Original Research Article

Prevalence of candida species and antifungal drug sensitivity in tertiary care hospital - A cross sectional study

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Abstract

Background: Fungal infections are a major cause of morbidity and mortality in immunocompromised individuals and *Candida* are among the most common pathogens in these patients. Candidaemia increases mortality rates in the range of 20-49%, Emergence of *Candida* species resistance is on the rise especially to triazoles and amphotericin B has led to use echinocandins, mostly caspofungin in the management of invasive candidiasis. There has been published reports of caspofungin resistance in *Candida* species especially *C.glabrata*. **Aim and Objective:** 1. Prevalence of *Candida* species and its antifungal drug sensitivity 2. To study predisposing factors among Candida infection cases.3. To study antifungal susceptibility pattern of *Candida* species by disk diffusion and Minimum Inhibitory Concentration by E strip. **Methods:** Hospital based Cross sectional study, **Study setting:** Microbiology Department of tertiary care centre. **Study population:** The study population included all the Candida species sample positive cases admitted at a tertiary care center. **Sample size:**150 **Results:** In this study, majority of Candida isolates (40%) obtained were from age group >60 years, followed by 20.67% from age group 21-30 years, 18.67% from 31-40 years age group. Majority of *Candida* isolates, 52(34.67%) isolates were from patients who had immunosuppression and chronic drug therapy as predisposing factor. The second common predisposing factor was diabetes mellitus 38(25.33%), followed by pregnancy 22(14.66%), pre-term and LBW babies 18(12.00%) and undetermined factors 20(13.34%). A total 150 *Candida* isolates, 106(70.67%) were susceptible, 13(8.66%) were susceptible dose dependent and 31(20.67%) were resistant to fluconazole. Of the total 150 *Candida* isolates, 138(92%) were susceptible, 2(1.33%) were susceptible dose dependent and 10(6.67%) were resistant to voriconazole. majority of the cases were found in females e.g 60 (54.60%) and Males were 50 (45.40%), proportion of acute abdomen with dengue illness was statistical significant in age group 7 and above ($p < 0.05$), majority of cases had Dengue fever e.g 98 (89.09%, followed by Dengue Hemorrhagic 8, (7.27%) and Dengue shock syndrome was found in 4 cases (3..64%). **Conclusions: 1.** Females were found to be commonly affected than males.2. *Candida* infection was found to be more in >60 years age group.3. In the present study, immunosuppression and chronic drug therapy and LBW (12.00%) and undetermined factors (13.34%). 4.candida species 70.67% of *Candida* were susceptible to fluconazole, 92% were susceptible to voriconazole and 80.67% were susceptible to ketoconazole

Keywords: Antifungal susceptibility, Susceptible Dose dependent, candida species anti fungal resistant

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INTRODUCTION

Fungal infections are a major cause of morbidity and mortality in immunocompromised individuals and *Candida* are among the most common pathogens in these patients. Candidaemia increases mortality rates in the range of 20-49%.[1,2] The unmet medical needs surrounding candidaemia and invasive candidiasis are defined in general- from diagnosis to prophylaxis, empiric and pre-emptive strategies to treatment.[3] *Candida* is an asexual,

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diploid, dimorphic fungus. *Candida* species belong to normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina[4] and are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections.[5] candidiasis has emerged as an alarming opportunistic disease.[6] more than 90% of invasive infections are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*.[7] The emergence of non-albicans *Candida* spp. Has however been well recognized during the past decade.[8,9] *Candida* spp. Have been shown 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.[10] The potential clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of putative virulence factors and antifungal susceptibility.[11,12] Emergence of *Candida* species resistance is on the rise especially to triazoles and amphotericin B has led to use echinocandins, mostly caspofungin in the management of invasive candidiasis. There has been published reports of caspofungin resistance in *Candida* species especially *C.glabrata.*[13] The present study was designed to identify the spectrum of *Candida* species in clinical infections and to identify their susceptibility pattern to available antifungal agents.

