

# Nasopharyngeal Bacterial Flora in Healthy Preschool Children during Winter-Spring Months

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## ABSTRACT

The paper aimed to determine the incidence of colonization of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b and *Neisseria meningitidis* in the nasopharynges of healthy children in two preschool institutions during winter and spring months, without using antimicrobial treatment or serotyping of these bacteria. In addition to colonization of the above bacteria, the research that continued for 3 months monitored the length of their persistence in and disappearance from children's nasopharynges, children's health statuses, and provision of adequate medical interventions in children demonstrating clinical signs of disease. The ultimate aim of the paper was based on contributing to clearer and more accurate determination of a medical procedure in case of a positive result for bacteria intended to be found in the nasopharynx of a healthy child who spends time in a preschool institution.

**Key words:** Bacteriological flora, nasopharynx, healthy children, preschool institutions

## Introduction

This paper monitored the incidence of colonization of *Streptococcus pyogenes* (also referred to as beta hemolytic group A *Streptococci* or group A *Streptococci* – GAS), *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis* in nasopharynx swabs in healthy children in two preschool institutions during winter and spring months, without serotyping these bacteria or using antimicrobial treatment in healthy germ carriers.

The nasopharynges of preschool children are often colonized by potentially pathogenic bacteria such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b and *Neisseria meningitidis*, in the form of an asymptomatic carrier, without any clinical symptoms of disease. Healthy carriers are the most important source of infections caused with these pathogens. Nasopharyngeal colonization is most prevalent among children attending communities such as daycare

centres<sup>1–6,24,29</sup> and has an important effect on the spreading of these potentially pathogenic bacteria.

*Streptococcus pyogenes* (SP) is a strictly Gram-positive human pathogen that remains a major cause of human morbidity and mortality worldwide<sup>8,9,12,16,17,23</sup>. *Streptococcus pyogenes* normally colonizes the throat or skin without causing disease but it can also cause in human being from mild to several diseases, including pharyngitis (strep throat), tonsillitis, skin infections such as primarily non-bullous impetigo, erysipelas, and other forms of pyoderma, cellulitis and scarlet fever<sup>5,6,8,11,14</sup>. Most morbidity arises from autoimmune sequelae due to *Streptococcus pyogenes* diseases such as poststreptococcal glomerulonephritis (PSGN) and acute rheumatic fever (ARF). Rheumatic carditis with characteristic heart murmur as a result of valve deformation is the most severe manifestation of all five of the streptococcal sequelae. Other manifestations of ARF are polymigrating arthritis

and Sydenham chorea as single neurologic manifestation or associated with carditis and polymigrating arthritis. A possible variant of Sydenham chorea is pediatric autoimmune neuropsychiatric disorder associated with *Streptococci* – PANDAS<sup>19</sup>. Skin manifestation of ARF are subcutaneous nodules and erythema marginatum<sup>8,9,12,14,19</sup>. Invasive infections of *Streptococcus pyogenes* such as postanginal septicemia-Lemierre syndrome<sup>20</sup>, purulent pericarditis, puerperal fever (childbed fever)<sup>17</sup> are now rare. Severe infections such as sepsis, meningitis, streptococcal toxic shock syndrome (STSS)<sup>28</sup> or necrotizing fasciitis (NF- flesh-eating disease) occur worldwide and are associated with high mortality<sup>5</sup>. Fortunately, they occur sporadically in most cases<sup>20,32,33</sup>. It has been reported that *Streptococcus pyogenes* was responsible for at least an estimated 616 million cases of throat infection (pharyngitis, tonsillitis) worldwide per year, and 111 million cases of skin infection (primarily non-bullous impetigo) in children of less developed countries (Carapetis et al., 2005).

Despite intensive research, the molecular mechanisms of *Streptococcus pyogenes* disease remain unclear<sup>10,13,15,18,21,22,23</sup>.

*Streptococcus pneumoniae* is an important pathogen with significant morbidity and mortality in both the pediatric and adult populations worldwide, especially among children under the age of 5 years old. *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia and acute otitis media<sup>8,10,30,32,35,36</sup>, but also a common colonizer of the upper respiratory tract.

*Haemophilus influenzae* type b (Hib) causes invasive infections such as otitis media acuta, mastoiditis, sepsis and meningitis worldwide. The highest rates of infection are in children below the age of five (5)<sup>2,6,7,31,34</sup>. Hib is responsible for hundreds of thousands of deaths each year<sup>7</sup>.

