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

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**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.


## 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

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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 facilitate the transmission of these bacteria among children [1]. In Sweden, more than 80% of children 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 prescribe 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 outbreaks 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 negative 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 nasopharynx in unforeseen ways. Thus, it is important to understand the interaction among various bacteria 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.

## Methods

### Population

This study included all children who attended one DCC in a village in southern Sweden during the spring of 2008. On two occasions, in January and April, a child with a respiratory tract infection harbored PNSP. The index child was not the same on the two occasions. As required by the local infectious disease control department's policy, a nasopharyngeal specimen was taken from each child attending this DCC to identify other carriers of PNSP.

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 closely, both outdoors and indoors, during the day. The staff provided a list of the children in attendance, 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 regulations, 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 bacterial specimens from the nasopharynx of each child registered at the DCC at the time of sampling. The specimens were transported in modified Stuart medium and were analyzed by the Department 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 exposures. The model can be used to describe many different relationships because the curve has a sigmoid shape, as do many dose—response relationships. 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 continuous 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,