CHARACTERIZATION AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA SPP. ISOLATED FROM CLINICAL SPECIMENS
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ABSTRACT: BACKGROUND: With the changing health scenario fungal infections have increased significantly, contributing to morbidity, mortality and health care cost. Candida is major human fungal pathogens that cause both superficial and deep tissue infections. With emergence of non-albicans Candida species, availability of advanced identification methods and antifungal resistance, the spectrum of candidiasis has changed. OBJECTIVE: The aim of our study was to identify the distribution of Candida species among clinical isolates, risk factors associated with candidiasis and their sensitivity pattern for common antifungal drugs. MATERIALS AND METHODS: One hundred thirty nine different clinical isolates of Candida were collected from indoor patients of a tertiary care centre of Gujarat from May 2009 to June 2010. Identification of Candida species and antifungal susceptibility testing was performed with miniAPI (Analytical Prophylactic Index) (Biomerieux, France) which is an automatic identification and susceptibility testing instrument. RESULTS: We found that the non-albicans Candida were more prevalent than Candida albicans. Candida tropicalis (48.9%) was the most common Candida spp. and also more resistant than that of C.albicans. C.albicans showed resistance against fluconazole (3.5%) and itraconazole (8.8%) whereas C.tropicalis were resistant to amphotericin B (10.3%), fluconazole (20.7%), itraconazole (32.3%), and voriconazole (23.5%) and flucytosine (5.8%). Overall resistance rates of Candida for amphotericin B, fluconazole, itraconazole, and voriconazole and flucytosine were 6.4%, 15.2%, 22.3%, 12.9%, 5% respectively. CONCLUSION: To achieve better clinical results species-level identification of Candida spp. and their antifungal sensitivity testing should be performed. KEYWORDS: Yeast, Candida, C. albicans, C. tropicalis, Antifungal resistance.

INTRODUCTION: Fungi are present ubiquitously in the environment and are known to mankind since centuries. But fungi were treated as a step child in clinical microbiology.¹ But with the changing scenario fungi are now emerging as a major human pathogen in both immunocompetent and immunocompromised persons leading to prolonged hospitalization and additional cost.¹-³ The annual incidence of fungal infection was increased by 207% in between 1979 to 2000.²,⁴ Candida are the most common fungal pathogen in these patients.⁵ Candida is sixth most common pathogen isolated from hospital worldwide and fourth most frequent pathogen in intensive care unit (ICU).⁶,⁷ Incidence of candidemia has ranged from 0.3 to 28 per 10,000 admissions worldwide with 19-24% attributable mortality rate.⁷,⁸ Candida is a part of normal flora of human alimentary canal and mucocutaneous regions but becomes pathogenic when certain predisposing conditions prevail. Although more than 20 different species of Candida are known to cause candidiasis, more than 90% of invasive infections are caused by C. albicans, C. tropicalis, C. parapsilosis, C. glabrata and C. krusei.⁹-¹¹ C. albicans was previously responsible for 80% of candidiasis but now there is a shift in distribution of infections, with non-
albicans Candida spp. being increasingly detected. These non-albicans Candida cause a diverse spectrum of diseases, ranging from superficial candidiasis to invasive candidiasis but with a difference in severity and therapeutic option. Similar to C.albicans, non-albicans Candida are also associated with significant morbidity, mortality and antifungal resistance.

Prolonged, wide spread and inappropriate empirical use of antifungal drugs leads to emergence of antifungal resistant strain of Candida particularly to azoles. Moreover, some of the non albicans Candida like C.krusei are inheritantly resistant to azoles.4,9,12

Therefore, the potential clinical importance of species-level identification has been recognized as the need of the time as Candida spp. differ in the expression of virulence factors and antifungal susceptibility. Rapid identification of Candida spp. also helps in early appropriate antifungal therapy in clinical set up and in reducing morbidity, mortality and health care cost.

The aim of the present study was to identify the spectrum of Candida spp. in clinical infections, risk factors associated with it and to identify their sensitivity pattern to available antifungal agents.

MATERIALS AND METHODS: The study was conducted prospectively in department of Microbiology between May 2009 to June 2010, in Pramukhswami Medical College and Shree Krishna Hospital, Karamsad, Anand which is a tertiary health care centre of Gujarat. Approval of Human Research Ethical Committee of the institution and written informed consents of patients had been taken. This study included all indoor patients from whom candida was isolated from various clinical specimens submitted to Microbiology laboratory. Whenever required second specimen was collected.

Blood culture sample collected in blood culture bottles were incubated in BacT Alert (Biomerieux, France) automated blood culture system and upon getting growth signal, gram stain of blood culture brooth was done. After getting gram positive budding yeasts on gram stain, it was sub – cultured onto Sabouraud dextrose agar (Hi Media, India) and blood agar plates.

