Passive Smoking as a Risk Factor for Upper Respiratory Tract Colonization by *Haemophilus influenzae* in Healthy Pre-School Children

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**Abstract**

A total of 1,035 swabs from 345 healthy pre-school children - 268 attending day care centers (DCC group) and 77 staying at home were taken. The statistically significant difference in *Haemophilus influenzae* colonization was observed among children exposed and unexposed to tobacco smoke in the total population ($p=0.042$) and in the DCC group ($p=0.034$). *H. influenzae* prevalence among passive smoking children prone to recurrent respiratory infections was higher compared to unexposed children in the total population ($p=0.084$) or in DCC group ($p=0.032$). This suggests that passive smoking and attending DCC may predispose *H. influenzae* colonization of the upper respiratory tract in pre-school children.

**Keywords:** *Haemophilus influenzae* colonization, passive smoking, healthy pre-school children

**Introduction**

Exposure to environmental tobacco smoke (also known as second-hand or passive smoking) is defined as the involuntary or passive breathing of air contaminated with tobacco smoke by non-smokers [1]. When non-smokers breathe in second-hand smoke, besides nicotine they take in other toxic chemicals [2, 3]. Chemical compounds from tobacco smoke are known or suspected to cause cancer or cardiovascular and respiratory diseases. Passive smoking appears to be an important factor that results in damage of mucosa within airways and disturbance of host defense mechanisms [4]. This may cause serious problems with health or deterioration of quality of life. Some of the consequences that have been or are frequently attributed to passive smoking include increased risk of several diseases, chronic illness, or behavior problems, not only among adults, but especially among children [1, 5-9]. However, there are controversial literature data concerning passive smoking as a risk factor predisposed to change in natural flora of the upper respiratory tract and to colonization of mucous membranes by various potentially pathogenic bacteria [4, 10-12].

The ecosystem of nasopharyngeal microflora may be the reservoir of bacterial pathogens, e.g. *Haemophilus influenzae*, responsible mainly for community-acquired respiratory infections, especially in young children [13, 14]. The nasopharyngeal carriage of potentially pathogenic microorganisms in children during the first years of life depends on various epidemiologic and socioeconomic factors.

The aim of our studies was to investigate if passive smoking increased the rate of overall colonization by *H. influenzae* in the upper respiratory tract of healthy pre-school children.

**Experimental Procedures**

During our study 345 pre-school children (3-5 years old) were included; they were divided into two groups: 268...
children attending a day care center (DCC group: DCC1, n=86; DCC2, n=63; DCC3, n=44, DCC4, n=75) and 77 staying at home (home group). In this study, children exposed to tobacco smoke were defined as those in the presence of which one or more cigarettes per day were smoked. Children prone to recurrent respiratory infections (RRI) were defined as having ≥6 episodes per year. According to parents’ information, 163 (47.25%) children were exposed to tobacco smoke and 147 (42.6%) individuals were with RRI.

The *Haemophilus influenzae* isolates were selected from a total of 1,035 specimens – swabs from throat and both nostrils (three samples from one child). The specimens were immediately placed onto the selective media (*Haemophilus* chocolate agar, HAEM Oxoid) and then incubated in the atmosphere with increased CO2 concentration (appropriate for microaerophilic bacteria) for 18-48 hours at 35ºC. The isolated microorganisms were identified on the basis of routine methods (macroscopic, microscopic or biochemical assays by API NH microtest, bioMérieux).

The results obtained were analyzed statistically using StatSoft (1997) Statistica for Windows. Risk factors associated with *H. influenzae* colonization were evaluated on the basis of p value, odds ratio (OR), and their 95% confidence intervals (CI); multivariate analysis was performed to determine independently significant risk factors for *H. influenzae* colonization using logistic regression stepwise. p values less then 0.05 were considered statistically significant.

