

Speciation and antifungal susceptibility testing of *Candida* species isolated from clinical samples



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ABSTRACT

Background: The importance of epidemiological monitoring of yeasts involved in pathogenic processes is unquestionable due to the increase in trend of infections caused by various species of *Candida* over the last decade; so are the changes observed in species causing Candidiasis and empirical antifungal treatment. **Aims and Objective:** To speciate the clinically isolated *Candida* species by phenotypic methods and to estimate the antifungal susceptibility of the isolated species against fluconazole, ketoconazole, voriconazole, itraconazole by disc diffusion method. **Materials and Methods:** A cross-sectional study conducted in 2018 in the Department of Microbiology, J. N. Medical College, KAHER, Belagavi Karnataka. Ethical Clearance was obtained from institutional ethical committee J.N. Medical College. **Results:** Out of 59 *Candida* isolates, *Candida tropicalis* was the predominant species 41(69.49%), followed by *Candida glabrata* 5 (8.47%), *Candida parapsilosis* and *Candida lusitanae* 4 (6.78%) respectively, *Candida guilliermondii* and *Candida kefyr* 2 (3.39%) respectively and the least one was *Candida krusei* 1 (1.69%). Voriconazole showed the highest level of sensitivity whereas Itraconazole has shown the least sensitivity pattern by disk diffusion method. Out of 59 *Candida* species, 52 (88.13%) were sensitive to Voriconazole, 44 (74.57%) were sensitive to Fluconazole, 40 (67.79%) were sensitive to Ketoconazole and the least sensitivity was shown by Itraconazole 30 (50.84%). *Candida krusei* and *Candida guilliermondii* showed 100% sensitive to Fluconazole, Voriconazole, Ketoconazole and Itraconazole respectively. **Conclusion:** *Non-albicans Candida* species are being common isolates from cases of candidiasis. *Candida tropicalis* is the predominant isolate, followed by *Candida glabrata*, *Candida lusitanae*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida kefyr* and *Candida krusei*. Most of the isolates were sensitive to Voriconazole.

Key words: Disk diffusion method; NAC; Sensitive; Predominant species

INTRODUCTION

Importance of an epidemiological monitoring of yeasts involved in pathogenic processes is unquestionable due to the increase in trend of infections caused by various species of *Candida* over the last decade; so are the changes observed in species causing Candidiasis and empirical antifungal treatment.¹ There is a rise in *non-albicans Candida* species being more prevalent with varied antifungal susceptibility. There is need for continuous surveillance of prevailing species and to

know their antifungal susceptibility so as to aid in treatment of affected patients². The present study is taken to know the prevalent species isolated from various clinical specimens and their antifungal susceptibility testing by disc diffusion technique against various antifungal agents such as fluconazole, ketoconazole, voriconazole and itraconazole. *C. albicans* is the common and major pathogen considered to cause Candidiasis among *Candida* species, more frequently the immunocompromised individuals are affected suffering from HIV, using long-term immunosuppressive therapy in

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cancer and organ transplant patients, Azole resistance were seen more commonly in *non-albicans Candida* than the *Candida albicans*, therefore, antifungal susceptibility testing will help to choose the empirical antifungal treatment and to identify to species level; it will also help the epidemiology of *Candida* species particularly the source and mode of transmission so that we can prevent and control the transmission and resistant pathogens.²⁻⁵ Over the past 2-3 decades increasing numbers of incidence due to *non-albicans Candida* species is significant although considered as normal flora of human beings at the different sites such as skin, female genital tract, gastrointestinal tract, respiratory tract etc. Due to variety of predisposing factor it alters the host immune status of the individuals which leads to invasive Candidiasis. Virulence factors of *Candida* species plays as essential role to facilitate the pathogenesis of Candidiasis such as; **Adhesion:** that interact with the surface of endothelial and epithelial cells. **Enzymes:** Phospholipase, Esterase, Proteases, Lipase plays an important role to initiate the infection. **Toxins:** These are like endotoxin in bacteria which induce the anaphylactic shock, Virulent to the patients such as-glycoproteins. **Phenotyping Switching:** In which the organisms has the ability to grow in various morphological features such as unicellular budding (blastospore) to filaments pseudo hyphae and sometimes true hyphae which helps the organisms to survive in the host at different conditions or temperature to facilitate the pathogenicity.⁶⁻⁹