Specimens other than blood (pus, sputum, urine etc) were inoculated onto Sabouraud dextrose agar in duplicates to exclude the laboratory contamination, in addition to blood agar, chocolate agar and MacConkey agar (Hi Media, India). Suspected candida colonies were confirmed by gram stain, germ tube test, corn meal test and then identified by miniAPI (Biomerieux, France).

Antifungal susceptibility testing for Fluconazole (FCA), Itraconazole (ITR), Voriconazole (VRC), Amphotericin B (AMB) and flucytosine (5-FC) was done by minimum inhibitory concentration determination and clinical susceptibility categorization in miniAPI following manufacturer instructions. For quality control American type culture collection (ATCC) C.glabrata 64677 strain was used.

RESULTS: During the study period, from the specimens received in the Microbiology laboratory with the culture request, 1342 specimens were reported culture positive for bacteria and fungus. Out of it 139 isolates were of Candida spp. (10.2%).
### Table 1: Age distribution of patients included in the study (n=139)

| Age interval | C.albicans | Non-albicans candida | Total | P value |
|--------------|------------|----------------------|-------|---------|
| 0-20         | 8          | 20                   | 28    | <0.05   |
| 21-40        | 8          | 09                   | 17    |         |
| 41-60        | 19         | 16                   | 35    |         |
| 61-80        | 15         | 28                   | 43    |         |
| 81-100       | 7          | 09                   | 16    |         |
| Total        | 57         | 82                   | 139   |         |

### Table 2: Sex distribution of patients included in the study (n=139)

| Sex       | C.albicans | Non-albicans Candida | Total | P value |
|-----------|------------|----------------------|-------|---------|
| Male      | 25         | 62                   | 87    | <0.05   |
| Female    | 32         | 20                   | 52    |         |
| Total     | 57         | 82                   | 139   |         |

In the study, majority of *Candida* isolates were from patients of more than 50 years of age group. Maximum 43 patients were in the age group of 61-80 years, 35 patients in 41-60 years. Out of 28 patients of 0-20 Years age group, twenty patients were infants. From the above distribution, candida infection appears to be more frequent at extreme of age group.

This study showed isolation of *Candida* from clinical specimen had male preponderance. Out of 139 patients 87 were male and 52 were female making male: female ratio 1.67:1.

### Table 3: Distribution of *Candida* spp. in different clinical isolates (n=139)

| Specimen     | Urine | Sputum | Invasive devices | Blood | Pus | Other* | Total |
|--------------|-------|--------|------------------|-------|-----|--------|-------|
| C.albicans   | 08    | 23     | 11               | 06    | 05  | 04     | 57(41%)|
| C.tropicalis | 27    | 10     | 12               | 10    | 07  | 02     | 68(48.9%)|
| C.glabrata   | 03    | 00     | 01               | 02    | 00  | 06     | 06(4.3%)|
| C.krusei     | 00    | 00     | 00               | 02    | 00  | 00     | 02(1.4%)|
| C. famata    | 00    | 00     | 00               | 02    | 00  | 00     | 02(1.4%)|
| C. kefyr     | 00    | 01     | 00               | 00    | 00  | 00     | 02(1.4%)|
| Other Candida| 00    | 00     | 01               | 01    | 00  | 00     | 02(1.4%)|
| Total        | 38    | 35     | 24               | 22    | 12  | 06     | 139   |

(* Other specimen include are bodyfluids, endotracheal secretion, oral swab.)

As shown in table no 3, infection due to non-*albicans Candida* was more common than *C.albicans*. *C.tropicalis* was most common isolate. Out of 139 specimens, *C.albicans* isolated was 41% (n=57) whereas *C.tropicalis* was 48.9% (n=68), *C.glabrata* 4.3% (n=6). Among rare species two each of *C. famata, C.krusei* and *C. kefyr* were also isolated. Two *Candida* spp. were unidentified. Maximum isolates were from urine which constituted 27.3% (n=38), followed by sputum 25.2% (n=35).
Predisposing factors | Number (n=139) | Percentage |
--- | --- | --- |
Age>50 years | 82 | 60.6% |
Age<1 year | 16 | 11.7% |
ICU | 108 | 77.7% |
Diabetes mellitus | 60 | 43.6% |
Indwelling devices | 124 | 89.3% |
Steroids | 53 | 38.3% |
Surgery | 43 | 30.8% |
Neoplasia | 18 | 12.8% |
Antibiotic use | 96 | 69.1% |
Others | 47 | 34% |

Table 4: Distribution of risk factors present in patients included in the study (n=139)

