Bacterial interactions in the nasopharynx —
Effects of host factors in children attending
day-care centers

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Microbial interactions; Child day-care center; Streptococcus pneumoniae; Haemophilus influenzae; Moraxella catarrhalis

Summary  The nasopharynges of preschool children are often colonized by potentially pathogenic bacteria. The interactions between these common pathogens and certain host factors were investigated in healthy preschool children 1–6 years of age. Nasopharynx samples were collected from all 63 children attending a day-care center that experienced an outbreak of penicillin-resistant Streptococcus pneumoniae. The samples were analyzed for S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Group A Streptococci. A model for the risk of carrying these bacteria was established using logistic regression. S. pneumoniae and H. influenzae antagonize each other, whereas M. catarrhalis and S. pneumoniae have a positively association. The risk of carrying M. catarrhalis decreases with age. The time spent in day care each week was not shown to influence the rate of carriage of any of these pathogens. The negative effect of H. influenzae on S. pneumoniae is discussed in relation to the carriage of penicillin-resistant S. pneumoniae, and possible mechanisms involved in this interaction are presented.
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Introduction

The nasopharynges of preschool children are often colonized by potentially pathogenic bacteria, such as Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Group A Streptococci [1]. These bacteria can cause severe infections, such as otitis media, pneumonia or...
meningitis [2]. In a crowded place such as a child
day-care center (DCC), a large number of preschool
children are in close contact. Therefore, DCCs facili-
tate the transmission of these bacteria among
children [1]. In Sweden, more than 80% of chil-
dren 1–5 years of age attend a DCC, because both
parents are working [3].

The spread of penicillin-resistant S. pneumoniae
(PNSP) has prompted the development of different
approaches to counteract the phenomenon, such as
informing parents that viral infections cannot be
treated by antibiotics and teaching doctors to pre-
scribe antibiotics less often or more precisely [4].
For more than 10 years, there has been an epidemic
of PNSP among children attending DCC (1–6 years
of age) in the south of Sweden [5]. Several out-
breaks of PNSP in DCCs have been reported [6]. To
combat the epidemic, each child carrying PNSP is
banned from the DCC until two sequential negative
specimens have been isolated [6]. Even though the
incidence of PNSP has decreased since the adoption
of this approach, the approach also bans healthy
carriers of PNSP from the DCC, which has a nega-
tive effect on the children and their families [5].
The direct effect of banning PNSP-positive children
from DCCs relative to the effect of reducing the
frequency with which antibiotics are prescribed is
unclear [7].

Colonized pre-school children may function as a
reservoir for the spread of PNSP in the community
[8]. Antibiotic treatment and immunization could
influence the ecological balance in the nasophary-
inx in unforeseen ways. Thus, it is important to
understand the interaction among various bacte-
ria species in the nasopharynx. It has earlier been
reported that multiple pathogens interact during
an upper respiratory infection [2]. The aim of this
study was to explore the interactions among S.
pneumoniae, H. influenzae, and M. catarrhalis and
host factors in healthy children attending a DCC.

There were 42 children at the DCC in January
and 63 children in April. The children were divided
to three smaller groups, but they all interacted
closest, both outdoors and indoors, during the day.
The staff provided a list of the children in atten-
dance, which included each child’s name, age, gender,
time since enrollment at that DCC, and the
number of hours at the DCC per week (Table 1).
The large number of children attending the DCC
for 15–20 h a week can be explained by local reg-
ulations, which allow the children of parents at
home on parental leave with another child or due
to unemployment to stay at the DCC for a maximum
of 15 h a week.

Nasopharyngeal specimens

On both occasions, one of the authors took bac-
terial specimens from the nasopharynx of each
child registered at the DCC at the time of sam-
ping. The specimens were transported in modified
Stuart medium and were analyzed by the Depart-
ment of Microbiology at Lund University Hospital.
In January, the specimens were inoculated on blood
agar and were analyzed for the presence of S.
pneumoniae and β-hemolytic group A Streptococci
only. In April, the specimens were inoculated on
blood agar and hematin agar and were analyzed
for the presence of M. catarrhalis and non-typable
H. influenzae in addition to S. pneumoniae and β-
hemolytic group A Streptococci. S. pneumoniae and
H. influenzae were tested for resistance to selected
antibiotics. Only the PNSP isolates were serotyped.
All analyses were performed using EUCAST
methods.