MATERIALS AND METHODS

A cross sectional study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi. In the year 2018 from January 2018 to December 2018, isolates were collected from clinical samples by Universal sampling procedure where all the Isolates from patients with Invasive Candidiasis, Suspected cases of Candidiasis were included. Once the isolates of Gram positive yeasts were identified in the sample, the smears of the samples were reviewed by the investigator. The sputum samples were graded by Bartlett's scale. If the sample was graded between '1' to '2' the samples were included in the study. The patient's clinical diagnosis was also considered before including the isolate¹⁰. The vaginal samples' smear was graded as per Nugent's criteria. Score of 7-10 was taken as diagnostic of Bacterial Vaginosis *Candida* spp. Thus isolated was regarded as co-infecter.¹¹ Germ tube test is done for presumptive identification of *Candida* species which differentiate between *Candida albicans* (positive) from *non-albicans Candida* species (negative) based on the production of tube like filaments at the point of origin, are not constructed in case of *Candida albicans* whereas constriction is seen in *non-albicans Candida*.¹² Rapid method for cultivation and identification of *Candida* species. Distinguished between divers *Candida* species based on morphological features.^{8,13,14} Antifungal susceptibility testing

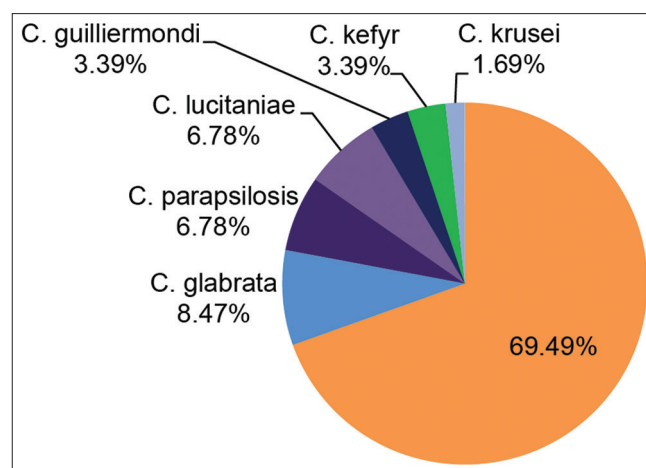
plays an essential role to establish an appropriate antifungal drug to treat specific fungal infection. This was done for all the 59 isolated *Candida* species, antifungal disc were used from Hi-Media (India), which were commercially prepared. Antifungal susceptibility testing was performed as per CLSI for antifungal (formerly NCCLS) M44-A2 guideline which recommends Muller Hinton Agar, 2% Glucose with 5µg/ml Methylene blue. After 48 hours of incubation the zone of inhibition around the disc is measured.^{3,15,16}

RESULTS

In Graph 1, among 59 *Candida* isolates *Candida tropicalis* was the predominant species 41 (69.49%), followed by *Candida glabrata* 5 (8.47%), *Candida parapsilosis* and *Candida lusitanae* 4 (6.78%) respectively, *Candida guilliermondii* and *Candida kefyr* 2 (3.39%) respectively and the least one *C. krusei* 1 (1.69%).

In Table 1, our study out of 59 clinical samples sputum was 28.81% followed by vaginal swab 27.11% and the least collected samples cervical swab, Broncho-alveolar lavage and stool were 3.38% followed by Sub hepatic fluid, oral tissue and skin scrapping were 1.69%.

In Tables 2 and 3, our study Voriconazole showed the highest level of sensitivity whereas Itraconazole had shown the least sensitivity pattern by disk diffusion method. Among all the isolated 59 *Candida* species, 52 (88.13%) were sensitive to Voriconazole, 44 (74.57%) were sensitive to Fluconazole, 40 (67.79%) were sensitive to Ketoconazole and the least sensitivity was shown by Itraconazole 30 (50.84%). Among 41 (69.49%) isolates of *Candida tropicalis* 37 (90.24%) were sensitive to Voriconazole, 31 (75.61%) were sensitive to Fluconazole, 28 (68.29%) were sensitive to Ketoconazole and 22 (53.22%) were sensitive to Itraconazole. *Candida krusei* and *Candida guilliermondii*



Graph 1: Distribution of isolates in percentage

showed 100% sensitive to Fluconazole, Voriconazole, Ketoconazole and Itraconazole respectively.

to our study.²¹ Various study in the past has shown the increasing numbers of non-albicans *Candida* species were

Figure 1 and 2 Antifungal Susceptibility testing.

