



Original Article

Species Distribution and Antifungal Susceptibility of *Candida* spp among Superficial Candidiasis in Outpatients at RMCH

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Abstract

Background: The increase in the incidence of fungal infections, especially those caused by *Candida albicans* and non albicans *Candida* species, necessitates the understanding and treatment of *Candida*-associated infection. Due to frequent use of antifungal drugs, a shift is observed towards non-*albicans* *Candida* species and change in the pattern of susceptibility of antifungal drugs. The aim of this study was to isolate and identify the *Candida* species from different superficial candidal infections in adult patients and to observe their antifungal susceptibility patterns.

Materials and Methods: A cross sectional study was performed over a period of one year in at tertiary care hospital that involved 180 patients. The candidal species were isolated, their antifungal susceptibility patterns were determined from vaginal swab, oral swab and skin appendages.

Results: Commonest age group involved was of 30-39 years age (30%, 54/180). Among 67 culture positive samples, *C. albicans* was the most common isolates and found in 39/67(58.21%) cases. Non-*candidal* species like *C. tropicalis* and *C. krusei* were isolated in 16/67(23.88%), 09/67 (13.43%) cases respectively. Caspofungin was found to be the most sensitive and fluconazole was found to be the least sensitive antifungal drugs. Non-*albicans* *Candida* species showed more antifungal resistance than *C. albicans*.

Conclusions: *Candida albicans* were predominant isolated from the superficial candidal infection but *C. tropicalis* was the most frequently isolated among non albicans species. They showed a wide range of susceptibility towards different antifungal agents and fluconazole was found to be less sensitive drugs.

Key words: *Candida albicans*; Non-*albicans* *Candida*, Caspofungin, Fluconazole, antifungal susceptibility.

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Introduction

Fungal infections caused by different *Candida* spp. has become one of the most frequent health care associated infections showing an increasing trend.¹

Candida spp. are normal commensals of skin, the mucosa or gastrointestinal tract of human and often cause common but easy to treat

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mucocutaneous candidiasis in immunocompetent patient.²

In these last decades, the increasing of patients susceptible to invasive candidiasis is related to the larger number of immunocompromised hosts, the widespread use of indwelling medical devices and invasive techniques, the prolonged hospitalization in the intensive care units (ICU) and the misuse of broad spectrum antimicrobial agents.³

Although *Candida albicans* has been reported as the most predominant *Candida* species that frequently causes invasive fungal infections, a significant increase in non-*C. albicans* *Candida* (NCAC) species such as *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis* in human candidiasis has also been notably detected and their emerging role in human infections became of interest over the last decade.^{4,5,6} Apart from the above *Candida* species *C. auris* has emerged as a serious global health threat to humankind now a days.⁷

In a study, patients attending a dermatology clinic in a rural area of Bangladesh in 2017 showed that 17.5% were diagnosed with superficial fungus infections. It was also estimated that 8100 cases of *Candida* infections occurred in Bangladesh each year.⁸

Antifungal resistance is an increasing problem with *Candida* species as a result candidal infections may resist antifungal drugs, making them difficult to treat.⁹ The emergence of multiple drug resistant *Candida* strains has been increasingly reported in recent years. Patients with *Candida* infections that are resistant to both fluconazole and echinocandin drugs have very few treatment options.¹⁰ Frequent and indiscriminate use of antifungal drugs as prophylaxis and also for treatment purpose in patients infected with *Candida* species have resulted in measurable rates of acquired or innate resistance to the antifungal agents.

Candida albicans showed less resistance compared to non albicans group. Resistance rates for *C. auris* are much higher than for other *Candida* species.¹¹

Based on these remarks, accurate diagnosis with antifungal susceptibility is essential. Currently used methods for the isolation and identification of *Candida* species are microscopic examination, different conventional culture media specially chromogenic culture media, germ tube test, manual and automated methods of assimilation of carbohydrates and nitrogen (auxanogram), carbohydrates fermentation test and molecular techniques. Culture in Sabouraud dextrose agar media supplemented with chloramphenicol and gentamicin takes 24-48 hours to grow. The germ tube test is the gold standard of laboratories for the identification of *C. albicans* because it is cheap, quick and simple.¹²

So the aim of this study was to isolate and identify different *Candida* species from different specimens and to see their antifungal susceptibility pattern in adult patients attending in Rajshahi Medical College Hospital.

