

Original Research Article

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A study of Identification and Antifungal Susceptibility Pattern of Candida species Isolated from Various Clinical Specimens in a Tertiary care Teaching Hospital, Chinakakani, Guntur, Andhra Pradesh, South India

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ABSTRACT

The global incidence of Candidiasis has risen steadily over the past few decades. Candida species are now among the most common health care –associated pathogens. Most of the studies show that the predominant species *C. albicans* has been replaced by non albicans Candida with the use of antifungal agents. As various species differ in susceptibility pattern to the newer antifungal agents, antifungal susceptibility test should be performed before administration of antifungal agents. To isolate and identify different Candida species in various clinical specimens, to know the predominant species, to study the risk factors, to compare the advantage of rapid method such as use of Chrom agar over conventional methods for identification and confirmation of Candida species and to detect their antifungal susceptibility pattern. All the clinical samples collected from cases of candidiasis were processed initially using blood agar, MacConkey bile salt lactose agar and SDA with chloramphenicol. Cultures that are identified as candida species were processed using standard yeast identification protocol and chrom agar. Patient detailed clinical history including risk factors was noted. Antifungal susceptibility testing of the yeast isolates was assessed using agar disc diffusion method according to CLSI guidelines. The antifungal drugs used for disc diffusion method are - amphotericin B, Nystatin, fluconazole, Voriconazole, ketaconazole & clotrimazole. A total of 150 Candida isolates were subjected to speciation & antifungal susceptibility testing. Candida isolates were present in 94 (62.6%) males and 56 (37.3%) females. Prolonged use of antibiotics was the major risk factor 41 (27.3%) followed by Biomedical devices 32 (21.3%), Medical causes 21(14%), Diabetes mellitus 17 (11.3%), Trauma 14 (9.3%), Surgical causes 11 (7.3%), Pregnancy 7(4.7%), birth asphyxia 5 (3.3%) and HIV 2 cases (1.3%) respectively. A total of 90 (60%) isolates were obtained from urine, respiratory samples 26 (17.3%), Blood 16 (10.7%), High vaginal swabs 14 (9.3%), wound swabs 4 (2.7%) respectively. In our study Non albicans candida species are more in number over *C. albicans*. Among Non albicans Candida, *C.tropicalis* isolation is more, 86 (57.33%). The sensitivity and specificity of CHROM agar was found to be 100% for *C.albicans*. For *C.tropicalis* the sensitivity was 100% & specificity was 95.3%. All the isolates of Non albicans Candida were susceptible to Amphotericin B, Nystatin, Voriconazole, Clotrimazole, Itraconazole & Fluconazole. But some species of NAC like *C.guilliermondii* and *C.lipolytica* which showed 40% and 33.4% resistance. 22% of strains of *C.tropicalis* showed resistance against Fluconazole. *C.famata* showed complete resistance to fluconazole, clotrimazole, Itraconazole, whereas *C.dubliniensis* & *C.guilliermondii* showed 33.4% and 40% resistance to Fluconazole. *C.albicans* showed 100% susceptibility to Amphotericin B & nystatin followed by Ketoconazole (83.3%), Voriconazole (76.6%), Itraconazole (75.8%), Clotrimazole (71.6%) and Fluconazole (63.3%) respectively. Isolation of non – candida albicans species was more than *Candida albicans*. The predominant species isolated in our study was *C.tropicalis*. Chrom agar candida medium was found to be useful in identification of candida species. All candida isolates were sensitive to amphotericin & nystatin The antifungal resistance was more in the non albicans species than in *Candida albicans*. Hence there is a need for speciation, susceptibility testing & periodic surveillance of candida species.

Keywords

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Nonalbicans
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Introduction

The incidence of Nosocomial fungal infection has been increased due to advancement in medical management, and change in patient profile (Fridkin SK. *et al.*, 2006). The candida species are a part of normal flora of the alimentary tract and mucocutaneous membranes of humans. Candida species that are a part of normal flora can invade tissues and cause life threatening diseases in patients whose cell mediated immunity is decreased by disease or iatrogenic intervention (Odds FC.1988)

Due to variable clinical presentation of Candida infections, it becomes very important to identify these pathogens from all the clinical specimens. *C. krusei* and *C. glabrata* are known for their innate resistance to fluconazole (Chander J., 2009). The genus Candida includes several species implicated in human pathology such as *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. guilliermondii* and *C. dubliniensis* (Sullivan DJ. *et al.*, 1995)

The increase in the incidence of Candida species over the past two decades is significant and non-albicans Candida species continue to replace *Candida albicans* at most of the clinical sites like bloodstream infections (Chander J., 2009). Since 1995, Candida species have become the fourth most common cause of nosocomial bloodstream infection and are associated with a crude mortality rate of 39%, which is the highest mortality rate associated with any cause of nosocomial bloodstream infections. In intensive care units (ICUs), infection with Candida species is the third most frequent cause of nosocomial bloodstream infection and is associated with a crude mortality rate of 47% (Anurag Malani, *et al.*, 2005).

The incidence and associated mortality due to Candidaemia can be influenced by several factors including characteristics of the population at risk, standard of the healthcare facilities available, distribution of candida species and prevalence of resistance. Hence, epidemiological information available for one centre or geographic region may not be applicable to others (Hobson RP., 2003)

Candidiasis is a primary or secondary infection involving a member of the genus *Candida*. The clinical manifestations of disease are extremely varied, ranging from acute, sub acute and chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicaemia, endocarditis, and meningitis. *Candida*-associated denture stomatitis is a very common inflammatory process affecting about 60 % of the subject's carrier of a prosthesis. Oral carriage of *Candida* species was significantly higher in diabetic patients compared with healthy individual (Salerno C. *et al.*, 2011; Soysa C.*et al.*, 2006).

The frequency of vaginal candidiasis during pregnancy was found to be 38%, in which 27% were symptomatic and 11% were asymptomatic group. Increased ratio of infection was observed in multigravida and diabetic women (Parveen N.*et al.*, 2008).

Conditions that increase the rates of genital *Candida* colonization are known risk factors for candiduria including diabetes mellitus, antibiotic use, immunosuppressive therapy, prolonged hospitalization, extremes of age, and female sex (Uma Chaudhary. *et al.*, 2009). Recent studies indicate that *Candida* may be isolated from the hands of 15-54% of health care workers in the intensive care unit setting and that the strain of *Candida* carried on the hands may be

shared by infected patients (Pfaller, 1995).

Recently, the incidence of life-threatening fungal infections has been the rise, and rapid identification of pathogenic yeasts and detection of polyfungal infections has become mandatory. The most widely used medium for the isolation of *Candida* species and other fungi is Sabouraud's dextrose agar. As the identification of yeasts may take several days, employing chromogenic media can help to reduce the time of isolation and identification as well as the detection of mixed cultures by 48-72 hours.

Little or no similar work has been carried out in Medical Colleges more so in Andhra Pradesh. So, the present study has been taken up to isolate and identify the *Candida* species using conventional & rapid methods. Such identification gives us prognostic & therapeutic significance, allowing in the selection of appropriate antifungal agents by clinicians and to prevent the emergence of drug resistance.

