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Original Research Article

Prevalence of Azole Resistance in Various *Candida* Species in Various Sample Along with Its Antifungal Susceptibility Pattern in a Tertiary Care Hospital, Vadodara

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Abstract:

Introduction: *Candida spp*. is the fourth most common cause of nosocomial bloodstream infections and the third leading cause of urinary catheter related infections. In recent years there has been an increasing trend in the emergence of *Candida non albicans* as a potential pathogen responsible for nosocomial infections as well. The in vitro 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 Antifungal agents.

Aims and Objectives: To study the prevalence of Azole resistance in various *Candida species*. To study the antifungal susceptibility pattern of various *Candida species*.

Material & Method: Various samples like urine, blood, cerebrospinal fluid, pus, tissue, sputum, body fluids, vaginal swab, broncho alveolar lavage fluid were taken from clinically suspected cases for culture and antifungal sensitivity testing was done according to CLSI guideline. A total of 90 *Candida spp.* were isolated from these samples, which were included in this study.

Result: Out of 3137 culture positive isolates, 90 were *Candida species*. Prevalence rate of *candida spp*. is 2.86%. Out of total 90 isolates, 77% (69 isolates) were *Candida non albicans* and 23% (21 isolates) were *Candida albicans*. The rate of isolation of Candida was more in Males (51%) than Female (49%). The most frequently isolated species was *Candida krusei* (32%). Highest number of *Candida* was isolated from the Urine (51%) & in the infants (44%). Maximum *Candida* species were isolated from the Extramural-NICU (39%). All *Candida* species were highly sensitive to Voriconazole and highly resistant to Amphotericin B.

Conclusion: The changing epidemiology of Candidiasis, therefore highlights the need for close monitoring of *Candida* species distribution and susceptibility in order to optimize therapy and outcome. We should also develop the guideline for empirical therapy based on epidemiology of India. *Candida non-albicans* species were more resistant to azoles compared to *C.albicans*, information that can be useful for clinicians dealing with non - responding cases.

Keywords: *Candida albicans, Candida non-albicans,* Antifungal Susceptibility Pattern.

Introduction

Candidiasis is the commonest fungal disease affecting mucosa, skin, nails and internal organs. It is caused by various species of yeast like fungi belonging to genus *Candida*.

The infection may be acute or chronic, superficial or deep and its clinical spectrum is wide. It is found mainly as secondary infection in individuals with some underlying immunocompromised condition and very rarely as the primary disease.[1]

Candida species was initially known only to be a part of normal flora of humans, however due to certain factors, the incidence of pathogenic *Candida* species have been increased. The various predisposing factors which precipitate these infections are:

- Natural receptive states like infancy, old age, pregnancy.
- Changes in local bacterial flora secondary to antibiotics.
- Changes in epithelial surface due to moisture, trauma.
- Primary or Secondary T-lymphocytes defects due to underlying disease e.g immunosuppression or AIDS.
- Endocrine diseases like Diabetes Mellitus
- Miscellaneous condition like Zinc or Iron deficiency.[1]

In recent years there has been an increasing trend in the emergence of *Candida non-albicans* as a potential pathogen responsible for nosocomial infections as well. These *Candida nonalbicans* species are found to be more resistant than *Candida albicans* to Antifungal drugs. [2,3,4,5]

There are 163 anamorphic species of genus *Candida* with telemorphs in at least thirteen genera. Nearly twenty of these species are considered to be significant pathogen causing various infections in the human beings and out of these seven are well-known opportunistic pathogens.[1] Some of these species are *Candida albican, Candida tropicalis, Candida krusie, Candida glabrata, Candida guilliermondi, Candida parapsilosis, Candida keyfer, Candida dubliniensis, Candida rugosa, Candida viswanathii, Candida lusitaniae.*

In vitro 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, flucytosine, azoles group of drugs and nystatin.[6] Systemic infection due to *candida* and resistance to antifungal agent is on rise in Indian Hospitals.[7] *Candida* albicans accounts for 40-60% isolated in developed countries[8] whereas in Indian report show increased predominance of *Candida non albicans* isolates.[7]Study from India has shown very high resistance to voriconazole and fluconazole. Some study from India have shown that the *Candida albicans* isolates were 100% susceptible to Amphotericin B and fluocytosine and Certain species like *Candida* krusei and *Candida glabrata* had shown high degree of resistance to Fluconazole[9,10].