### Results

The overall prevalence of *H. influenzae* in the upper respiratory tract of the assayed children was 19.71%, similarly in DCC group and in home group – 20.15% and 18.18%, respectively (Table 1). However, there were differences among the frequency of colonization in DCC subgroups – from 9.3% (DCC1) to 32.0% (DCC4). From 68 colonized children we selected a total of 71 isolates of *H. influenzae* – 57 in DCC group and 14 in home group.

As presented in Table 1, the statistically significant difference in the rate of colonization of the upper respiratory tract by *H. influenzae* among children exposed and unexposed to tobacco smoke was observed, both in the total population (12% vs. 8%, p=0.042, OR=1.79, CI 1.04-3.06) or in children from DCC group (12% vs. 8%, p=0.034, OR=1.93, CI 1.05-3.56). There was no statistically significant difference in *H. influenzae* colonization between passive smoking and nonsmoking children in the home group (9% vs. 9%, p=0.768, OR=1.33, CI=0.42-4.26).

As presented in Table 2, univariate analysis revealed that DCC1’ attendance didn’t predispose to *H. influenzae* colonization of the upper respiratory tract (OR=0.34), while DCC4’ attendance increased risk (OR=2.42) of prevalence of this microorganism. According to the multivariate analysis model, statistically significant independent factors predisposed to colonization of the upper respiratory tract by *H. influenzae* appeared to be male sex, age 5 years, and passive smoking (Table 2).
Next, we examined the effect of passive smoking on the prevalence of *H. influenzae* colonization among children prone to recurrent respiratory infections – RRI (Fig. 1). These infections included pneumonia, pharyngitis, sinusitis, and bronchitis (≥6 episodes per year, according to parent information). There were 147 children with recurrent infections – 121 (82.31%) in the DCC group (DCC1 – 29, DCC2 – 25, DCC3 – 26, DCC4 – 41) and 26 (17.68%) in the home group. The rate of *H. influenzae* prevalence among passive smoking children prone to RRI was higher compared to unexposed population – it was noted in 17 (65.38%) and 9 (34.62%) children, respectively; however, this difference was not statistically significant (p=0.084, OR=2.27, CI=0.94). The *H. influenzae* colonization in DCC group was found in 6 (28.57%) unexposed children and 15 (71.43%) exposed children; this difference was statistically significant (p=0.032, OR=3.06, CI=1.10-8.52). In the home group the number of children prone to RRI was too small to perform statistical analysis.

### Discussion

The health hazards of environmental tobacco smoke (ETS) exposure are well documented. According to many organizations, for example the World Health Organization [1], Action on Smoking and Health [2], the American Academy of Pediatrics [15], the Centers for Disease Control and Prevention [16], the U.S. Department of Health and Human Services [17], and others [9, 18, 19], young children’s exposure to environmental tobacco smoke (also known as second-hand smoke or passive smoking) is very high and is a worldwide problem. It is estimated that about 33 to 50% of children are exposed to tobacco smoke, similar to our results (47%). The summary of WHO [1], based on the studies, suggest that 30-50% of children are exposed to ETS at home, the proportion of exposed older children aged 13-15 years is higher, e.g. in Poland (about 88%). The largest source of environmental tobacco smoke for younger children, especially <5 years old, is using cigarettes by parents (major maternal smoking) or other household members.

