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Candida Species Isolated from Various Clinical Samples and Their Susceptibility Patterns to Antifungals

Sankarankutty Jaya[1, 2], Vipparti Harita[1, 3]

[1]Department of Microbiology and Serology, Narayana Hrudayalaya Hospital, Bangalore, India; [2]Department of Microbiology, Shridevi Institute of Medical Sciences and Research Centre, Rajiv Gandhi University, Tumkur, Karnataka; [3]Department of Microbiology, Malla Reddy Institute of Medical Science, Dr. NTR University, Hyderabad, Andhra Pradesh.

Candida **is an asexual, diploid, dimorphic fungus that is present on human body and his environment. Nowadays the number of patients, who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation, is on increase, and therefore *candidiasis* emerged itself as an alarming opportunistic disease. The aim of this study is to identify the most common *Candida* species in clinical samples, and their antifungal susceptibility patterns. During a cross-sectional study performed in the Department of Microbiology and Serology, Narayana Hrudayalaya Hospitals (India) from January to December 2012, some 213 fungal isolates from various samples were collected. All the isolates were identified to the species level, using Vitek 2 YST identification card (bioMerieux, France). Antifungal sensitivity was performed against amphotericin B (AMB), 5 flucytosine (5-FC), fluconazole (FLU) and voriconazole (VOR) using ASTYS06 (bioMerieux, France). The majority of the isolates were from urine (48%) followed by respiratory (17%) and blood samples (16%). The most common species among the 213 isolates were *Candida tropicalis* (56%) followed by *Candida albicans* (33%). Non-albicans *Candida* species are emerging as the major pathogens and mainly seen in patients on prolonged ventilation and central lines. Antifungal agents should be used cautiously due to increased resistance seen in these agents.**

Keywords: **Candidiasis, antifungals, clinical samples, susceptibility patterns**.

INTRODUCTION

Candida is an asexual, diploid, dimorphic fungus that is present on human body and his environment. A relatively small number of *Candida* species are pathogenic for humans. These organisms are capable of causing a variety of superficial and deep-seated mycoses such as cutaneous, mucocutaneous, subcutaneous, or systemic candidiasis. *Candida* fungus are commensals, therefore to act as pathogens, interruption of normal host defense system is necessary. As a result, general risk factors for *Candida* infections are associated with compromised immune system, diabetes mellitus, and iatrogenic factors like antibiotic use, indwelling devices, intravenous drug use, and hyperalimentation fluids. There has been an increase in number of patients who are immunocompromised, aged, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation; therefore, candidiasis has emerged as an alarming opportunistic disease [1]. In the 1980s, *Candida* species were reported as the seventh most common nosocomial pathogens, ranking fourth in intensive care units (ICUs) [2]. The aim of this study is to identify the most common *Candida* species in clinical samples and their antifungal susceptibility patterns.

Corresponding author: Sankarankutty Jaya, Department of Microbiology, Shridevi Institute of Medical Sciences and Research Centre, Rajiv Gandhi University, Tumkur, Karnataka.
Email: drsjaya@gmail.com

MATERIALS AND METHODS

During a one-year cross-sectional study, which was performed in the Department of Microbiology and Serology, Narayana Hrudayalaya Hospitals (India) from January to December 2012, fungal isolates were collected from various clinical samples obtained from inpatients and outpatients of the hospitals. The clinical samples included sputum, endotracheal secretions, tracheal aspirates, broncho-alveolar lavages, blood, urine, fluids (cerebrospinal, pleural, and ascetic fluids), and pus from post-surgical wounds, vaginal swabs, tissues, and CVP tips. The specimens processed according to the standard microbiological procedures. A Gram stain was performed on all the samples and streaked on Blood, Chocolate, MacConkey agars, and SDA when fungal cultures were required.

The colonies grown on the agar were subjected to Gram stain and the yeasts were further identified. All the isolates were identified to the species level using Vitek 2 YST identification card (bioMerieux, France). Antifungal sensitivity was performed against amphotericin B (AMB), 5 flucytosine (5-FC), fluconazole (FLU) and voriconazole (VOR) using ASTYS06 (bioMerieux, France) by Vitek2. Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) M27-A2.