Materials and Methods

A cross-sectional descriptive study was conducted during the period from July 2021 to June 2022. Total 180 adult respondent with clinically suspected superficial candidiasis attending outpatient departments of Rajshahi Medical College Hospital (RMCH) were included in this study. Microbiological works were done in the laboratory of the department of Microbiology, Rajshahi Medical College (RMC). Vaginal swab from adult female patient, skin scraping, nail clipping, hair plucking and oral swab from adult male and female patients have been collected as specimens. Data were compiled in a partially structured pre-tested data sheet. Isolation of *Candida* species is done by direct microscopy with wet mount, gram stain, LPCB stain preparation and culture in SDA media incorporate with chloramphenicol and gentamicin. Identification of different *Candida* species were done by germ tube test, subculture in chromogenic agar media and carbohydrate fermentation test. Antifungal drug susceptibility test of *Candida* species was done by disk diffusion method according to CLSI M44-A2 document.¹³

Results

A total of 180 specimens were collected from clinically suspected superficial candidial infections. The highest age group was 30-39 years (54; 30%) with the mean age of 37.4 years (Table 1). Overall female predominance was observed with a male: female ratio of 1:1.9. Among them, total culture positive cases were 67 (37.22%). Highest specimens were 100 (55.56%) from vaginal swab, where 39(39%) were culture positive. Culture positive cases were 11 (27.5%) and 17 (42.5%) from oral swab and skin appendages respectively (Figure 1). Among culture positive specimens, 64 (95.52%) were single isolates and 3 (4.48%) mixed infections. Among the single isolates, 39 (58.21%) were *C. albicans*. Out of 25 (37.31%) non *albicans Candida* species, 16 (23.88%) were *C. tropicalis*, 9 (13.43%) were *C. krusei* (Table 2).

Casposfungin was the most sensitive drug for *C. albicans*, *C. tropicalis* and *C. krusei* with 100% susceptibility. *C. albicans* showed less resistance to antifungal drug compared to non albicans groups. *C. tropicalis* showed good sensitivity to nystatin (89.5%) and amphotericin B (84.2%) but less sensitivity to ketoconazole (52.6%) and fluconazole (52.6%). In case of *C. krusei*, sensitivity to all antifungal drugs were poor and the sensitivity pattern to nystatin, amphotericin B, voriconazole, itraconazole, fluconazole and ketoconazole were 70%, 60%, 60%, 50%, 50% and 30% respectively (Table 3).

Table 1: Demographic data of the suspected superficial candidiasis cases (N=180)

Variables	n%
Age (Years)	(30-39, highest age group)
Mean age	37.4 years
Gender	
Male	61 (33.89%)
Female	119 (66.11%)
Presentation (Sample 180)	
Vaginal swab	100 (55.56%)
Oral Swab	40 (22.22%)
Skin Appendages (Skin, Hair, Nail)	40 (22.22%)