Materials and Methods

A cross sectional study was carried out in the Department of Microbiology, NRI Medical College and General Hospital (NRIMC & GH), Chinakakani, Guntur (Dt) Andhra Pradesh. This is a tertiary care hospital and Super speciality centre. The study was undertaken for a period of 1 year from 1st January 2014 to 30th December 2014.

The specimens were collected under strict aseptic precautions from clinically suspected cases of candidiasis in MICU, SICU, NICU, PICU, CCU, KTU, wards and outpatient departments of NRI General Hospital. Detailed clinical history of all Patients was recorded.

Sample Size: A total of 150 *Candida*

species isolated from various clinical specimens were taken for the study.

Inclusion Criteria

All patients male and female of all age groups were taken into study and all the specimens showing *Candida* isolation in two consecutive samples were included in the study.

Specimens Collection

The specimens for the study were collected from the clinically suspected cases of candidiasis under strict aseptic precautions. The various clinical specimens collected were throat swabs, ear swabs, vaginal swabs, CSF, sputum, blood, pus, nail scrapings etc. Test tube containing two swabs or sterile bottles was used to collect each sample. The specimens were transported immediately to the laboratory. One of the swabs was used for direct examination and the other for the culture. The specimens were processed by using a set of preliminary tests like wet mount, Gram stain, and culture on SDA with Chloramphenicol and incubated at 25°C for 2 days to 3 weeks. Cultures were identified by the colony characters and by gram's stain. Once the colonies were confirmed speciation done by the following methods (Segal E. *et al.*, 2007; Rippon JW. 1988), (Milne, 2007).

- a. Germ Tube Test: (Reynolds Braude Phenomenon) (Milne LJR. 2007),(Forbes BA. *et al.*, 2007)
- b. CHROM (HICROME) Agar *Candida*.
- c. Corn meal agar inoculation (Dalmau plate culture)
- d. Sugar Fermentation Test (Arunaloke Chakrabarti . *et al.*,2008)
- e. Sugar Assimilation Test(Arunaloke Chakrabarti. *et al.*, 2008),(Larone DH., 2002)
- f. Urease test .

Antifungal susceptibility testing of the yeast isolates was assessed using the agar diffusion method¹⁶ according to CLSI guidelines. The discs were supplied by Hi-Media, Mumbai. Muller-Hinton agar supplemented with 2% glucose and 0.5µg/ml methylene blue was used for the sensitivity testing. The addition of the glucose speeds up growth of the yeasts and the addition of the methylene blue enhances edge-definition of zones. Disc diffusion method was used in the present study. The antifungal agents used for disc diffusion method are: Amphotericin B -100 units, Nystatin-100 units, Fluconazole – 10µg, Clotrimazole-10µg, Voriconazole -1 µ g, Ketaconazole-10 µg, Itraconazole- 10 µg. ; the diameters of the zones of inhibition obtained were compared with the standard zones interpretive breakpoints published by CLSI M44-A guidelines.(CLSI., 2009) The following quality control strains were obtained from PGIMER, Chandigarh. *C.albicans* -ATCC90028, *C. parapsilosis* - ATCC22019, *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258.

Statistical Analysis

Data analysis done by using SPSS software version 20. Descriptive statistics such as mean, standard deviation (SD), proportions and graphs were used to describe the data. Differences between proportions were analyzed using chi square test or by Fisher's exact test.

Ethical Clearance was obtained from institutional ethical committee.

Results and Discussion

During the study period, all the clinical specimens showing Candida isolation in two consecutive samples were included in the study, thus a total number of 150 Candida isolated from various clinical specimens

were subjected to Speciation and antifungal susceptibility testing. The prevalence of Candida species were studied in relation to age, sex, site of isolation, underlying risk factors, different species of Candida isolated and their antifungal susceptibility pattern. In our study, the highest incidence was seen in the age group above 60 years (29.3%), and the least incidence was seen in the 0 -10 years of age group (6%). Candida isolates were present in 94 (62.6%) males and 56 (37.3%) females. Male : Female ratio is 2 :1 (Table 1).

In the study, prolonged use of antibiotics was the major risk factor constituting 41(27.3%) followed by Biomedical devices 32(21.3%), Medical causes 21(14%), which include - pleural effusion, bronchopneumonia and tuberculosis. Diabetes mellitus 17(11.3%), Trauma 14(9.3%), Surgical causes 11(7.3%), Pregnancy 7(4.7%), Birth asphyxia 5(3.3%) and HIV 2(1.3%). In all these patients with above predisposing factors, the incidence of non albicans Candida 120 (80%) is more than *C.albicans* 30 (20%). It is statistically not significant (P > 0.005) (Table 2). Total of 90 (60%) isolates were obtained from urine (candiduria in patients with risk factors) followed by respiratory samples 26 (17.3%) (which include sputum - 17, Throat swab - 3, Tracheal aspirate – 4, Bronchial alveolar lavage – 20), Blood 16 (10.7%), High vaginal swabs (vulvovaginitis) 14 (9.3%) and wound swabs 4 (2.7%) respectively (Table 3).

In the present study nine species of Candida were characterised using standard conventional method. *Candida tropicalis* was the main species 86 (57.33%) followed by *C. albicans* 30 (20%). The following non-albicans species were isolated. *C.dubliniensis* 12 (8%), *C.krusei* 7 (4.7%), *C.guilliermondii* 5(3.3%), *C.kefyr* 4 (2.7%), *C.lipolytica* 3 (2%), *C.glabrata* 2(1.3%) and

C.famata 1(0.6%). Higher incidence of *C. tropicalis* was found in urine 63 (70%) followed by blood 10 (62.5%), respiratory samples 7 (26.9%), vaginal swab 5(35.7%) and wound swab 1 (25%). *C.albicans* were more frequently isolated from urine 17(18.8%) followed by respiratory samples 9(34.5%), and Vaginal swabs 4(28.5%), *C.dubliniensis* was isolated from respiratory samples 6(23%), urine 2(2.2%), blood 2(12.5%), vaginal swab 2(14.2%). *C.krusei* was found in urine 2(2.2%), respiratory samples 2(7.6%), vaginal swabs 2(14.2%) and 1(25%) from wound swab.

C.guilliermondii was isolated from urine 1(1.1%), blood samples 3(18.7%), and 1(25%) from wound swab. *C.kefyr* was frequently isolated from urine 2(2.2%), respiratory samples 1(3.8%), Blood 1(6.2%). *C.lipolytica* was isolated from urine 2 (2.2%), respiratory samples 1(3.8%). *C.glabrata* was found in urine 1(1.1%) and vaginal swab 1(7.1%). 1 (25%) of *C.famata* was isolated from wound swab (Table 4).

In our study, all the isolates of *C.albicans*, *C.dubliniensis*, *C.krusei*, and *C.guilliermondii* and *C.glabrata*, were easily identified by chrom agar. Four strains which were identified as *C. tropicalis* by chromogenic agar were identified as one mixed growth of *C. tropicalis* and *C. kefyr*, 2 strains were identified as *C.lipolytica* and one as *C.famata* by conventional method. Among the 5 isolates of *C. kefyr* on chromogenic agar one strain turned to be *C.lipolytica* by conventional method. However, conventional method identification was taken into final consideration (Table 5). The sensitivity and specificity of CHROM agar was 100% for *C. albicans*, *C.dubliniensis*, *C.krusei*, *C. guilliermondii*, *C. lipolytica*, *C.glabrata* and *C. famata*. For *C. tropicalis* the sensitivity was 100% and specificity was 95.31% and

for *C. kefyr* the sensitivity was 100% and specificity was 99.3% (Table 6).