Isolates with minimum inhibitory concentration (MIC) of <8 μg/ml for fluconazole, <4 μg/ml for flucytosine, and <1 μg/ml for amphotericin B and voriconazole were considered as sensitive.

RESULTS

A total of 213 *Candida* isolates were obtained from various clinical specimens. The majority of the isolates were from urine (48%) followed by respiratory samples (17%) including sputum, endotracheal secretions, tracheal aspirate, and bronchoalveolar lavage fluids, and blood (16%). Pus from surgical site and vaginal swabs constituted around 9.85% of the samples. Fluid samples included pleural fluids from thoracic surgery cases and peritoneal fluids and CSF from head injury cases. Miscellaneous samples included samples from suction tips, CVP catheter tips, tissues (cardiac valve vegetations, debridement of vascular wound) and etc. (Table 1).

Table 1. Distribution of *Candida* isolates in different clinical samples

Sample	No. of *Candida* isolates
Urine	102 (48%)
Respiratory samples	37 (17%)
Pus	21 (9.85%)
Blood	34 (16%)
Fluids	6 (2.8%)
Miscellaneous	13 (6%)
Total	213

The most common species among the 213 isolates were *Candida tropicalis* (56%) followed by *Candida albicans* (33%) (Table 2).

Table 2. Distribution of *Candida* species collected from different clinical samples

Sample	*Candida albicans*	*Candida tropicalis*	*Candida parapsilosis*	*Candida Guilliermondii*	*Candida Kefyr*	*Candida haemulonii*	*Candida lusitaniae*	*Candida krusei*
Urine	27	62	4	2	2	4		1
Respiratory samples	12	24	1					
Pus	7	11					1	
Blood	15	14	4	2		1		
Fluids		6						
Miscellaneous	9	3	1					
Total	70	120	10	4	2	5	1	1

Table 3 shows the sensitivity pattern of various candida species, isolated from clinical samples, to various antifungals. *C. tropicalis* showed 100% sensitivity to voriconazole and 97.5% to amphotericin B and fluconazole. *C. albicans* shows 100% sensitivity to flucytosine, 97% to voriconazole, and 88% to amphotericin B. The species *C. haemulonii* showed 100% resistance to amphotericin B and 100% sensitivity to flucytosine and voriconazole.

Table 3. Sensitivity patterns of *Candida* isolates

***Candida* species**	**Amphotericin B**			**Flucytosine**			**Fluconazole**			**Voriconazole**		
	S	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Candida tropicalis	117(97.5%)	1	2	97(80%)		23	117(97.5%)	2	1	120(100%)		
Candida albicans	62(88.5%)	6	2	70(100%)			68(97%)	1	1	68(97%)		2
Candida parapsilosis	10(100%)			10(100%)			10(100%)			10(100%)		
Candida guilleirmondii	4(100%)			3(75%)		1	4(100%)			4(100%)		
Candida kefyr	2(100%)			2(100%)			2(100%)			2(100%)		
Candida haemulonii			5	5(100%)			1	4		5(100%)		
Candida krusei	1(100%)				1			1		1(100%)		

(S-sensitive, I-Intermediate, R-resistant)

DISCUSSION

Candida species are among the gut flora, i.e., the many organisms which live in the human mouth, and gastrointestinal tract. Up to 75% of healthy individuals carry the yeast *Candida* as part of their normal commensal oral microflora. In the past 3 decades *candida* infections have increased dramatically [3]. Several studies have shown a considerable increase in the non-albicans *Candida* infections. A study by Saldhana *et al.* [4] showed that non-albicans *Candida* were isolated more frequently (53%) than *C. albicans* (47%). These results were in agreement with the findings of the study by Mokaddas *et al.* [5] who also showed the non-albicans *Candida* incidence (60.5%) was higher than that of *C. albicans* (39.5%). These findings suggest that non-albicans *Candida* species are emerging as important pathogens. Similarly, in our study we found that *C. tropicalis* was the predominant patho-gen (47.8%) followed by *C. albicans* (32.86%).

