

Distribution Factors of *Candida* species in Respiratory Tract of Elderly Population of Kathmandu.

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ABSTRACT

Though normal flora of upper respiratory tract, *Candida* species are a significant clinical problem for a variety of immune compromised patients worldwide. Impaired salivary gland function, drugs, dentures, high carbohydrate diet, age, smoking, diabetes mellitus, Cushing's syndrome, malignancies, and immunosuppressive conditions are the associated risk factors for the Candidiasis. The study was designed with the objectives of describing the distribution pattern of *Candida* species and some molds in the elderly and non elderly population randomly sampled from the three Oldage Care Centers. A total of 304 samples each of sputum and throat swabs were collected from the study objects having age 20-80 years. The specimens were cultured on Sabouraud's Dextrose Agar (SDA). *Candida* isolates were identified on the basis of Gram stain, germ tube test, production of chlamydospore, sugar fermentation and assimilation test. The mold isolates were identified by cultural and microscopic characteristics. Out of total 304 samples of each sputum and throat swabs 16.45% and 5.3% samples respectively were growth positive for fungal culture. Among elderly population, 32.87% (47/143) of sputum and 9.8% (14/143) throat swabs showed culture positivity. In case of non elderly population however only 1.9% (3/161) sputum and 1.24% (2/161) throat swab samples were culture positive. In total, 56 *Candida* isolates (41 from sputum and 14 from throat swabs) were obtained. Significantly higher number of isolates was obtained from elderly population (38 from sputum and 12 from throat swabs) than non elderly population. Among three antimycotic drugs used, the *Candida* isolates were highly susceptible against Ketoconazole and resistant towards Fluconazole. *Candida* distribution was significantly associated with the age, respiratory tract problem and smoking habits of the study population. Elderly population having age above 50 years are in the high risk of drug resistant *Candida* infection.

Key Words: *Candida*, elderly population, sputum, throat swab, antimycotic susceptibility.

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INTRODUCTION

Candida is an opportunistic fungus that normally inhabits the mouth, throat, gastrointestinal tract and vagina.¹ In the last two decades a steady increase in nosocomial infections caused by *Candida* species has been seen due to its endogenous origin. *Candida* species have been the third most common pathogen isolated from patients with lower respiratory tract infection.^{2,3} There are several types of *Candida* infections, like candidiasis involving primarily the mucosal surfaces (thrush) of the throat, respiratory tract and urogenital tract, candiduria, *Candida* infection of gastrointestinal tract, invasive candidiasis, including candidemia and disseminated candidiasis with deep organ involvement.^{4,5}

Risk factors of candidial infection include impaired salivary gland function, drugs, dentures, high carbohydrate diet, and extremes of life, smoking, diabetes mellitus, Cushing's syndrome, malignancies, and immunosuppressive conditions.⁶ *Candida* species

are prevalent in the oral cavity, among elderly population and immunosuppressed patients.⁷ Currently, there are more than 150 known species of *Candida*. However only 15 of these species are isolated from patients as infectious agents. Among these in 95% of infections, the pathogens involved are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*.^{8,9,10,11}

Since concurrent with the increase in fungal infections, a large variety of antifungal drugs are available with different spectrum of activity and a high variability in the susceptibility of clinical isolates to antifungal agents has been documented.^{2,12} Recent reports indicate non-albicans infections are on the rise, often accounting for more than 50 % of candidiasis found in the infected population. Additionally, drug resistance may vary by species.¹³

The present study is based upon the hypothesis that colonization of *Candida* and molds in respiratory tract of elderly population is higher than non elderly population. Therefore the present study has been

designed with objective to describe distribution pattern and risk factors of different species of *Candida* and in upper and lower respiratory tract of selected elderly and non elderly population of Kathmandu. Furthermore the antimycotic susceptibility testing of *Candida* isolates have also been described. The study possibly highlights importance of screening of *Candida* in elderly population.

MATERIALS AND METHODS

Altogether 304 populations with age above 20 years were taken for the study. Population above 50 years was categorized as ‘elderly population’ and population having age equal or less than 50 years was categorized as ‘non elderly population’. For elderly population three Old age Care Centers (Samaj Kalyan Centre, Pashupati, Nisaya Sewa Sadan, Baneshwor and Birddha Vewasthapan Kendra, Teku) were selected and non elderly populations were selected from different regions of Kathmandu. Similarly, a structured questionnaire on name/code, age, demographic data, health problem, respiratory tract problem, smoking and drinking habits, disease history, symptoms and other information were recorded with taking verbal consent, by interviewing with them or their care-takers.