In the present study, 98.7% of *Candida* species were susceptible to nystatin and only 1.3% were resistant. *C.albicans* showed 100% susceptibility to nystatin. Among NAC, *C.lipolytica* showed higher resistance (33.4%) to Nystatin than *C. tropicalis* (1.2%) respectively. The results of susceptibilities to Amphotericin B indicated that most of the tested isolates 97.3% were sensitive and only 2.7% of isolates were resistant. *C.albicans* showed 100% susceptibility to Amphotericin. Among NAC, *C.guilliermondii*, *C.lipolytica* and *C. tropicalis* showed 40%, 33.4% and 1.2% resistance to Amphotericin B respectively. In the present study, 90% of tested isolates showed susceptibility to Ketoconazole. *C.albicans* showed 83.3% susceptibility. Among NAC, *C.krusei*, *C.guilliermondii*, *C.kefyr*, *C.glabrata* and *C.famata* showed 100% susceptibility. *C. tropicalis*, *C.dubliniensis* and *C.lipolytica* showed 91.8%, 91.6% and 33.4% susceptibility respectively. Voriconazole was the next effective drug with 82% susceptibility followed by Clotrimazole 73.3%, Itraconazole 72.7%, and Fluconazole 69.3% respectively. The susceptibility of *C.albicans* to Clotrimazole & Itraconazole was 71.6% and 75.8% respectively.

C.albicans showed 76.6% and 63.3% susceptibility to voriconazole & Fluconazole. Among NAC, *C.krusei*, *C.guilliermondii*, *C.kefyr*, *C.glabrata* and *C.famata* showed 100% susceptibility to Voriconazole. *C.krusei* shows innate resistance to fluconazole. *C.kefyr*, *C.glabrata* showed 100% susceptibility to fluconazole. *C. guilliermondii* showed 60% susceptibility & only 40% of isolates were resistant. The susceptibility of *C. tropicalis* to Voriconazole and fluconazole was 81.3%

and 77.9% respectively. The susceptibility of *C.dubliniensis* and *C.lipolytica* to Voriconazole was 75% & 66.6% and for fluconazole was 66.6% & 33.4% respectively (Table 7).

Fungal infections due to *Candida* spp., especially those that are caused by non-*albicans* species, increased enormously (Colombo, 2003).

Major factors Causing Candidiasis include advances in medicine, expansion of human life span & immunodeficiency (Filloti.*et al.*,2007), (Cuetara, *et al.*,2006). *Candida* species colonise the mucosal surfaces of all humans soon after birth. In the immunocompetent host several conditions predispose fungal infections like prolonged antibacterial therapy, corticosteroid use, integumentary breach as in intravenous or intra arterial catheters, surgical procedures, poor nutritional status and metabolic derangements (Chander, 2009). The extensive use of antimycotic drugs for prolonged therapeutic courses led to change in the relative prevalence of various species of *Candida* (Rippon JW. 1988), (Merz WG. *et al.*, 2005).

The present study identified the specific species of *Candida* that causes infections among patients who attended NRI Medical College and General Hospital. The antifungal agents tested include Amphotericin B, Nystatin, Fluconazole, Clotrimazole, Voriconazole, Itraconazole, and Ketoconazole.

Candida albicans and non-*albicans* species are closely related but differ from each other with respect to epidemiology, virulence characters and antifungal susceptibility. All *Candida* species have been known to cause a similar spectrum of disease ranging from oral thrush to invasive disease, yet

differences in disease severity and susceptibility to different antifungal agents have been reported (Vazquez JA.*et al.*, 2002), (Fridkin MA. *et al.*, 1996).

Many studies in the past decade showed the isolation of various species of *Candida* and increase in isolation of non *albicans Candida* in these situations and similar results were obtained in the present study.

In our study, it was found that candidiasis can occur at all ages and in both sexes. The youngest in our study was one week old baby while the oldest was 83 years old man and the mean age was 44.5 years. The majority of the patients were in the age group of 21-60 years. Sahni *et al.*, (Sahni V *et al.*, 2005) in their study, the mean age was 43.4 years. In another study (Mirdha B R *et al.*, 1998) the age range for funguria was 1-75 years. The age and sex distribution of the patients in our study correlates with the observation of other researchers like (Sachin *et al.*, 2012, Verma *et al.*, 2003, Hajjeh *et al.*, 2004 and Ragini *et al.*, 2011).

Sex distribution in present study showed that males are more commonly effected than females with an incidence of 94 (62.6%) and 56 (37.3%) respectively in a ratio of 2:1 (M:F). In our study, females are more commonly affected in the age group of 21-30 years 15 (26.7%), this might be related to the fact that this is the child bearing age group when a greater hormonal variation occur. In a similar study conducted by Lata R patel, *et al.*, (2012), had a male preponderance with an overall male: female ratio being 2:1, observed that male sex is a risk factor for developing candidemia. Ragini *et al.*, 2011 also reported male preponderance, with an overall male: female ratio being 1: 0.81. Singh SC. *et al.*, 2004 PGIMER Chandigarh observed that male sex is a risk factor for developing

fungaemia. Yashawanth, *et al.*, 2013 and Shivaprakasha, *et al.*, 2007, also reported male preponderance.

Candidiasis occurs in the presence of risk factors (Table - 2) prolonged Medication for >7 days was the leading risk factors in this study. In this group 33 (27.5%) isolates were NAC. The antibiotics that were used were cephalosporins, carbapenems and gentamicin. Chakrabarthy, *et al.*, 2005, observed that patients with antibiotics >7 days, patients on immunosuppressants/steroids, patients receiving 3 or more antibiotics, had infections with *Candida* species. Administration of broad-spectrum antibiotics suppresses the endogenous micro flora, permitting fungal overgrowth and any impairment of mucosal immunity is a potential threat for dissemination of *Candida* (Chakrabarthy, *et al.*, 1999).

Biomedical devices was the second commonest risk factor noted 27 (22.5%) with NAC. Devices such as stents, shunts, prosthesis, implants, endotracheal tubes, pacemakers, and various types of catheters, to name a few, have all been shown to support colonisation and biofilm formation by *Candida* (Kojic, *et al.*, 2004) Biofilm production found to be of more significance to NAC than *Candida albicans*. Catheter related infections are the major cause of morbidity and mortality among hospitalised patients and catheter microbial biofilms are associated with 90% of these infections (Sardi, *et al.*, 2013), Medical causes were the third commonest risk factor in our study. 21 (14%) which include pleural effusion, bronchopneumonia and tuberculosis. The findings correlate with the study conducted by Jha, *et al.*, 2006).

In the present study, Diabetes mellitus was the fourth most commonest risk factor. In this group 13 (10.8%) were NAC. Diabetes

mellitus decreases the chemotactic factors and impairs phagocytosis hence such patients are prone to infections by *Candida* species (Singhi, *et al.*, 2004).

Trauma was the fifth commonest predisposing factor. In this group, 12(10%) were NAC. Trauma patients usually have prolonged ICU stay and therefore are more prone to the hospital-acquired infections (HAIs).

