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Breastfeeding and Nasopharyngeal Colonization With Common Respiratory Pathogens Among Children

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Background: Breastfeeding has been associated with decreased frequency of otitis media in children. It might be due to lower prevalence of nasopharyngeal colonization with the respiratory pathogens among breast-fed infants compared to those who are formula-fed.
Objectives: This study was performed to determine the influence of breastfeeding on *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* colonization rates in children.
Patients and Methods: In this cross-sectional study, 2 - 6 years old children in 10 randomly selected daycare centers in northeast of Iran (Mashhad) were studied. We obtained nasopharyngeal specimens to culture for *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* from 1125 children. Carriage rates for aforementioned bacteria were determined and analyzed on the basis of feeding type during infancy.
Results: A total of 885 children were breast-fed (78.7%), 69 children were formula-fed (6.1%), and 171 children were mixed-fed (15.2%). Carriage rates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* among children were 13.1%, 9.1%, and 4.3%, respectively. Although lower incidence of nasopharyngeal colonization was found in breast- versus formula-fed children, it was not significant ($P > 0.5$).
Conclusions: It seems that feeding in infancy has no influence over the prevalence of nasopharyngeal colonization with respiratory pathogens during childhood.

Keywords: Breastfeeding; Nasopharyngeal Carriage; *Moraxella catarrhalis*; *Streptococcus pneumoniae*; *Haemophilus influenzae*

1. Background

Based on the results of several studies, breastfeeding is associated with decreased frequency or duration of otitis media episodes (1, 2). Even if a causal relationship exists, the mechanism of breastfeeding protection has not been exactly established yet.

However, it has been postulated that breastfeeding may provide protection against acute otitis media by interfering with the attachment of bacterial pathogens to nasopharyngeal epithelial cells (3, 4). Various protective factors of breast milk, including secretory IgA antibodies, lactoferrin, and oligosaccharides functioning as receptor analogues are thought to provide passive protection against nasopharyngeal colonization (1, 4-6). However, clinical and epidemiological studies have not yet confirmed the influence of breastfeeding on the prevalence of nasopharyngeal colonization with common bacterial pathogens.

In young children, *Streptococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis* are the most common bacterial pathogens causing respiratory infections such as acute otitis media, sinusitis, and pneumonia, as well as invasive infections like bacteremia and meningitis (7-9). The nasopharynx of children occasionally becomes the reservoir of such potential pathogens and serves as ports of entry to both adjacent mucosal tissues and the bloodstream for them (10-12).

2. Objectives

The objectives of the present study were to determine the influence of breastfeeding vs. non-breastfeeding during infancy on *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* carriage rates during childhood.

3. Patients and Methods

3.1. Study Design and Population

The study was conducted from September through

April at the capital city of Khorasan Province, Mashhad in northeast of Iran. Among 98 daycare centers in Mashhad, 10 centers, with regard to their geographical distribution, were randomly selected and all children aged 2 - 6 years were enrolled in the study. The study was approved by the ethics committee of the university and signed informed consents were obtained from parent or guardian of each child. Written questionnaires on demographics data and medical history were completed by the parents. Age, sex, type of feeding during infancy (such as breast-, formula-, or mixed-feeding), number of family members, economic status of the family, duration of attendance in daycare center, history of recent infection and antibiotic usage within the month before enrollment, history of recent infection or any underlying disorders such as allergic rhinitis were considered in this questionnaire.

Children with respiratory problems (such as an acute asthma attack, acute infection), chronic illnesses (e.g. malignancies), anatomical abnormalities of the mouth or nose (e.g. cleft palate) or history of vaccination for *S. pneumoniae*, and or *H. influenzae* were excluded from the study. Moreover, all children receiving antibiotic treatment during the last 30 days as of the enrollment were also excluded. In addition to what parents recalled, the interviewing physician examined each child's health statues in the daycare center. All information was gathered without knowledge of the child's carrier status.

Then, children were divided into 3 groups according to their feeding status: 1) breast-fed defined as mainly breastfed during first two year of life, 2) breast/formula-fed, and 3) formula fed.

3.2. Microbiology Procedures

Nasopharyngeal specimens were obtained by a single trained investigator. For this purpose, a thin flexible wire swab with the bending tip of approximately 30 degree, was inserted through the mouth and placed 2 to 4 cm into the nasopharynx, taking care not to touch the uvula or the tongue, and maintained there for at least 3 seconds. Children were not allowed to eat or drink within one hour before specimen collection. The swabs were inoculated immediately onto culture media containing appropriate supplements and antibiotics for isolation and then transported to the clinical microbiology laboratory of the Ghaem University Hospital, within 1 to 3 hours. Next, the plates were incubated in an atmosphere containing 5% CO_2 at 35°C for 48 hours. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were isolated and identified according to standard laboratory procedures. Identification was performed by conventional microbiological methods as follows: 1) *H. influenzae*: Gram staining, colony morphology, growth on chocolate agar with bacitracin, catalase test and X (hemin), V (NAD) and combined XV factors dependency, 2) *S. pneumoniae*: Gram staining, morphology, catalase and hemolysis reaction, optochin susceptibility, and bile solubility, 3) *M. catarrhalis*: Gram staining, morphology, growth on chocolate agar containing vancomycin, trimethoprim, and amphotericin B, catalase, oxidase, and DNase reaction. Antibiotic susceptibility of the strains was determined using disk diffusion according to clinical laboratory standards institute (CLSI) recommendation.