The isolation of *Candida* species from the respiratory secretions is common in mechanically ventilated patients. This occurs as a result of seeding of the lungs following hematogenous dissemination, or it may follow the aspiration of colonized oropharyngeal or gastric contents [6]. In this study we obtained around 37 isolates from respiratory samples (17%), mostly originated from endotracheal secretions and sputum from patients with prolonged mechanical ventilation.

Bloodstream infections (BSIs) caused by various *Candida*s species, which are a significant cause of morbidity and mortality in hospitalized patients, have been reported from many countries worldwide. Candidemia is associated with many risk factors such as long-term hospitalization, antibiotic therapy, use of intravascular catheters, and underlying diseases like diabetes and malignancy. *C. albicans* is the most common *Candida* species isolated from BSIs worldwide [7]. Mokkadas *et al.* [5] in a study showed that *C. albicans* was the predominant species in bloodstream infections (39.5%), followed by *C. parapsilosis* (30.6%), *C. tropicalis* (12.4%), *Candida glabrata* (5.6%) and *Candida krusei* (1.6%). In our study *C. albicans* and *C. tropicalis* were seen in 44% and 41.1% of patients, respecttively, with bloodstream infection. Bloodstream infections were most commonly seen in patients with prolonged central lines and antibiotic therapy. *Candida* has been reported in up to 44% of urine samples collected for culture. *C. albicans* was the most commonly reported pathogen (including bacte-

ria) isolated from urine (21%), constituting more than half of the fungal isolates. Also, *C. albicans* was more commonly reported in catheter-associated UTIs than in non-catheter-associated infections. Fungal urinary infections are more common in patients with urinary catheters [8]. In our study, urine isolates constitute around 48% of the isolates and the most common species were *C. tropicalis* (60.7%) and *C. albicans* (26.7%).

Saldanha *et al.* [4] reported 7% of isolation from pus and 38% from vaginal swabs. In our study 21 isolates (10%) were obtained from vaginal swabs (n=6) and post-surgical wounds (n=15).

Most of the central nervous system infections are associated with *C. albicans*; however, *C. parapsilosis* and *C. tropicalis* have been isolated in some cases [9]. We isolated *C. tropicalis* from CSF of a patient with a prolonged external ventricular drain hospitalized due to head injury following a car crash.

In our study *C. albicans* showed 88.5% sensitivity to amphotericin B, 97% sensitivity to fluconazole and voriconazole, and 100% sensitivity to flucytosine. *C. tropicalis* also showed 97.5% sensitivity to amphotericin B and fluconazole, 80% sensitivity to flucytosine, and 100% sensitivity to voriconazole.

The new species *C. haemulonii*, previously known to cause an epidemic disease in laboratory animals and onychomycosis in humans, has emerged as an opportunistic fungal pathogen capable of causing outbreaks of fungaemia. *C. haemulonii* has shown increased MICs and resistance to both amphotericin B and fluconazole resulting to clinical failures [10]. We isolated *C. haemulonii* from urine samples of the patients with prolonged catheterization in intensive care unit. Also, an isolate was obtained from blood of an autoimmune disease patient receiving immunosuppression therapy and prolonged central line. *C. haemulonii* was resistant to amphotericin B (MIC>8) and fluconazole (MIC>32).

Kim *et al*. [11] reported the emergence of *C. haemulonii* from five Korean hospitals during 2004 to 2006, with genotypes suggesting intra-and inter-hospital transmission of a clonal strain. Oberoi *et al.* [12] reported first isolation of this species at Sri Ganga Ram Hospital, New Delhi, India in 2006. Isolation of this species increased significantly so that it became the third most common species collected from 2006 to 2008. Non-albicans *Candida* species are among the major pathogens seen in patients on prolonged ventilation and central lines. Regarding the increased resistance of some of these species to antifungals, administration of these agents should be used with precaution.

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