Candida is becoming a very important pathogen among critically ill hospitalised patients. Surgical causes were the sixth most common predisposing factor in our study. In this group 9 (7.5%) were NAC. The widespread use of invasive monitoring and therapeutic equipment in critically ill surgical patients increases the risk of fungal colonisation and infection.

In the present study, pregnancy was the seventh most common predisposing factor. The increased prevalence of genital candidiasis in pregnancy is due to the increase in the glycogen content of the vagina and thus increasing the colonization of *Candida*. Occurrence of candidial vulvovaginitis in pregnant women causes risk of transmission to newborn infants (Narain, *et al.*, 2003).

Birth asphyxia was the eight common predisposing factor. Disrupted mucosal barrier promotes adhesion of yeasts to the underlying epithelium (Oksala E. 1990). At the same time birth asphyxia may interfere with normal salivary flow which is integral to the maintenance of normal micro flora in mouth. Disturbed micro flora and mucosal breaks provide opportunities for fungal growth in the oral cavity (Piyush Gupt. MMA *et al.*, 1996).

The present study showed that in patients

who were HIV positive, NAC 2(1.6%) were isolated. The reason for increased Candidiasis in HIV positive individuals is due to the decrease in CD4 T lymphocytes and hence a decrease in the amounts of IL-2, IFN-gamma and other lymphokines which cause major damage to the cell mediated immunity. Humoral immunity is also affected. The data of this study showed that even in different risk factors groups, it was the NAC that were isolated more frequently (Table-2).The commonest being *C. tropicalis*. In the present study, *Candida* species was isolated from various clinical samples and the highest was from urine in patients with risk factors 90 (60%) followed by respiratory samples 26 (17.3%), Blood 16(10.7%), High vaginal swabs 14(9.3%) and wound swabs 4(2.7%).

In our study, maximum numbers of *Candida* species were isolated from urine samples. *Candida* spp. are reported as seventh most common nosocomial pathogen in hospital settings causing 25% of all the urinary tract infections in some of previous studies. Roy R.C.*et al.*, 2013, Lata R Patel *et al.*, 2012, Pfaller, *et al.*, 1996, Almeida, A.A *et al.*, 2013 had also reported most of *Candida* spp. from urine samples in there study (62%).

In this study, we observed that Non-albicans *Candida* species had predominance over *C.albicans*. Among the Non-albicans *Candida* species, *C. tropicalis* showed higher prevalence followed by *C.dublinsiensis*, *C.krusei*, *C.guilliermondii*, *C.kefyr*, *C.lipolytica*, *C.glabrata*, *C. famata*, which is consistent with the published reports from different parts of the world (Bandi *et al.*, 2014, Tajashree *et al.*, 2014,Geeta S.H., 2014, Lata R Patel. *et al.*, 2012, Saroj Golia.*et al.*, 2013, Agarwal, *et al.*, 2011). *C. tropicalis* was the most common isolate, followed by *C.albicans*. A relatively greater proportion of *C. tropicalis*

isolates in our study is concordant with other studies from India (Bandi *et al.*, 2014, Ragini *et al.*, 2011. This is represented in the (Figure- 1).

Furthermore, Invasive candida infection was mostly caused by Non-albicans *Candida*. Non-albicans *Candida* was alone more prevalent in paediatric and older patients groups than in patients of young age group. The findings are also in agreement with the studies conducted by Chakrabarti A.*et al.*,1996 and 2002, Agarwal J. *et al.* 2004,Singhi SC.*et al.*,2004.In some of the other studies the percentage of *C.albicans* was higher than NAC which is in contrast to the present study. St.Germian, *et al.*,2001, Amar, *et al.*,2013, Basu, *et al.*, 2003,Vijaya, *et al.*,2011 and Saroj Golia, *et al.*, 2013.

For differentiation between different species of candida conventionally Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests are being used which are laborious and time consuming and may take 72 hrs to 2 weeks. Chrom agar helps in rapid identification of most of the species of candida. It can serve a primary isolation and differentiation medium for clinical specimens likely to contain yeast and also an adjunctive differential medium for identification of yeasts isolated on other media. A major advantage of CHROM Agar (HICROME Agar, HI MEDIA, Mumbai) is the ability to detect mixed cultures of yeast in clinical specimens (Baradkar, *et al.*, 2010).

The Varying sensitivities and specificities have been reported by several authors for identification of *Candida* species. Sensitivities ranged from 66.7% to 100%, specificity from 78.8 to 100% in a study done by Amar, *et al.*, 2013, Syamala, *et al.*, 2012. The present study showed sensitivity 100% and specificity 95.31% which is in

between the rates reported by Amar, *et al.*, 2013, Willinger, *et al.*, 1999, Yucesoy, *et al.*, 2003, Peng, *et al.* 2007 and Shyamala, *et al.*, 2012. We found it difficult to objectively identify colony characters like pink to lavender and steel blue or *met allic* blue colour. Other test like CMA and SAT need to be done in case of ambiguity.

Similarly varying sensitivities and specificities have been reported by several authors for identification of *C.albicans*. Shyamala, *et al.*, 2012 who used Hicrome agar reported a good sensitivity (97.05%) and specificity (98.07%), Bharadkar, *et al.*, 2010 reported a sensitivity of 96.55% and specificity of 96.42%. Willinger, *et al.*, 1999 reported 98.8% and 100%, Peng, *et al.*, 2007, 100% and 94.6% and Yucesoy, *et al.*, 2003 reported 99.4% and 100% of sensitivity and specificity. In our study we found 100% sensitivity and specificity for *C.albicans* which is in agreement with Amar C S. *et al.*, 2013 and Yucesoy M. *et al.*, 2003.

The present study had 12 isolates of *C.dublinsiensis* which was easily identified by their dark green colouration. Thus for *C.dublinsiensis* sensitivity and specificity on Hicrome agar was found to be 100%. In a study conducted by Baradkar *et al.*, 2010 showed low sensitivity (60%) and specificity (96.55%).

Jabra-Rizk, *et al.*, 2005 and Faller, *et al.*, 1996 suggested the same colour for *C.dublinsiensis*. The present study shows 100% sensitivity and specificity. The isolate was easily identified by their rough, fuzzy, spreading large pink colonies on CHROM agar, which is in concordance with Yucesoy *et al.*, 2003 and Amar *et al.*, 2013 revealed that all *C.krusei* isolates produced rough, fuzzy spreading big pink colonies on CHROM agar. Yucesoy *et al.*, 2003 opines that although there are many yeast species

giving the same colour, the morphology of the colonies of *C.krusei* is distinctly different (fuzzy, rough, large, pink).

In our study *C.guilliermondii* and *C.glabrata* was easily identified by their colour, pink to lavender and dark violet colour for *C.glabrata*. Hence, the sensitivity and specificity of both species was found to be 100%.

Rapid identification of *C. glabrata* has a special importance because *C. glabrata* is less sensitive than other species to ketoconazole and fluconazole (Abi-Said D.*et al.*, 1997, Khan ZU.*et al.*, 2004), Baradkar VP *et al.*, 2010 reported, a sensitivity of 90.90% and specificity of 88.23% were reported.

