methods. Only one sample proved additionally sensitive to fluconazole by disc diffusion method. In a similar study conducted in Spain by Flo´rez et al., 2009, C. albicans was found the most commonly isolated species (49.2%). In non albicans group C. parapsilosis (17.3%), C. tropicalis (15.2%), C. glabrata (13.7%) and C. krusei (3.6%) were implicated. A total of 8 isolates (4.1%) were found to be resistant to fluconazole and 7 (3.6%) resistant to itraconazole. However, all the isolates were found susceptible to other fungal agents. C. krusei and C. glabrata were implicated in over 18% cases of candidemia (Flórez et al., 2009). In another study conducted by Wang et al., 2004, low resistance (3.7%) against fluconazole was observed in 230 blood isolates of Candida as compared to isolates of other origins. The proportion of fluconazole resistance observed was high in non albicans species than Candida albicans.

In the present study, we examined the susceptibility of these isolates only to flucoanzole. However, we have examined the fluconazole resistant species for their susceptibility to ergosterol pathway inhibitors such as aminobisphosphonates alone and or in combination with fluconazole. These results are discussed under combination therapy below.

In a similar study antifungal agents like terbinafine and fenpropimorph which targets other enzymes of the ergosterol pathway also exhibited synergistic antifungal activity against wildtype
C. albicans. However, many other antifungal agents are also known to act as ergosterol biosynthetic pathway inhibitors (Bossche et al., 1987). The inhibitory action of these inhibitors can be increased by using these in combination (Onyewu et al., 2003). Such studies can be undertaken to know the antifungal efficacy of other drugs which affect the pathway at other level i.e. upstream or downstream of the 14-alpha-demethylation step. Also the combined effects in different combination of different antifungal agents can also be further studied in order to get an insight into the therapeutic potentials.

The drug resistant strains of Candida tend to be more pathogenic due to various biochemical and physiological changes that take place during the development of drug resistance. These changes may be with regard to alteration in the composition of cell wall polysaccharides, increased and extensive hypha formation, increase in adherence to cells and increased biofilm formation by the drug resistant species (Nailis et al., 2010, Angiolella et al., 2008).

Candida albicans is the prototype of Candida species and has been reported to have determinants of pathogenicity such as adherence to cells, pseudohyphae formation, phenotypic switching, protease production and alterations in the antigen make-up. Strains other than Candida albicans are reported to produce aspartylprotease enzymes, which play an important role in the pathogenesis of infections due to these strains ((Kuleta et al., 2009, Chakrabarti et al., 2008). Hyphae formation, surface recognition molecules, phenotypic switching and extracellular hydrophobic enzyme production have been considered to be some of the virulence traits for Candida species studied in details in recent years (Calderone and Bran 1991).

In the present study, most strains have been obtained from candidemia cases; we proceeded to investigate their virulence traits so that the same could be correlated to fluconazole resistance by comparative analysis of the sensitive and resistant strains. Of the 25 resistant and sensitive strains tested, we observed gelatinase activity in most (22/25) strains followed by phospholipase (16), hemolysin production (11). Biofilm production was seen in 17 isolates and production of protease on skimmed milk agar by 8 strains.

The protease activity was not observed in sensitive and sensitive dose dependent strains of C. albicans as well as C. tropicalis (Table-4.8). This activity was also not observed in 2 sensitive as well as resistant strains of C. glabrata, C. parapsilosis (4) C. tropicalis (2) and C. guillermondii (2). Gelatinase activity was found in 22 out of 25 isolates examined, those which did not exhibit this activity included; one strain of fluconazole sensitive C. albicans (MCG 10B) and one strain each of C. parapsilosis FR-10 and C. guilermondii (CG-12). These results suggest that the
fluconazole resistant strains may have maximum protease production activity owing to increase in their virulence. Hemolysin activity was also observed for fluconazole resistant and sensitive isolates of *Candida* species. This activity was found in 10 isolates; *C. albicans* (5), *C. tropicalis* (2) and *C. parapsilosis* (2) (Table-4.8). Our results suggest that fluconazole resistant species tend to have maximum phospholipase, protease and hemolysin production.