Therefore, the species identification of the *Candida* isolates, along with their anti-fungal susceptibility patterns can influence the treatment options for the clinician and have a beneficial impact on the patient care.

Material & Methods

A descriptive-cross sectional study was carried out at Department of Microbiology, Medical College Baroda & S.S.G. Hospital, Vadodara from ethical approval to September 2022.A total of 90 fungal culture positive isolates from all the samples received from different wards and ICUs of various department of SSG Hospital and Medical College Baroda, Vadodara were included in this study. As this study starts from Ethical approval to September 2022, 3137 samples showing growth in culture media from the various samples were screened for Candidiasis.

Inclusion Criteria: Various clinical specimens-Blood, urine, and Miscellaneous samples like (vaginal swabs, body fluids, wound, pus) of all age patients having clinical infection, received for culture & sensitivity test in Department of Microbiology, Medical College Baroda.

Exclusion Criteria [12]:

- Unlabeled& improperly labelled specimen.
- Specimen that have leaked out of container.
- Specimen received in non-sterile container.
- Sputum specimen with >25 squamous epithelial cells/low power filed.
- A dried-out swab is received or material collected is insufficient in volume.
- 90 *Candida* species isolated from various samples like urine, blood, Cerebrospinal fluid, pus, tissue, sputum, body fluids, vaginal swab, Broncho alveolar lavage fluid were taken from clinically suspected cases.

All specimens were transported to the laboratory as soon as possible and process promptly. Well mixed specimen is inoculated on Brain Heart Infusion agar & MacConkey agar incubated at 37°C aerobically. All inoculated plates were observed next day. Growth on Brain heart infusion agar sub cultured on Sabouraud's dextrose agar.

Colony Morphology: On Brain heart infusion agar the colonies of *Candida* were large creamy moist & white in color when incubated for 24-48 hours at 37°C. On Sabouraud's dextrose agar *Candida* produce creamy moist colony when incubated for 24-48 hours at 37°C. The isolates of *Candida* were confirmed by Gram stain, Germ tube formation, Color on CHROM agar Candida medium, Clamydospore formation on Corn meal agar, Sugar fermentation test, Sugar assimilation test, Urease test.