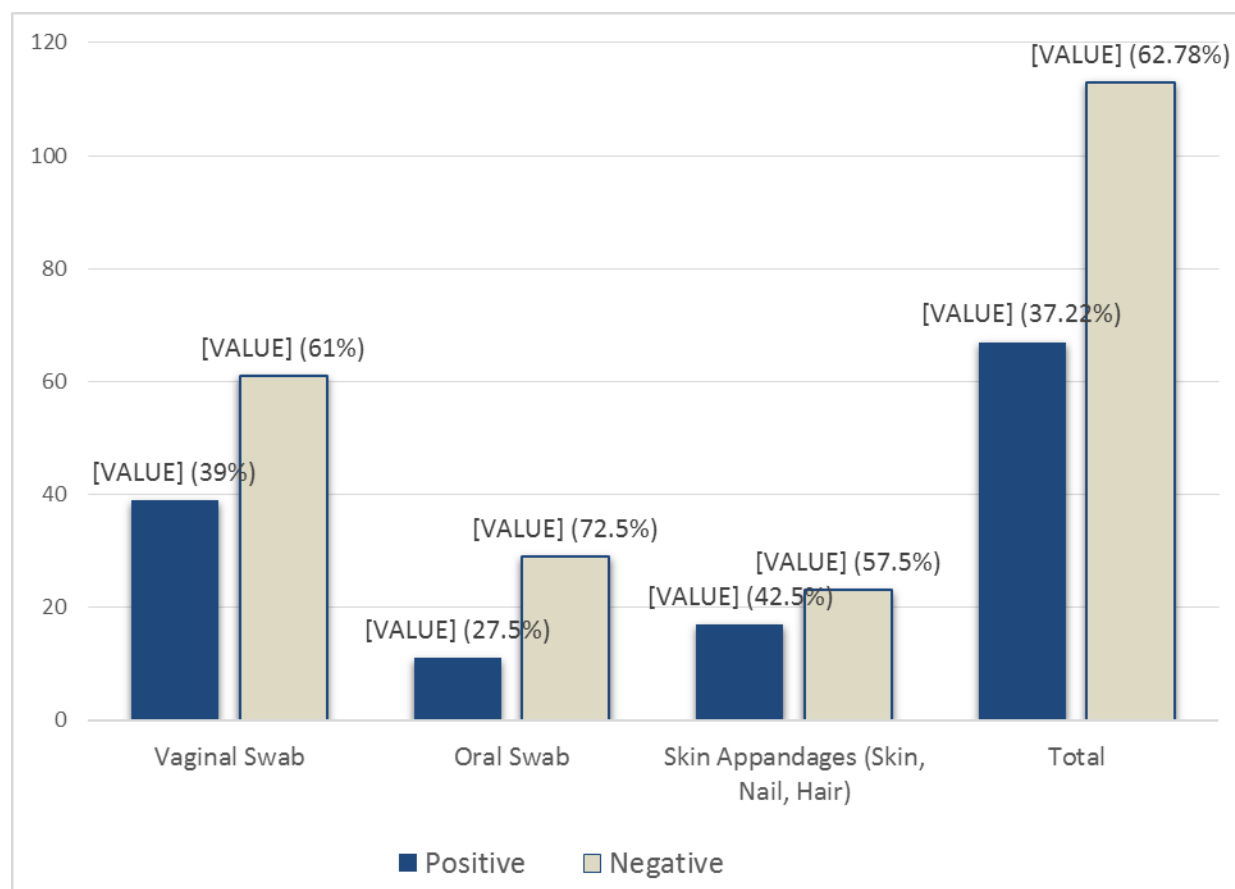


Figure 1: Total isolation of *Candida* species by culture from different specimen (N=180).

Table 2: Distribution of isolated *Candida* Species from different specimen: (n=67)

Name of species	Number	Percentage
<i>C. albicans</i>	39	58.21
Total non <i>albicans candida</i>	25	37.31
<i>C. tropicalis</i>	16	23.88
<i>C. krusei</i>	9	13.43
Total mixed	3	4.48
(<i>C. albicans</i> + <i>C. tropicalis</i>)	1	
(<i>C. albicans</i> + <i>C. tropicalis</i>)	1	
(<i>C. tropicalis</i> + <i>C. krusei</i>)	1	
Total	(39+25+3) =67	100

Table 4: Antifungal sensitivity pattern among *Candida albicans*, *Candida tropicalis* and *Candida krusei*(n=70).

Name of drugs	Name of fungus	Sensitivity pattern		χ^2 value	df	p-value
		Sensitive	Resistant			
Nystatin	<i>Candida albicans</i>	40 (97.60%)	1 (2.40%)	6.54	2	0.04
	<i>Candida tropicalis</i>	17 (89.50%)	2 (10.50%)			
	<i>Candida krusei</i>	7 (70.00%)	3 (30.00%)			
Amphotericin B	<i>Candida albicans</i>	39 (95.10%)	2 (4.90%)	7.70	2	0.02
	<i>Candida tropicalis</i>	16 (84.20%)	3 (15.80%)			
	<i>Candida krusei</i>	6 (60.00%)	4 (40.00%)			
Voriconazole	<i>Candida albicans</i>	39 (95.10%)	2 (4.90%)	14.70	2	0.01
	<i>Candida tropicalis</i>	11 (57.90%)	8 (42.10%)			
	<i>Candida krusei</i>	6 (60.00%)	4 (40.00%)			
Itraconazole	<i>Candida albicans</i>	38 (92.70%)	3 (7.30%)	14.07	2	0.01
	<i>Candida tropicalis</i>	11 (57.90%)	8 (42.10%)			
	<i>Candida krusei</i>	5 (50%)	5 (50%)			
Fluconazole	<i>Candida albicans</i>	32 (78.00%)	9 (22.00%)	5.34	2	0.07
	<i>Candida tropicalis</i>	10 (52.60%)	9 (47.40%)			
	<i>Candida krusei</i>	5 (50%)	5 (50%)			
Ketoconazole	<i>Candida albicans</i>	34 (82.90%)	7 (17.10%)	12.66	2	0.002
	<i>Candida tropicalis</i>	10 (52.60%)	9 (47.40%)			
	<i>Candida krusei</i>	3 (30.00%)	7 (70.00%)			