METHODOLOGY

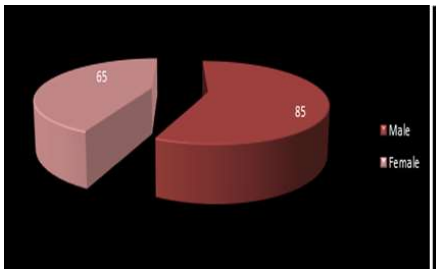
Study design: Hospital based Cross sectional study. **Study setting:** Microbiology department of tertiary care centre.
Study duration: 1 years (from……to……). **Study population:** The study population included all the Candida species sample positive cases admitted at a tertiary care center
Inclusion criteria: 1. All *Candida* isolates from various clinical samples.
Exclusion criteria: 1. *Candida* isolates from stool and sputum samples.
Approval for the study:
Written approval from Institutional Ethics committee was obtained beforehand. Written approval of Microbiology department was obtained. After obtaining informed verbal consent from all patients with the definitive diagnosis of Candida species infections admitted to tertiary care centre included in this study.
Sample size: Sample size was calculated using software nMaster2.0 and sample size of 150 *Candida* was estimated.
Sampling technique:
Total population sampling technique used for data collection. All patients admitted in tertiary care center with Candida species infections. Explained the purpose of study and who gave consent and detailed history of fungal illness such cases included in this study.
Methodology: Predesigned and pretested questionnaire was used to record the necessary information. Questionnaires included general information, such as age, sex, religion, occupation of parents, residential address, socioeconomic status and date of admission. Medical history- chief complain, past history, past medical history, immunosuppressant drug history, general examination, systemic examination. Explained the purpose of study and who gave consent and detailed history of fungal illness such cases included in this study. After obtaining informed verbal consent from all patients with the definitive diagnosis of candida species admitted to tertiary care centre such cases were included in the study. The data were entered in Microsoft Excel and data analysis was done by using SPSS demo version no 21 for windows. Susceptibility of antifungal agents was performed using Chi-square test, $p < 0.05$ was considered as statistical significance.

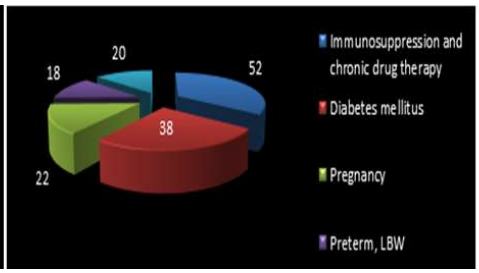
OBSERVATIONS AND RESULTS

Table 1: Distribution of study patients according to age

Sr No	Age In Years	Candida isolates	Percentage
1	0-10	11	7.33%
2	11-20	3	2.00%
3	21-30	31	20.67%
4	31-40	28	18.67%
5	41-50	11	7.33%
6	51-60	6	4.00%
7	>60	60	40.00%
	Total	150	100

Table 1 shows age wise distribution of patients from whom *Candida* isolates were obtained. The maximum isolates (40%) obtained were from age group >60 years, followed by 20.67% from age group 21-30 years, 18.67% from 31-40 years age group.





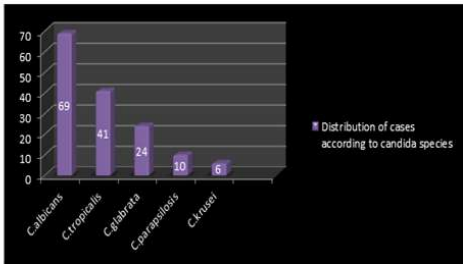


Figure 1 **Figure 2** **Figure 3**

Figure 1: Distribution of Candida species cases according to Sex**; Figure 2:** Distribution of cases according to predisposing factors; **Figure 3:** Distribution of cases according to *candida* species

The above figure 1 shows majority of the cases were found in males 85 (56.67%) and 65 (43.33%) were from females. Figure 2 shows predisposing factors in various patients from whom *Candida* species were isolated. Out of total 150 *Candida* isolates, 52(34.67%) isolates were from patients who had immunosuppression and chronic drug therapy as predisposing factor. The second common predisposing factor was diabetes mellitus 38(25.33%), followed by pregnancy 22(14.66%), pre-term and LBW babies 18(12.00%) and undetermined factors 20(13.34%).