*Neisseria meningitidis* remains to be the most frequent cause of serious invasive disease such as sepsis and meningitis in children with high morbidity and mortality and can present as isolated cases or outbreaks. Endemic or epidemic meningococcal infection occurs with a global distribution. It was reported that 500 000 cases of invasive disease occur worldwide every year with more than 50 000 deaths<sup>4</sup>. Patients who survive the disease (9–11%) are left with sequels such as deafness, seizures, mental retardation and limb amputations<sup>4,24–27</sup>.

In winter and spring months, preschool children often have acute upper respiratory tract infections (URI) caused by viruses and bacteria. Children who leave their protected family environments for the first time to join nursery and daycare center groups, normally in autumn, are the most affected ones. These children have their microflora in the nasopharynxes and pharynxes, while children who spend more time in a group have theirs. URI is transmitted by saliva – as children in these groups are in very close contact with each other (they play, kiss, whisper in each other's ear), the most affected children are new arrivals who are not resistant to the microflora of children who have spent time there for months and who are clinically healthy.

Affected children are given antimicrobial treatment and they undergo microbiological tests, to it may take weeks for a child to be cured and re-included in the group.

Pediatric practices have demonstrated that antibiotics are also prescribed to children who are healthy germ carriers. This practice is normal in our country and worldwide. Unfortunately, control swabs following antimicrobial treatment have shown bacterial re-colonization in most children, which largely indicates failure of such treatment. This has also been confirmed by numerous authors<sup>1–8,14,33,37</sup>.

## Subjects and Methods

Both preschool institutions where pharynx swabs were taken are facilities that meet the required standards, but the difference between them is in the fact that one facility was built for a specific purposes (herein referred to as Facility b in the paper), while the other one has been adapted to meet the required standards (herein referred to as Facility a).

Nasopharynx swabs were taken on 6 occasions over 3 months, from mid-January to late March, twice monthly, exclusively from children included in the study. A total of one hundred (100) randomized healthy children were included. Approximately 40 children from nursery groups and approximately 60 from daycare groups were tested. Swabs were taken from 2 nursery (20 subjects) and 3 daycare (30 subjects) groups in each institution, with 10 subjects from each group.

One hundred (100) children participated in the first collection of nasopharynx swabs, 79 children were present during the second collection of nasopharynx swabs, 85 during the third collection, 86 children during the fourth collection, and 91 during the fifth and sixth collection.

The healthy children who were absent from their nursery and daycare groups on the nasopharynx swab collection date were absent for various reasons.

The health statuses of the children were monitored during the research and it was found that a total of 16 subjects in the two Facilities fell ill – 9 from the nursery groups and 7 from daycare groups. The children who fell ill between specific nasopharynx swab dates were treated and followed up with at the ENT Clinic where new swabs were taken from them. After recovering, the children re-joined their respective groups and control nasopharynx swabs were taken on the 10<sup>th</sup> day following the end of therapy.

## Results and Discussion

This paper compared the number of nasopharyngeal carriers of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis* germs in nursery and daycare groups at two (2) preschool institutions during winter and spring months without using antimicrobial treatment.

The completed research showed that the colonization of the relevant bacteria in nasopharyngeal swabs keeps changing and that there is a difference in the colonization of all four bacteria types during the study period. Each relevant bacterial cause presented its occurrence curve (Figures 1–5).

