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 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.

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%.

MATERIALS AND METHODS
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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. krusei - white, blue and pink colonies respectively.

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].
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.

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Percentage of colonized children</th>
<th>26.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage of specimens with microbial count</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^4$</td>
<td>71.4%</td>
</tr>
<tr>
<td></td>
<td>$10^5$</td>
<td>28.6%</td>
</tr>
</tbody>
</table>

Eleven (29.7%) of the saliva specimens from the control group (37 in number) grew 2 different yeast species, all demonstrating low microbial counts – $\leq10^3$ (up to 18 colonies per unit of culture) (table 2).

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

<table>
<thead>
<tr>
<th>Candida spp.</th>
<th>Percentage of colonized children</th>
<th>29.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichosporon spp.</td>
<td>Percentage of specimens with microbial count</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\leq10^3$</td>
<td>29.7%</td>
</tr>
</tbody>
</table>

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. guiliermondi (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 presented by C. tropicalis (9%), C. guiliermondi (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] – 43.3% 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 (9.1%) and C. tropicalis (9.1%) were isolated. 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].

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%) 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].

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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 (10³–10⁵), whereas healthy children demonstrated mono infestation and lower microbial counts – fewer than 10³. It is generally considered that counts of 10² are clinically significant, counts of 10¹ – are clinically equivocal and 10⁰- 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.

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