Figure 3 shows distribution of *Candida* species among various clinical samples. Out of 150 *Candida* species isolated from various clinical samples, 69(46%) were *C.albicans*, followed by 41(27.33%) *C.tropicalis,* 24(16%) *C.glabrata,* 10(6.67%) *C.parapsilosis* and by 6(4%) *C.krusei*.

Table 2: Antifungal susceptibility pattern of total candida species by disk diffusion method

Drug	Susceptible	Susceptible dose dependent	Resistant
FLUCONAZOLE	106	13	31
(n=150)	(70.67%)	(8.66%)	(20.67%)
VORICONAZOLE	138	2	10
(n=150)	(92%)	(1.33%)	(6.67%)
KETOCONAZOLE	121	16	13
(n=150)	(80.67%)	(10.67%)	(8.66%)

Table 2 shows antifungal susceptibility of total *Candida* species to fluconazole and voriconazole, ketoconazole by disk diffusion method. Of the total 150 *Candida* isolates, 106(70.67%) were susceptible, 13(8.66%) were susceptible dose dependent and 31(20.67%) were resistant to fluconazole. Of the total 150 *Candida* isolates, 138(92%) were susceptible, 2(1.33%) were susceptible dose dependent and 10(6.67%) were resistant to voriconazole. Of the total 150 *Candida* isolates, 121(80.67%) were susceptible, 16(10.67%) were susceptible dose dependent and 13(8.66%) were resistant to ketoconazole.

Table 3: Antifungal susceptibility of different *candida* species to fluconazole by disk diffusion

species	Susceptible	Susceptible dose dependent	Resistant
C.albicans	58	13	31
(n=69)	(84.06%)	(8.66%)	(20.67%)
C.tropicalis	24	2	10
(n=41)	(58.54%)	(1.33%)	(6.67%)
C.glabrata	14	16	13
(n=24)	(58.33%)	(10.67%)	(8.66%)
C.parapsilosis	10	-	-
(n=10)	(100%)		
*C.krusei**	-	-	6
(n=6)			
TOTAL	**106**	**13**	**31**
(n=150)	**(70.67%)**	**(8.66%)**	**(20.67%)**

Table 3 shows antifungal susceptibility of different *Candida* species to fluconazole by disk diffusion. Out of 69 *C.albicans* 58(84.06%) were susceptible, 6(8.69%) were susceptible dose dependent and 5(7.25%) were resistant to fluconazole. Out of 41 *C.tropicalis* 24(58.54%) were susceptible, 6(14.63%) were susceptible dose dependent and 11(26.83%) were resistant. Out of 24 *C.glabrata*14(58.33%) were susceptible, 1(4.17%) was susceptible dose dependent and 9(37.5%) were resistant. All the 10(100%) *C.parapsilosis* isolates were susceptible to fluconazole.

Table 4: Antifungal susceptibility pattern of different *candida* species to voriconazole by disk diffusion

species	Susceptible	Susceptible dose dependent	Resistant
C.albicans (n=69)	64 (92.76%)	-	5 (7.24%)
C.tropicalis (n=41)	39 (95.12%)	-	2 (4.88%)
C.glabrata (n=24)	20 (83.33%)	1 (4.17%)	3 (12.5%)
C.parapsilosis (n=10)	09 (90%)	1 (10%)	-
*C.krusei** (n=6)	6 (100%)	-	-
TOTAL (n=150)	**138 (92%)**	**2 (1.33%)**	**10 (6.67%)**

Table 4 shows antifungal susceptibility of different candida species to voriconazole by disk diffusion method. Out of 69 *C.albicans,* 64(92.76%) were susceptible and 5(7.24%) were resistant to voriconazole. Out of 41 *C.tropicalis,* 39(95.12%) were susceptible and 2(4.88%) were resistant. Out of 24 *C.glabrata,* 20(83.33%) were susceptible, 1(4.17%) was susceptible dose dependent and 3(12.5%) were resistant. Of the 10 *C.parapsilosis,* 9(90%) isolates were susceptible, 1(10%) was susceptible dose dependent. All (100%) *C.krusei* were sensitive to voriconazole.