**Table 2. Risk factors affecting the upper respiratory tract colonization by *Haemophilus influenzae*.**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>No. (%) of children</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonized n=68</td>
<td>Uncolonized n=277</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Male gender</td>
<td>40 (58.8)</td>
<td>129 (46.6)</td>
<td>1.64 (0.96-2.81)</td>
</tr>
<tr>
<td>Age of 5 years</td>
<td>25 (36.8)</td>
<td>89 (32.1)</td>
<td>1.22 (0.71-2.14)</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>39 (57.4)</td>
<td>122 (44.0)</td>
<td>1.71 (1.0-2.92)</td>
</tr>
<tr>
<td>Attendance to:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCC1</td>
<td>8 (11.8)</td>
<td>78 (28.2)</td>
<td>0.34 (0.16-0.74)</td>
</tr>
<tr>
<td>DCC2</td>
<td>15 (22.1)</td>
<td>48 (17.3)</td>
<td>1.35 (0.70-2.59)</td>
</tr>
<tr>
<td>DCC3</td>
<td>7 (10.3)</td>
<td>37 (13.4)</td>
<td>0.74 (0.32-1.75)</td>
</tr>
<tr>
<td>DCC4</td>
<td>24 (35.3)</td>
<td>51 (18.4)</td>
<td>2.42 (1.35-4.33)</td>
</tr>
<tr>
<td>RRI</td>
<td>26 (38.2)</td>
<td>121 (43.7)</td>
<td>0.80 (0.46-1.38)</td>
</tr>
</tbody>
</table>

DCC – day care center, RRI – recurrent respiratory tract infections, OR – odds ratio, CI – confidence interval

Fig. 1. Passive smoking and the upper respiratory tract colonization by *Haemophilus influenzae* in children prone to recurrent respiratory infections.

Col(-) – children without *Haemophilus influenzae* colonization; Col(+) – children with *Haemophilus influenzae* colonization; DCC – day care center; unexposed children (n=75): DCC1 (n=12), DCC2 (n=15), DCC3 (n=16), DCC4 (n=18), home group (n=14); exposed children (n=72): DCC1 (n=17), DCC2 (n=10), DCC3 (n=10), DCC4 (n=23), home group (n=12)
It is also known that the presence of children in large groups, as can be the case in day care centers, may expose children to an indoor environment that can favor the development of various infections and induce various diseases [20, 21]. Several social or environmental factors, such as day care centers attendance, parental or household smoking, represent important risk factors for respiratory tract diseases and may determine the incidence of recurrent respiratory tract infections [22].

Most strains of *Haemophilus influenzae* are opportunistic pathogens colonizing some regions of the human body, especially in infants and young children. That is, they usually live in their host without causing disease, but cause problems when other factors (such as a viral infection or reduced immune function) create an opportunity. *H. influenzae* is a significant pathogen, causing otitis media, sinusitis, conjunctivitis, pneumonia, epiglotitis, and occasionally invasive infections [23-26]. The pathogenesis of *H. influenzae* infections is not clearly understood, although the presence of the capsule in type b (Hib) is one of the major factors in virulence and protection by the host immune. Some nonencapsulated *H influenzae* strains (NTHi) are almost always less invasive, but they can also produce an inflammatory response in humans, which can lead to many symptoms and cause a lot of mucosal infections [24]. Approximately one-third of episodes of recurrent infection, e.g. otitis media, are caused by nontypeable *H. influenzae*.

The composition of the oral microbial communities, essential for the normal development of the host and contributing to the host defense by excluding exogenous microorganisms, remains relatively stable over time. However, the stability of natural microflora can be perturbed by different risk factors, e.g. passive smoking. Indeed, our results showed that passive smoking increased the rate of the overall *H. influenzae* colonization of the upper respiratory tract in healthy pre-school children attending DCCs. Our results are partially in contrast to the observation with pediatric patients by Greenberg et al. [11], and with healthy children by Principi et al. [27]. These authors found that exposure to tobacco smoke did not influence the *H. influenzae* carriage in the upper respiratory tract in children aged <5 years. These discrepancies between our observations and literature data suggest that the effect of passive smoking on *H. influenzae* prevalence in the nasopharynx may be associated with other predisposing factors, e.g. age, gender, staying in a close population (e.g. DCC), chronic respiratory infections, previous antibiotic treatment, allergy, and geographic area [28, 29].

**Conclusion**

Our results suggest that chronic exposure of children to tobacco smoke may have consequences on the upper respiratory tract microflora composition, e.g. increased *H. influenzae* colonization, especially in children attending DCC.

**References**