Throat swabs and sputum of individuals were collected as samples for the study. These samples were examined macroscopically as well as microscopically. Both swabs and sputum samples were cultured on Sabouraud’s Dextrose Agar (SDA) separately and were incubated at 37°C and examined after 48 hrs. Growth of cream coloured pasty colonies was suggestive of *Candida* species and they were further identified by simple staining, germ tube test, sugar fermentation test and Sugar assimilation test, Chlamyospore detection test. The antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute recommended Kirby-Bauer susceptibility testing method.¹⁴ The data obtained from questionnaire and laboratory findings were interpreted by using statistical package for social science (SPSS version 11.5)

RESULTS

Out of total 304 samples of each sputum and throat swabs, 16.45% and 5.3% samples respectively were growth positive for fungal culture showing comparatively higher growth positivity in sputum samples. Out of 143 elderly population, 47 (32.86%) showed sputum

culture positive while in 96 (67.0%) sputum specimen culture was negative. Of 143 total target population of age 50 years and above, 4.6 % (14/143) were swab cultures positive for fungi. Statistically the relationship between age category and throat swab/sputum culture was significant ($p < 0.001$).

Table 1: Fungal growth positivity in sputum specimens of study population

Growth pattern	Population under study		Total	p-value
	=<50 Years	Above 50 Years		
Growth	3(1.9%)	47(32.87%)	50(16.45%)	p< 0.001
No growth	158(98.13%)	96(37.13%)	254(83.55%)	
Total	161(100.0%)	143(100.0%)	304(100.0%)	

Table 2: Fungal growth positivity on throat swab culture in the study population

Growth Pattern	Population under study		Total	p-value
	=< 50 years	Above 50 years		
Growth	2 (1.24%)	14 (9.8%)	16 (5.3%)	p< 0.001
No growth	159 (98.75%)	129 (42.43%)	288 (94.73%)	
Total	161 (100.0%)	143 (100.0%)	304 (100.0%)	

C. albicans was most frequently encountered in sputum samples of study population. In the case of population of age above 50 years (143), highest number i.e. 23 showed presence of *C. albicans* followed by *C. tropicalis* (5), *C. stelloide* (3), *C. pseudotropicalis* (3), *C. guilliermondii* (2), *C. krusei* (1) and *C. parapsilosis* (1). However only two species of *Candida*, *C. albicans* (2) and *C. krusei* (1) were isolated from the sputum specimen of total 161 objects of age less or equal to 50 years (Table 3). The distribution of *Candida* species was significant with age category of the study population ($p < 0.001$). In the case of throat swab specimen, prevalence of *Candida* species was higher in elderly population as compared to non elderly population. Only four species of *Candida* i.e. *C. albicans*, *C. tropicalis*, *C. guilliermondii* and *C. krusei* were isolated. Comparatively *C. albicans* presence was highest (8) (Table 4). The prevalence of *Candida* species in swab cultures was insignificantly related with the age group of the population ($p > 0.001$).

Table 3: Distribution of *Candida* species obtained in sputum samples of study population

Candida species isolated	Study population of age		Total	p-value
	=<50 years	Above 50 years		
No growth	158(98.14%)	105(73.43%)	263(86.51%)	p< 0.001
<i>Candida albicans</i>	2(1.24%)	23(16.08%)	25(8.22%)	
<i>Candida tropicalis</i>	0(0.0%)	5(3.5%)	5(1.64%)	
<i>Candida pseudotropicalis</i>	0(0.0%)	3(2.1%)	3(0.98%)	
<i>Candida stelloide</i>	0(0.0%)	3(2.1%)	3(0.98%)	
<i>Candida krusei</i>	1(0.62%)	1(0.7%)	2(0.65%)	
<i>Candida guilliermondii</i>	0(0.0%)	2(1.39%)	2(0.65%)	
<i>Candida parapsilosis</i>	0(0.0%)	1(0.7%)	1(0.32%)	
Total	161(100.0%)	143(100.0%)	304(100.0%)	

Table 4: Distribution of *Candida* species isolated from throat swab samples of study population

<i>Candida</i> species	Study population		Total	p- value
	=<50 years	Above 50 years		
No growth	159(98.76%)	131(91.60%)	290(95.39%)	(p>0.001)
<i>Candida albicans</i>	1(0.62%)	8(5.6%)	9(2.96%)	
<i>Candida tropicalis</i>	0(0.0%)	2(1.39%)	2(0.65%)	
<i>Candida guilliermondii</i>	0(0.0%)	2(1.39%)	2(0.65%)	
<i>Candida krusei</i>	1(0.62%)	0(0.0%)	1(0.32%)	
Total	161(100.0%)	143(100.0%)	304(100.0%)	

Among three antimycotic drugs used, *Candida* isolates was highly susceptible to Ketoconazole and resistant to Fluconazole (Table 5). *Candida albicans* was found resistant towards Fluconazole as compared to other two antimycotic drugs used.

