ORIGINAL ARTICLE

Isolation of *Candida* species on human mucosal surfaces

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ABSTRACT

Objectives: Fungi are part of the healthy microbiota of the human mucosal surfaces. Fungal microbiota is often altered in disease states, especially when there is an imbalance in the host. (1). *Candida* is one of the most commonly isolated yeasts in mucosal fungal microbiota (2). When considering the role of mucosal surfaces as a reservoir from which infection of a susceptible host can occur, determining the fungal microbiome, especially *Candida*, is important. Therefore, in the current study, mucosal surfaces related to three different anatomical sites of the body were tested in 223 healthy people for the presence of *Candida spp.*

Results: Children had the highest incidence of *Candida* isolated in the mucosa surfaces (n=43, 46.2%) and the lowest was among the adults (n=22, 24.2%). *Candida albicans* (n=53, 58.2%) was the predominant fungal species isolated from mucosal surfaces, followed by *C. glabrata* species complex (n=18, 19.8%), *C. parapsilosis* species complex (n=10, 11.0%), *Pichia kudriavzevi* (formerly *C. krusei)* (n=6, 6.%) and *C. tropicalis* (n=4, 4.%). In this study the isolation of *Candida* species was similar between males (n= 37, 40.%) and females (n= 54, 59.3%).

Conclusions: There was a direct association between the age of subjects and the frequency of mucosal *Candida* isolates. *C. albicans* was the predominant species isolated from all age groups. This study showed no statistically significant relationship between subjects gender and the frequency of mucosal *Candida* isolates.

Keywords: Mucosal *Candida* isolates, normal flora, age, gender, DNA-sequencing, culture.

N Z J Med Lab Sci 2021; 75: 33-37

INTRODUCTION

The genus *Candida* was created during the IX International Botanical Congress. *Candida* species are opportunistic fungal pathogens found as part of the normal microflora in human skin and mucosal surfaces and exist harmlessly in these anatomical sites (7-9). Mucous membrane surfaces constitute the largest interface between the host and the environment. There are several defense mechanisms that protect and combat commensal and opportunistic microorganisms from causing infection in a healthy individual (10).

Researchers have shown that *Candida* species are human and warm-blooded animal reservoirs (11,12). Since *Candida* species are commonly found in the human body, colonisation or infection occurs when this normal balance is disrupted (10). An example of this is long-term use of broad-spectrum antibiotics which may interrupt this balance. Other factors that can affect this imbalance include immunological functions, age, physiological changes, physical and mental disabilities, debilitating diseases, diabetes, the clinical use of immunosuppressive drugs, occupation, obesity, vascular disease, alcoholism, and avitaminosis. Damage to the mucus membranes and to the salivary glands also allow for *Candida* colonisation (10-15). They can be the agents of local or systemic opportunistic infections in hospitalized patients, those under intensive treatment, immunocompromised patients, and even in healthy individuals. Infections caused by opportunistic pathogens such as *Candida* spp. are frequent, especially in immunodeficiency disorders such as neutropenia, neoplasia, decompensated diabetes mellitus, malnutrition, organ transplantation, and AIDS (6,16 -19). Two of the most common factors influencing the microbiome of mucosal surfaces are gender (13) and age (15).

There is little information about *Candida* distribution inhabiting human mucosal surfaces. This is the first study on this topic in Iran. The purpose of this study was to determine the type and percentage of *Candida* population residing on mucosal surfaces (the mouth, nose, and vagina). In addition, to determine the potential influences of age and gender on the prevalence of *Candida* isolation on mucosal surfaces.

MATERIALS AND METHODS

Ethics

This study was approved by the ethical committee of Tehran University of Medical Science (Ethics Committee protocol: IR.TUMS.SPH.REC.1395.1759). Written informed consent was obtained from all subjects or their guardians prior to sample collection.

Sampling

A total of 223 healthy individuals (including 93 males and 130 females) categorised into three age groups were included. The distribution of subjects in each group was as follows:

- 103 children aged between 1 and 12 years old. Most were drawn from schools and kindergartens.
- 63 healthy adults aged between 18 and 45 years old. This group was composed of students of Tehran University, factory workers, health workers, and housewives
- 57 adults greater than 60 years old, which mainly consisted of retiries.