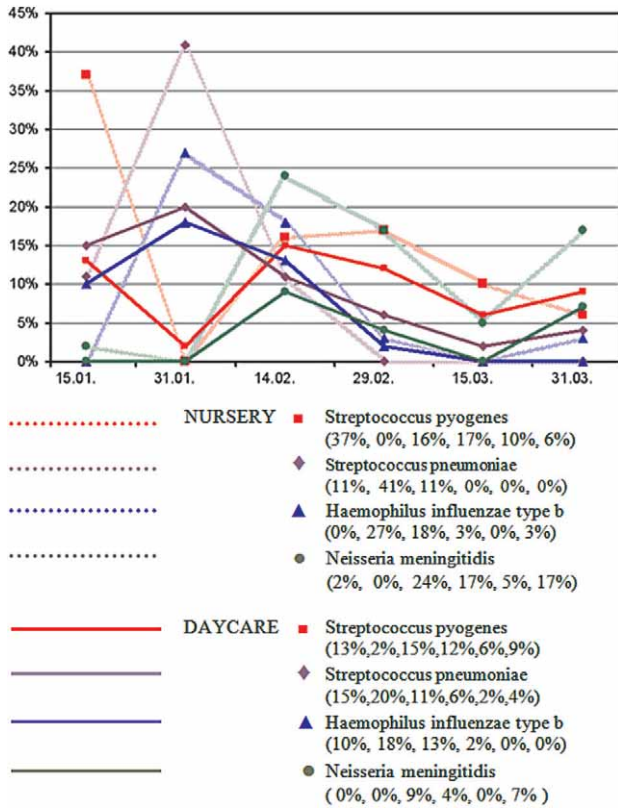


Fig. 1. Colonization curves for the tested bacteria – a comparison between nursery and daycare groups of both Facilities, which show that the nursery groups have more nasopharyngeal germ carriers.

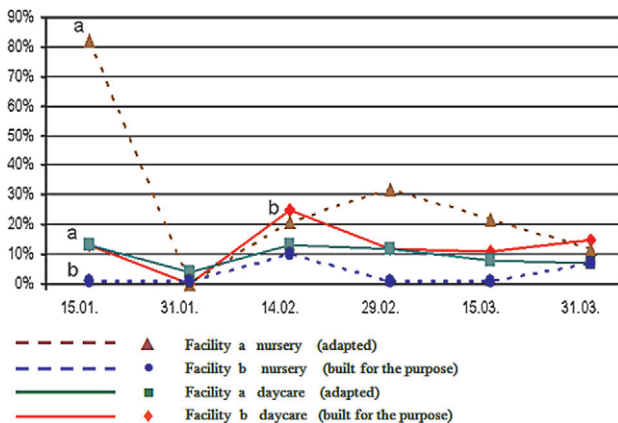


Fig. 2. Streptococcus pyogenes colonization curves in the nursery and daycare groups of Facility a and Facility b. The bacterial colonization rate was much higher in the nursery groups of Facility a, especially during the 1<sup>st</sup> and 4<sup>th</sup> nasopharynx swab collection.

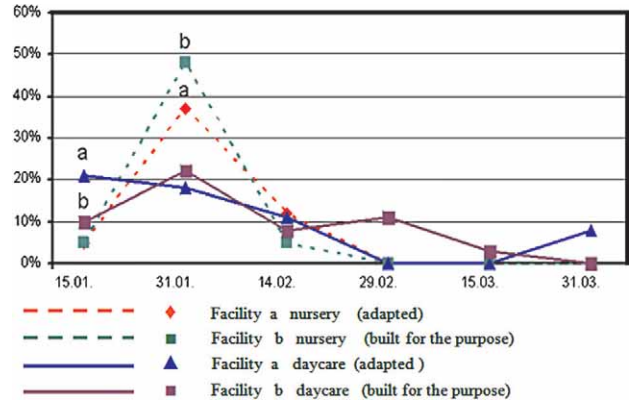


Fig. 3. Streptococcus pneumoniae colonization curves in the nursery and daycare groups of Facility a and Facility b. The bacterial colonization rate was much higher in the nursery groups of both Facilities during the 2<sup>nd</sup> nasopharynx swab collection.

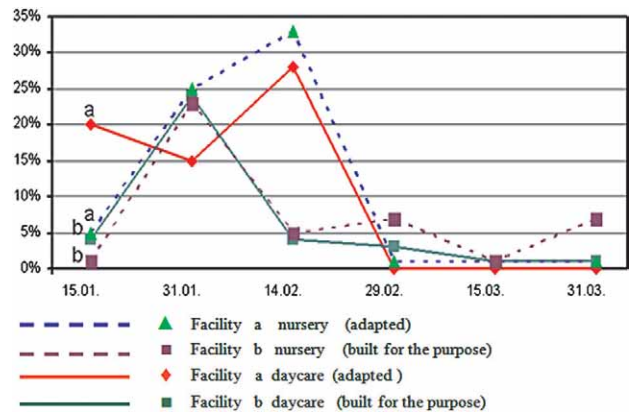


Fig. 4. Haemophilus influenzae type b colonization curves in the nursery and daycare groups of Facility a and Facility b. The bacterial colonization rate was much higher in the nursery and daycare groups of Facility a during the 3<sup>rd</sup> nasopharynx swab collection.