DISCUSSION

A total of 59 isolates were processed from the samples received in the Department of Microbiology of Jawaharlal Nehru Medical College, KAHER University, Belagavi. Among isolates *Candida tropicalis* was found to be the most predominant species whereas *Candida krusei* is the least to be isolated. In our study *Candida tropicalis* being the most predominant species 41 (69.49%). Similar studies done by Bhawana et al,³ Singh R et al¹⁷ have reported *Candida tropicalis*, ranging between 26-59%, being the major isolate.^{3,14,17}

A study carried out by Khadka. S et al¹⁸ 2014-15 Nepal has found that *Candida tropicalis* is the major isolate among non-albicans *Candida* followed by *Candida glabrata* which is similar

Table 1: Distribution of collected numbers of samples among the clinical specimens

S.no.	Clinical specimens	Numbers	Percentage (%)
1	Sputum	17	28.81
2	Vaginal swab	16	27.11
3	Urine	9	15.25
4	Wound swab	4	6.67
5	Long line tip	3	5.08
6	Cervical swab	2	3.38
7	Broncho-alveolar lavage	2	3.38
8	Stool	2	3.38
9	Sub hepatic fluid	1	1.69
10	Oral tissue	1	1.69
11	Skin scrapping	1	1.69
12	Nail clipping	1	1.69
Total	12	59	100%

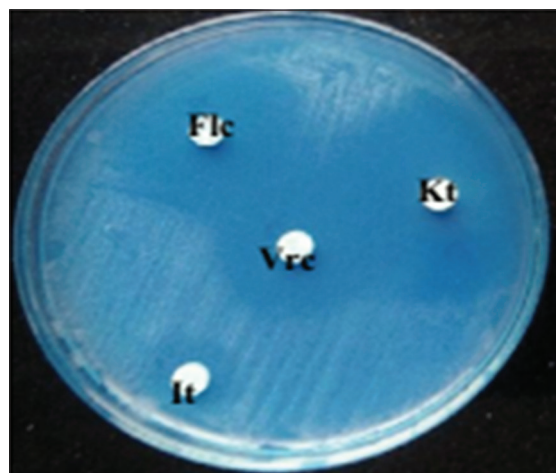


Figure 1: Sensitive to Fluconazole, Voriconazole, Ketoconazole, Resistant to Itraconazole

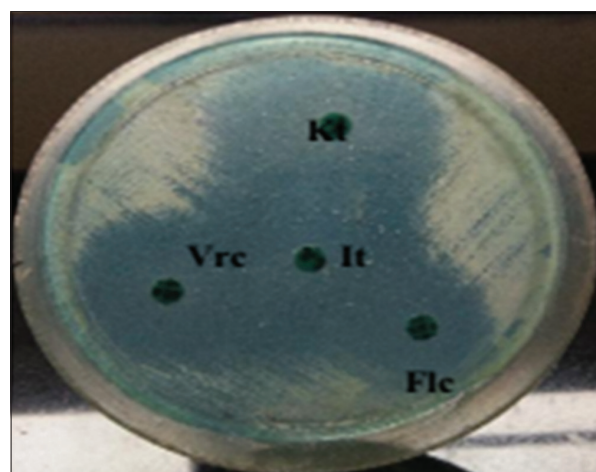


Figure 2: Susceptible to Fluconazole, Voriconazole, Ketoconazole, Itraconazole

Table 2: Antifungal susceptibility pattern of all the isolated *Candida* species

Species of candida	Fluconazole S	Voriconazole S	Ketoconazole S	Itraconazole S
<i>C. tropicalis</i> (n=41)	31 75.61%	37 90.24%	28 68.29%	22 53.66%
<i>C. glabrata</i> (n=5)	3 60%	3 60%	2 40%	1 20%
<i>C. parapsilosis</i> (n=4)	4 100%	4 100%	3 75%	2 50%
<i>C. lusitaniae</i> (n=4)	2 50%	3 75%	3 75%	2 50%
<i>C. guilliermondii</i> (n=2)	2 100%	2 100%	2 100%	2 100%
<i>C. kefyr</i> (n=2)	1 50%	2 100%	1 50%	0 0.00%
<i>C. krusei</i> (n=1)	1 100%	1 100%	1 100%	1 100%
TOTAL	44	52	40	30
PERCENTAGE	74.57%	88.13%	67.79%	50.84%

Table 3: Interpretation of zone size as per CLSI guideline¹⁶

Antifungal agents	Concentration	Zone diameter inmm	Quality control limits
S R	C. parapsilosis ATCC22019		
Fluconazole	25µg	≥19 ≤14	22 - 33
Voriconazole	1 µg	≥17 ≤13	28 - 37
Ketoconazole	15µg	≥28 ≤20	20 - 26
Itraconazole	10 µg	≥23 ≤13	26 - 35