Willinger *et al.*, 1999 reported 98% sensitivity and 95.7% specificity for *C. glabrata* on chrom agar. Peng *et al.*, 2007 reported sensitivity and specificity values of chrome agar for *C. glabrata* as 90.2 and 95.4% respectively. *C. kefyri*, *C. lusitanae*, *C. guilliermondii* produced similar colonies as described by many authors (Freydiere AM., 1996) (Koehler AP. *et al.*, 1999). In our study sensitivity and specificity of *C.kefyri* was found to be 100% and 99.3%. These strains can be easily differentiated from *C. glabrata* as it doesn't produce even pseudo hyphae on cornmeal agar. Thus the combination of cornmeal agar and chrome agar can be used for early identification of *C. glabrata*.

In our study, chromogenic medium and morphology on CMA did not enable the differentiation of *C.famata*, *C.lipolytica*. However these isolates could be clustered as those providing white to pink colonies on Chrom agar and providing only yeast cells (yeast cells without pseudohyphae) on CMA.

In the present study these two species can be easily identified by sugar fermentation and sugar assimilation test. The present findings are in concordance with Sanjeev kumar *et al.*, 2013, who opined that all such isolates could be successfully identified only through automated systems i.e. the V2C system. Thus Vitek 2 ID could be an additional help in those situations where CHROM agar could not give identification (LO, *et al.*, 2001).

However V2C is expensive and its use in most of the hospitals in developing countries like India cannot be justified. CHROM agar was certainly the more efficient method over Vitek 2 for identification of most commonly isolated species from our hospital. However there were certain limitations in our study such as very few isolates of *C. famata* and *C. lipolytica* for comparison with other authors.

The invitro susceptibility testing of antifungal agents is becoming increasingly important because of the introduction of new antifungal agents and the recovery of clinical isolates that exhibit inherent or developed resistance to Amphotericin B, Nystatin and the Azole group of drugs during chemotherapy.

In the present study we performed disc diffusion method for antifungal susceptibility testing of candida isolates. In our study, 27.3% showed resistance to Itraconazole, 26.7% showed resistance to clotrimazole, 26% showed resistance to Fluconazole, 18% showed resistance to voriconazole, 10% showed resistance to ketoconazole, 2.7% showed resistance to Amphotericin B, 1.3% showed resistance to Nystatin. The comparative study of fluconazole resistance is shown in figure –

2. In the present study, isolation of NAC is more than *C.albicans*. Identification of candida species is important as non-albicans Candida are more resistant to azoles compared to *C.albicans*.

All the isolates of candida were susceptible to Amphotericin B and Nystatin except *C.guilliermondii* and *C.lipolytica* which showed 60% and 66.6% susceptibility. Next to Amphotericin B & Nystatin, NAC were susceptible to ketoconazole. Only *C.lipolytica* showed higher resistance to Ketoconazole (66.6%) in our study. Jayalakshmi *et al.*, 2014 from South India reported similar finding.

In this study, all the species of candida except *C.kefyr* were susceptible to Amphotericin B & Nystatin. Ketoconazole showed 83.8% susceptibility followed by Itraconazole and Clotrimazole 75.23% and 71.42%.

Fluconazole is 69.3% susceptible, of which 22% strains of *C. tropicalis* showed resistance against Fluconazole and 77.9% were susceptible. *C.famata* showed complete resistance to Fluconazole, Clotrimazole, Itraconazole whereas *C.dublinsiensis* & *C.guilliermondii* showed 33.4% and 40% resistance to Fluconazole.

C.glabrata showed 100% susceptibility to Fluconazole in our study which is in contrast to Farooqi *et al.*, 2013 showed higher resistance to fluconazole by *C.glabrata*. Itraconazole and Clotrimazole showed highest resistance 27.3% and 26.7% respectively among NAC. Our study is in concordance with the study done by Geeta S *et al.*, 2014, and Jayalakshmi *et al.*, 2014.

Table.1 Distribution of Patients according to age and sex in culture positive cases

AGE	Culture positive cases		Total No. of Cases
	Sex		
	Males	Females	
0-10	5 (5.3%)	4 (7.1%)	9 (6%)
11-20	3 (3.1%)	8 (14.2%)	11 (7.3%)
21-30	11 (11.7%)	15 (26.7%)	26 (17.3%)
31-40	9 (9.5%)	9 (16.0%)	18 (12%)
41-50	18 (19.1%)	5 (8.9%)	23 (15.3%)
51-60	17 (18.0%)	2 (3.5%)	19 (12.7%)
>60	31 (32.9%)	13 (23.2%)	44 (29.3%)
TOTAL	94 (62.6%)	56 (37.3%)	150(100%)
Mean age : 44.5 years		SD – 20.4	

Table.2 Candida species among various predisposing factors.

Predisposing factors	<i>C.albicans</i> (%)	Non-albicans candida (%)	Total
Prolonged Medication	8 (26.6)	33 (27.5)	41(27.3%)
Biomedical devices	5 (16.6)	27 (22.5)	32(21.3%)
Medical causes	6 (20)	15 (12.5)	21(14%)
Diabetes mellitus	4 (13.3)	13 (10.8)	17(11.3%)
Trauma	2 (6.6)	12 (10)	14(9.3%)
Surgical causes	2 (6.6)	9 (7.5)	11(7.3%)
Pregnancy	3 (10)	4 (3.3)	7(4.7%)
Birth asphyxia	0	5 (4.1)	5(3.3%)
H I V	0	2 (1.6)	2(1.3%)
Total	30 (20)	120 (80)	150(100%)

Table.3 Distribution of *Candida* isolates in clinical specimens.

Clinical Specimens	Total No. of Isolates	Percentages
Urine	90	60
Respiratory samples	26	17.3
Blood	16	10.7
Vaginal swab	14	9.3
Wound swab	04	2.7
Total	150	100

Table.4 Distribution of different species of *Candida* among various clinical specimens by conventional method (CMA and SAT)

Species	From Urine (candiduria in patients with risk factors) (%)	Respiratory samples (%)	From Blood (%)	Vaginal swab (%)	Wound Swab (%)	Total No. of isolates (%)
<i>C. tropicalis</i>	63 (70)	7(26.9)	10(62.5)	5(35.7)	1(25)	86(57.3)
<i>C.albicans</i>	17(18.8)	9(34.6)	0	4(28.5)	0	30(20.0)
<i>C.dubliniensis</i>	2(2.2)	6(23)	2(12.5)	2(14.2)	0	12(8.0)
<i>C.krusei</i>	2(2.2)	2(7.6)	0	2(14.2)	1(25)	7(4.7)
<i>C.guilliermondii</i>	1(1.1)	0	3(18.7)	0	1(25)	5(3.3)
<i>C.kefyr</i>	2(2.2)	1(3.8)	1(6.2)	0	0	4(2.7)
<i>C.lipolytica</i>	2(2.2)	1(3.8)	0	0	0	3(2.0)
<i>C.glabrata</i>	1(1.1)	0	0	1(7.1)	0	2(1.3)
<i>C.famata</i>	0	0	0	0	1(25)	1(0.66)
Total	90(60)	26(17.3)	16(10.7)	14(9.3)	4(2.7)	150 (100)
Chi square test : 294.12			P valve <0.05, Significant.			