(N.B.- Caspofungin was not included here because it was sensitive against 100% fungal agents)

Discussion

This study included 180 suspected superficial candidiasis patients and specimens were taken as vaginal swab, oral swab and skin appendages (100+40+40=180) (Table 1). In the current study, most of the respondents (54; 30%) were within the age group of 30-39 years with a mean age of 37.4 years (Table 1), which is similar to an Indian study South Korean study.^{14,15} Female (66.11%) were predominant subject in superficial candidial infection (Table 1). Egypt and South Korea and Egypt also reported alike with present study.^{15,16} People with adult age group have more job activities, female was predominant as it is child bearing age and they suffer from malnutrition and iron deficiency, so they have more chances of exposure to fungal agents.

In this study (Figure 1), total culture positive cases were 67 (37.22%) similar with the study of Iran,¹⁷ but dissimilar with the study of China and Brazil.^{18,19} Highest specimens were 100 (55.56%) from vaginal swab (Figure 1) where 39(39%) were culture positive. Both oral swab and skin appendages contributed separately 40 (22.22%) from which 11 (27.5%) and 17 (42.5%) were culture positive, respectively (Table-2). These findings were in accordance with a study done in Turkey.²⁰

In this study (Table 2), out of 67 culture positive specimen 64 (95.52%) were single isolates and 3 (4.48%) mixed infections. Among the single isolates 39 (58.21%) *C. albicans*, 25 (37.31%) non *albicans Candida* species were found. Out of 25 non *albicans Candida* species, 16 (23.88%) was *C. tropicalis*, 9 (13.43%) was *C. krusei*. Among the 3 mixed infections 6 *candida* were found and they were *C. albicans* + *C. tropicalis* in one mixed infection, *C. albicans* + *C. tropicalis* were in another one and *C. tropicalis* + *C. krusei* were in rest another one. Nearly similar findings were found in Bangladesh, India and Turkey.^{21,22,23}

Candida albicans and non *albicans* species are closely related but differ from each other with respect to epidemiology, virulent characteristics and antifungal susceptibility. Concern is rising

about the high incidence of infections caused by non *albicans* species and the emergence of antifungal resistance.²⁴

In this study (Table 3), data revealed that caspofungin was the most sensitive drug for *C. albicans*, *C. tropicalis* and *C. krusei* with 100% susceptibility. *C. albicans* was (Table 3) sensitive to nystatin in 97.6%, followed by amphotericin B in 95.1% and voriconazole in 95.1% of the isolates. But fluconazole had the poorest antifungal activity, sensitive in 78% of the cases. We found *C. tropicalis*, which showed 89.5%, 84.2% sensitivity to nystatin and amphotericin B respectively. Both ketoconazole and fluconazole were sensitive separately in 52.63% of the cases. In case of *C. krusei*, sensitivity pattern was poor to all antifungal drugs, Nystatin and voriconazole were sensitive in 70% and 60% of the isolates respectively. Fluconazole, itraconazole and ketoconazole were resistant in 50%, 50% and 70% of the isolates respectively. Table 3 revealed that voriconazole and itraconazole were highly sensitive against *Candida albicans* than *Candida tropicalis* & *Candida krusei* and it was statistically highly significant ($p=0.01$). Similarly, antifungal sensitivity pattern of nystatin, Amphotericin B and Ketoconazole were also statistically significant among *Candida albicans*, *Candida tropicalis* and *Candida krusei* ($p < 0.05$). On the other hand, only fluconazole was not statistically significant among *Candida albicans*, *Candida tropicalis* and *Candida krusei* ($p > 0.05$). Some studies found near similar to this study.^{22, 25, 26} Our results were contradictory with some studies also.^{27,28}

Conclusion

Fungal infections, such as candidiasis is a growing medical concern now a days. This time demanding study provides a glimpse of current situation of the problem. In this study, *Candida albicans* is the most common isolate followed by *C. tropicalis*, *C. krusei*. Among the seven antifungal drugs used in this study, caspofungin is the most sensitive (100%) for all *Candida* species, followed by nystatin, amphotericin B, voriconazole, itraconazole, ketoconazole. Among them,

fluconazole is found to be the least sensitive drug. *Candida albicans* showed less resistance compared to non *albicans* group. So to establish an effective treatment protocol and to reduce the alarming increase of antifungal resistance, it is advised to ensure regular culture practice and antifungal susceptibility testing for candidiasis.

Conflict of interest: None declared

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