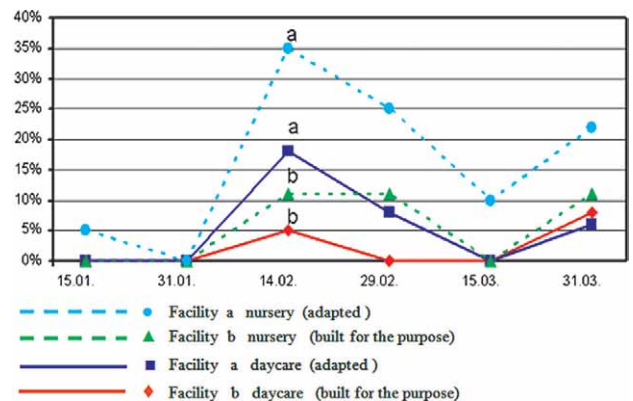


Fig. 5. Neisseria meningitidis colonization curves in the nursery and daycare groups of Facility a and Facility b. The bacterial colonization rate was much higher in the nursery and daycare groups of Facility a during the 3<sup>rd</sup> nasopharynx swab collection.

*Streptococcus pyogenes* reached its colonization peak at the beginning of the research and then the colonization rate dropped toward the end of the research (Figure 2). *Streptococcus pneumoniae* reached its colonization peak at the end of January (Figure 3). *Haemophilus influenzae* type b had its colonization peak between the end of January and mid-February (Figure 4), while *Neisseria meningitidis* had its colonization peak in mid-February

A statistically significant difference appeared in the colonization of *Streptococcus pyogenes* after the 1<sup>st</sup> nasopharyngeal swab collection in the nursery groups of Facility a compared to the nursery groups of Facility b (Figure 2). *Haemophilus influenzae* type b demonstrated a statistically significant difference after the 3<sup>rd</sup> nasopharyngeal swab collection because the bacterial colonization rate was much higher in the nursery and daycare groups of Facility a (Figure 4).

The colonization of the relevant bacteria progressed at a certain pace and was more expressed in the nursery groups than the daycare groups in both Facilities (Figure 1). Colonization was more expressed in the adapted Facility a, which was to be expected because, although it meets the prescribed standards, the surface areas of its

rooms is less than the surface areas of the rooms in the Facility built for the relevant purpose, so bacteria spread more quickly (Figures 1 and 2).

Although most children had the same bacteria in their nasopharyngeal swabs, each subject had his/her own colonization curve. Bacteria colonized the nasopharynx of each child in different time intervals, spontaneously disappeared or recolonized the nasopharynx after some time.

During the research, two (2) different bacteria were isolated in a single nasopharyngeal swab in each of the 18 children in the nursery and daycare groups of both Facilities (Table 1).

It is important to underline that three (3) different bacteria were isolated in a single swab in five (5) of the children in the daycare groups of both Facilities after the 3<sup>rd</sup> nasopharyngeal swab collection and none of the children had any clinical signs of disease (Tables 1 and 2).

In the 5<sup>th</sup> and 6<sup>th</sup> nasopharyngeal swab collections, each child had one (1) bacterium isolated in each swab (Table 1).

During the research sixteen (16) children fell ill, nine (9) from the nursery groups and seven (7) from the daycare groups of both Facilities (Tables 3–6).

TABLE 1

NASOPHARYNGEAL GERM CARRIERS IN THE NURSERY AND DAYCARE GROUPS OF BOTH FACILITIES IN WHOM 2 OR 3 DIFFERENT BACTERIA WERE ISOLATED IN A SINGLE NASOPHARYNGEAL SWAB

Nasopharyngeal swab collection	I 15.01.	II 31.01.	III 14.02.	IV 28.02.	V 15.03.	VI 31.03.
Facility a nursery/daycare	2**	3**	3***	2**	Each subject has one bacterium in the swab	Each subject has one bacterium in the swab
Facility b nursery/daycare	1**	2**	2***	3**	Each subject has one bacterium in the swab	Each subject has one bacterium in the swab

\*\* – 2 different bacteria were isolated in a single nasopharyngeal swab; \*\*\* – 3 different bacteria were isolated in a single nasopharyngeal swab; 2, 3 – the number of children in whom 2 or 3 different bacteria were isolated in a single nasopharyngeal swab

