



MICROBIOLOGICAL STUDY ON CANDIDA PRESENCE IN SALIVA OF CHILDREN WITH ASTHMA

Mariela Georgieva¹, Ani Belcheva², Eli Hristozova³, Neli Genkova⁴, Tanya Nihtianova², Mariana Murdjeva³

1) Student, Faculty of Dental Medicine, Medical University - Plovdiv

2) Department of Paediatric Dentistry, Faculty of Dental Medicine,

3) Department of Microbiology and Immunology, Faculty of Pharmacy,

4) Department of Paediatrics and Medical Genetics, Faculty of Medicine, Medical University - Plovdiv, Bulgaria.

ABSTRACT:

Asthma is a chronic disease with increasing frequency that can affect medical and oral health. During the prolonged treatment mainly with corticosteroids and anticholinergic drugs, changes in the oral environment may occur.

The aim of this study is to investigate Candida presence in saliva collected from children with asthma and healthy coevals.

The study is comprised of 52 children with asthma and 37 controls. Saliva specimens from all the children were prepared and inoculated into selective chromogenic medium to establish colonies growth. Microbial counts determination followed inoculation. Medium-sized plates were cultured under aerobic conditions. Colony forming units (CFU) were counted and biochemical identification was performed.

Nearly one third of the examined children were colonized with yeasts on their oral mucosa without significant difference between the asthmatic and controls (26.9% of asthmatic patients and 29.7% of the controls).

Children with asthma had higher microbial counts of Candida (10^4 - 10^5), whereas healthy children demonstrated mono infestation and lower microbial counts – fewer than 10^3 . *C. albicans* was the most frequently isolated fungal species from the oral cavity in children with asthma (in 69% of the samples) and in healthy children (in 46% of the samples). Some non-*albicans* species were also isolated in both groups.

Keyword: children, asthma, candida, saliva,

INTRODUCTION

Asthma is a major global health problem and its prevalence is increasing in most countries, especially among children [1]. Asthma is a chronic inflammatory condition medicated usually by bronchodilators, corticosteroids and anticholinergic drugs. Oropharyngeal candidiasis is often associated with the use of inhaled corticosteroids [2]. This adverse effect may be attributed to the topical effect of these

drugs on the oral mucosa, as only 10%-20% of the inhaled drug reaches lungs, rest remains in oropharynx. This is seen mainly among patients who use high dose of inhaled corticosteroids regularly [3]. Asthmatic patients taking medication show higher risk of developing dental caries, dental erosion, periodontal diseases and oral candidiasis [4].

Aim to investigate Candida presence in saliva collected from children with asthma and healthy coevals.

MATERIALS AND METHODS

The study is comprised of 52 children diagnosed with asthma and 37 controls. Eighty-nine saliva specimens were collected and frozen at -80°C prior to testing. Morning fasting samples were collected in sterile containers following thorough mouth washing with phosphate buffered saline.

Non-centrifuged saliva specimens were inoculated into selective chromogenic medium - Chromagar Candida (Bio Merieux) in order to establish colonies growth qualitatively and quantitatively. Due to its low pH and antibiotic impregnation, the medium is designed to inhibit contamination with oral flora. Thus, fungal species identification (based on the color of the colonies) and the count of grown colonies are performed simultaneously.

Microbial counts determination followed inoculation with non-calibrated loop, corresponding to a volume of 1.76 μl . Medium-sized plates were cultured under aerobic conditions for 24-48 at 37°C . Candida species formed predominantly smooth, creamy, convex colonies, which could be clearly identified based on their color. *C. albicans* presented with green colored colonies whereas *C. tropicalis*, *C. glabrata* and *C. kruzei* - with white, blue and pink colonies respectively.

Colony forming units (CFU) were counted using automated BIOMIC V3 system (Giles Scientific, USA). Subsequent biochemical identification was performed using API 20C AUX (BioMerieux) system on BIOMIC V3. All isolates were verified with a high reliability of a minimum 97.2%.

