

# Prevalence and Antifungal Susceptibilities of *Candida* spp from a South Indian Tertiary Care Hospital

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## Abstract

**Introduction:** *Candida* sp. is increasingly being implicated as the cause of both community acquired and Nosocomial infections. With development of new resistance patterns versus the inherent resistance present in some of the species isolated from critical care areas, the complications resulting from long term antibiotics, surgical and medical interventions and environmental colonization by the *Candida* sp. is becoming more prevalent. **Method:** Prevalence of different species and their antifungal susceptibilities grown from various samples received in 20 months were studied using semi-automated MiniAPI kits. Results of tests done on 356 isolates were collated and analysed retrospectively. **Results:** Of the 356 samples, *C. tropicalis* showed prevalence of 37.07% as compared to *C. albicans* 31.1%, *C. guilliermondii* (11.8) and *C. parapsilosis* (6.74%). *Candida* sp were found to sensitive to 5-Flucytocine and Amphotericin B. Resistance to Itraconazole was noted more than to Fluconazole. **Conclusions:** Infections with *Candida* spp are usually of low virulence and are associated with a few well-defined risk factors as immunocompromised state, Malignancy and steroid therapy. Understanding these risk factors, identifying the species with changing trends in antifungal resistance, instituting infection control practices to reduce morbidity and mortality in critical care areas can improve outcomes.

**Keywords:** Antifungal susceptibilities, Azole resistance *Candida* species, Species distribution,

## Background

Opportunistic infections by *Candida* sp are becoming quite common in hospitals today with antifungal resistance, an increasing problem in many wards. With interventions and management of diseases becoming more invasive and complicated, patients' immunity can be affected due to various reasons. Apart from immunosuppression and immunodeficiencies from underlying causes such as chemotherapy, corticosteroids, surgery, malignancy, any manipulation, intervention or prolonged hospital stay increases the risk of infections due to *Candida* sp. and other fungal pathogens [1, 2].

*Candida* is the commonest fungal pathogen and also can be a contaminant in our laboratories. It causes both invasive and mucosal infections especially in women and in patients with immunity disorders/immunity-

challenged patients. They have been isolated from a wide variety of samples especially blood sputum pus and urine. *Candida* species rarely cause infection in physiologically healthy individuals however, a host of factors can lead to *Candida* infections involving the superficial skin (cutaneous), mucosa (oral oesophageal or vaginal) or organ/ blood stream (systemic or invasive infections).

In Hospital acquired infection Candidiasis is the one of the leading causes with mortality noted between 15-35% [3]. Due to the growing size of the population at special risk (neutropenia, immunosuppression, metabolic dysfunction), *Candida* sp continues to be threat in the critical care patients with increasing number of Non-*albicans* *Candida* (NAC) being isolated.

In a study NAC species which accounted for 10%– 40% of invasive candidiasis between 1970 to 1990 has now increased to 35%–65% in the recent years [4, 2].

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A majority of infections are commonly attributed to five species—*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, the species varying from region to region in its predominance. Speciating the *Candida* isolates is very critical in the management of these diseases as different species vary in their susceptibilities to the antifungal antibiotics tested in vitro [5]. A high level of resistance toward azoles is well known for *C. krusei*, and few other species while *C. parapsilosis* and *C. guilliermondii* are noted for their decreased susceptibility to echinocandins [5,6].

*C. guilliermondii* and *C. lusitanae* are slowly emerging as new causative agents of invasive candidiasis, while *C. kefyr*, *C. dubliniensis*, and *C. sake*, are occasionally associated in few patients in the recent years [7]. Interestingly, significant geographic variation in the frequency of NAC species occurs. While *C. glabrata* and *C. parapsilosis* were being reported from North America and Europe, recent reports have established them as emerging pathogens in the critical care patients from India too [7]. This compounded with the fact that a few of these fungal pathogens are already inherently resistant or developing resistance further highlights the need for studies in the epidemiological shifts or trends in fungal infections [8-10].