TABLE 2

NASOPHARYNGEAL GERM CARRIERS IN THE DAYCARE GROUPS OF BOTH FACILITIES IN WHOM 3 DIFFERENT BACTERIA WERE ISOLATED IN A SINGLE NASOPHARYNGEAL SWAB

Daycare	3 <sup>rd</sup> nasopharyngeal swab collection 14.02.		Clinical signs of disease
Facility a	M.B., 4 years and 5 months old	K.B., 5 years and 4 months old	M.I., 4 years and 7 months old
3 children *** from the same group	<i>Streptococcus pyogenes</i> , <i>Haemophilus influenzae</i> type b and <i>Neisseria meningitidis</i>	<i>Streptococcus pneumoniae</i> , <i>Haemophilus influenzae</i> type b and <i>Neisseria meningitidis</i>	<i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> type b
Facility b	I.K., 5 years and 9 months old	S.Z., 6 years and 3 months old	
2 children*** from the same group	<i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> and <i>Neisseria meningitidis</i>	<i>Streptococcus pyogenes</i> , <i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> type b	Not present in any of the children

**TABLE 3**  
PRESENTATION OF DIAGNOSES, TREATMENTS AND NASOPHARYNGEAL SWAB RESULTS BEFORE, DURING AND AFTER THE DISEASE

Facility a, nursery Patient's diagnosis/age	Child's period of disease	Treatment	Results of the nasopharyngeal swabs collected in the nursery/daycare before the disease	Result of the nasopharyngeal swab collected during the disease at the ENT Clinic	Nasopharyngeal swab collected 10 days after the end of treatment / the child is / is not clinically healthy
M.K., 1 year and 7 months old <i>Rhinitis purulenta</i> , <i>Tonsillitis ac.</i>	Between the 1 <sup>st</sup> and 2 <sup>nd</sup> nasopharyngeal swab collection	Antibiotic for 7 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	<i>Haemophilus influenzae</i> type b / the child is clinically healthy
M.B., 2 years and 10 months old <i>Angina streptococcica</i>	Between the 1 <sup>st</sup> and 2 <sup>nd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	Physiological flora	<i>Streptococcus pyogenes</i>	<i>Streptococcus pyogenes</i> / antibiotic for 10 more days / physiological flora for the next swab collection.
M.I., 2 years and 5 months old <i>Rhinitis purulenta</i> , <i>Tonsillitis ac.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 7 days	Physiological flora	<i>Haemophilus influenzae</i> type b	<i>Neisseria meningitidis</i> / the child is clinically healthy
I.B., 2 years and 6 months old <i>Otitis media ac.bil.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Haemophilus influenzae</i> type b	<i>Streptococcus pneumoniae</i> and <i>Haemophilus influenzae</i> type b	<i>Streptococcus pneumoniae</i> / the child is clinically healthy
K.B., 2 years and 8 months old <i>Angina streptococcica</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pyogenes</i>	Physiological flora / the child is clinically healthy

**TABLE 4**  
PRESENTATION OF DIAGNOSES, TREATMENTS AND NASOPHARYNGEAL SWAB RESULTS BEFORE, DURING AND AFTER THE DISEASE

Facility a, daycare Patient's diagnosis/age	Child's period of disease	Treatment	Results of the nasopharyngeal swabs collected in the nursery/daycare before the disease	Result of the nasopharyngeal swab collected during the disease at the ENT Clinic	Nasopharyngeal swab collected 10 days after the end of treatment / the child is / is not clinically healthy
I.K., 4 years and 3 months old <i>Angina streptococcica</i>	Between the 1 <sup>st</sup> and 2 <sup>nd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pyogenes</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus pneumoniae</i> / the child is clinically healthy
B.I., 3 years and 7 months old <i>Tonsillitis ac.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	Physiological flora	<i>Haemophilus influenzae</i> type b	<i>Haemophilus influenzae</i> type b / the child is clinically healthy
S.F., 5 years and 4 months old <i>Angina streptococcica</i>	Between the 3 <sup>rd</sup> and 4 <sup>th</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pyogenes</i>	Physiological flora / the child is clinically healthy
I.Z., 4 years and 6 months old <i>Sinuitis ac.</i> , <i>Otitis media bil.</i>	Between the 3 <sup>rd</sup> and 4 <sup>th</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Haemophilus influenzae</i> type b	<i>Haemophilus influenzae</i> type b and <i>Streptococcus pneumoniae</i>	<i>Neisseria meningitidis</i> / the child is clinically healthy