Biofilm formation is an important virulence trait of *Candida* species which plays a significant role in persistence of candidal infections. In the present study, biofilm formation was detected in 17/25 (68%) fluconazole resistant isolates with visual as well as spectrophotometric methods.

Negri and Faria (2010) assessed the virulence factor profile and *in vitro* antifungal susceptibility of 27 hospital isolates of *C. albicans* isolated from various sources; urinary tract infections, bloodstream infections and other sites of colonization such as hands of health professionals and central venous catheters. The isolates recovered from infections produced more hemolysin and germ tubes than those isolated from colonization sites. These workers did not find significant differences in the production of other virulence traits between isolates from the two sources. In their studies, maximum susceptibility of all the isolates was observed to Amphotericin and maximum resistance was observed against azoles. Their results suggested that the potential of *C. albicans* to produce hemolysins and germ tubes may be related with its virulence.

By visual detection method, a total of 5 isolates *Candida albicans* 1 GMC-6 *C. tropicalis* 3 FOD-8, CT-35, FOD-9 and *C. guillermondii* 1 B-1343/09 were strongly positive for biofilm production. In a similar study conducted by Hasana and co-workers 2009, 107 isolates of *Candida* recovered from 32 candidemia patients were assessed for biofilm production and compared with the biofilm forming ability of those isolates of *Candida* recovered from oropharyngeal lesions of 19 AIDS patients. They reported variation among various *Candida* species in respect of biofilm formation. *C. albicans*, *C. lusitaniae* and *C. krusei* produced more biofilm than the other species. High biofilm formation slows down the growth rate of the organism but not adherence. Studies on murine model suggest that the degree of biofilm formation was associated with increase in virulence (Hasana et al., 2009). Thus, the biofilm formation is a stable virulence attribute, although is strain specific that can vary among *C. albicans* and non-albicans strains and contributes to virulence. Our results also suggest that the fluconazole resistant isolates appear to be more potent biofilm producers.

During recent years, hyphae formation, phenotypic switching and extracellular hydrophobic enzyme production have been considered among the virulence traits of *Candida* species.
In the agar invasion test, we found 13/25 (52%) isolates positive; the majority belonged to *C. albicans* (28%). In the non albicans group, *C. tropicalis* and *C. parapsilosis* strains were positive for agar invasion. Cell morphology has been linked to agar invasion. The *rad54Δ/rad54Δ* mutants of *Candida albicans* were defective in invasion of Spider agar because of the altered cell morphology mediated by gene mutation. However, altered morphology had no effect on fluconazole susceptibility (Hoot *et al.*, 2011). In other words, the agar invasion is not necessarily linked to fluconazole resistance.

The hypha formation is a major virulence trait of *Candida* species. We selected 26 isolates for hyphae production randomly which included *C. albicans* (11) (5 Resistant, 2 SDD and 4 sensitive), *C. tropicalis* 4, *C. guillermondii* 2, *C. glabrata* 1, and *C. parapsilosis* 7. Hypha formation was maximum in fluconazole resistant *C. albicans* isolates (AGK-3, B-1599) followed by one strain of *C. guillermondii* (CG-12) and five *C. parapsilosis* strains. Among SDD strains two strains *C. tropicalis* (CT-44) and *C. albicans* (0/074/37) showed maximum hyphal growth. However some fluconazole sensitive strains belonging to *C. albicans* 2 (03/074/37, CA-29) and one strain of *C. parapsilosis* also showed maximum hyphal growth. Tsang and co-workers (2012) did qualitative examination of hyphal formation by *Candida albicans* using scanning electron microscopy (SEM) and correlated it with hypha-specific genes and hyphal regulators (Wai-Kei *et al.*, 2012). However, we did not conduct such studies.