Table.5 Identification of species by CHROM agar & Conventional method

Species	CHROM agar	Conventional method (CMA & SAT)
<i>C. tropicalis</i>	89 (59.3%)	86 (57.3%)
<i>C.albicans</i>	30 (20%)	30 (20%)
<i>C.dubliniensis</i>	12 (8%)	12 (8%)
<i>C.krusei</i>	07 (4.7%)	07 (4.7%)
<i>C.guilliermondii</i>	05 (3.3%)	05 (3.3%)
<i>C.kefyr</i>	05 (3.3%)	04 (2.7%)
<i>C.lipolytica</i>	0	03 (2.0%)
<i>C.glabrata</i>	02 (1.3%)	02 (1.3%)
<i>C.famata</i>	0	01 (0.66%)
Total	150(100%)	150 (100%)

Table.6 Sensitivity and specificity of CHROM agar for identification of various Candida species

Species	Total Candida species identified using CHROM agar	No. of Candida species identified by conventional method (CMA & SAT)	Sensitivity of CHROM agar	Specificity of CHROM agar
<i>C. tropicalis</i>	89 (59.3%)	86 (57.3%)	100%	95.31%
<i>C.albicans</i>	30 (20%)	30 (20%)	100%	100%
<i>C.dubliniensis</i>	12 (8%)	12 (8%)	100%	100%
<i>C.krusei</i>	07 (4.7%)	07 (4.7%)	100%	100%
<i>C.guilliermondii</i>	05 (3.3%)	05 (3.3%)	100%	100%
<i>C.kefyr</i>	05 (3.3%)	04 (2.7%)	100%	99.3%
<i>C.lipolytica</i>	0	03 (2.0%)	0%	100%
<i>C.glabrata</i>	02 (1.3%)	02 (1.3%)	100%	100%
<i>C.famata</i>	0	01 (0.7%)	0%	100%

Table.7 Antifungal susceptibility of Candida species

Species	FLU (10µg)		CLO (10µg)		VOR (1µg)		ITR (10µg)		KETO (1µg)		NYS (100 U)		AMB (100U)	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
<i>C. tropicalis</i> (n=86)	67 77.9 %	19 22%	59 68.6 %	27 31.3 %	70 81.3 %	16 18.6 %	70 81.3 %	16 18.6 %	79 91.8 %	7 8.1 %	85 98.8 %	1 1.2 %	85 98.8 %	1 1.2 %
<i>C.albicans</i> (n=30)	19 63.3 %	11 36.6 %	24 80%	6 20%	23 76.6 %	7 23.4 %	18 60%	12 40%	25 83.3 %	5 16.7 %	30 100 %	0	30 100 %	0
<i>C.dubliniensis</i> (n=12)	8 66.6 %	4 33.4 %	10 83.3 %	2 16.7 %	9 75 %	3 25%	7 58.3 %	5 41.7 %	11 91.6 %	1 8.4 %	12 100 %	0	12 100 %	0
<i>C.krusei</i> (n=7)	0	0	5 71.4 %	2 28.4 %	7 100 %	0	4 57.1 %	3 42.9 %	7 100 %	0	7 100 %	0	7 100 %	0
<i>C.guilliermondii</i> (n=5)	3 60%	2 40%	4 80%	1 20%	5 100 %	0	5 100 %	0	5 100 %	0	5 100 %	0	3 60%	2 40 %
<i>C.kefyr</i> (n=4)	4 100 %	0	4 100 %	0	4 100 %	0	3 75%	1 25%	4 100 %	0	4 100 %	0	4 100 %	0
<i>C.lipolytica</i> (n=3)	1 33.4 %	2 66.6 %	2 66.6 %	1 33.4 %	2 66.6 %	1 33.4 %	1 33.4 %	2 66.4 %	1 33.4 %	2 66.6 %	2 66.6 %	1 33.4 %	2 66.6 %	1 33.4 %
<i>C.glabrata</i> (n=2)	2 100 %	0	1 50%	1 50%	2 100 %	0	1 50%	1 50%	2 100 %	0	2 100 %	0	2 100 %	0
<i>C.famata</i> (n=1)	0	1 100 %	0	1 100 %	1 100 %	0	0	1 100 %	1 100 %	0	1 100 %	0	1 100 %	0
Total	104 69.3 %	39 26%	110 73.3 %	40 26.7 %	123 82 %	27 18%	109 72.7 %	41 27.3 %	135 90%	15 10 %	148 98.7 %	2 1.3 %	146 97.3 %	4 2.7 %
χ^2	3.166		7.28		3.696		15.39		14.7		24.2		40.0	
P value	.530,Not significant		0.608, not significant		0.26,not significant		0.08,not significant		0.09,not significant		0.004,signi ficant		0.000,Signi ficant	

FLU – Fluconazole, CLO-Clotrimazole, VOR- Voriconazole, ITR-Itraconazole, KETO - Ketoconazole, NYS-Nystatin, AMB - Amphotericin B.

Fig.1 Distribution of patients according to age & sex in culture positive cases

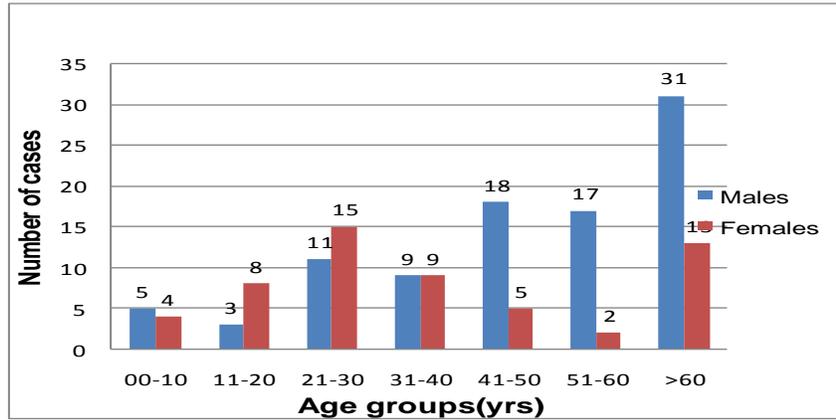


Fig.2 Candida species among various predisposing factors.