Table 5: Antifungal susceptibility pattern of different *candida* species to ketoconazole by disk diffusion method

species	Susceptible	Susceptible dose dependent	Resistant
C.albicans (n=69)	59 (85.5%)	07 (10.15%)	03 (4.35%)
C.tropicalis (n=41)	32 (78.05%)	03 (7.32%)	06 (14.63%)
C.glabrata (n=24)	19 (79.17%)	03 (12.5%)	02 (8.33%)
C.parapsilosis (n=10)	07 (70%)	03 (30%)	-
*C.krusei** (n=6)	04 (66.67%)	-	02 (33.33%)
TOTAL (n=150)	**121 (80.67%)**	**16 (10.67%)**	**13 (8.66%)**

Table 5 shows antifungal susceptibility of different *Candida* species to ketoconazole by disk diffusion method. Out of 69 *C.albicans,* 59(85.5%) were susceptible, 7(10.15%) were susceptible dose dependent and 3(4.35%) were resistant to ketoconazole. Out of 41 *C.tropicalis,* 32(78.05%) were susceptible, 3(7.32%) were susceptible dose dependent and 6(14.63%) were resistant. Out of 24 *C.glabrata,* 19(79.17%) were susceptible, 3(12.5%) were susceptible dose dependent and 2(8.33%) were resistant. Of the 10 *C.parapsilosis,* 7(70%) isolates were susceptible, 3(30%) were susceptible dose dependent. Out of 6 *C.krusei,* 4(66.67%) were sensitive and 2(33.33%) were resistant to ketoconazole.

Table 6: Minimum inhibitory concentration (MIC) of fluconazole for *Candida* by Etest method

Sr.No	Sensitivity pattern	MIC range	Frequency
1	**Susceptible**	**≤ 8 µg/ml**	**0**
2	**Susceptible dose dependent**	**16-32 µg/ml**	**4**
3	**Resistant**	**≥ 64 µg/ml**	**21**
	Total		**25**

Table 6 shows MIC of fluconazole for *Candida* by Etest method. Out of total 25 *Candida* isolates which were resistant to fluconazole by disk diffusion, 4 were showing MIC between16-32µg/ml (susceptible dose dependent) and 21 were showing MIC greater than or equal to 64µg/ml (resistant) by Etest method.

DISCUSSION

Gender distribution: In the present study, out of 150 *Candida* isolates, 85 (56.67%) were isolated from males and 65 (43.33%) were from females. Pahwa *et al.* (2014)3 reported male preponderance of 59.91% while More SR *et al.* (2016)[14] reported 66.96% of *Candida* in males. Our study correlates with their studies. This could be due to higher number of samples which were collected from male

patients. Incontrast, Dharwad and Saldhana (2011)[15] reported rate of isolation of *Candida* species as more in females (64%) than in males. Also, Guru P *et al.* (2016)[16] reported isolation of *Candida* species in females (54.5%) than males (45.5%).

Age wise distribution of patients from whom *Candida* species were isolated: In the present study, 40% of *Candida* were isolated from >60 years age group followed by 20.67% from 21-30 years. 18.67% *Candida* were from 31-40 years age group, followed by 7.33% from age group 0-10 years and 41-50 years and 4.00% from 51- 60 years and 2.00% from 11- 20 years. Similar finding observed in A study by Pahwa *et al.* (2014)3 reported peak incidence of 39.66% in age group >60 years which corresponds to findings in our study. Guru P *et al.* (2016)[16] also reported higher incidence of *Candida* in the age group >60 years accounting for 24%.