RESULTS

Nearly one third of the examined children (26.9% of asthmatic patients and 29.7% of the controls) were colonized with yeasts on their oral mucosa.

Saliva samples from asthmatic children grew yeasts belonging to the genus *Candida*. 10 of the specimens demonstrated microbial counts of 10^4 , corresponding to 18 to 176 colonies per sample, and 4 specimens (28.6%) had a microbial count of 10^5 *Candida*, which amounts to ≥ 176 colonies per sample (table 1).

Table 1. Percentage of asthmatic children colonized with *Candida* and microbial count.

<i>Candida spp.</i>	Percentage of colonized children	26.9%
	Percentage of specimens with microbial count	
	10^4	71.4%
	10^5	28.6%

Eleven (29.7 %) of the saliva specimens from the control group (37 in number) grew 2 different yeast species, all demonstrating low microbial counts – $\leq 10^3$ (up to 18 colonies per unit of culture) (table 2).

Table 2. Percentage of control children colonized with *Candida* and microbial count.

<i>Candida spp.</i>	Percentage of colonized children	29.7%
<i>Trichosporon spp.</i>	Percentage of specimens with microbial count	
	$\leq 10^3$	29.7%

Diagram 1 presents distribution of different *Candida* species in children with asthma.

C. albicans was the most frequently isolated fungal species from the oral cavity in children with asthma. It represents 69% of all the species isolated. The other part is randomly distributed between other species *C. guilliermondii* (8%), *C. famata* (8%), *C. dubliniensis* (8%), *C. kefir* (8%) (diagr. 1).

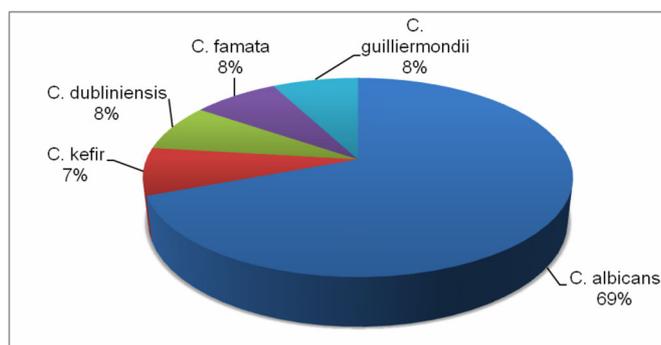


Diagram 1. Distribution of *Candida spp.* isolated from saliva of children with asthma

Results from clinical isolates of healthy children’s saliva are presented on Diagram 2. *C. albicans* was again the most frequently isolated fungal species - 46% of all the isolates. *Trichosporon mucoides* was identified in 27.8% of the specimens. The other non-albicans species were pre-

sented by *C. tropicalis* (9%), *C. guilliermondii* (9%) and *C. famata* (9%) (diagr. 2.).

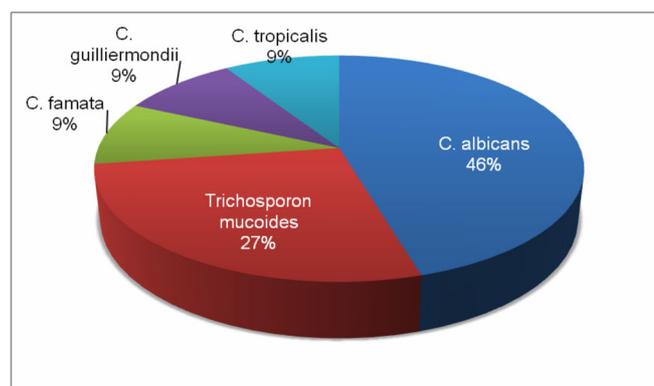


Diagram 2. Distribution of *Candida spp.* isolated from saliva of healthy children

