Assessment of *Candida* spp. frequency in the oral cavity ontocenosis of healthy individuals in different age groups

Jolanta Szymańska¹, Agnieszka Wójtowicz², Anna Malm²

- ¹ Chair and Department of Paedodontics, Medical University of Lublin, Poland
- ² Chair and Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Medical University of Lublin, Poland

Szymańska J, Wójtowicz A, Malm A. Assessment of *Candida* spp. frequency in the oral cavity ontocenosis of healthy individuals in different age groups. J Pre-Clin Clin Res. 2016; 10(2): 91–94. doi: 10.5604/18982395.1227563

Abstract

Introduction. Among the microorganisms colonizing the oral cavity ontocenosis, an important role is played by fungi: mainly yeast-like fungi of the *Candida* genus, and especially by the species *C. albicans*.

Objective. The aim of this study was to evaluate the frequency of oral colonization by *Candida* species in healthy individuals of different age groups, and to identify the species of the isolates.

Materials and method. The fungi were isolated from the oral cavities of 654 healthy individuals of both genders, representing different age groups, and the species of the isolates were determined. Initial identification of the yeast-like fungi was made based on the macroscopic appearance of the colonies on Sabouraud's medium, and the growth of coloured colonies on ChromAgar Candida medium. API 20 C AUX microtest (bioMerieux) was used to identify the most frequently found species of *Candida* spp.

Results. The frequency of *Candida* isolation from the oral cavity ontocenosis in healthy individuals of different age groups was assessed as 30.6%. The oral cavity ontocenosis was colonized mainly by the yeast-like fungi of *C. albicans* species: they were found in 24.5% of the tested population, while the frequency of oral cavity colonization by non-albicans *Candida* spp. was 6.1%. Seven species of non-albicans *Candida* spp. were identified: *C. glabrata*, *C. inconspicua*, *C. famata*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, and *C. kefyr*.

Conclusions. In the Polish population, 30.6% of the oral cavity ontocenosis is colonized by *Candida* yeast-like fungi, while *C. albicans* is the fungus that colonizes oral cavity ontocenosis most frequently. The frequency of oral cavity ontocenosis colonization with *Candida* spp (including *C. albicans*) increases with age.

Key words

fungi, Candida spp, Candida albicans

INTRODUCTION

The oral cavity is a specific ecosystem populated with numerous aggregations of various microbial species. Fungi, mainly yeast-like fungi of *Candida* genus, including especially *Candida albicans*, have an important place among microorganisms colonizing the oral cavity ontocenosis [1, 2, 3]. Yeast-like fungi are microorganisms very well adapted to overcome the defence mechanisms of the human organism. Among various *C. albicans* virulence factors, the most important role is attributed to its biofilm-forming abilities [4, 5, 6].

The aim of this study was to evaluate the frequency of oral colonization by *Candida* species in healthy individuals of different age groups, and to identify the species of the isolates.

MATERIALS AND METHOD

The study material included *Candida* yeast-like fungi strains isolated from the oral cavity ontocenosis of healthy individuals in different age groups. Swab samples from the oral cavity were taken from 654 individuals of both genders,

Address for correspondence: Jolanta Szymańska, Chair and Department of Paedodontics, Medical University of Lublin, Poland E-mail: szymanska.lublin@gmail.com

Received: 11 December 2016; accepted: 16 December 2016

residents of south-eastern Poland (Central-Eastern Europe). The study did not include patients undergoing orthodontic treatment with fixed and removable appliances, nor those using fixed or removable dental prostheses.

It was shown that such appliances affect the microbiological environment and cause an increase in the prevalence of *Candida* in the mouth [7, 8]. The examined persons were divided into groups according to age (Tab. 1). Immediately after sampling, or after sampling and placing on transport medium, the study material (swab samples from the oral cavity) was inoculated into Sabouraud's medium with chloramphenicol and into Chrom Agar Candida. The inoculates were incubated