Statistical analysis

Logistic regression was used to investigate how
the risk of being a carrier of one bacteria species
was affected by the presence of other bacteria
species and certain host factors. The analysis was
performed with SPSS Statistics 17.0 software. The
logistic model is often used to relate a binary
outcome to one or more continuous or binary ex-
posures. The model can be used to describe many
different relationships because the curve has a
sigmoid shape, as do many dose–response rela-
tionships. For binary exposures, the model produces
an estimate of the odds ratio (OR) for a given outcome
in someone who has been exposed. For a continu-
ous exposure, the OR is calculated for a one-unit
increase in exposure.

Each type of bacteria, S. pneumoniae, H.
influenzae, and M. catarrhalis, was analyzed using
“Binary Logistic: Backward LR” with gender, age,
time at the DCC per week, time since enrollment at the DCC and the presence of the other bacteria species as predictors. The program ran repeated logistic regressions and excluded one variable after each run based on a Likelihood Ratio (LR) test. By removing variables, the confidence intervals of the others may become smaller, at the cost of a larger risk of confounding. In one analysis, the three other tested bacteria were the only predictors; in another, the host factors were the only predictors. A separate analysis including only children younger than 4 years old was also performed. In some of the regressions, a few children had to be excluded because data for one or more variables were missing.

Results

The presence of various bacteria species is shown in Table 2. There was a large difference in colonization by S. pneumoniae with only a few children colonized in January and a majority colonized in April. In Fig. 1, the portion of the children colonized by each bacterium is shown for each age group.

In January, there was no child with PNSP other than the index case. In April, PNSP was isolated from six children. Five resistance patterns were found for S. pneumoniae, including resistance to penicillin V. All PNSP isolates belonged to serogroup 19. Four different resistance patterns were found in the analysis for H. influenzae, two of which included resistance to penicillin V. Resistance to multiple antibiotics was common.

The results of the logistic regression for the risk of being colonized with S. pneumoniae, H. influenzae, or M. catarrhalis are shown in Table 3 with

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
</tr>
<tr>
<td>1 to &lt;2</td>
<td>21 (33.3)</td>
</tr>
<tr>
<td>2 to &lt;3</td>
<td>17 (27.0)</td>
</tr>
<tr>
<td>3 to &lt;4</td>
<td>12 (19.0)</td>
</tr>
<tr>
<td>4 to &lt;7</td>
<td>13 (20.6)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>36 (57.1)</td>
</tr>
<tr>
<td>M</td>
<td>27 (42.9)</td>
</tr>
<tr>
<td>Time since enrollment at the DCC center, mo&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0 to &lt;9</td>
<td>41 (68.3)</td>
</tr>
<tr>
<td>9 to &lt;18</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>18 to &lt;27</td>
<td>15 (25.0)</td>
</tr>
<tr>
<td>Time at the DCC center per week, h&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10 to &lt;15</td>
<td>2 (3.7)</td>
</tr>
<tr>
<td>15 to &lt;20</td>
<td>20 (37.0)</td>
</tr>
<tr>
<td>20 to &lt;25</td>
<td>2 (3.7)</td>
</tr>
<tr>
<td>25 to &lt;30</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>30 to &lt;35</td>
<td>5 (9.2)</td>
</tr>
<tr>
<td>35 to &lt;40</td>
<td>11 (20.4)</td>
</tr>
<tr>
<td>40 to &lt;45</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>45 to &lt;50</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>50 to &lt;55</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>55 to &lt;60</td>
<td>1 (1.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data for three children were missing. The percentage of children for whom records were available is presented.

<sup>b</sup> Data for nine children were missing. The percentage of children for whom records were available is presented.

Figure 1 Colonization by different bacteria of children different age groups at the day-care center in April 2008.
Table 2  Potential pathogenic bacteria in children attending one day-care center in the south of Sweden in January and April, 2008.