*Candida* spp. has been associated with poor outcome in 50-60% of cases of invasive diseases [11-12]. Despite the increasing developments in the field of antifungal agents, the fungal infections especially the yeast pathogens continue to cause increased morbidity and mortality in the affected populations. With the rising trend in organ transplantations and increasing presentations of malignancy and use of potent immunosuppressive drugs, the vulnerable patient population keeps expanding [10, 13, 14].

For early management and better outcomes, identifying the yeast species is essential for monitoring hospital infection rates [15]. *Candida* spp is one of the most common isolates from both community acquired and nosocomial infection and with the increasing resistance

## Results

Between July 2008 and February 2011, 388 yeast isolates from various samples were collected and submitted for identification and antifungal sensitivity testing in our lab. After clinical correlation, 32 were identified as contaminants and discarded. Of the 388 isolates, 356 were further processed for susceptibility testing. Amongst the yeast isolates, *C. tropicalis* 132(37.07%) was the most prevalent followed by *C. albicans* 111(31.1%) and *C. guilliermondii* 42(11.8%). Other isolates as *C. krusei*, *C. kefyr* and *C. sake* were found in much lower frequency. Table 1 shows the distribution of the isolates.

to standard therapeutic agents [16] and evidence of cross resistance [17], standardized methodology and reliable and more rapid reporting becomes essential.

## Materials and Methods

A total of 356 clinically significant *Candida* isolates were obtained from various samples of patients attending Lakeshore hospital, Kochi, a tertiary care hospital in South India. After ethical clearance from the hospital committee, a retrospective analysis was performed to review records of all patients with positive cultures for *Candida* during the period and to assess the prevalence of in-vitro resistance amongst the isolates. All samples from which *Candida* spp was isolated as sole or in co-infection with other organisms were included in our study.

Patients with other fungal infections or of age below 18 years were excluded. Age, sex, date of hospital admission/ surgery /intervention, antibacterial or antifungal treatment history presence of indwelling catheter and underlying conditions were noted. Other risk factors for candidiasis were considered, such as presence of any other co-infection or history of Diabetes mellitus, neoplasias, presence of CVC catheter, recent surgery, or immunosuppression (chemotherapy, HIV infection, and steroidal therapy) were noted.

**Method-** Various samples were collected and treated according to standard microbiological methods. All fungal isolates were processed by routine methods i.e germ tube tests for identification of *Candida Albicans*, morphological growth patterns on the corn meal/Tween 80 agar, growth at 35° C and 43° C and speciation was done by MiniAPI ID 32C (BioMérieux Diagnostics, Lyon, France). Susceptibility testing was conducted using MiniAPI ATB Fungus 2 kit (BioMérieux Diagnostics, Lyon, France) according to the manufacturer's instructions complying with CLSI guidelines [18] Data was analysed using MS excel 2010 version.

**Table-1: Distribution of Candida spp according to samples and clinical conditions.**

Candida	Blood	Urine	Sputum	Pus	Fluids	Others	Underlying conditions	Co-infections
C albicans	7	53	25	16	8	2	DM, Renal failure COPD Procedures Hepatitis urosepsis	CONS MRSA Gram negative bacteria
C tropicalis	17	65	22	18	8	2	Cirrhosis DM urosepsis neutropenia, 2 <sup>nd</sup> line antibiotics	Gram negative bacteria, CONS, Enterococci
C guilliermondii	10	19	5			8	TCP, malignancy neutropenia, dialysis	Aspergillus sp
C.dubliniensis	5			2	3		Neutropenia, SLE malignancy transplant	
C parapsilosis	16	2			6		CVC catheter, CAD neutropenia, long stay	
C glabrata	2	4	7				Neutropenia Catheter	
C.krusei	0	4				2	HIV, transplant	
C.kefyr	1			2			NHL	
C.sake	2						ALL, Transplant	
Tricho.asahii	2						DM nephropathy	Pseudomonas
Total (356)	60	144	59	38	25			