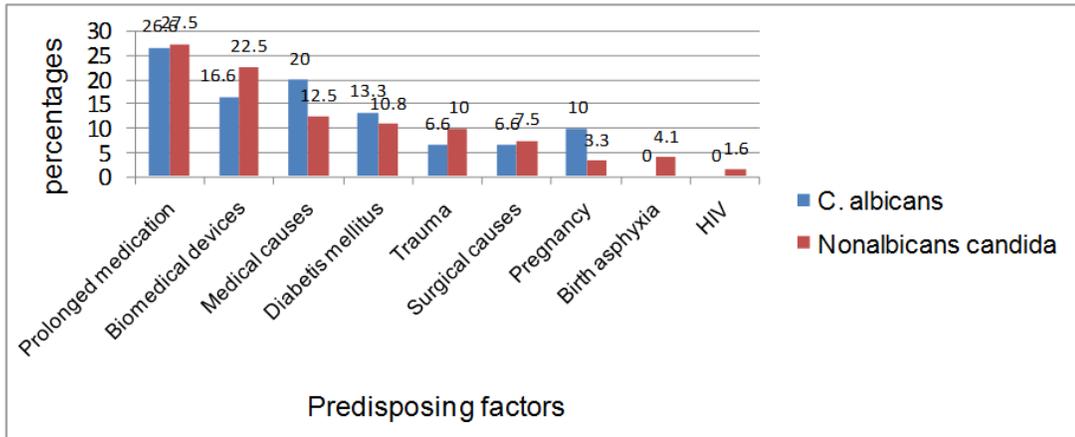


Fig.3 Distribution of Candida isolates in clinical specimens

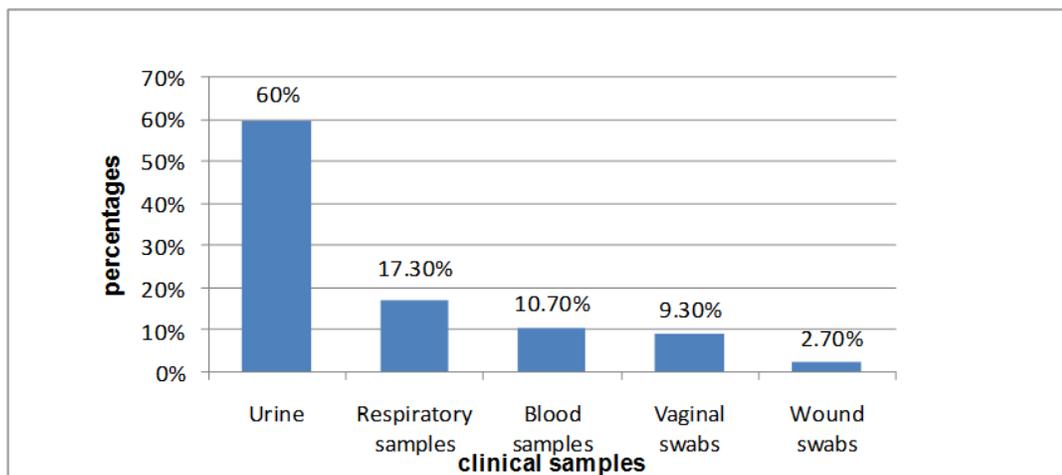


Fig.4 Distribution of different species of *Candida* among various clinical specimens by conventional methods (CMA & SAT)