DISCUSSION

Yeasts from the genus *Candida* belong to the indigenous flora of the human body. They contaminate the mucosa of healthy adults in 3 to 48% of the cases and the mucosa of healthy children in 45 to 68% of the cases. [5]. The percentage of oropharyngeal candidiasis in children and adults with asthma on steroid therapy ranges from 0 to 77%, based on different diagnostic criteria [6, 7, 8]. In our study, *Candida* was identified in 26.9% of asthmatic children and

in 29.6% if the healthy controls; no statistically significant difference was found between the two groups. Authors from Brazil have published similar findings [9] – 43.33% colonization in patients and 30% in controls. Lenander-Lumirakiet al. [10] also do not establish a statistically significant difference in the percentage of healthy subjects colonized with *Candida* and the percentage of infested asthmatic children on inhaled steroids. The percentages, documented in this study are lower, compared to those, established by Rueda-Gordillo et al. [11] - 50% and those, established by Samaranayake [5]. Similarly to other data, in our study, *C. albicans* was the most frequently isolated fungal species from the oral cavity in children with asthma (78.6%) [12]. Besides *C. albicans*, other non-*albicans* species as *C. famata* (7.1%), *C. dubliniensis* (7.1%) and *C. kefir* (7.1%) were isolated although in much lower percentage. These findings are similar to the observation of Williams [13]; On the contrary Rueda-Gordillo et al. identified only *C. glabrata* apart from *C. albicans* [11].

In our study, children from the control group were colonized with yeasts in 29.7% of the cases. Interestingly, apart from *C. albicans* (45.5%) and other non-*albicans* species as *C. famata* (9.1%), *C. guilliermondii* (9.1%) and *C. tropicalis* (9.1%), *Trichosporon mucoides* was identified in 27.8% of the specimens. *Trichosporon* is widely spread in the environment. In humans, it is normally found in the gastrointestinal tract and in the oral cavity and could transiently infest skin and respiratory tract [14].

The administration of steroids is considered a direct

risk factor for oral candidiasis and seems dependent on the frequency of steroid inhalation. This fact correlates with the microbial counts established in both asthmatic children and controls. In this study, children with asthma had higher microbial counts of *Candida* (10^4 - 10^5), whereas healthy children demonstrated mono infestation and lower microbial counts – fewer than 10^3 . It is generally considered that counts of 10^5 are clinically significant, counts of 10^4 – are clinically equivocal and 10^3 - non-significant.

Recently, there is a trend to establish more than one *Candida* species in the oral cavity (10). For this reason, the isolation of non-*albicans* species is of growing significance. However, in our study, the children were colonized with only one *Candida* species in their saliva.

CONCLUSIONS:

- *C. albicans* is the predominantly isolated yeasts in the oral cavity of both asthmatic and healthy children.
- The difference in the percentages of *Candida* colonization in healthy and asthmatic children is non-significant.
- The percentage of isolated *Candida* in our study is comparable to that found in other studies.
- Asthmatic children had higher microbial counts in their oral mucosa compared to healthy controls.
- None of the tested subjects were infested with more than one species of *Candida*.

ACKNOWLEDGMENTS:

This study was presented and awarded at the 25-th Jubilee Annual Assembly of International Medical Association Bulgaria, 14 - 17 May 2015, Varna, Bulgaria. The publication is sponsored by the publisher of the Journal of IMAB.

REFERENCES:

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention. GINA executive summary. *Eur Respir J*. 2008 Jan;31(1):143-78. [[PubMed](#)] [[CrossRef](#)]
2. Roland JN, Bhalla RK, Earis J. The local side effects of inhaled corticosteroids: current understanding and review of the literature. *Chest*. 2004 Jul;126(1):213-9. [[PubMed](#)] [[CrossRef](#)]
3. Kurt E, Yildirim H, Kiraz N, Orman A, Metintas M, Akgun Y, Erginel S. Oropharyngeal candidiasis with dry-powdered fluticasone propionate: 500 microg/day versus 200 microg/day. *Allergol Immunopathol* 2008 Jan-Feb;36(1):17-20. [[PubMed](#)] [[CrossRef](#)]
4. Stensson M, Wendt LK, Koch G, Oldaeus G, Ramberg P, Birkhed D. Oral health in young adults with long term, controlled asthma. *Acta Odontol Scand*. 2011 May;69(3) 158-64. [[PubMed](#)] [[CrossRef](#)]
5. Samaranayake YU, Samaranayake LP. Experimental oral candidiasis in animal models. *Clin Microbiol Rev*. 2001 Apr;14(2):398-429. [[PubMed](#)] [[CrossRef](#)]
6. Dubus JC, Margurt C, Deschildre A, Mely L, Leroux P, Brouard J, et al. Local side-effects of inhaled corticosteroids in asthmatic children: influence of drug, dose, age and device. *Allergy* 2001 Oct;56(10):944-8. [[PubMed](#)] [[CrossRef](#)]
7. Toogood JH, Jennings B, Greenway RW, Chuang L. Candidiasis and dysphonia complicating beclomethasone treatment of asthma. *J Allergy Clin Immunol*. 1980 Feb; 65(2): 146-53. [[PubMed](#)] [[CrossRef](#)]
8. Williamson IJ, Matusiewicz SP, Brown PH, Greening AP, Crompton GK. Frequency of voice problems and cough in patients using pressurized aerosol steroid preparations. *Eur Respir J*. 1995 Apr;8(4): 590-2. [[PubMed](#)]
9. Komiyama EY, Ribeiro PR, Junqueira JC, Koga-Ito CY, Cardoso Jorge AO. Prevalence of yeasts in the oral cavity of children treated with inhaled corticosteroids. *Braz Oral Res*. 2004 Jul-Sep;18(3):197-201. [[PubMed](#)] [[CrossRef](#)]
10. Lenander-Lumikari M, Laurikainen K, Kuusisto P, Vilja P. Stimulated salivary flow rate and composition in asthmatic and non-asthmatic adults. *Arch Oral Biol*. 1998 Feb;

43(2):151-6. [[PubMed](#)] [[CrossRef](#)]

11. Rueda-Gordillo F, Hernández-Solís SE, Rodríguez-Fernandez M, Lama-Gonzalez EM. Oral Cavity *Candida* spp. Prevalence in Asthmatic Children. *ICAAC*. 2014; on-line POM-029.

12. Walsh TJ, Groll A, Hiemenz J, Fleming R, Roilides E, Anaissie E.

Infections due to emerging and uncommon medically important fungal pathogens. *Clin Microbiol Infect*. 2004 Mar;10 Suppl 1:44–66. [[PubMed](#)] [[CrossRef](#)]

13. Colombo AL, Padovan AC, Chaves GM. Current Knowledge of *Tri-*

chosporon spp. and Trichosporonosis. *Clin Microbiol Rev*. 2011 Oct;24(4):682–700. [[PubMed](#)] [[CrossRef](#)]

14. Williams DW, Lewis MA, Isolation and identification of *Candida* from the oral cavity. *Oral Dis*. 2000 Jan;6(1):3-11. [[PubMed](#)]

Please cite this article as: Georgieva M, Belcheva A, Hristozova E, Genkova N, Nihtianova T, Murdjeva M. MICROBIOLOGICAL STUDY ON CANDIDA PRESENCE IN SALIVA OF CHILDREN WITH ASTHMA. *J of IMAB*. 2015 Oct-Dec;21(4):941-944. DOI: <http://dx.doi.org/10.5272/jimab.2015214.941>

Received: 30/09/2015; Published online: 23/11/2015

Address for correspondence:

Assoc. Prof. Ani Belcheva, DMD, MSc, PhD
Department of Paediatric Dentistry, Faculty of Dental Medicine,
Medical University, Plovdiv.
3, Hristo Botev Bvd., Plovdiv, Bulgaria.
E-mail: abeltcheva@gmail.com