Predisposing factors of patients from whom *Candida* species were isolated: In the present study, the predisposing factors associated with candidiasis included immunosuppression and chronic drug therapy (34.67%), followed by diabetes (25.33%), pregnancy (14.66%), pre-term and Low Birth Weight (LBW) (12%) and undetermined factors (13.34%).The present finding of immunosuppression and chronic drug therapy with candidiasis was close with those of Dharwad and Saldhana (2011)[15] who reported 22% of *Candida* isolates from patients with history of drug intake and secondary to disease. Similarly, Dharwad and Saldhana (2011)[15] in their study reported diabetes mellitus as predisposing factor accounting for 22%.

Species distribution of *Candida*: In our study, *C.albicans* (46%) was the commonest species isolated followed by *C.tropicalis* (27.33%), *C.glabrata* (16%), *C.parapsilosis* (6.67%), and *C.krusei* (4%).similar finding observed in the study of Dharwad *et al.* (2011)144 He found that the *C.albicans* (47%) was the commonest species isolated followed by *C.tropicalis* (30%), *C.glabrata* (9%) and *C.krusei* (14%).

Antifungal susceptibility of total *Candida* species to Fluconazole by disk diffusion method:

Authors	Year	Susceptible
Pfaller *et al.*[22]	2005	89.6%
Ooga *et al.*[23]	2011	75%
Dharwad and Saldhana[15]	2011	60
Guru P *et al.*[16]	2016	73.5%
Present study	2019	73.33

Susceptibility of different *Candida* species to fluconazole by disk diffusion method:

a) Susceptibility of *C.albicans* to fluconazole by disk diffusion: In present study out of 69 *C.albicans* isolates, 58 (84.06%) were susceptible, 6 (8.69%) were susceptible dose- dependent and 5 (7.25%) were resistant to fluconazole by disk diffusion method.The susceptibility pattern of *C.albicans* for fluconazole by disk diffusion method in our study was in agreement with those of studies of Guru P *et al.* (2016),150 Mondal *et al.* (2013)[25] who reported 78.2%, 80% and 84.2% respectively. However, Pfaller *et al.* (2005)[22] Pfaller *et al.* (2007)[7] Pfaller *et al.* (2010)[24] Jaya and Harita(2013)[19] respectively in their studies.

Antifungal susceptibility of total *Candida* species to voriconazole by disk diffusion method: In our study, 138 (92%) *Candida* isolates were susceptible, 2 (1.33%) were susceptible dose- dependent and 10 (6.66%) were resistant to voriconazole by disk diffusion method. Jaya and Harita (2013)[19] reported 99.05% susceptibility to voriconazole by ASTY06. A study by Pahwa *et al.* (2014)[3] reported 96.6% of susceptibility to voriconazole by Vitek2 "Fungal Susceptibility Card (AST YS01) which is close to the present finding.

Antifungal susceptibility of total *Candida* species to ketoconazole by disk diffusion method: In our study of total 150 *Candida* isolates, 121(80.67%) were susceptible, 16(10.67%) were susceptible dose dependent and 13(8.66%) were resistant to ketoconazole. The present findings were close to the findings of Mondal *et al.* (2013)148 who reported 73.4% susceptible, 14.8% susceptible dose- dependent and 11.7% resistant *Candida* isolates to ketoconazole. However, Sukumaran J *et al.* (2012)[18] reported 100% susceptibility to ketoconazole in their study.

Minimum inhibitory concentration of fluconazole by E test method: Of total 25 *Candida* isolates which were resistant to fluconazole by disk diffusion, 4 were showing MIC between 16-32µg/ml (susceptible dose dependent) and 21 were showing MIC ≥64µg/ml (resistant) by Etest method. Madhavan *et al.* (2010),158 showed that out of total 41 *Candida* isolates, 71% of *Candida* isolates were susceptible to fluconazole. All strains of C. krusei were resistant to fluconazole and 50% were susceptible in a dose-dependent manner to voriconazole. There were 66% of *C. glabrata* that were resistant to fluconazole by E- test method.

CONCLUSION

Candidiasis is one of the major fungal infections.

Candida albicans is by far the most common species causing infections in humans.

The increase in the predisposing conditions like immunosuppression, chronic drug therapy and diabetes mellitus in recent years has resulted in a concurrent increase in patients who suffer from candidiasis.

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