S: susceptible; SDD: dose-dependent susceptible; DM: Diabetes mellitus; NHL: Non-Hodgkins Leukemia; ALL: Acute lymphoid leukemia; CONS: Coagulase negative Staphylococcus

**Table-2: Distribution and Antifungal susceptibilities of the Candida isolates.**

Candida	Total isolates	Fluocytosine %	Amphotericin B %	Fluconazole %	Itraconazole %	Voriconazole %	Antifungal therapy (No. of isolates)
C albicans	111	100	100	86.48+3 S-DD	81.8 3 S-DD	97.29	70
C tropicalis	132	100	98.11	96.22	92.45	98.48	79
C guilliermondii	42	90.47	90.47	76.19 2-SDD	49.88 2 S-DD	97.6	33
C.dubliniensis	10	100	100	78.57	64.28	100	8
C parapsilosis	24	100	100	95.8	83.3	100	15
C glabrata	13	100	80	60 1 S-DD	20 1 S-DD	100	2
C.krusei	6	100	100	-	50	100	
C.kefyr	3	100	100	50	100	100	
C.sake	2	100	100	100	100	100	
Tricho.asahii	2	100	100	100	100	100	

9.8% patients had underlying diabetes mellitus, 5% with transplant history and 8.4% with cirrhosis. 43.1% of the patients with candidaemia had a central venous catheter in place while, 95/144(65.9%) patients had urinary catheter. 207/356 (58.14%) patients received antifungal therapy within 1 week of collecting sample. All patients had history of antibacterial treatment. Cephalosporins, Piperacillin Tazobactam, Amoxicillin-clavulanic acid and Carbapenem were commonly prescribed agents.

The results of the susceptibility testing for the isolates are presented in the Table 2. Overall fluconazole exhibited better activity against most species as compared to Itraconazole. All the species had very good sensitivity to Flucytosine Amphotericin B and Voriconazole. Fluconazole results were valid for ketoconazole and miconazole. Sensitive or Intermediate results for some of the species with Fluconazole were also predictive as a susceptible result with Ravuconazole. For Fluconazole, Itraconazole and Voriconazole, the Intermediate category corresponded to S-DD (Susceptible-dose dependent) Category of CLSI which is Susceptible if dose administered is increased. The category includes isolates with MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates) [18].

For all *Candida* sp. isolates, MICs of Voriconazole were <0.5 µg/ml, except in 3 *C. albicans* isolates for which the MIC > 4 µg/ml and four *C. guilliermondii* and 2 *C. tropicalis* isolates for which the MIC was >8µg/ml.

Highest resistance to antifungal agents was seen in *C.glabrata* followed by *C.guilliermondii* and *C. tropicalis*. Relative low resistance to fluconazole and Itraconazole were also observed in *C.dublinsiensis*, *C.albicans* and *C. parapsilosis*. *C. krusei* was found to be resistant to fluconazole.

## Discussion

*Candida* sp is one of the most common fungal pathogens isolated from various samples collected from patients with immunological dysfunctions and also forms one of the identifying criteria as marker of this dysfunction in patients with AIDS and immunosuppression.

*Candida* species are environmental contaminants and also present as endogenous flora in most patients. Often in major complications with multifactorial and polymicrobial attributes, it becomes imperative to include antifungal agent coverage especially in patients presenting with neutropenia. Shift in the causal pattern from *C.albicans* to Non-albicans *Candida* and increasing reports of resistant species emphasizes the need for a standardized reliable and easy to interpret methodology based on CLSI guidelines which also provides rapid results. The isolation rate from blood culture was low as compared to other studies. Many factors are known to decrease the growth rates in blood culture. Therefore in case of high suspicion of fungal focus elsewhere and worsening clinical parameters, negative blood culture should not preclude fungemia [19]. In urine samples, *C. tropicalis* was the most common yeast isolated (45.1%) followed by *C. albicans* 38.6% and *C. guilliermondii* (13.1%). and *C. parapsilosis*. This finding was in agreement with many other reports [19-20].