At the time of sampling, the age and gender of each subject were recorded. The mouth, nose, and vagina were sampled by means of a cotton-tipped swab moistened with sterile serum physiology.

Mycological techniques

All swabs were inoculated on Sabouraud Chloramphenicol Agar (SC, Merck, Germany) by swab-streak technique (3). All cultures were incubated at 25°C for 4 days. Isolated colonies were identified by growth on Corn Meal Agar Tween 80 (Micro Media, Hungary). Using a straight wire, we picked a colony off the surface from the SC medium and made a deep cut in the Corn Meal Agar (a horizontal furrow). Then we placed a flamed sterile coverslip over the line of inoculum. After incubation for 24 to 48 hours at 22°C the streaks were examined. Along with such streaks, *Candida albicans* produces characteristic mycelium-bearing ball-like clusters of budding cells and thick-walled round chlamydospores (4) which is distinct from other *Candida* species.

In this study, we picked several colonies from each SC medium and plated onto CHROMagar *Candida* medium (Paris, France). This medium contains chromogenic substrates which react with enzymes secreted by the target microorganisms to yield colonies of varying colors (5). CHROMagar *Candida* medium is species-specific, allowing the organisms to be identified to the species level by their color and colonial characteristics. It is able to detect and differentiate three species types: *C. albicans* by growth as light to medium green colonies, *C. tropicalis* by growth as steel blue colonies accompanied by purple pigmentation diffused into surrounding agar, and *C. krusei* by growth as large, fuzzy, rose-colored colonies with white edges, after incubation for 48 hours at 37°C. Other yeasts may develop either light to dark mauve or cream colors (e.g., *C. glabrata* comlex) on isolation media (5).
In this study, DNA sequencing was performed on isolates that were not identified by mycological techniques.

DNA extraction

An aliquot of 100 µL of yeast cell suspension was transferred to microtubes and incubated at 100°C in a boiling water-bath for 10 min, then centrifuged at 5,000*g* for 5 minutes. The supernatant (containing the DNA) was carefully transferred to a clean tube and was used for PCR.

RESULTS

Among the studied population, including children, adults and the elderly, divided into three age groups, 91 subjects were positive for *Candida* isolation. The highest prevalence of *Candida* isolation was related to the children age group (n=43, 46.2) and the lowest prevalence was related to the adults (n=22, 2.17%), and showed there was a direct association between the age of subjects and the frequency of mucosal *Candida* isolates ($p < 0.001$). *Candida albicans* (n=53, 58.2%) was the predominant isolated species from all age groups followed by *C. glabrata* species complex (n=18, 19.8%), *C. parapsilosis* species complex (n=10, 11.0%), *Pichia kudriavzevi* (formerly *C. krusei)* (n=6, 6.6%), and *C. tropicalis* (n=4, 4.4%). It should be noted that there was not a significant difference between the individual's age and the isolated organism (Table 2 and Figure 1).

In this study, 223 healthy subjects (93 males and 130 females) were examined. The isolation of *Candida* species was significantly similar between males (n= 37, 40.6%) and females (n= 54, 59.4%) and there was no significant difference in the mucosal *Candida* population between genders ($p<0.999$). *Candida albicans* (n= 53, 58.2%) was the most frequently isolated species and *Candida tropicalis* (n=2, 2.%) showed the lowest prevalence in both genders (Table 3 and Figure 2).

Considering *Candida* isolation based on anatomical sites, from 91 positive sites for *Candida* isolation, the highest prevalence of *Candida* isolation was related to the oral mucosa (n=62, 68.1%) and the lowest prevalence was related to the vagina (n= 10, 11.0 %) in females (Table 4 and Figure 3).