Table 1. Studied populations

Studied group	Age brackets (years)	Number of persons
0 (neonates, infants, children attending day nurseries)	0-3	102
1 (children attending kindergartens)	4-6	82
2 (primary school children)	7-14	91
3 (secondary school adolescents)	15-18	101 92
4 (students)	19-25	
5 (professionally-active individuals)	26-45	79
6 (professionally-active individuals, pensioners, oldage pensioners)	46-65	53
7 (wards of social welfare homes)	≥ 66	54

for 48 h at 35°C. The initial identification of yeast-like fungi was based on the macroscopic appearance of the colonies on Sabouraud's medium, and of the growth of coloured colonies on ChromAgar Candida medium. Further tests were performed on the isolates that formed cream-coloured, smooth or slightly wavy, concave, glossy, smelling of yeast, and creamy-textured colonies. Yeast-like fungi were isolated on Sabouraud's medium. The inoculates were incubated for 48–72 h at 35°C. In the microscopic preparations stained with Gram's method, Gram-positive thin-walled, spherical, cylindrical or egg-shaped 4–6µm balstospores were found. The identification of the most common species of *Candida* spp. was performed with API 20 C AUX test (bioMerieux).

The frequency of different species of *Candida* spp. in the oral cavity in the studied population of healthy individuals from various age groups is presented in Figure 1. The percentage share of *C. albicans* in the isolated yeast-like fungi varied from 71.4% (Group 7) to 87.4% (Group 2). In total, 7 species of non-albicans *Candida* spp. were identified: *C. glabrata*, *C. inconspicua*, *C. famata*, *C. tropicalis*, *C. parapsilosis*, *C. lusitaniae*, and *C. kefyr*; the species composition of those yeast-like fungi differed in various age groups. The percentage share of non-albicans *Candida* spp. varied from 12.6% (Group 2) to 26% (Group 0). In individual groups, from 3–5 species were isolated. The simultaneous presence of two species of *Candida* genus was found in 16 (8%) persons.

RESULTS

According to the data presented in Table 2, the frequency of isolating *Candida* yeast-like fungi from the oral cavity ontocenosis of the study population of healthy individuals in different age groups was 30.6%; in all age groups, this index varied from 22.5% in the youngest group (Group 0) to 52.0% in the oldest group (Group 7). Group 6, composed of 3 mature people, was characterized with a high level of oral cavity colonization, reaching 45.3%. The results for the remaining age groups were similar, from 25.7%–30.4%.

Table 2. Frequency of *Candida* spp., including *C. albicans*, in the oral cavity ontocenosis of healthy individuals in different age groups

Studied group	Number of persons	Age bracket	Number (percentage) of persons colonized by <i>Candida</i> spp.	Number (percentage) of persons colonized by <i>C. albicans</i>	Number (percentage) of persons colonized by non-albicans Candida spp.
0	102	0-3	23 (22.5)	17 (16.7)	6 (5.8)
1	82	4-6	25 (30.4)	20 (24.4)	5 (6.0)
2	91	7-14	24 (26.4)	21 (23.0)	3 (3.3)
3	101	15-18	26 (25.7)	21 (20.8)	5 (5.0)
4	92	19-25	26 (28.3)	22 (24.0)	4 (4.3)
5	79	26-45	24 (30.4)	20 (25.3)	4 (5.0)
6	53	46-65	24 (45.3)	19 (35.8)	5 (9.5)
7	54	≥66	28 (52.0)	20 (37.0)	8 (14.8)
Total	654	-	200 (30.6)	160 (24.5)	40 (6.1)

The oral cavity ontocenosis in the study population was colonized mainly by *C. albicans* yeast-like fungi; they were found in 160 (24.5%) persons. The level of *C. albicans* in all the studied groups varied from 16.7% in Group 0 to 37.0% in Group 7. A high colonization level was found also in Group 6, reaching 35.8%. In the remaining age groups, the results were similar and varied from 20.8%–25.3%.

The frequency of oral cavity colonization with non-albicans *Candida* spp. yeast-like fungi in the study population was 6.1%; their presence was found in 40 (20%) persons. In different age groups, the frequency of those yeast-like fungi varied from 3.3% (Group 2) to 14.8% (Group 7). A high level of colonization with those yeast-like fungi was also found in Group 6 (9.5%). The frequency of isolating non-albicans *Candida* spp. in the remaining age groups was similar and varied from 4.3%-6%.

DISCUSSION

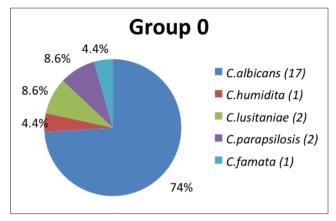
According to literature data [1, 3, 9–22], *Candida* yeast-like fungi can occur in healthy people with frequency from 10%-100%. The results obtained in this study, both concerning the frequency of *Candida* spp. (30.6%) and of *C. albicans* (24.5%), in the examined population fall into the range indicated by other authors [1, 3, 22].