In the adherence assay of the 21 fluconazole resistant and sensitive strains of *Candida* strains, we observed maximum adherence in five strains which were resistant to fluconazole *C. albicans* (3) and one each of *C. tropicalis* (1) and *C. parapsilosis*. However *C. albicans* (3/028/14) showed least adherence. Angiolella and co-workers (2008) finding which suggest that drug resistant strains produced more hyphae than those generated by the drug-susceptible parent strain after 24 hrs of incubation. These workers also tested the strains for agar invasion and observed that drug-resistant strains showed long branching filaments radiating into the agar on the surface after incubating the culture for 7 days. However, agar invasion by the drug-susceptible strain was completely absent. In our study, we also report that drug resistant strains have maximum hyphal production and adherence. Thus, due to development of multiple to antifungal agents, treatment of such fungal infections is becoming very difficult (Nailis *et al.*, 2010). The studies are required to be conducted to determine the susceptibility of isolates of different *Candida* species which are recovered from clinical cases from time to time which might further be correlated to phenotypic traits and virulence traits.
For treating cases of candidiasis, four classes of antifungal drugs, polyenes, azoles, allylamines and echinocandins are in use as described in section 2.8 under review of literature (Chen and Sorrell 2007). Due to prolonged use of a single drug, resistance to that drug may be developed, hampering the therapeutic outcome. One of the strategies to combat such infections an approach of combination antifungal therapy may be attractive and useful step for treating such complications. The interaction of several antifungal agents with other drugs has been tested previously several times. Nitrogenous bisphosphonates were shown to cause macrophage apoptosis by inhibiting enzymes in the biosynthetic pathway leading from mevalonate to cholesterol. Fisher and co-workers (1999) suggests that bisphosphonates affect geranylgeranyl diphosphate which is asubstrate for prenylation of most GTP binding proteins (Fisher et al., 1999). Bisphosphonates are very important antiresorptive drugs and used for the treatment of metabolic bone diseases. Nitrogen-containing bisphosphonates such as alendronate, ibandronate, and risedronate inhibit post-translational modification of proteins. These proteins include the GTP-binding protein Ras, with farnesyl or geranylgeranyl isoprenoid groups. It is suggested that bisphosphonates may also inhibit bone resorption by preventing protein prenylation. Also, the enzymes of the mevalonate pathway or prenyl protein transferases may act as molecular targets of the nitrogen-containing bisphosphonates (Luckman et al., 1998). Chemically, bisphosphonates are analogues of inorganic pyrophosphate (P Pi). The most important nitrogen-containing bisphosphonates including pamidronate, alendronate, risedronate, ibandronate and zoledronate inhibit essential enzymes of the mevalonate biosynthetic pathway by targeting enzyme targeting farnesyl pyrophosphate synthase (FPPS). The inhibition of FPPS enzyme prevents the biosynthesis of isoprenoid compounds. Bisphosphonates are currently used in the treatments of various diseases of excessive bone resorption (Russell, 2011). In the present study we tested two nitrogenous bisphosphonates (Alendronate sodium and Pamidronate disodium) for antifungal activity alone and in combination with fluconazole against Candida species with reduced susceptibilities to this drug.

In the present study, the combination of both fluconazole and pamidronate disodium (FLC/PAM) was synergistic only against four strains C. albicans (B-1599/09), C. tropicalis (SHD-34) and C. guillermondii (CG-12, CG-13). The MIC values for fluconazole ranged from 0.5 to ≥ 64 µg/ml, pamidronate disodium (PAM) alone were 4 to ≥ 64 µg/ml and fluconaozole and pamidronate disodium (FLC/PAM) were 2 to ≥ 64 µg/ml. The FICI values for FLC/PAM combination ranged from 0.00024 to 6. The same drug combination results were indifferent for 12 strains and antagonistic for five strains. The MIC values for Alendronate sodium (ALN) ranged from 16 to ≥ 64 µg/ml when tested alone and 1 to ≥ 64 µg/ml
for fluconazole and alendronate combination (FLC/ALN). The FCI I values for FLC/ALN ranged from 0.03128 to 8. The synergistic effect for FLC/ALN combination was observed only for two strains belonging to *C. guillermondii* (CG-12,CG-13). The drug interaction for FLC/ALN combination was indifferent for 15 antagonistic for four strains. The *in vitro* and *in vivo* interactions of most of drug combinations are not clear because of the limited clinical trials conducted in this regard (Ostrosky-Zeichner, 2008). The results of these drug combinations are not clearly conclusive, although some synergistic action has been observed in some cases further studies are therefore required before they can be used as effective therapeutic agents.