Out of 139, 82 isolates were from the patients of more than 50 years of age and 16 were less than one year of age. The most common associated predisposing factor was indwelling catheters (89.3%), followed by previous antibiotic therapy (69%). Other associated factors were presence of diabetes mellitus (43.6%), steroid users (38.3%), post-surgical patient (30.8%) and neoplasia (12.8%). Other risk factors were pregnancy, premature delivery, low birth weight babies, babies with congenital anomalies and endocrineopathies other than diabetes mellitus like hypothyroidism and hypoparathyroidism. In the present study, maximum isolates were from different intensive care units of the hospital. 77.7% (n=108) patients were from ICU whereas 30.8% (n=33) were from general wards.

| Antifungal Agent | Candida albicans (57) (a) | Candida Tropicalis (68) (b) | Other non-albicans Candida (14) (c) | Non-albicans Candida (b+c)(82) | Total | Results |
|--- | --- | --- | --- | --- | --- | --- |
| **Amphotericin B** | | | | | | |
| Resistant | 0 | 7(10.3%) | 2(14.2%) | 9(11%) | 9(6.4%) | |
| Sensitive | 57 (100%) | 61(89.7%) | 12(85.8%) | 73(89%) | 130(93.6%) | p<.05 |
| Intermediate | 0 | 0 | 0 | 0 | 0 | Significant |
| **Fluconazole** | | | | | | |
| Resistant | 2(3.5%) | 14(20.7%) | 6(42.9%) | 20(24.4%) | 22(15.2%) | p<.05 |
| Sensitive | 55(96.5%) | 52(76.4%) | 6(42.9%) | 58(70.7%) | 113(81.8%) | Significant |
| Intermediate | 0 | 2(2.9%) | 2(14.2%) | 4(4.9%) | 4(2.9%) | |
| **Itraconazole** | | | | | | |
| Resistant | 5(8.8%) | 22(32.3%) | 4(28.6%) | 26(31.7%) | 31(22.3%) | |
| Sensitive | 52(91.2%) | 41(60.3%) | 9(64.3%) | 50(61%) | 102(73.4%) | p<.05 |
| Intermediate | 0 | 5(5.9%) | 1(7.1%) | 6(7.3%) | 6(4.3%) | Significant |
As shown in Table 5, two (3.5%) of 57 Candida albicans was resistant to Fluconazole and five (8.8%) were resistant to Itraconazole whereas all Candida albicans were sensitive to Amphotericin B, Voriconazole and Flucytosine. Two isolates of Candida albicans had a dose dependent susceptibility to both Voriconazole and Flucytosine.

In contrast to this seven (10.3%) of 68 Candida tropicalis isolated were resistant to Amphotericin B, 14(20.6%) were resistant to Fluconazole, 22(32.3%) were resistant Itraconazole, 16(23.5%) were resistant to Voriconazole and four (5.8%) were resistant to Flucytosine. Out of the remaining isolates of Candida tropicalis, two had dose dependent susceptibility to Fluconazole and Flucytosine respectively whereas five had dose dependent susceptibility to Itraconazole.

Among the other Candida isolates, out of 14 AmphotericinB was resistant in 2(14.2%), Fluconazole in 6(42.9%), Itraconazole 4(28.6%), Voriconazole 2(14.2%) and Flucytosine in 3(21.4%) cases.

**DISCUSSION:** In the present study it was observed that candida infection is most common in people above 50 years of the age (60.6% as shown in table 4) which match with the findings of the study conducted by Pinto Resende et al who described that this infection were prevalent in population above 60 year of age.13

In the present study there was a male preponderance with a male: female ratio of 1.67:1 which is comparable to various other studies which have shown male: female ratio ranging from 2.3-3.14,15

It is very important to carry out the correct species identification of clinical candida isolates because of variation in both distribution and susceptibility profiles according to the hospital, underlying diseases, clinical specimen analyses and geographical region in which the studies were conducted.8,9,12

In this present study, we observed that prevalence of non-albicans Candida spp. was more than that of C. albicans, which is consistent with other published reports from different parts of world.16,17,18 This is in contrast to the earlier studies in which C. albicans was predominant over non-albicans Candida.19,20 This indicates emergence of non-albicans Candida as human pathogen.

In this study, C. tropicalis was the most common isolate, followed by C. albicans which is concordant with the other studies (24). C. tropicalis has been emerging as a new opportunistic pathogen to cause severe invasive disease owing to greater capacity to invade deeper tissue. Several studies have shown that positive culture of C. tropicalis to be highly predictive of subsequent systemic infection.22
After C. tropicalis and C. albicans, C. glabrata was the third most common Candida spp. isolated (4.2%) which is similar to that of other studies.\textsuperscript{19,23} But it was also very low in comparison to other studies done outside India which reported 8.9% to 36% of C. glabrata.\textsuperscript{20,24}

In the present study most of the candida isolates were from urine specimen than from other specimens (Table-3), which was comparable with study done by Capoor et al\textsuperscript{16} and Yang et al\textsuperscript{20}. In most of the studies C. albicans was the most predominant species isolated from urine followed by C. tropicalis.\textsuperscript{23} But in this study C. tropicalis was most common isolates from urine (27 out of 38 isolates).