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>January (n = 42)</th>
<th>April (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae, n (%)</td>
<td>4 (9.5)</td>
<td>45 (71.4)</td>
</tr>
<tr>
<td>Group A Streptococci, n (%)</td>
<td>2 (4.8)</td>
<td>2 (3.2)</td>
</tr>
<tr>
<td>Haemophilus influenzae, n (%)</td>
<td>Not analyzed</td>
<td>16 (25.4)</td>
</tr>
<tr>
<td>Moraxella catarrhalis, n (%)</td>
<td>Not analyzed</td>
<td>52 (82.5)</td>
</tr>
</tbody>
</table>

*Data for two children were missing. The percent of all children is shown.*

Table 3  Logistic regression for the risk of being colonized with different bacteria using host factors and other bacteria as predictors.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.74 (1.17–3.19)</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.85 (0.47–1.55)</td>
</tr>
<tr>
<td>Time per week, h</td>
<td>1.01 (0.95–1.07)</td>
</tr>
<tr>
<td>Time since enrollment, mo</td>
<td>1.00 (0.92–1.09)</td>
</tr>
<tr>
<td><strong>Haemophilus influenzae</strong></td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.73 (1.18–2.93)</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.93 (0.49–1.77)</td>
</tr>
<tr>
<td>Time per week, h</td>
<td>1.03 (0.98–1.09)</td>
</tr>
<tr>
<td>Time since enrollment, mo</td>
<td>1.03 (0.95–1.13)</td>
</tr>
<tr>
<td><strong>Moraxella catarrhalis</strong></td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.29 (0.07–1.25)</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.29 (0.07–1.25)</td>
</tr>
<tr>
<td>Time per week, h</td>
<td>2.43 (0.31–19.13)</td>
</tr>
<tr>
<td>Time since enrollment, mo</td>
<td>2.43 (0.31–19.13)</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, confidence interval.*

all known possible predictors. The results from the regression with only the other bacteria as predictors are presented in Table 4.

There was a tendency for an increased risk of being colonized by *S. pneumoniae* among children colonized by *M. catarrhalis* (Table 3). This connection was significant in the model that used only the other bacteria as predictors (Table 4). Carriage of *H. influenzae* was inversely correlated with the risk of being a carrier of *S. pneumoniae* (Table 3). This correlation was not significant in the model with all predictors but was significant in the last step of the model that used only the other bacteria as predictors. In this instance, the results were as follows: *H. influenzae* 0.27 (95% CI 0.07–1.00); *M. catarrhalis* 5.63 (95% CI 1.19–26.65); constant 1.04. When only children younger than 4 years old were included, *H. influenzae* was the only significant predictor at 0.10 (95% CI 0.02–0.59).

The risk of being colonized by *H. influenzae* was inversely correlated with the risk of colonization by *S. pneumoniae* (Table 3). This correlation was only significant in the second step of the model that used only the other bacteria as predictors.

Table 4  Logistic regression for the risk of being colonized with different bacteria using only bacteria as independent variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>0.27 (0.07–1.02)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>0.27 (0.72–1.01)</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>5.48 (1.13–26.58)</td>
</tr>
<tr>
<td>Group A Streptococci</td>
<td>0.77 (0.04–14.06)</td>
</tr>
<tr>
<td>Constant</td>
<td>1.068</td>
</tr>
<tr>
<td><strong>Haemophilus influenzae</strong></td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>5.23 (1.09–24.94)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>2.17 (0.35–13.56)</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>2.55 (0.36–17.92)</td>
</tr>
<tr>
<td>Group A Streptococci</td>
<td>3.23 (0.17–61.07)</td>
</tr>
<tr>
<td>Constant</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Moraxella catarrhalis</strong></td>
<td></td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>1.83</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>1.06</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>1.06</td>
</tr>
<tr>
<td>Group A Streptococci</td>
<td>1.06</td>
</tr>
<tr>
<td>Constant</td>
<td>1.06</td>
</tr>
</tbody>
</table>

*OR, odds ratio; CI, confidence interval. Significant ORs and CIs are shown in boldface.*
The coefficients were as follows: *S. pneumoniae* 0.27 (95% CI 0.07–1.00); *M. catarrhalis* 2.15 (95% CI 0.34–13.47); constant 0.46. This connection was also significant with a value of 0.09 when only children younger than 4 years old were included (95% CI 0.01–0.59).

