



ORIGINAL RESEARCH PAPER

Microbiology

Spectrum of *Candida* species in sputum samples – a laboratory based study**KEY WORDS:** *Candida*, speciation, sputum**Dr.Naga Srilatha Bathala**

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ABSTRACT

Introduction: *Candida* species are components of normal microbial flora of human body inhabiting mouth, intestines and vagina. Even though these are commensals, they have been reported frequently as pathogens because of risk factors. As *Candida* species are commensals in oral cavity there is an increased chance of invading to gastrointestinal tract and/or respiratory tract. The rates of non-albicans species such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis* have been increasingly reported. Identification of *Candida* to the species level has become mandatory for selection of appropriate antifungal agents.

Aims and objectives:

1. To know various species of *Candida* isolated from sputum samples.
2. To know the most common species of *Candida* in sputum samples.

Material and methods:

All sputum samples received for routine culture and sensitivity were inoculated on Mac Conkey's medium, blood agar medium and incubated overnight at 37°C. Smears prepared from white colonies on blood agar were subjected for gram stain. Gram positive, yeast like budding cells were considered as *Candida* species. 100 strains of *Candida* species were isolated during the study period. Each isolate was tested for germ tube formation and chlamydospore production. All *Candida* isolates were inoculated on HiCrome *Candida* Differential Agar for *Candida* speciation.

Conclusions:

1. *Candida* species identified were *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. kuzei*.
2. *C. albicans* was the most common and more than 60% of *Candida* species were non albicans *Candida*.
3. More isolates were from 61- 70 years.

Introduction:

Candida species are components of normal flora of human body inhabiting mouth, intestines and vagina [1, 2]. Survival of the organisms in these sites depends on a various factors, including their ability to adhere to mucosal cells and compete with commensal organisms. Invasive fungal infections have been reported in recent years in 26% of chronically and intensively immuno suppressed patients [3]. Even though *Candida* species are commensals, they have been reported frequently as pathogens because of risk factors such as increased use of broad-spectrum antibiotics, underlying malignant diseases, HIV/AIDS, organ transplantation, prolonged hospital stay, and exposure to invasive procedures [4]. Due to the commensal nature of *Candida* species, majority of the times it is found to be a cause of endogenous infection [5]. Candidiasis is a primary or secondary infection involving a member of the genus *Candida* [6]. Although there are over 150 *Candida* species in nature, only 15 of them are human pathogens [7]. The clinical manifestations of disease are extremely varied, ranging from acute, sub acute and chronic to episodic. Involvement may be localized to mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, gastrointestinal tract or become systemic as in septicemia, endocarditis, and meningitis [6]. Though *Candida* is not a significant clinical factor in pulmonary diseases, it causes serious complication as co infection with other chronic illness [8]. As *Candida* species are commensals in oral cavity there is an increased chance of invading further to gastrointestinal tract and/or respiratory tract whenever there is presence of underlying one or more predisposing factors. In such an instance it is important to report *Candida* species if the growth is obtained from sputum samples as it could able to cause even though criteria for diagnosis of pulmonary candidiasis is still controversial [9]. *Candida albicans* is responsible for around 50 % of systemic infections caused by *Candida* species, making it the most common infectious *Candida* agent. The changes in the distribution of *Candida* species have been observed and the rates of non-albicans species such as *C. glabrata*, *C. tropicalis* and *C. parapsilosis* have been increasingly reported [4]. Identification of *Candida* to the species level has become mandatory to aid the selection of appropriate antifungal agents in treatment of invasive candidiasis because most of the Non *Candida albicans* *Candida* [NCAC]

usually exhibit a reduced susceptibility to the common antifungal agents especially *Candida glabrata* which exhibits reduced fluconazole susceptibility [10]. Isolation of *Candida* species has been increased in various clinical samples in recent years. Hence the present study was conducted to know various species of *Candida* isolated from sputum samples.

Aims and objectives:

1. To know various species of *Candida* isolated from sputum samples.
2. To know the most common species of *Candida* in sputum samples.
3. To know the most common age group in which *Candida* isolates are more prevalent.