S- Sensitive R- Resistant

isolated which is comparable to our study, moreover these findings suggest that the *non-albicans Candida* species taking over *Candida albicans* day by day as an important pathogens since past 2-3 decades. *Non albicans Candida* has got direct impact on choosing the empirical antifungal treatment.¹⁸

Other *non-albicans Candida* isolated in our study were, *Candida glabrata*, *Candida lusitanae*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida kefyr*, *Candida krusei*. It is proved in many studies that distribution of *Candida* species follows geographic distribution. Thus our study adds-up to the observation of, *Candida tropicalis*, being prevalent in causing Candidiasis, in Eastern Asia.

In our study, isolates showed highest sensitivity to Voriconazole whereas were least sensitive to Itraconazole by disk diffusion method. Of all the isolated *Candida* species, sensitivity for Voriconazole was 88.13% (52) followed by Fluconazole 44(74.57%), ketoconazole 40(67.79%) and the least sensitivity was against Itraconazole 30(50.84%). *Candida guilliermondii* and *Candida krusei*, both being single isolates were sensitive to all the tested antifungals.

In a study done by Jangla *et al*⁹ *Candida tropicalis* and *Candida parapsilosis* were 100% sensitive to Fluconazole and Voriconazole. Sebastian. A *et al*²⁰ have shown 62.2%, 58.8%, 47% sensitivity against Fluconazole, Itraconazole, Ketoconazole respectively which is nearly similar to that our study which shows 74.57% to fluconazole, 67.79% to Ketoconazole, 50.84% to itraconazole respectively.^{19,20}

Our study showed Voriconazole is an effective drug 52(88.13%) where as 100% sensitive to Voriconazole was found in a similar study done by Deepthi. T *et al*,²¹ 60% by Dharwad. S *et al*,²² 83% by Shafi F T *et al*²³ respectively.²¹⁻²³ Though Voriconazole is found to be effective in the study, the observation has to be practiced with a guard. The results need to be validated by another study where in clinical correlation would be appropriate. In our study *Candida glabrata* had shown the highest level of resistant against Itraconazole followed by Ketoconazole whereas *Candida tropicalis* had shown the highest level of

resistant to Itraconazole among all the tested antifungal agents. *Candida kefyr* showed 100% resistant to Itraconazole.

In our study, among various clinical specimens increasing numbers of *Candida* isolates were isolated from sputum samples followed by vaginal swabs and urine samples whereas the least were isolated from sub hepatic fluid, oral tissue respectively. Several studies in the past indicates the increasing growing rates of *non-albicans Candida* over *Candida albicans* is an essential clue to proper monitoring in the distribution of antifungal agents for the pathogenic *Candida* species.²⁴ *Candida* was isolated from a neonatal case repeatedly twice. The baby had diarrhoea and was on broad spectrum antibiotics thus the isolate was included in the study. The isolate was *C. tropicalis*. *Candida* is known to cause antibiotic induced diarrhea.²⁵

CONCLUSION

Candida non-albicans species are being common isolates from cases of Candidiasis. *Candida tropicalis* is the predominant isolate, followed by *Candida glabrata*, *Candida lusitanae*, *Candida parapsilosis*, *Candida guilliermondii*, *Candida kefyr* and *Candida krusei*. Most of the isolates were sensitive to Voriconazole and resistant to Itraconazole. Resistance was most commonly seen in *Candida glabrata*, *Candida kefyr* had shown 100% resistant against Itraconazole. Thus the study emphasizes the need for speciation of *Candida* isolates and recommends antifungal susceptibility for all the clinical isolates. To identify various isolates of *Candida* to their species level along antifungal sensitivity pattern is clinically significant for the appropriate choice of antifungal therapy.

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ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of Jawaharlal Nehru Medical College, KLE Academy Higher Education and Research, Karnataka, India.

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Authors Contribution:

LS- Concept and design of the study, data collection, analyzed and interpreted, reviewed the literature, manuscript preparation and critical revision of the manuscript; **SUH-** Concept, and review of literature and helped in preparing first draft of manuscript; **BB-** Prepared first draft of manuscript and critical revision of the manuscript; **KP-** Conceptualized study, literature search, statistically analyzed and interpreted, prepared first, draft of manuscript and critical revision of the manuscript and data collection.

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