*M. catarrhalis* carriage was strongly associated with *S. pneumoniae* carriage. This association was not significant in the model that included all predictors (Table 3) but was significant in the model that used only the other bacteria as predictors (Table 4). The risk of being colonized by *M. catarrhalis* decreased with increasing age. This trend was not significant when all predictors were included in the model (Table 3) but was significant in the model that used only the host factors as predictors. The coefficients in the last step of this analysis were as follows: age 0.59 (95% CI 0.36–0.98) and constant 26.17. For children younger than 4 years of age, no predictor was significantly associated with the presence of *M. catarrhalis*.

### Discussion

This study shows that the risk of being colonized by *S. pneumoniae* is negatively associated with colonization by *H. influenzae* but positively associated with colonization by *M. catarrhalis*. The risk of being colonized by *H. influenzae* was also negatively associated with colonization by *S. pneumoniae*, and these two species seem to antagonize each other. The risk of being colonized by *M. catarrhalis* was associated with the presence of *S. pneumoniae* but decreased with increasing age. The large difference in colonization between January and April may indicate that the transmission of bacteria was reduced in January because many children had just returned from a long holiday. Another factor could be the presence of many children new to the DCC, which also led to a reduction in the mean age of study participants.

### Effects of host factors on bacteria

In this study, no significant association between gender and colonization by any of the bacteria species was found. Earlier studies have found a higher risk of colonization by *H. influenzae* among males [2,9] but no connection between gender and colonization by *S. pneumoniae* [1,2] or *M. catarrhalis* [2].

The occurrence of *M. catarrhalis* decreased with increasing age. This finding is in accordance with the results of an earlier study [2]. A negative effect of age on the risk of colonization by *S. pneumoniae* has been found in earlier studies [2,10] but was not found in this one. No connection between age and the risk of being colonized by *H. influenzae* was found in this or a previous study [2].

The time per week that the children spent at the DCC was not associated with the risk of colonization by any of the bacteria, except for *H. influenzae*, which was more common among children who spent more time at the DCC each week. An earlier study found a non-significant increase in the risk among children attending a DCC full-time relative to children attending part-time [9]. No previous study has compared the time spent at the DCC per week and infection by *S. pneumoniae* or *M. catarrhalis*. Thus, there was no evidence for a reduction in bacterial transmission corresponding to a reduction in time spent at the DCC.

No connection between time since enrollment at the DCC and the presence of any of the bacteria was found in this or any previous study.

### Interactions among bacteria

#### Earlier epidemiological investigations

Because *S. pneumoniae* and *H. influenzae* seem to antagonize each other and *M. catarrhalis* seems to increase the risk of infection with *S. pneumoniae*, there are likely interactions among these species. Only a limited number of studies have investigated this phenomenon. Jacoby et al. [11] studied how often these bacteria co-occurred compared with the rate predicted by an independent random distribution. They found significant positive associations among all of these bacteria. Their study had a longitudinal setup and the analysis was different. The age group also differed; all children were younger than two years. Pettigrew Melinda et al. [2] investigated how the bacteria affected each other during upper respiratory tract infections and found a significant negative effect of *H. influenzae* on the presence of *S. pneumoniae*. The presence of *M. catarrhalis* was positively associated with the existence of *S. pneumoniae*, but this trend was not significant. When both *M. catarrhalis* and *H. influenzae* occurred together, the effect of *H. influenzae* on *S. pneumoniae* reversed and became significantly positive; thus, *H. influenzae* supported the occurrence of *S. pneumoniae*. The first two results were also observed in this study, but the last was not. The influence of an ongoing infection, as opposed to carriage, on these bacteria may explain these discrepancies.

#### Underlying mechanisms

In vitro, *H. influenzae* is inhibited by *S. pneumoniae* [12]. Some strains of *S. pneumoniae* produce neuraminidase A [13], which makes *H. influenzae*...
susceptible to the complement system. *S. pneumoniae* also produces hydrogen peroxide, which can inhibit *H. influenzae* [12]. Research in a mouse model has shown that both *S. pneumoniae* and *H. influenzae* can colonize and be sustained, but when both species are present in the same animal, the *S. pneumoniae* population is rapidly cleared [14]. This effect is observed in both immune-deficient mice (SCID) and immune-competent mice, indicating that the effect is independent of the adaptive immune system. Antigens on *H. influenzae* activate neutrophil granulocytes, which kill *S. pneumoniae* but not *H. influenzae* [14]. According to studies using mice, the adaptive immune system also protects against colonization by potentially pathogenic bacteria, mostly through secreted IgA [15]. This IgA-mediated protection has been observed for *H. influenzae* in children [16]. The IgA response has an important effect on the duration of the colonization and is also important in preventing re-colonization by the same strain of *H. influenzae* [16]. A non-serotype specific defense against *S. pneumoniae*, which decreases the risk of being colonized by a new strain after earlier colonization by a different strain, has been described [17].