Figure 1: Colony of C. albicanson Sabouraud's Dextrose Agar

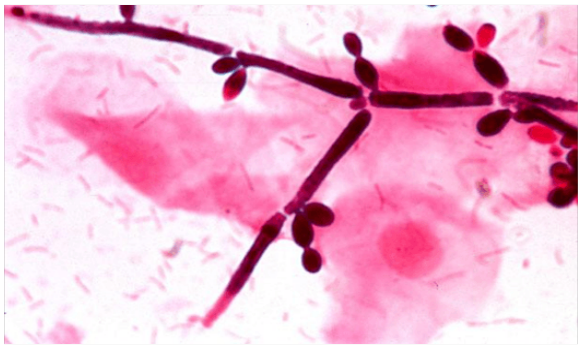

Figure 2: Gram Staining ofC.albicans

Figure 3: Chlamydospore formation of C.albicnas

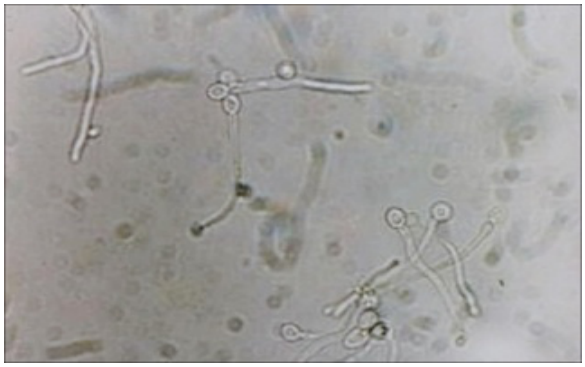

Figure 4: Germ Tube Formation

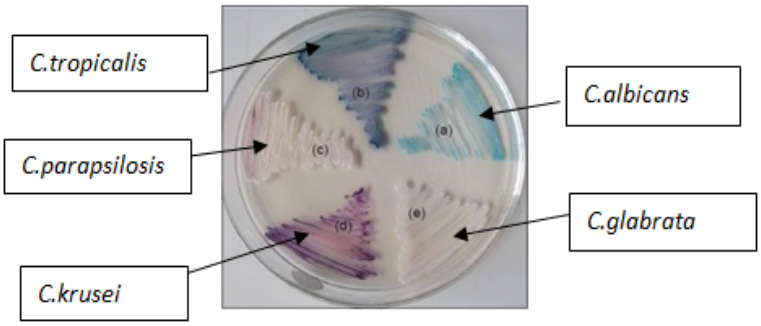

Figure 5: *Candida* species on CHROM Agar Medium

Sugar fermentation test: Colonies of *Candida* species were inoculated in tubes containing sugar medium and incubated for 24 hours at 30°C. Sugar fermentation was noted by change in colour of sugar medium and gas production was noted by production of gas bubbles in the inverted Durham's tube.

Sugar assimilation test: The organisms were sub cultured on Nutrient agar 24 hour before performing the test. Yeast Nitrogen Base agar plates were prepared using Yeast Nitrogen Base agar containing 6.7% Nitrogen and 2% agar.

Disc diffusion testing of antifungal drug was performed as described in CLSI [document M44-A(2)]. Antifungal drugs used for *candida* isolates are antifungal drug disc Ketoconazole (10ug), Fluconazole (10ug), Clotrimazole (10ug)

Voriconazole(1ug), Amphotericin B(50ug), Nystatin (50ug).

The diameters of inhibition zone were measured by use of zone measuring scale or by placing the plate against a ruler and report the results as "sensitive "and "resistant". The interpretive criteria for antifungal drug's disk diffusion testing are those published by the CLSI.

Results

In the present study the total number of clinical specimens processed in the Microbiology laboratory of Medical College Baroda & S.S.G Hospital was 10,716 during the study period of December 2021 to September 2022.

Out of which 3137 culture positive isolates were obtained. Out of 3137 culture positive isolates,90 were *Candida* species. Thus prevalence of *Candida* spp. in the present study is 2.86%.

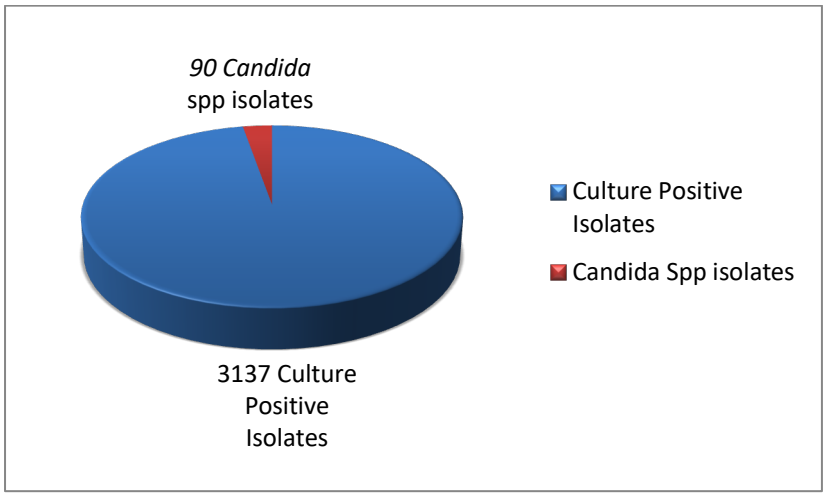

Chart 1: Isolation of *Candida spp* among culture positive isolates

Table 1: Distribution of *Candida albicans* and *Candida non albicans* in various clinical samples

Candida isolates	No. of Candida positive samples	Percentage (n=90)
Candida albicans	21	23%
Candida non albicans	69	77%

Out of total 90 isolates, 77% (69 isolates) were *Candida non albicans* and 23% (21 isolates) were *Candida albicans*.