Non-albicans *Candida* causes 67 to 90% of nosocomial *Candidaemia* [21, 22]. Non-albicans *Candida* were responsible for 87.93 % of *candidaemia* in our study. There are many risk factors reported for *Candidaemia*. Isolation of *candida* from non-sterile sites/samples such as urine and wound swabs can significantly improve outcomes in critical care patients if measures are instituted to control its spread or complications [11, 23].

A total of 21 *candida* isolates were identified from Ventilator associated pneumonia cases, with co-infection in 3 cases of multi-drug resistant *Klebsiella*, 1 case of *Pseudomonas aeruginosa* and 1 case of *Staphylococcus aureus*. The species recovered were *C.albicans* 11, *C. tropicalis* 5 *C.glabrata* 3 and *C.krusei* in one case. This correlates well with other study findings [24].

While *Candidemia* due to *C.tropicalis* and *C.albicans* have been reported in many studies, *C.guilliermondii* has also been gaining importance as causative agent in urosepsis especially in hospitalised patients on urinary catheters diagnosed with malignancy. The incidence of *Candidaemia* due to *C. guilliermondii* in many studies ranged from 1% - 3%, based on the geographic region [25]. *C. guilliermondii* has been known to be associated with malignancy, abdominal procedures and neutropenia. This was in agreement with our findings in

most patients presenting with fungemia due to this species. *C. guilliermondii* has been known to be relatively more resistant to antifungal agents than other species which has been associated with administration of antifungal drugs [25] 8 of the patients who grew *C.guilliermondii* from two different sites were treated with Caspofungin. Two of these patients died of other complicating causes (AIDS, Chronic Liver disease).

While all strains of *C.albicans* were susceptible to Amphotericin B and Flucytosine, 16 of the isolated species were resistant to both Fluconazole and Itraconazole, of which 3 isolates were resistant also to Voriconazole. Resistance in *C. albicans* to Azoles has been noted in many reports [8, 26 27].

27.5% of isolates from patients with candidaemia grew *C. parapsilosis*. All the isolates showed good sensitivity to Flucytosine, Amphotericin B and Voriconazole. CVC tips showed low isolation rate of *C. parapsilosis* as compared to blood collected from the central and peripheral lines when collected simultaneously.

This agreed favourably with findings from other studies. The method of transporting and processing the tips were associated with either low growth rate or with growth of contaminants [8, 28].

Many studies regarding sensitivity of *C.glabrata* and *C.krusei* to Fluconazole have emphasized the inherent resistance as in case of *C.krusei* or varying sensitivity with respect to *C.glabrata*. In these patients, either Amphotericin B or an echinocandin must be advised [7, 29]. *C.glabrata* showed higher resistance to Itraconazole as compared to fluconazole and Amphotericin B, consistent with other studies [8 28].

In 13/356 fatal cases, the death was related in part to the complications of the underlying condition as malignancy, surgery, chemotherapy or interventions in post-transplant cases and in part due to ventilator acquired pneumonia [1] due to *C.krusei* and 2 due to *C.tropicalis*.

In one patient, fungaemia in a ventilator assisted patient was complicated by Multi-drug resistant *Acinetobacter baumannii* not responding to treatment. Drug resistances being low comparatively with respect to other studies, the mortality rates were found to be low in our study [11-12].

## Conclusion

Surveillance of the rates of *Candida* infection in critical areas, reporting of outbreaks and continuous monitoring of antifungal susceptibility patterns will help in choosing the best therapeutic management of complicated cases. Comparison of trends in infection rates amongst hospitals between various Indian cities and their resistance patterns can reveal vital information regarding the breakdown of infection control measures. Most *Candida* infections are of low virulence and only become significant in the vulnerable critical care areas. With the rise in prevalence of inherently azole resistant species and rising use of echinocandins in ICUs, identifying risk factors and controlling the infection early can improve the patient outcome.

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