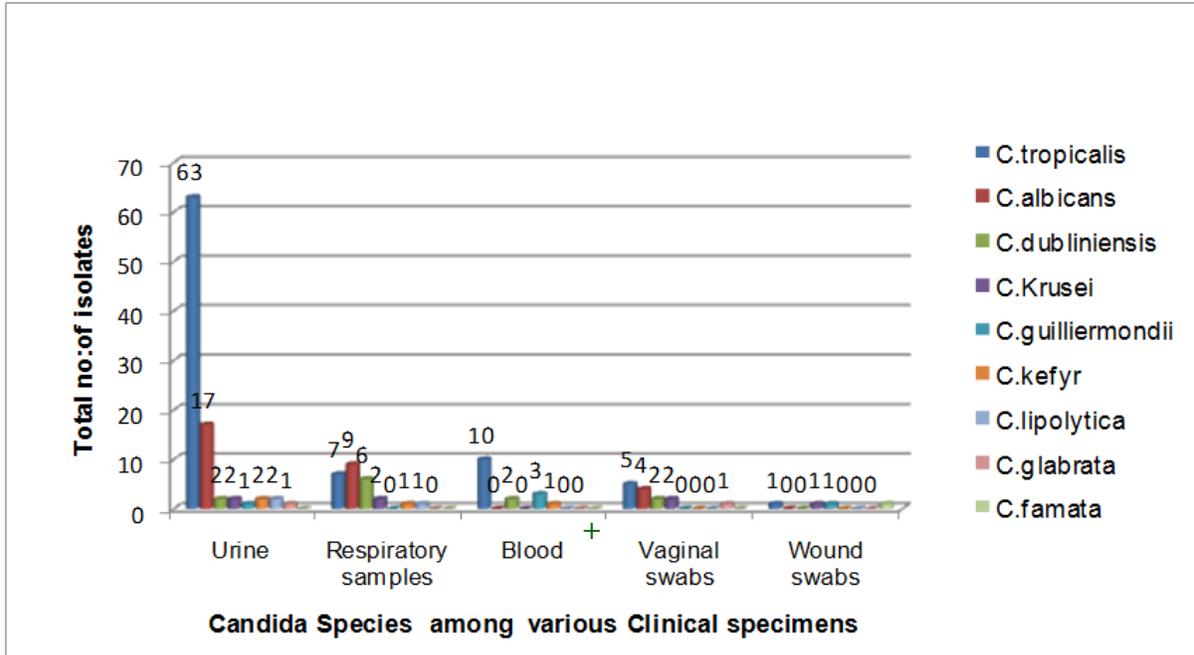


Fig.5 Identification of species by CHROM agar & Conventional method

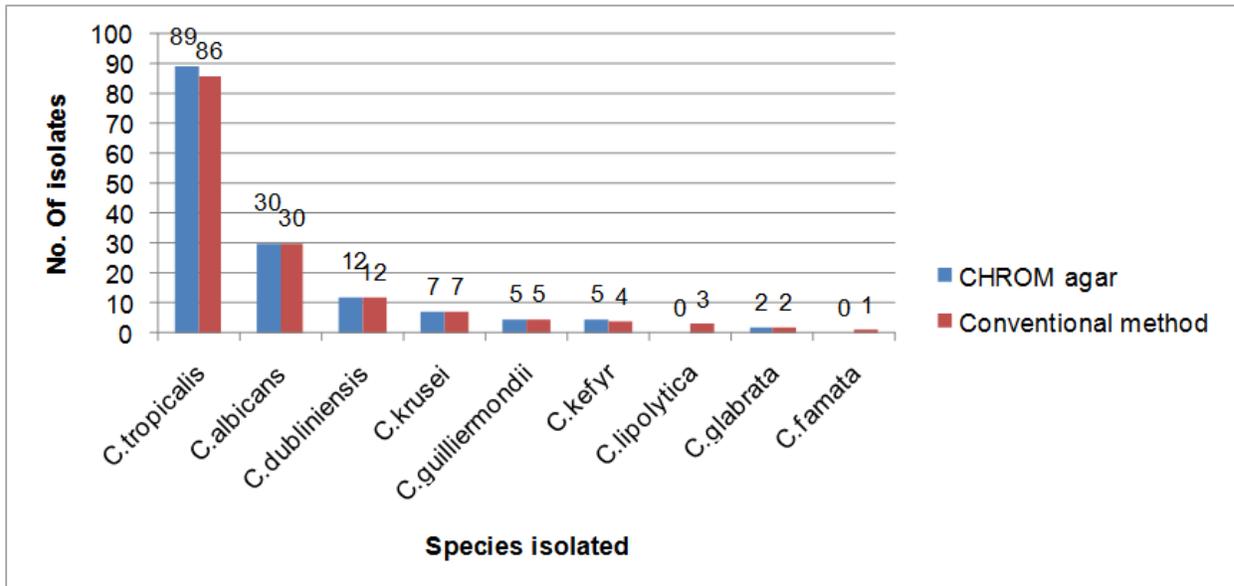


Fig.6 Antifungal susceptibility pattern of Candida species

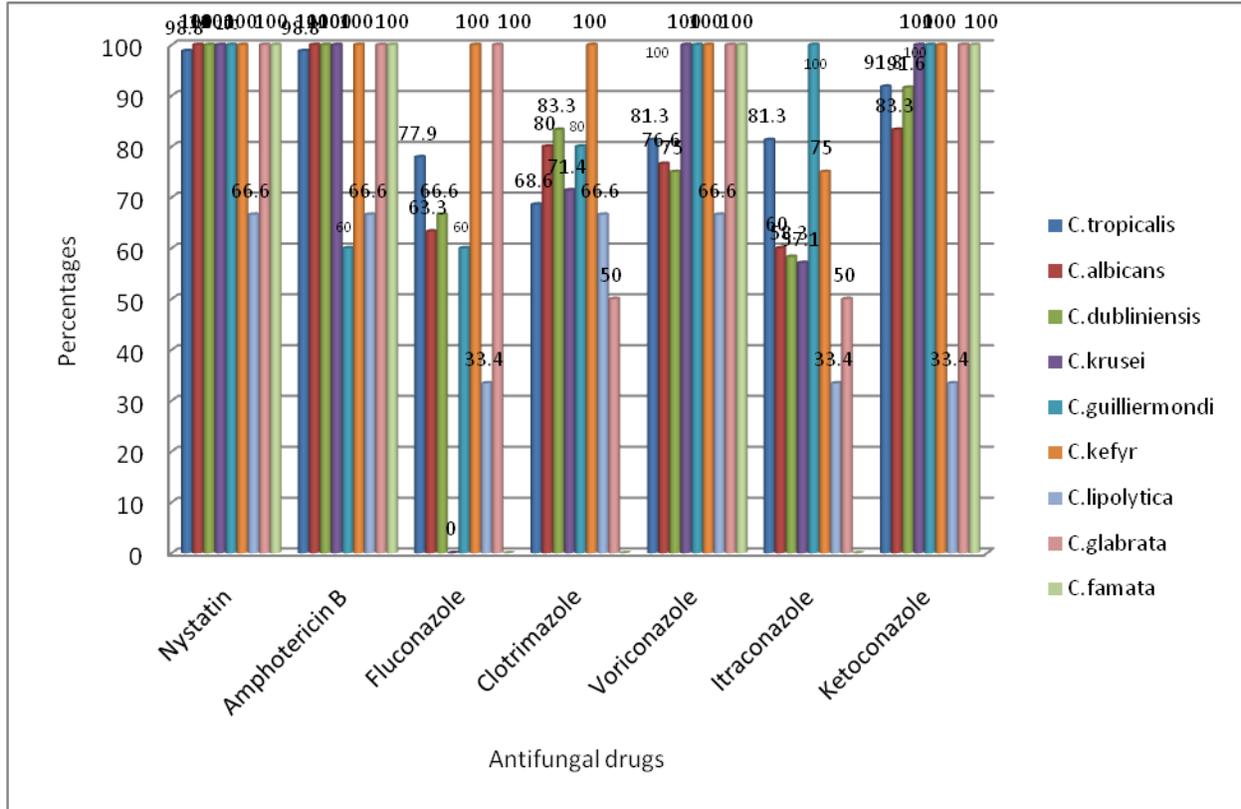
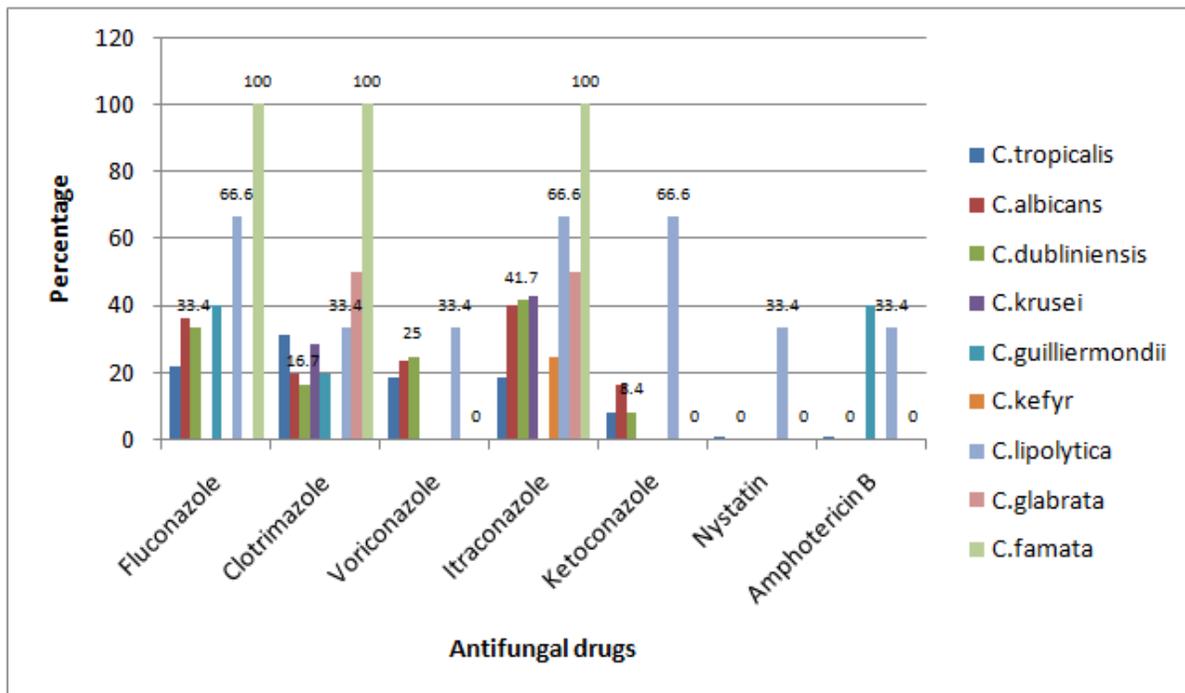


Figure.7 Antifungal resistance pattern of candida species



In the present study, *C.albicans* showed 100% susceptibility to Amphotericin B & Nystatin which is in concordance with Vijaya *et al.*, 2011, Shivanand *et al.*, 2011, Ragini *et al.*, 2011, Mondal *et al.*, 2013, Ayesha Aslam *et al.*, 2015, Parvez Anwar Khan *et al.*, 2015.

C.albicans showed 36.6% resistance to Fluconazole which is in concordance with Ragini *et al.*, 2011, Jayalakshmi *et al.*, 2014 and Jayapriyasu kumaran *et al.*, 2012.

To conclude, the present study showed that incidence of non-albicans *Candida* were higher from various clinical specimens. An increase in the predisposing conditions has resulted in an increasing incidence of non-albicans *Candida* infections. Some of the Non-albicans *Candida* species is intrinsically resistant to commonly used antifungal drug like Fluconazole. Therefore, early speciation of *Candida* isolates along with their antifungal susceptibility tests not only will restrict the empirical use of antifungal agents but also greatly influence the treatment options for the clinicians and thus will be beneficial for the patients. The present study highlights the need for periodic surveillance of antifungal susceptibility pattern of the prevalent *Candida* species, as it would enlighten the judicious use of antifungal drugs in patients and thus preventing the emergence of drug resistance.

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