**TABLE 5**  
PRESENTATION OF DIAGNOSES, TREATMENTS AND NASOPHARYNGEAL SWAB RESULTS BEFORE, DURING AND AFTER THE DISEASE

Facility b, Nursery Patient's diagnosis/age	Child's period of disease	Treatment	Results of the nasopharyngeal swabs collected in the nursery/daycare before the disease	Result of the nasopharyngeal swab collected during the disease at the ENT Clinic	Nasopharyngeal swab collected 10 days after the end of treatment / the child is / is not clinically healthy
G.G., 2 years and 11 months old <i>Angina streptococcica</i>	Between the 3 <sup>rd</sup> and 4 <sup>th</sup> nasopharyngeal swab collection	Antibiotic for 10 days	Physiological flora	<i>Streptococcus pyogenes</i>	<i>Haemophilus influenzae</i> type b / the child is clinically healthy
J.V., 2 years and 3 months old <i>Tonsillitis ac. Otitis media ac.bil.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i> / the child is clinically healthy
M.I., 2 years and 11 months old <i>Tonsillitis ac. Bronchitis ac.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Haemophilus influenzae</i> type b	Physiological flora / the child is clinically healthy
B.M., 2 years and 7 months old <i>Tonsillitis ac. Otitis media ac. bil.</i>	Between the 3 <sup>rd</sup> and 4 <sup>th</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Haemophilus influenzae</i> type b	<i>Haemophilus influenzae</i> type b	<i>Streptococcus pneumoniae</i> / the child is clinically healthy

**TABLE 6**  
PRESENTATION OF DIAGNOSES, TREATMENTS AND NASOPHARYNGEAL SWAB RESULTS BEFORE, DURING AND AFTER THE DISEASE

Facility b, daycare Patient's diagnosis/age	Child's period of disease	Treatment	Results of the nasopharyngeal swabs collected in the nursery/daycare before the disease	Result of the nasopharyngeal swab collected during the disease at the ENT Clinic	Nasopharyngeal swab collected 10 days after the end of treatment / the child is / is not clinically healthy
D.S., 6 years and 4 months old <i>Angina streptococcica, Otitis media ac.l.dex.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Haemophilus influenzae</i> type b	<i>Streptococcus pyogenes</i>	Physiological flora / the child is clinically healthy
M.M., 5 years and 9 months old <i>Angina streptococcica, Otitis media bil.</i>	Between the 2 <sup>nd</sup> and 3 <sup>rd</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pyogenes</i>	<i>Streptococcus pyogenes</i> / antibiotic for 10 more days / physiological flora for the next swab collection.
K.B., 5 years and 4 months old <i>Otitis media bil.</i>	Between the 3 <sup>rd</sup> and 4 <sup>th</sup> nasopharyngeal swab collection	Antibiotic for 10 days	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	Physiological flora / the child is clinically healthy

These children fell ill between specific nasopharyngeal swab collection dates. Among the sixteen (16) children who fell ill, twelve (12) of them had tested positive for bacteria in their nasopharyngeal swabs before they fell ill without showing any clinical signs of disease

It still remains unclear why these particular children fell ill during that period rather than earlier or later. Please note that our research only monitored the colonization of these causes, while the issue of their invasiveness exceeds the scope of this paper.

The children who fell ill were treated and followed up with at the ENT Clinic where nasopharyngeal swabs were collected from them and treatment was provided according to their antibiograms. The nasopharyngeal swab results in the children who fell ill showed that the same cause they had had before the onset of disease was isolated in seven (7) of the children, while a different cause was found in nine (9) of the children, and in two (2) of the children were isolated 2 different bacteria in a single nasopharyngeal swab (Tables 3–4). This means that »new« bacteria colonized the nasopharyngeal swabs of these children, while the »old« bacteria spontaneously disappeared.

After treatment, the children rejoined their respective nursery and daycare groups. A follow-up nasopharyngeal swab was collected from these children on the 10<sup>th</sup> day following the antibiotic therapy.

The same bacteria that existed during the disease were isolated in five (5) of the children, different causes were isolated in six (6) of the children, while five (5) of the children had physiological flora. As *Streptococcus pyogenes* was re-isolated in two (2) children as it had been during the disease, the 10-day antibiotic treatment was repeated. The next nasopharyngeal swab showed physiological flora in both children (Tables 3 and 6).