Table 2: Distribution of *Candida* Species within different age groups

Age groups	*C.albicans*	*C.tropicalis*	*C.krusei*	*C.parapsilosis*	*C.glabrata*	Total
Infant	1	5	23	2	9	40(44%)
Children	6	2	1	2	6	17(19%)
Adults	14	8	4	0	7	33(37%)
Total	21	15	28	4	22	90

In this study, *Candidiasis* was most common in the infants (44%) followed by adults (37%) followed by children (19%).

Table 3: Gender wise distribution of patients with *Candida* isolates

Gender	*C.albicans*	*C.tropicalis*	*C.krusei*	*C.parapsilosis*	*C.glabrata*	Total
Male	7	10	15	2	12	46(51%)
Female	14	5	13	2	10	44(49%)
Total	21	15	28	4	22	90

The rate of isolation of *Candida* was more in Males(51%) than Female (49%).

Table 4: Ward wise distribution of *Candida spp.* isolates

Ward	*C.albicans*	*C.tropicalis*	*C.krusei*	*C.parapsilosis*	*C.glabrata*	Total (n= 90)
NICU	0	3	22	2	8	35(39%)
PICU	4	2	0	2	6	14(16%)
Pediatric ward	3	1	2	0	1	7(8%)
Medicine	9	7	3	0	1	20(22%)
TB ward	2	2	0	0	1	5(6%)

ICCU	0	0	0	0	1	1(1%)
MICU	1	0	1	0	2	4(4%)
SICU	0	0	0	0	1	1(1%)
Surgical ward	0	0	0	0	1	1(1%)
Obstetrics & Gyne-cology Ward	2	0	0	0	0	2(2%)

Maximum Candida species isolated from the Extramural-NICU (39%) followed by Medicine ward(22%), Pediatric ICU (16%), Pediatric ward (8%), TB ward (6%), MICU (4%) and Gynecology ward (2%). In present study maximum number of Candida were isolated from Intensive Care Units (61%). C.albicans & *C.tropicalis* were maximum isolated from Medicine ward. *C.krusei* & *C.glabrata* were maximum isolated from Extramural-NICU whereas *C.parapsilosis* were maximum isolated from the pediatric –ICUs.

Table 5: Distribution of *Candida* species among various types of specimens

Specimen	***C.albicans***	***C.tropicalis***	***C.krusie***	***C.parapsilosis***	***C.glabrata***	**Total**
Blood	0	3	20	2	10	35(39%)
Urine	18	11	6	2	9	46(51%)
Sputum	3	1	1	0	0	5(6%)
CSF	0	0	0	0	1	1(1%)
Tracheal secretion	0	0	1	0	0	1(1%)
Pleural fluid	0	0	0	0	1	1(1%)
Ear discharge	0	0	0	0	1	1(1%)
Total	21	15	28	4	22	90(100%)

In the present study, highest numbers of *Candida* were isolated from the Urine (51%) followed by Blood (39%), sputum (6%) and other specimens.