Material and methods:

The present study was conducted prospectively from November 2016 to March 2017 in clinical microbiology laboratory, Rajiv Gandhi Institute of Medical sciences, Kadapa, Andhra Pradesh. All sputum samples received for routine culture and sensitivity were inoculated on Mac Conkey's medium, blood agar medium and incubated overnight. Smears were prepared from colonies those were white in colour on blood agar and subjected for gram stain. Organisms those appeared as gram positive, yeast like budding cells were considered as *Candida* species and included in the present study. In the same manner 100 strains of *Candida* species were isolated during the study period. Each isolate was tested for germ tube formation by inoculating the colony in 0.5 ml. of human serum using sterile loop. It was inoculated at 37°C for 2-4 hrs. A wet mount was prepared with a drop of serum yeast culture and observed under high power objective (40X) presence of sprouting yeast cells was considered as germ tube test positive and the isolate was identified as *Candida albicans*. The isolated *Candida* species was also sub cultured on corn meal agar and incubated at 25°C for 48-72 hrs to test chlamydospore production and observed microscopically. The strain that showed chlamydospore production was identified as *Candida albicans* [11]. Both albicans and non albicans *Candida* species were further inoculated on HiCrome *Candida* Differential Agar which is selective and differential medium for *Candida* speciation and incubated over

night at 37 °C. as per the manufacturer's instructions speciation was performed. Depending on the colour on HiCrome Candida Differential Agar, isolates were differentiated into Candida albicans (light green), Candida tropicalis (blue to purple), Candida glabrata (cream to white) and Candida kruzei (purple, fuzzy). The obtained results were analysed.

Results:

Out of 100 Candida isolates 57 were from Outpatient department and 43 from Inpatient department. Candida isolates were more common in males (66) than females (34) as shown in table 1. It was observed that as the age increases Candida isolates number was also increased. Candida isolates were more in age group 61-70 years (33) followed by 51-60 years (26). In the age group of < 20 years only 2 isolates of Candida were there as shown in table 2. Among the Candida isolates Candida albicans (38) was the most common isolate followed by Candida tropicalis (32). From Outpatient department isolates of Candida albicans and Candida tropicalis were equal in number (17). Not only from Inpatient department but also among overall Candida isolates, the most common species was Candida albicans as shown in table 3. 68.42 % of Candida albicans, 62.5% of Candida tropicalis, 70.59% of Candida glabrata and 61.53% of Candida kruzei were from males as shown in table 4.

Discussion:

Candida species are components of normal flora of human beings. They are commonly found on skin, gastro intestinal tract and vagina [1,2]. Survival of the organisms in these sites depends on a various factors, including their ability to adhere to mucosal cells and compete with commensal bacteria. The first step in the development of a Candida infection is colonization of the mucocutaneous surfaces [10]. Factors which disturb this balance favour either elimination or growth and subsequent invasion by the organism [12]. The prolonged antibacterial therapy may predispose to fungal infections even in immunocompetent host [2]. Candidiasis is a primary or secondary infection involving a member of the genus Candida [6]. With an increase in the number of individuals sensitive to invasive fungal infections, by both moulds and yeasts have begun to be reported more frequently as pathogens. When all at-risk groups are considered as a whole, it is seen that the leading pathogen is Candida species [13]. Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and antifungal susceptibility [14]. In the last 20 years, a change has been observed in the rates of Candida species isolated from patients with candidiasis [7]. Most infections are caused by C.albicans, the shift towards treatment resistant non albicans candida species is evident in recent years [15].

The present study was done on Candida isolates from sputum samples of all age groups of both outpatient (OP) and inpatient (IP) departments. Out of 100 Candida isolates 57 isolates were from OPD and 43 isolates were from IPD. Candida isolates were more from male individuals than females (66:34). This male preponderance was also observed in studies of Dey etal [5] (70:30), Mehta etal [1] (62:38), Jaggi etal [14] (55.2:44.8), Monika etal [16] (68:32), Naseem etal[6] (62.6:37.4) whereas in Rakesh etal[17] study female preponderance was observed (46:54). Among all Candida isolates Candida albicans was the most common species (38%). The same was observed in many studies with varied percentages- Dey etal [5] (70%), Keepeng etal [18] (70%), Monika etal [16] (50%), Jaggi etal [14] (44%), Rakesh etal [17] (44%), Naseem etal [6] (34.6%). Next to Candida albicans, Candida tropicalis was in high percentage (32%) followed by Candida glabrata (17) and Candida tropicalis (13) in sputum samples. It was also observed in studies by Dey etal [5] (13.33%), Naseem etal [6] (26.9%), Jaggi etal [14] (26.4%), and Rakesh etal [17] (30%). In a study by Mehta etal [1] on Candida isolates in various clinical specimens, among sputum samples Candida tropicalis (6/13) was the most common non albicans Candida species and in a study by Kee peng etal[18] 38.56% of non albicans Candida isolates were Candida tropicalis. In a study by Obga etal [9] after Candida albicans (80%), Candida tropicalis