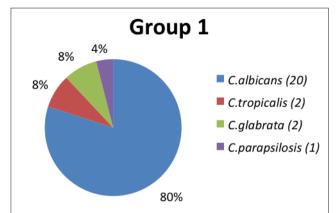
It seems important that these indices differ according to age group. In the presented study, the carriership of Candida spp. and C. albicans in all studied groups varied from 22.5% in the youngest age group to 52.0% in the oldest age group, and from 16.7%-37.0%, respectively. These data confirm that with age there is an increasing tendency in the level of oral cavity ontocenosis colonization with Candida spp. (including *C. albicans*), while in children, youths and professionally-active individuals, a certain plateau can be observed. According to the authors, old age has been shown as one of the factors related to Candida carriage. This is related to salivary glands hypofunction, reduced salivary flow rate, intensification of periodontal diseases with age, a decrease in manual dexterity connected with age, poor oral hygiene habits in the elderly, and metabolic diseases causing serious multiorgan disturbances (e.g. diabetes mellitus or decreasing immunity) [23].

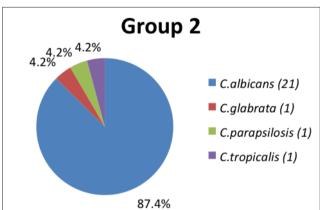
As the literature data shows [1, 3, 22], *C. albicans* continues to be a prevailing yeast-like fungi species that can be found in the oral cavity. This is confirmed by the results obtained in the present study. It was shown that 80% of all the isolated strains of *Candida* spp. belonged to that species. It should be noted, however, that a tendency to shift the balance towards non-albicans *Candida* spp. species occurs especially in patients with immunity disorders [1, 11, 12, 13, 14, 15]. In the presented study, this tendency could be observed in the oldest age groups, where the indices of non-albicans *Candida* spp. species isolation were significantly higher (14.8%) than in younger age groups (3.3%).

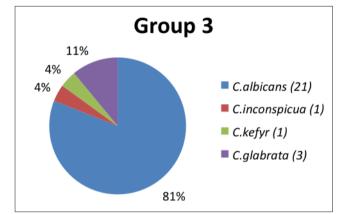
CONCLUSIONS

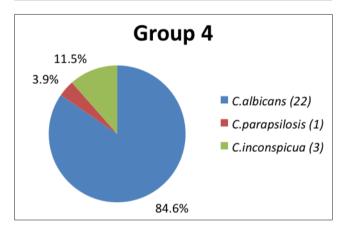
- 1. In the Polish population, 30.6% of the oral cavity ontocenosis is colonized by *Candida* yeast-like fungi.
- 2. C. *albicans* is a yeast-like fungus most frequently colonizing the oral cavity ontocenosis.
- 3. The frequency of oral cavity ontocenosis colonization with *Candida* spp. (including *C. albicans*) increases with age.

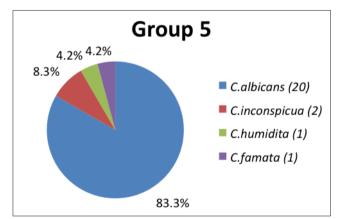


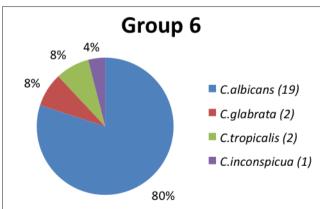


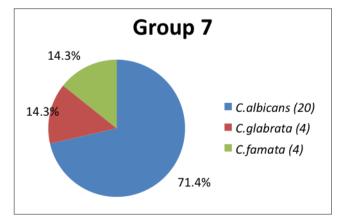












The number of isolates is given in parentheses.