In the present study Candidiasis was more common in extremes of the age group due to decrease immune status, which is in concurrence with other studies also.\textsuperscript{13,14} Diabetis mellitus is a known predisposing factor for candidiasis because hyperglycemia increases the adherence of yeast to mucoepithelial surface. In present study 43.6% were diabetic which is similar to several other studies.\textsuperscript{16,25}

Due to ability of the candida to form biofilm over indwelling devices, candidiasis most commonly occurs in patients with these devices. In the present study 89.3% candida isolates were associated with indwelling devices. Similar finding were reported with other studies.\textsuperscript{13,16}

In present study 69% of cases were associated with previous antibiotic use. As the antibiotics eliminate normal bacterial flora and allow candida to proliferate freely. Other studies\textsuperscript{13,16,25} shows similar finding. Neoplasia and corticosteroid usage and major surgical cases, prolonged ICU stays were found to be major risk factor for candida infection in this study (12.8%, 33.8%, 30.8% and 77% respectively) which was comparable with other studies.\textsuperscript{9,16,26,27}

**ANTIFUNGAL RESISTANCE:** Antifungal susceptibility is a comparatively newer concept which is not done widely in routine microbiology laboratory. In most of the cases clinicians are treating the patient empirically and indiscriminately due to easy availability of oral azoles which leads to development of antifungal resistance and shifting of classical C. albicans to non-albicans Candida.

In the presence study C. albicans isolates had a 100% susceptibility to Amphotericin B, a polene antifungal which was similar to the finding of Papas et al\textsuperscript{28} and Guisiano et al.\textsuperscript{29} In constrast to it, Yang et al\textsuperscript{20} and Capoor et al\textsuperscript{16} detected 0.2 % and 4.3% resistant of C. albicans to Amphotericin B respectively. The reason for high susceptibility in our study may be because of less prescription of Amphotericin B by clinician due to high toxicity and its availability in parenteral form. On the other hand, in the present study the resistance of C. tropicalis to Amphotericin B was 10.8% which was high in comparison to other studies by Yang et al\textsuperscript{20} and Capoor et al\textsuperscript{16} which reported 4.9% and 6.1% resistance respectively.

Over all resistance of Candida to AmphotericinB in the present study was 6.4% (9/139) as shown in table 5, which was comparable to other study which reported 2.5 to 16% resistance.\textsuperscript{16}

Fluconazole is most commonly used antifungal drug due to less toxicity and versatility of oral or intravenous administration. However, acquired or intrinsic resistance to it has been reported. In the present study, resistance of C. albicans to fluconazole was 2.6% which was comparable to other study in which 2.4% to 2.8% resistance was reported.\textsuperscript{16,24,30}

Higher degree of resistance was observed in C. tropicalis (21.7%) than C. albicans in this study where as other study reported 3.9% to 53.7% restistance.\textsuperscript{16,19,20,24} This is clinically significant as it develop resistance rapidly.\textsuperscript{19}
In the present study there was no resistant detected for *C. albicans* whereas *C. tropicalis* showed a resistance of 23.9% for voriconazole. This is in contrast to the previous study which showed candida was 20% resistant in India whereas the global prevalence reported was 0-2.2%.30

In our study resistance of Candida against Itraconazole was more in comparison to other azole. *C. albicans* was 10% and *C. tropicalis* was 33.3% resistant to Itraconazole which is comparable to other study which shows resistance of *C. albicans* ranging from 0-10% and *C. tropicalis* from 0-21.1%.16,24,31 These findings suggest emergence of Itraconazole resistance *C.tropicalis*.

In the present study the resistance to Flucytosine in *C.albicans* was 0%, *C. tropicalis* was 5.8% (4 out of 68) and other non *albicans Candida* was 21.4% (3 out of 14). The result shows that the candida isolates were less resistance than other study which describe 0-3% resistance in *C. albicans* and 7-30% resistance in *C.tropicalis.*31-34

**CONCLUSION:** Fungal infections and resistance to antifungal agents have been identified as an important problem. Extreme of ages, ICU stay, antimicrobial therapy, indwelling devices have been observed as important predisposing factors. Among yeast and yeast like pathogens non-*albicans Candida* seems to be an emerging pathogen. Drug resistance in *Candida spp.* especially to the azoles may be due to the prophylactic antifungal therapy and intrinsic resistance. So the continuous surveillance of fungal infections and their resistant pattern is recommended. Screening of fungal infection in hospitalized patients should be done. Strict enforcement and monitoring of antifungal prescribing policy is the urgent need of hour.

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