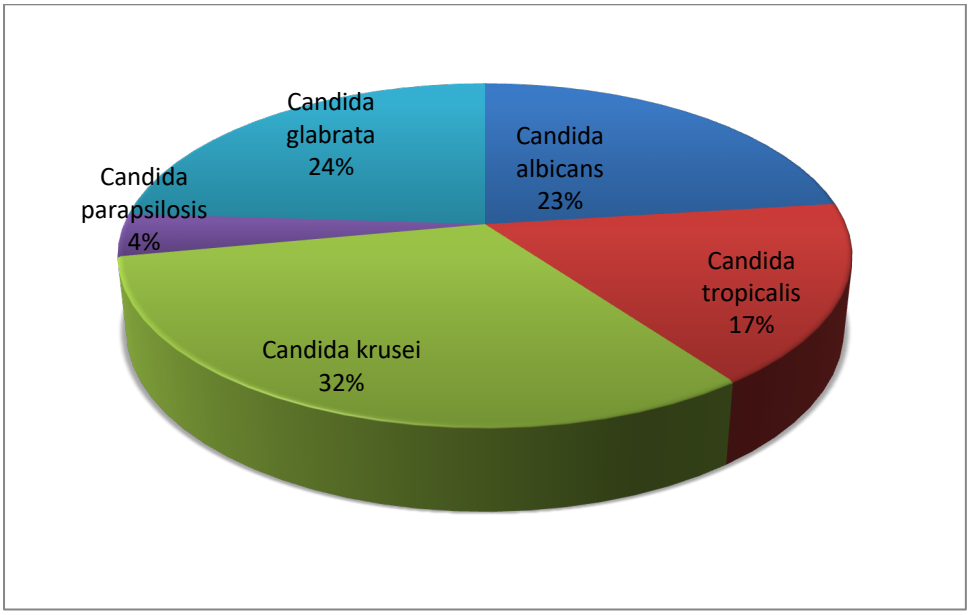

Chart 2: Distribution of Different species in Total Specimens

In this study, most frequently isolated species was *Candida krusei* (32%) followed by *C.glabrata* (24%), *C.albicans* (23%), *C.tropicalis* (17%) and *C.parapsilosis* (4%).

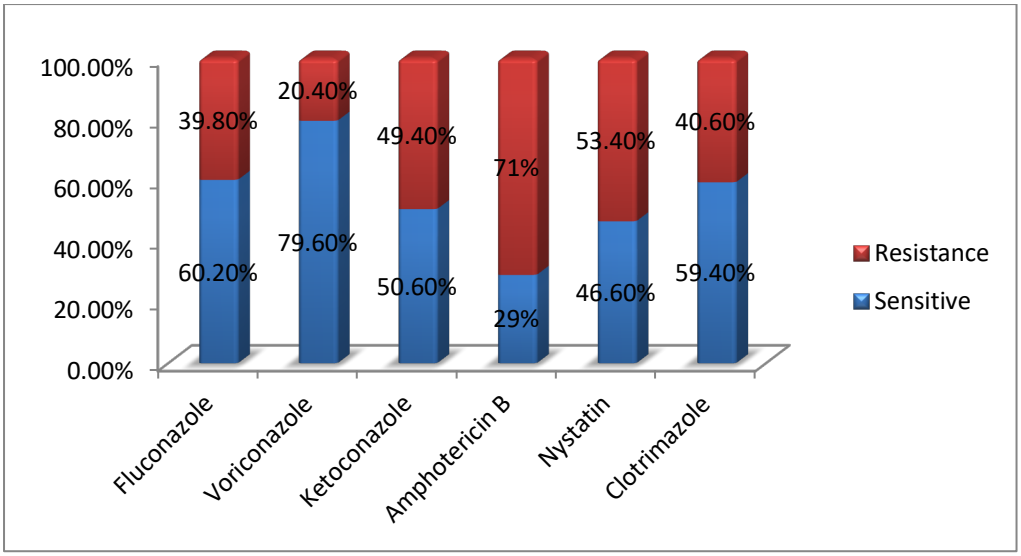

Chart 3: Antifungal Susceptibility pattern of all *candida* isolates

Azole resistance in our study was highest in Ketoconazole (49.4%) followed by Clotrimazole (40.6%), Fluconazole (39.8%) and Voriconazole (20.4%).

Discussion:

Candidiasis is a primary or secondary infection which involves members of the genus *Candida*. The clinical manifestations of the disease are extremely varied, ranging from acute, subacute and chronic to an episodic involvement. It may be localized to the mouth, lungs or the gastrointestinal tract, or may become systemic as in septicemia, endocarditis and meningitis.[1] The frequency of life-threatening fungal infections is rising worldwide. Considering that most patients infected with opportunistic fungal agents have AIDS or neoplastic and for degenerative diseases, it is Clear that effective antifungal therapy is critical[13].

Patients admitted at tertiary care hospitals have access to very intensive management modalities. This along with increasing number of immunocompromised patients have lead to rise in infections caused by *candida* especially by non albicans *candida*[11,14,15] to variable clinical presentation of *candida* infections, it becomes very important to identify this pathogens from all the routine culture specimens received at laboratory irrespective of clinician's suspicion. Thus identification of *candida* upto species level along with antifungal susceptibility becomes very essential.