Bisphosphonates have also been tested for the treatment of several other diseases. Dhodapkar and co-workers (1998) demonstrated the antimyeloma activity of bisphosphonates. These workers treated two patients with progressive myeloma with pamidronate disodium every 2-4 weeks and found that pamidronate therapy led to significant reduction of marrow plasmacytosis and plasma cell labelling index (PCLI) including stabilization of immunoglobulin (Ig) levels (Dhodapkar et al., 1998).

Pamidronate disodium and alendronate were also found effective against some parasites; the *in vivo* activities of three bisphosphonates were determined by Yardley and co-workers (2002) against *Leishmania donovani* and *Toxoplasma gondii*. The findings of these workers suggest that alendronate was inactive against both parasites whereas, intravenous administration of pamidronate was effective against *L. donovani*. Thus, bisphosphonates may be used in treatment of parasitic infections. Studies are therefore required on the application of these agents in fungal infections.

The combination therapy has been proven very effective against fluconazole resistant *Candida* spp. Tsutomu and co-workers 2011 reported the synergistic activity of lactoferrin and fluconazole against *Candida* spp. These workers observed that this combination of drugs inhibit hyphal formation in fluconazole-resistant strains of *Candida albicans*. However, they found variation in the susceptibility of drug combination among the strains. Iron-chelating function of lactoferrin may be contributing to the synergistic effect of this agent. However, radiolabeled studies suggest that lactoferrin did not alter intracellular concentrations of fluconazole, thus, these synergistic effects may presumably be due to the alteration in the uptake of the drug by the fungal cells (Tsutomu et al., 2011).

The drug interaction of fluconazole with some other compounds has been studied by several other workers. Huang and co-workers (2008) investigated *in vitro* interaction of fluconazole and baicalein (BE) against 30 fluconazole-resistant *Candida albicans* strains isolated from clinical cases. These workers observed synergistic activities when tested in combination. However, the 2
drugs also tested singly and in combination using time-kill methods. They observed weak activity when fluconazole and BE tested singly. This synergetic effect of fluconazole and BE may overcome drug-resistance in this fungus and may prove useful in treating candidiasis (Huang et al., 2008). However, we did not performed time-kill method in our study.

The drug interaction of azoles other than fluconazole has been studied by Turan and coworkers 2011 and observed the efficacy of combination therapy of liposomal amphotericin B and voriconazole against the neonates with positive blood culture. These workers demonstrated that in addition to conventional antifungal treatment, the doses of voriconazole can be added in treatment of fungal sepsis in neonates. However, more clinical data are required before voriconazole is used as drug of choice (Turan et al., 2011).

The development of azole resistance among fungi may arise due to increased levels of the target product and upregulation of drug efflux controlling genes. These agents have a role in induction expression of ERG11 mRNA. In the present study, ERG 11 genes from six fluconazole sensitive C. tropicalis (F0D-9) and resistant C. albicans (AGK-3, GMC-6), C. glabrata (1/018/9) and C. parapsilosis (FR-5) strains were amplified. The predicted amino acid sequences of the Candida species were compared with that of fluconazole sensitive C.albicans (ATCC 90028) strain which served as standard strain for comparison. The major amino acid substitutions were seen at positions 150, 154, 157, 158, 161, 217, 235, 255, 265 and 277 which are as follows: L150I (C. parapsilosis FR-5), L150I (C. albicans AGK-3), I154V (C. tropicalis FOD-8), I154 C (C. parapsilosis FR-5), I154V (C. albicans AGK-3) and I154C (C. albicans GMC-6), I157F (C. parapsilosis FR-5), I157H (C. albicans GMC-6, I158I (C. parapsilosis FR-5), F161I (C. parapsilosis FR-5), F161E (C. albicans AGK-3), F161V (C. albicans GMC-6), L217T (C. albicans GMC-6), Y235W (C. albicans AGK-3), Y235I (C. albicans GMC-6), K255I (C. albicans AGK-3), K255R (C. albicans GMC-6), S265I (C. parapsilosis FR-5), S265G (C. albicans AGK-3), S265F (C. albicans GMC-6), L277I (C. parapsilosis FR-5), L277F (C. albicans AGK-3) and L277G (C. albicans GMC-6) (Fig 4.38 & 4.39). These substitutions might be linked to fluconazole resistance in these species. Several other workers have also linked susceptibility of strains of different Candida species to azoles. Wang and co-workers (2005) isolated three fluconazole susceptible and 10 resistant C. albicans strains from different sites such as the urethra, vagina, oropharynx, respiratory tract, and prostate secretion and blood samples. The comparative analysis of ERG11 gene sequences demonstrated mutations at 21 sites among 13 strains including 17 same-sense and 4 missense mutations. These workers suggest that fluconazole resistance of C. albicans may be associated with point mutations of V437I and N440K occurring in ERG11 gene. Xu and co-workers (2008),
amplified and sequenced ERG11 genes of 23 isolates of *C. albicans* (8 susceptible and 15 resistant) and 6 type strains (4 susceptible and 2 resistant) and detected eighteen silent mutations and 19 missense mutations. Some fluconazole resistant isolates were having G487T and T916C mutations in ERG11 gene.