Table 5: Antimycotic susceptibility pattern of *Candida* isolates

<i>Candida</i> Isolate	Antifungal agent								
	Fluconazole (25 µg/ml)			Itraconazole (conc)			Ketoconazole (conc)		
	S	I	R	S	I	R	S	I	R
<i>Candida albican</i> (n=36)	1 (2.8%)	3 (8.3%)	32 (88.9%)	16 (44.4%)	4 (11.1%)	16 (44.4%)	24 (66.6%)	-	12 (33.3%)
<i>Candida tropicalis</i> (n=7)	6 (85.7%)	1 (12.3%)	-	3 (48.9%)	2 (28.7%)	1 (12.3%)	7 (100%)	-	-
<i>Candida guilliermondii</i> (n=4)	2 (50%)	-	2 (50%)	3 (75%)	-	1 (25%)	3 (75%)	-	1 (25%)
<i>Candida pseudotropicalis</i> (n=3)	2 (66.6%)	-	1 (33.3%)	2 (66.6%)	1 (33.3%)	-	3 (100%)	-	-
<i>Candida krusei</i> (n=3)	1 (3.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	-	2 (66.6%)	1 (33.3%)	-	2 (66.6%)
<i>Candida stelloide</i> (n=3)	-	-	3 (100%)	1 (33.3%)	-	2 (66.6%)	1 (33.3%)	-	2 (66.6%)
<i>Candida parapsilosis</i> (1)	-	-	1 (100%)	-	-	1 (100%)	-	-	1 (100%)

DISCUSSION

In this study, out of total 304 samples of each sputum and throat swabs 16.45% and 5.3% samples respectively were growth positive for fungal culture showing comparatively higher growth positivity in sputum samples. Sputum and swabs are the best specimens used for isolation of yeast and molds especially in case of fungal respiratory tract infection. However sputum sample is better for higher recovery of fungi.¹⁵ In present study in total, *Candida* species (55), *Aspergillus* species (8), *Penicillium* species (1) and *Rhizopus* species (1) were isolated from both sputum and throat swab samples with *Candida* species being the most prevalent. However molds like *Aspergillus*, *Penicillium* and *Rhizopus* are also frequently encountered in sputum and throat swab cultures.^{15,16}

In our study, fungal culture positivity of both sputum and swab were found higher in elderly people (having

age above 50 years) than those with age ≤ 50 years. Fungal prevalence is more in elderly population because their immune defenses have been altered by old age.¹⁶

In the present study, of the total 133 (43.75%) male respondents, growth was obtained in 12.78% (17/133) sputum samples while among 171 (56.25%) female respondents, the growth was obtained in 19.30% (33/171) sputum samples. The growth was more preponderance in the females. This may be due to higher number of female respondents than male respondents enrolled for the study. However distribution of fungal culture positivity with sex was statistically insignificant (p>0.001).

In the study total number of *Candida* species isolated was 55 (14 from the swab sample and 41 from the sputum sample). Seven species of *Candida* were obtained from sputum and throat swab culture. These include *C. albicans*, *C. tropicalis*, *C. stelloide*, *C. pseudotropicalis*, *C. guilliermondii*, *C. krusei* and *Candida parapsilosis*. Higher number of *Candida* species were observed in sputum sample than in throat swab. This findings is in accordance to the findings done in Kathmandu where higher number of *Candida* species were isolated from sputum sample.¹⁷ The study indicates higher susceptibility of higher age group for *Candida* or its infection. Similar to our observation, in a study by Jha *et al.*, the commonest species of *Candida* isolated from sputum was found to be *C. albicans* followed by *Candida tropicalis*. Non-albicans *Candida* may be of high clinical significance mainly in case of the patients with predisposing risk factors.^{17,18}

In this study different *Candida* species were tested for azole antifungal drugs. Among three antimycotic drugs used, Ketoconazole was highly susceptible by 68.42% against the *Candida* isolates where as fluconazole was more resistance by 70.17%. In present study antimycotic susceptibility test was performed by disc diffusion assay. However estimation of MICs is more definitive in interpreting the results. This study has indicated that ketoconazole is effective antifungal agent for the treatment of infection by *Candida* species. The yeast and molds positivity in the sputum culture was significantly associated with the presence of health problem in the study population while swab culture positivity had no significant association. Sputum of respondents above 50 years and having health problem showed high positivity towards the *Candida* species growth as well *Aspergillus* species growth. This might

indicates yeasts and molds can be health harshening agents in the people having age above 50 years. The yeast and molds positivity in the sputum culture was significantly associated with the presence of respiratory tract problem in the study population while swab culture positivity had no significant association. *Candida* and *Aspergillus* species were more prevalent in the above 50 years people with respiratory tract problem.

CONCLUSION

The populations with age above 50 years were more

susceptible to the *Candida* infection both in upper and lower respiratory tract. Among the *Candida* isolates, *C. albicans* were found predominant. Most of the isolated *Candida* species were resistant to fluconazole and susceptible to Ketoconazole. *Candida* and molds positivity in the sputum culture was significantly associated with the presence of respiratory tract problem. Smoking habits and the Carbohydrates diet can be the enhancing factor for the fungal infection in the respiratory tract with the associated symptoms of irritation and discomfort in the throat.

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