(12.5%) was the common isolate from sputum samples in individuals with HIV/AIDS. In the present study Candida albicans isolates were more in inpatient individuals (48.84%). It might be due to predisposing factors like hospital stay and administration of broad spectrum antibacterial. In a study by Mehta etal for all Candida isolates from sputum samples antibiotic usage was the predisposing factor [1]. Candida isolates were more in the age group of 61-70 years followed by 51-60 years in our study. The present study also observed that nearly 70% of Candida isolates were from more than 50 years. In a study by Dey etal [5] the most common age group was 71-80 years (30%) whereas in studies by Rakesh etal [17] and Naseem etal [6] Candida isolates were common in > 60 years group. In the age group of less than 20 years from only two individuals Candida species were isolated – (Candida albicans and Candida tropicalis each one) and these two isolates were from inpatients represents that not only colonization with Candida species increases with age but also colonized organisms might convert to pathogen in certain predisposing factors. 62% of Candida isolates were non albicans Candida species in the present study showed that Candida albicans is being replaced by non albicans even in community. The isolates of non albicans Candida species – C.tropicalis, C. glabrata and C. kruzei were more from sputum samples of outpatient individuals in our study. Nowadays there is increased usage of antibacterials even in outpatient departments which can further lead to emergence of resistance strains as well as increasing prevalence of fungal infections. This can slowly replace actual existing organisms in community. The frequency of Candida spp. infections has being increased worldwide in recent years. If Candida species is not reported as an isolate, even though commensal, especially in hospitalized patients leads to long time hospital stay which causes difficulty in the diagnosis, increases cost of treatment and also increases chance of getting hospital acquired infections.

Conclusions:

1. Candida species identified in the present study were Candida albicans, C. tropicalis, C. glabrata and C. kuzei.
2. Among all these C.albicans was the most common and but more than 60% of Candida species were non albicans candida.
3. More isolates were from the age group of 61- 70 years.

Limitations:

1. Immune status of individuals was not known.
2. History of antibiotic usage was not known.
3. Sample size was less

Conflicts of interest: nil
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Table 1 showing distribution of sputum samples-

Gender	Out patient	In patient	Total
Male	38	28	66
Female	19	15	34
Total	57	43	100

Table 2 showing gender wise distribution of Candida isolates in various age groups

Age group in years	Male	Female	Total
< 20	01	01	02
21-30	02	05	07
31-40	06	04	10
41-50	07	05	12
51-60	20	06	26
61-70	24	09	33
> 70	06	04	10
Total	66	34	100

Table 3 showing distribution of Candida isolates among Outpatients and Inpatients

Name of species	Out patient	In patient	Total
Candida albicans	17 (29.82%)	21(48.84%)	38

Candida tropicalis	17(29.82%)	15(34.88%)	32
Candida glabrata	12(21.05%)	05(11.63%)	17
Candida kruzei	11(19.30%)	02(4.65%)	13
Total	57(100%)	43(100%)	100

Table 4 showing gender wise distribution of Candida isolates

Name of species	Male	Female	Total
Candida albicans	26(39.39%)	12(35.29%)	38
Candida tropicalis	20(30.30%)	12(35.29%)	32
Candida glabrata	12(18.18%)	5(14.70%)	17
Candida kruzei	8(12.12%)	5(14.70%)	13
Total	66(100%)	34(100%)	100

Table 5 showing species of Candida in various age groups –

	Candida albicans		Candida tropicalis		Candida glabrata		Candida kruzei		Total
	OP	IP	OP	IP	OP	IP	OP	IP	
< 20	-	1		1	-	-	-	-	02
21-30	-	1	1	1	1	-	3	-	07
31-40	4	2	1	-	3	-	-	-	10
41-50	1	1	3	3	1	1	1	1	12
51-60	5	5	6	4	3	1	2	-	26
61-70	5	9	6	4	2	2	4	1	33
> 70	2	2	-	2	2	1	1	-	10
Total	17		17	15	12	05	11	02	100
	38		32		17		13		

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