Song *et al.*, (2004) cloned ERG11 promoter and induction of ERG11 gene expression was monitored by luciferase assays. Also, the effects of pH, carbon source and growth conditions on induction of the ERG11 promoter by azoles were assessed. Although, treatment with terbinafine and fenpropimorph also resulted in a delayed induction of ERG11 promoter, these agents targeted other enzymes in the ergosterol pathway.

Geber and co-workers (1995) suggested that cloning and sequencing of the structural genes ERG3 and ERG11 which encodes D5,6 sterol desaturase and the 14α-methyl sterol demethylase respectively might be useful to understand resistance mechanism of *Candida* species. The changes in the affinity of azoles to cytochrome P450 14α-demethylase, is due to the amino acid substitutions. Marichal and co-workers (1999), observed 12 different amino acid substitutions and 16 silent mutations in seven isolates. These workers further reported that mutations in the N-terminal regions were not important but only the change Y132H was found importance in azole resistance.

Besides ERG11 gene, ERG3 gene is responsible for *in vitro* resistance to azoles in *C. albicans* infections. Inactivation of this gene results in loss of filamentation along with attenuated virulence in disseminated candidiasis cases as demonstrated in animal models (Vale-Silva *et al.*, 2012). The reversion of the double base deletion in this gene proved that ERG3 inactivation is one of the mechanisms of azole resistance.

**5.1 Future directions**

The present study aimed at characterizing the known *Candida* isolates by conventional methods which shall be extended to clinical samples in order to see the involvement of various *Candida* species in different clinical conditions. The work in this direction has been initiated in our laboratory and the association of different *Candida* species in suspected cases of dermatophytosis and other filamentous fungi is currently under investigation. We plan to develop combination of conventional and molecular diagnostic methods which would help in rapid detection of *Candida* species with accuracy. An early diagnosis would facilitate the management of candidiasis. The work on the epidemiology of candidiasis shall also be extended further and this shall include larger collection of clinical samples particularly, blood stream infections from different geographical locations of the country. Keeping this in mind, a research project has been submitted to the Indian Council of Medical Research (ICMR), New Delhi for funding. This would give some
insight and clue about the resistance of the *Candida* strains to various antifungal agents. Also, more studies are required with regard to therapeutic use of new antifungal drugs or combinational antifungal therapy for better management of candidasis. Continuous monitoring of drug resistance *in vitro* and *in vivo* in experimental animals is required in order to understand the virulence and pathogenesis of resistant and sensitive strains. More studies are required in respect of ERG11 gene of different *Candida* species in order to correlate the mutations with the resistance to antifungal agents definitively. Also, other genes encoding other drug targets can also be included in the study. Further studies to elucidate the mechanisms of drug resistance such as efflux pump genes, and over expression of gene encoding drug target enzymes are also required in order to understand the pathogenesis of resistance with clarity.