In the children among 43 *Candida* species isolated from three different mucosal surfaces (the mouth, nose, and vagina), the highest prevalence of *Candida* isolation was related to the oral mucosa, with 35 positive cases (81.4%), and the nasal mucosa with eight positive cases (18.6 %) was in second place. In this age group, none of the vaginal specimens had positive results for *Candida* isolation. Also, *Candida albicans* with 23 isolates (54.8%) was the predominant species isolated from three different mucosal surfaces of each person in this age group (Table 2).

In adults, among 22 isolated *Candida* species, the highest prevalence of *Candida* isolation was from the vagina (n=10, 5%) followed by the mouth (n= 9, 40.9%), and the nasal mucosa (n=3, 13.4 %). Furthermore, in this age group, *Candida albicans* with 16 isolates (72.7 %) was the predominant species from the mucosal surfaces of each person (Table 2).

In the elderly age group, among 26 isolated *Candida* species, the highest prevalence of *Candida* isolation was related to the oral mucosa with 18 positive cases (69.2 %), and the nasal mucosa with eight positive cases was in second place (30.8%). All vaginal specimens in this age group had negative results for *Candida* isolation. Also, in this age group, *Candida albicans* with 14 positive isolates (53.8 %) was the predominant species (Table 2).

The oral mucosa of male participants had the highest prevalence of *Candida* isolation with 28 of 37 isolated *Candida* species (75.7%). Also, in the male gender, the nasal mucosa with nine *Candida* isolates (24.32%) was in second place. This finding was similar in the female gender with 32 out of 54 *Candida* species isolated from the oral mucosa (59.3 %), and the nasal mucosa with 12 isolates (22.2 %) was in second place.

Table 1. GenBank accession numbers of DNA sequences included in this study

Fungal elements	GenBank accession numbers used in the sequence analysis
Candida albicans	MG913256, KY996543, MF614725, MF614723, MH729024, MG599201, MN4193373, MN318604, MH729028, KC905069, MG818819, MG818824
Candida tropicalis	MK793225, MK547223
Candida glabrata species complex	KU992391, KU992392, KU992393, LC311497, LR757911
Pichia kudriavzevi (Candida krusei)	MH545928, FJ515204
Candida parapsilosis complex	MK394127, KY102205, KP131738, EU564209

Table 2. The frequency of *Candida* species isolated from mucosal surfaces in different age groups

***Candida* species**	**Children**		**Adults**		**Elderly**		**Total**	
	Number	%	Number	%	Number	%	Number	%
Candida albicans	23	54.8	16	72.7	14	53.8	53	58.2
Candida parapsilosis species complex	2	4.8	2	9.1	6	23.1	10	11.0
Candida glabrata species complex	15	35.7	3	13.6	0	0	18	19.8
Pichia kudriavzevi (Candida krusei)	2	4.8	0	0	4	15.4	6	6.6
Candida tropicalis	0	0	1	4.5	2	7.7	4	4.4
Total	43	100	22	100	26	100	91	100

Table 3. Frequency of *Candida* species isolated from mucosal surfaces based on gender.

***Candida* species**	**Men**		**Women**	
	Number	**%**	**Number**	**%**
Candida albicans	21	56.8	32	59.3
Candida parapsilosis species complex	4	10.8	6	11.1
Candida glabrata species complex	8	21.6	10	18.5
Pichia kudriavzevi (Candida krusei)	3	8.1	5	9.3
Candida tropicalis	1	2.7	1	1.9
Total	37	100	54	100

Table 4. Frequency of *Candida* species isolated from mucosal surfaces based on anatomic site.

Anatomical site	***Candida albicans***		***Candida parapsilosis* species complex**		***Candida glabrata* species complex**		***Pichia kudriavzevi (Candida krusei)***		***Candida tropicalis***		**Total**	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
Mouth	36	67.9	6	60.0	14	77.8	6	100	0	0	62	68.1
Nose	10	18.9	2	20.0	4	22.2	0	0	3	75.0	19	20.9
Vagina	7	13.2	2	10.0	0	0	0	0	1	25.0	10	11.0
Total	53	100	10	100	18	100	6	100	4	100	91	100