In our study, out of the total 90 isolates, 77% were of *Candida* non albicans and 23% isolates were *Candida* albicans which was in agreement with the findings of the studies by Mokaddas EM et al[16] who also showed that *Candida* non albicans incidence (60.5%) to be higher than that of C. albicans (39.5%) [16] and Barot N et al[17] study highest incidence was in *candida* non albicans (56%) than *candida* albicans (44%). C. non albicans was the most frequently isolated species. Many other species of *Candida* are also being reported in the current literature[18,19]. In this study, the most frequently isolated species was *C. krusei* (32%) of the infections, followed by *C. glabrata* (24%), followed by *C. albicans* (23%) and *C.tropicalis* (17%) and C. *Parapsilosis* (4%). Comparative studies on different *Candida* species which were isolated in their studies by different researchers showed that the isolation of C. albicans was the highest in Destidher SG et al[23] (72.8%), and Gupta N et al[22] (45.8%%), Mokaddas EM et al[16] (39.5%) study, except in Chakrabarti A et al[20] and Pethani JD et al[21] which showed that the isolation of *C.tropicalis* was the highest (42%) in Chakrabarti A et al[20]and (43%)in Pethani JD et al[21]

Though Candidiasis can occur at all ages, studies by Dalal PJ et al[24]at Mumbai showed the highest incidence of candidiasis to be in the age group of 21-40 years. In this study, Candidiasis is most common in the Infants (44%) followed by adults (37%) followed by Childrens (19%). Which is also differ from Saldenha Dominic S et al[6] & Pethani JD et al[21], in these studies Candidiasis was most common in adult.

In our study, the incidence of candidiasis was higher in male (51%) than in females (49%). Which is differ from study by Kandhari KC et al[25], the incidence was found to be higher in females (61.2%) than in males (38.8%). Maximum *Candida* species isolated from the Extramural-NICU (39%) followed by Medicine ward (22%) followed by Pediatric ICU (16%) followed by Pediatric ward (8%) followed by TB ward (6%) followed by MICU (4%) followed by Gynecology ward (2%). In our study maximum number of *Candida* isolated from Pediatric Intensive

Care Units(61%). In other studies (Dominic S et al[6] & Pethani JD et al[21]) similar result found that maximum number of *Candida* isolated from Intensive Care Units. In the present study highest number of *Candida* isolated from the urine samples (51%) followed by the blood samples (59%) followed by sputum samples (6%) followed by other specimens, which was different from other studies. In Dominic S et al[6] highest number of *Candida* isolated from High Vaginal Discharge followed by blood and followed by Urine. While in Pethani JD et al[21] highest number of *Candida* isolated from blood and urine followed by other specimen.

In our study, Antifungal Susceptibility patterns revealed that Voriconazole has excellent in vitro activity against various *Candida* species with 79.6% susceptibility. In our study *Candida* isolates shows 49.4% resistance to Ketoconazole, 40.6% resistance to Clotrimazole, 39.8% resistance to Fluconazole, 20.4% resistance to Voriconazole. In Barot N et al[17] 26.5% resistance to Fluconazole, 5.12% resistance to Voriconazole, only 0.3% resistance to Amphotericin B. In other studies (Dharwad S et al[26] & Oberoi JK et al[27]) similar result found that maximum resistance to Fluconazole 32% and 21.1% respectively.

Conclusion

Candida species was initially known only to be a part of normal flora of humans. But the incidence of the *Candida* albicans and *Candida* non albicans as the pathogen came into picture. Now according to recent studies it's quite clear that *Candida* non albicans is a major cause of pathogenicity. This Study highlights the emergence of non-albicans *candida* as major isolates and alarming Voriconazole resistance in C. non albicans.

The changing epidemiology of Candidiasis therefore highlights the need for close monitoring of *Candida* species distribution and susceptibility in order to optimize therapy and outcome. We should also develop the guideline for empiric therapy based on epidemiology of India. *Candida* non-albicans species were more resistant to azoles compared to albicans, information that can be useful for clinicians dealing with non - responding cases. Thus, eliciting history of exposure to these drugs may be important in choosing appropriate therapy.

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