*S. pneumoniae*, *H. influenzae* and *M. catarrhalis* are all known to exist in biofilms [18]. The biofilm environment can alter the susceptibility to antibiotics as well as to different aspects of the immune system. It is also possible that the interactions among bacteria species are affected by the biofilm environment.

**Models for host factor effects on the interactions**

Given that the occurrence of *H. influenzae* facilitates immune clearance of *S. pneumoniae*, the interactions between *S. pneumoniae* and *H. influenzae* could be independent of external factors. Because these two bacteria often coexist, the antagonization is not absolute, and other factors may have a significant impact. The variables investigated in this study were age and time at the DCC per week.

Generally, the risk of carrying potentially pathogenic bacteria decreases with age [1]. The interactions between these bacteria and the host depend on the innate and adaptive immune systems. *S. pneumoniae* and *H. influenzae* may, due to their differences, interact with the immune system in distinct ways during the different stages of immune system development. The adaptive immune system also reduces the risk of colonization by producing antibodies specific for an increasing number of strains. The logistic regression analysis used in this study was not suitable for finding a local maximum of colonization for a small age group. The results do not include any connections between the bacteria and age that were strong enough to verify this theory.

Another variable that could possibly affect the balance between *S. pneumoniae* and *H. influenzae* is how much time the child spends at the DCC per week. Such an effect could, for example, be due to differences in the colonization time for each strain and differences in the number of strains existing at the DCC. An investigation of the transmission rates of different strains among children at a DCC found a larger number of *H. influenzae* strains in circulation and a shorter maximal colonization time for *H. influenzae* strains than for *S. pneumoniae* strains [19]. Additionally, a few *S. pneumoniae* strains dominated over this period, while a larger number of *H. influenzae* strains exhibited substantial spread. A shorter time at the DCC decreases the exposure time and should accordingly decrease the risk of infection by circulating strains. Thus, a child who spends a short time at the DCC each week has a smaller risk of catching all of the *H. influenzae* strains, but the risk of catching each *S. pneumoniae* strain is not affected to the same degree. It is also possible that differences in the degree of infectiousness among the strains and species play a role.

Another possible effect of a longer time at the DCC per week is that the immune system matures more rapidly due to the extended exposure to different strains.

The interactions among the bacteria are probably, at least in part, independent of the effects of host factors because the interactions among the bacteria were found to be significant in this study, whereas most of the host factors had a very small and nonsignificant effect on the occurrence of various bacteria species.

**Limitations and consequences**

Although all of the children at one DCC were examined and included in the study, the small study population is an important limitation, especially when analyzing complex interactions. The age distribution may be a problem in extrapolating the results to other groups of children.

Since the beginning of 2009, one year after this study was carried out, a vaccine against seven selected serotypes of *S. pneumoniae* was added to the vaccination program of the Child Health Program [20]. The vaccination was available in the private market earlier but no data about the prevalence of its use at that time have been published. Public vaccination may affect the carriage rate of *S. pneumoniae* and the interactions among potential pathogens in the nasopharynges of preschool
children. This call for a new study including more host factors, such as current and earlier antibiotic treatment and the vaccinations received.

This type of study does not provide evidence of causality. If a causal relationship exists (i.e., H. influenzae has the ability to eliminate S. pneumoniae), this relationship may be used clinically as an alternative treatment to watchful waiting or antibiotic treatment in healthy children carrying a penicillin-resistant S. pneumoniae strain. The carrier stage of resistant S. pneumoniae can last for several months and is difficult to eradicate with antibiotics alone [6].

Conflict of interest

Funding: This work has not been supported by any external funding.

Competing interests: The collection of nasopharyngeal specimens was decided by the local infectious disease control department.

Ethical approval: Review by the ethics committee was not necessary for this analysis of the collected data.

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