Figure 1. Genetic grouping of Candida spp. isolated from oral cavity ontocenosis of healthy people of different age groups

Jolanta Szymańska, Agnieszka Wójtowicz, Anna Malm. Assessment of Candida spp. frequency in the oral cavity ontocenosis of healthy individuals in different age groups

REFERENCES

- 1. Grimoud AM, Marty N, Bocquet H, et al. Colonization of the oral cavity by Candida species: risk factors in long-term geriatric care. J Oral Sci. 2003; 45(1): 51–55.
- Kurnatowska AJ. Occurence of fungi in oral cavity ontocenosis and changes to the mucosa. Mikol Lek. 2003; 10(4): 295–298 (in Polish).
- 3. Siqueira J, Sen B. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 97: 632–641.
- 4. Karkowska-Kuleta J, Rapala-Kozik M, Kozik A. Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus. Acta Biochem Pol. 2009; 56 (2): 211–224.
- Ramage G, Martinez JP, Lopez-Ribot JL. Candida biofilms on implanted biomaterials: a clinically significant problem. Yeast Res. 2006; 6: 979– 986
- 6. Thein ZM, Seneviratne CJ, Samaranayake YH, et al. Community lifestyle of Candida in mixed biofilms: a mini review. Mycoses 2009; 52(6): 467–475.
- 7. Kyoko H, Wong RW, Hägg U, et al. The effect of orthodontic appliance on Candida in the human mouth. Int J Paediatr Dent. 2009; 19(5): 301–308.
- 8. Prakash B, Shekar M, Maiti B, et al. Prevalence of Candida spp. among healthy and nondenture wearers with respect to hygiene and age. J Indian Prosthodont Soc. 2015; 15(1): 29–32.
- Back-Brito GN, Mota AJ, Vasconcellos TC, et al. Frequency of Candida spp. in the oral cavity of Brazilian HIV-positive patients and correlation with CD4 cell counts and viral load. Mycopatholgia 2009; 167: 81–87.
- 10. Barros LM, Boriollo MFG, Alves ACBA, et al. Genetic diversity and exoenzyme activities of Candida albicans and Candida dubliniensis from the oral cavity of Brazilian periodontal patients. Arch Oral Biol. 2008; 53: 1172–1178.
- 11. Biedunkiewicz A. Yeast-like fungi isolated in students. Acta Mycol. 2007; 42(1): 141–149 (in Polish).

- 12. Ejdys E. Factors predisposing appearance of yeasts-like fungi in healthy school age girls and boys. Mikol Lek. 2008; 15(2): 84–88 (in Polish).
- Furlaneto-Maia L, Specian AF, Bizerra C, et al. In vitro evaluation of putative virulence attributes of oral isolates of Candida spp. obtained from elderly healthy individuals. Mycopathologia 2008; 166(4): 209–217.
- 14. Hu T, Qi QG, Zhou XD. Frequency, species and molecular characterization of oral Candida in host of different age in China. J Oral Pathol Med. 2005; 34: 352–356.
- 15. Qi QG, Wang WX, Zhou XD, et al. The distribution of Candida spp. in the oral cavities of normal children. Shanghai Kou Qiang Yi Xue 2003; 12(4): 288–291.
- 16. Qi QG, Hu T, Zhou XD. Frequency, species and molecular characterization of oral Candida in host of different age in China. J Oral Pathol Med. 2005; 34: 352–356.
- 17. Resende M, Franco de Sousa LVN, Oliveira RCBW, et al. Prevalence and antifungal susceptibility of east obtained from the oral cavity of elderly individuals. Mycopathol. 2006; 162: 39–44.
- Rożkiewicz D, Daniluk T, Zaremba ML, et al. Oral Candida albicans carriage in healthy preschool and school children. Adv Med Scn. 2006; 51(1): 187–190.
- 19. Ślebioda Z, Hemerling M, Błauciak M, et al. Occurence of yeast-like fungi of the Candida spp. in high school youth, healthy adults without systemic diseases. Dent Forum 2007; 35(1): 23–26 (in Polish).
- 20. Vale-Silva L, Goncalves M, Cavaleiro C, et al. Antifungal activity of the essential oil of Thymus x viciosoi against Candida, Cryptococcus, Aspergillus and dermatophyte species. Planta Med. 2010; 76(9): 882–888.
- Zaremba ML, Daniluk T, Rożkiewicz D, et al. Incidence rate of Candida species in the oral cavity of middle-aged and elderly subjects. Adv Med Sci. 2006; 51(1): 233–236.
- 22. Żurowski M, Szponar-Żurowska A, Jarzębowska M, et al. Occurence of yeast-like fungi of the Candida spp. in high school youth. Czas Stomatol. 1994; 47(4): 259–262 (in Polish).
- 23. Sardi JC, Almeida AM, Mendes Giannini MJ. New antimicrobial therapies used against fungi present in subgingival sites a brief review. Arch Oral Biol. 2011; 56(10): 951–959.