The results of repeated nasopharyngeal swabs showed that eleven (11) of the sixteen

(16) studied children remained germ carrier after being treated with antibiotics and recovering, without showing any clinical symptoms of disease (Tables 3–6).

During the observed period, 84 of 100 subjects (84%) had no clinical signs of disease. Although the issue of whether the antibiotic and chemotherapeutic interventions were justified arises from the kinetics of the incidence and colonization of these pathogens in the nursery and daycare groups, the results of our research demonstrated that positive bacteria test results in nasopharyngeal swabs in otherwise clinically healthy children are not an indication for antimicrobial treatment.

## Conclusion

Based on the results and statistical processing, as well as the health status of the studied children, we have come to the conclusion that antimicrobial treatment should not be provided to clinically healthy children who had positive microbiological nasopharyngeal swab results for conditionally pathogenic bacteria.

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## BAKTERIOLOŠKA FLORA NAZOFARINKSA U ZDRAVE PREDŠKOLSKE DJECE TIJEKOM ZIMSKO-PROLJETNIH MJESESCI

### SAŽETAK

Istraživanje je provedeno s ciljem da se odredi značenje pozitivnog nalaza bakterija *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b i *Neisseria meningitidis* u brisu nazofarinksa zdrave djece u predškolskim ustanovama tijekom zimsko – proljetnih mjeseci bez primjene antimikrobne terapije. Pratila se samo kolonizacija navedenih bakterija, bez serotipizacije istih, kao i zdravstveno stanje djece tijekom 3 mjeseca. U ispitivanje je bilo uključeno stotinu (100) nasumice izabrane zdrave predškolske djece, iz dvije predškolske ustanove. Jedna ustanova bila je namjenski građeni objekt, dok je druga bila adaptirani objekt. Brisevi nazofarinksa uzimani su u 6 navrata, od sredine siječnja do kraja ožujka, 2x mjesečno, isključivo samo u djece koja su bila uključena u ispitivanje. U svakoj predškolskoj ustanovi brisevi nazofarinksa uzimani su iz 2 jaslčke i 3 vrtičke grupe, iz svake grupe u 10 ispitanika, što znači da je tijekom istraživanja iz jaslčkih grupa ispitivano 40-ero djece, a iz vrtičkih grupa 60-ero djece. Tijekom svih 6 mjerenja broj ispitivane djece nije bio uvijek isti. Prvi bris nazofarinksa uzet je u 100-tinu djece, od tog broja bilo je pri drugom uzimanju brisa nazofarinksa prisutno 79-ero djece, pri trećem 85-ero djece, pri četvrtom 86-ero djece, a pri petom i šestom uzimanju brisa 91 dijete. Djeca koja su bila uključena u ispitivanje, a nisu bila prisutna u svojim jaslčkim/vrtičkim grupama na dan uzimanja brisa nazofarinksa, bila su u tom periodu zdrava, bez kliničkih znakova bolesti. Razlog izostanka bio je privatni. Tijekom istraživanja razboljelo se ukupno 16-ero djece. Sva djeca bila su pregledavana, liječena i kontrolirana na ORL klinici, gdje su im ponovno uzimani brisevi nazofarinksa, a terapija se odredila na temelju antibiograma. Nakon potpunog ozdravljenja djeca su bila ponovno uključena u svoje jaslčke i vrtičke grupe. Ovoj djeci, kao i ostalim ispitanicima uzimani su i nadalje brisevi nazofarinksa kako je planirano. Nalazi su pokazali da je od 16-ero oboljele djece koja su ozdravila nakon antibiotske terapije, u njih 11-ero i nadalje prisutna kolonizacija bakterija u nazofarinksu, ali bez kliničkih znakova bolesti. Provedeno istraživanje pokazalo je da se u brisu nazofarinksa kolonizacija traženih bakterija neprestalno mijenja, te da sve četiri tražene bakterije imaju svoje zasebne krivulje kretanja i pokazuju u različitom vremenskom periodu svoj maksimum javljanja i nestajanja. Kolonizacija traženih bakterija bila je zastupljena u većem postotku u nenamjenski građenom objektu, što je bilo i za očekivati. Analizirajući ukupne rezultate došlo se do zaključka da pozitivni nalazi bakterija u nazofarinksu zdrave djece, bez kliničkih znakova bolesti, ne pokazuju potrebu za davanjem antimikrobne terapije.