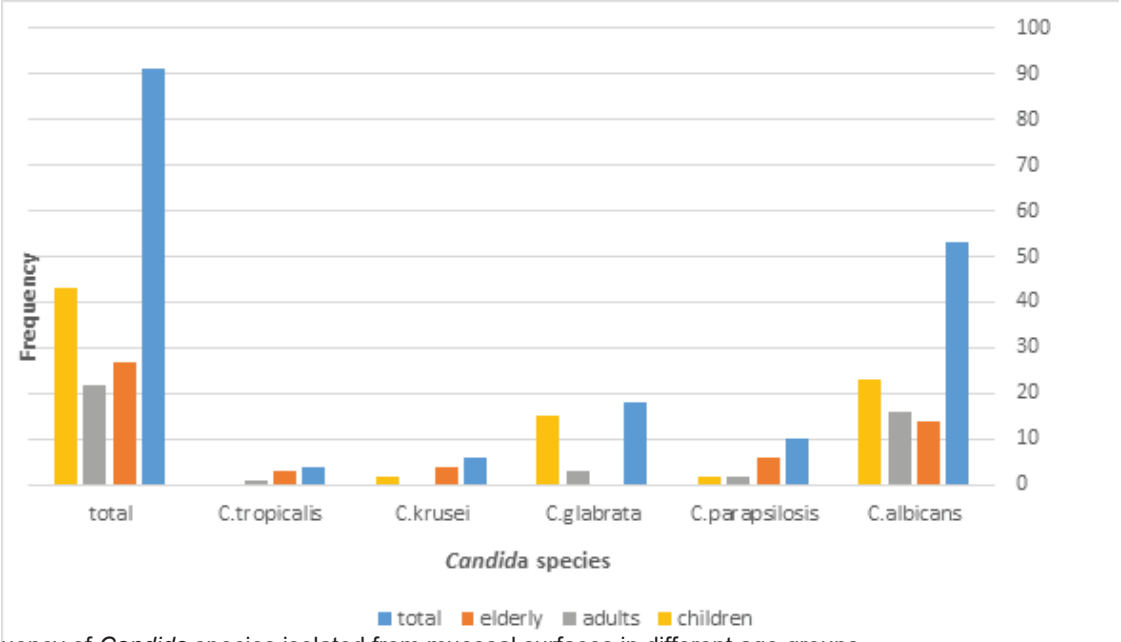

Figure 1. Frequency of *Candida* species isolated from mucosal surfaces in different age groups.

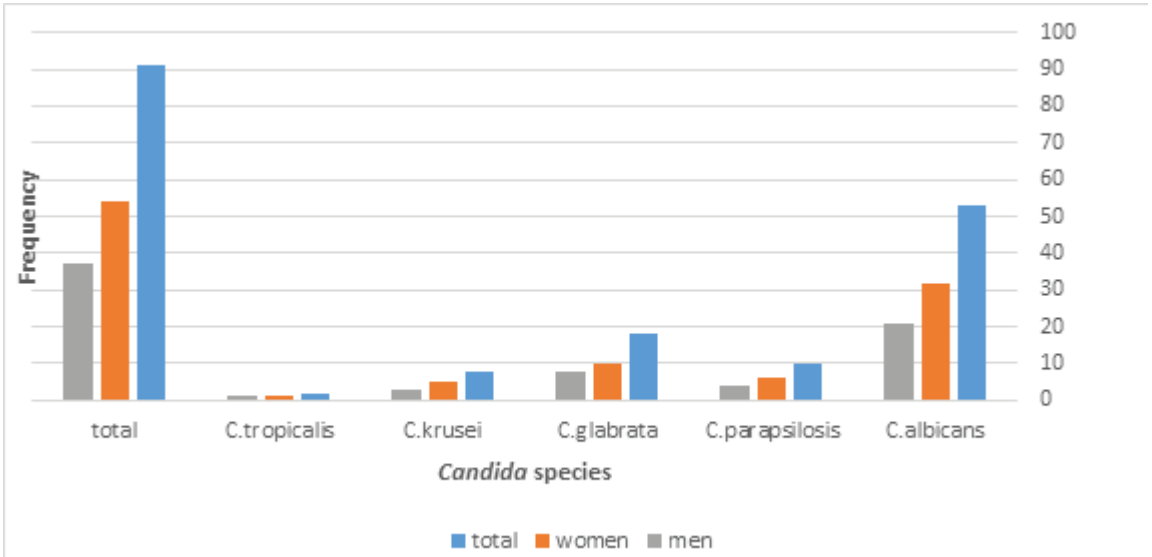

Figure 2. Frequency of *Candida* species isolated from mucosal surfaces based on gender.

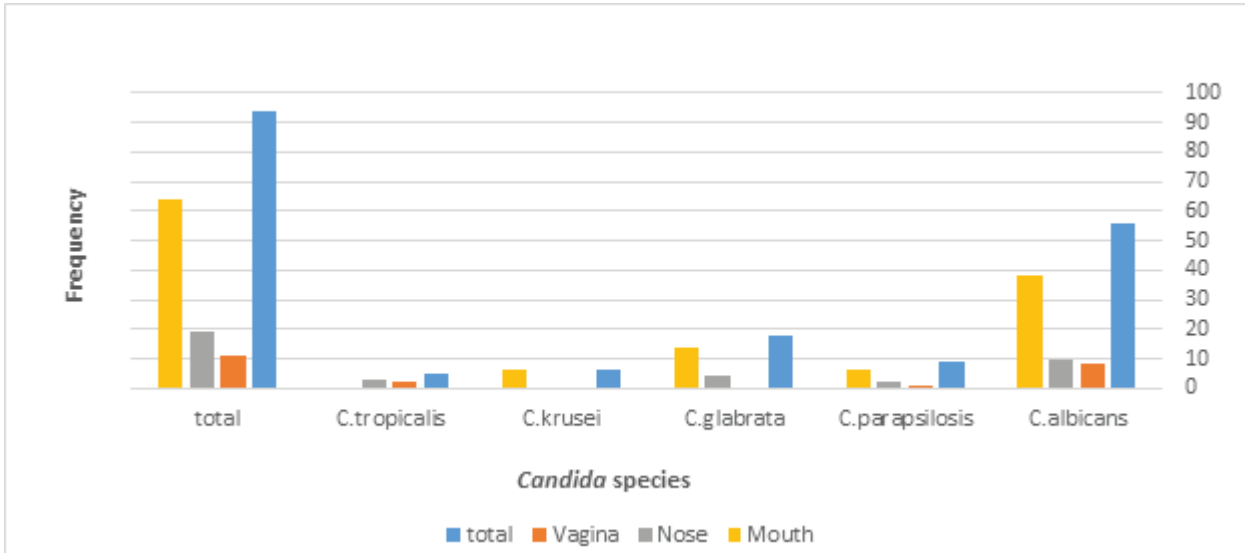

Figure 3. Frequency of *Candida* species isolated from mucosal surfaces based on anatomical site of the body.

DISCUSSION

Our study showed that *Candida* was most prevalent in children. This is mainly due to poor oral health among this age group (3-12 years). The elderly age group was the second most prevalent group. The main reason for this finding is due to the use of dentures in this age group (15, 21).

The mucosal *Candida* community was similar between males and females and there was no significant difference in *Candida* isolation between genders. Both groups share similar sociodemographic, lifestyle, and dietary conditions (20,23). The results of this investigation showed that the oral mucosa had the highest incidence of *Candida* isolation compared to the nasal mucosa and the vagina. This could be due to increased exposure of the mouth as the route of entry for a range of foods and other infecting microbes. Other factors that increase the microbial burden of mouth, such as poor hygiene, gingival disease, poor dentition and the effect of tooth decay and the use of dentures, the increased chance of yeast isolation from this anatomical site is explainable (15,25). *C. albicans* was the most frequently isolated organism (68.1%) from all mucosal surfaces in the present study (17,22). This finding is consistent with other published literatures (6,14).

ACKNOWLEDGMENTS

The authors appreciate the support of Tehran University of Medical Sciences, Tehran, Iran.

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