3.3. Statistical Analysis

Statistical analysis was performed using SPSS software, version 11.5 (SPSS). Univariate analyses were included in the multivariate logistic regression models. These potential risk factors were sex, age, allergic rhinitis, breastfeeding (of the child), number of siblings, and economic status of family. P value less than 0.05 was considered to be statistically significant.

4. Results

A total of 1125 children (528 males (46.9%) and 597 females (53.1%)) were enrolled in the study. The mean age of them was 5.05 ± 0.98 (range 2 to 6 years). About 15% of them had a smoker parent (considered as passive smoker) and 28.3% had a history of allergic rhinitis. The overall carrier rate of nasopharyngeal pathogens was 29.3% (330 of 1125). Most of the children (297, 90%) carried only one pathogen. No child carried 3 respiratory pathogens simultaneously (Table 1).

There were different kinds of feeding during infancy and 885 (78.7%) infants were breast-fed (Table 2). Univariate analysis of factors potentially associated with nasopharyngeal carriage of respiratory pathogens showed no significant difference between feeding type and the colonization of the nasopharynx by *S. pneumoniae*, *M. catarrhalis*, *H. influenzae*, or polygerms ($P > 0.05$) (Table 2).

In multiple logistic regression models controlling for sex, antibiotic treatment in the month before sampling, age, health versus illness, economic status, passive smoking, number of siblings, allergic background and ethnicity, the increased rates of *M. catarrhalis* carriage among younger children ($P < 0.001$) and those with history of multiple antibiotic usage ($P < 0.001$) remained independently significant, whereas rates of *H. influenzae* and *S. pneumoniae* were not significant (13).

Table 1. The Carriage Rate of Nasopharyngeal Pathogens

Pathogen	No (%)
Colonization with *S. pneumoniae*	146 (13.0)
Colonization with *H. influenzae*	102 (9.1)
Colonization with *M. catarrhalis*	48 (4.3)
Two bacteria (mixed)	34 (3.0)
None	795 (70.7)

Table 2. The Different Kinds of Feeding During Infancy

Pathogens	Feeding Type			P Value
	Breast-Fed	Formula-Fed	Breast/Formula-Fed	
No pathogen	633	48	114	N/A
S. pneumoniae	111	5	30	0.091
H. influenzae	78	9	15	0.542
M. catarrhalis	39	3	6	0.939
Polygerms	24	4	6	0.319
Total	885	69	171	

5. Discussion

The prevalence of nasopharyngeal carriage of respiratory pathogens in our study was about 29%. Previous studies reported nasopharyngeal colonization rates ranging from 20% to over 50% for all respiratory pathogens (14-17). However, low carriage rates had been reported for single respiratory pathogens by other researchers (18, 19). Our result is lower than many other studies. There are several explanations for this difference: 1) our study like some other studies was performed only among healthy children (20, 21); It has been shown that during episodes of respiratory illness, in particular otitis media, nasopharyngeal flora remarkably increases (7, 22), so the exclusion of sick children may result in a lower carriage rate, 2) Season may also be an important factor, so that studies in the different season may give different results (9, 15). In this survey, children were sampled during a very short period of time, from mid-autumn until mid-spring, in contrast to most studies, in which sampling was performed in different seasons, 3) We studied a very large number of children, which is probably representative of the all age groups in contrast to most previous studies, which covered only selected groups of children or small samples, so our results can unquestionably be generalized (23-25), and 4) In our study in addition to above mentioned reasons the Iranian genetic traits might also play some role.

In the present study, we have shown that breastfeeding is associated with decreased nasopharyngeal carriage rate of all pathogens, especially *S. pneumoniae*, though these low rates were not significant. Many studies showed that risk of otitis media reduced significantly in infants mainly breastfed until 6 month of age (5, 26). Several meta-analytic studies were published recently indicating the beneficial effect of breastfeeding on reducing episodes of acute otitis media (AOM) in infancy (1-4). Considering our study, the protective effect of breastfeeding may be explained by several mechanisms, including improved nutrition and bioactive or antibacterial effects of human milk (3, 4). Along with these, the increased risk for AOM and otitis media with effusion episodes during the first 2 years of life among formula-fed infants may be explained by keeping these children in daycare centers outside home, which in addition to depriving them from being breastfed, exposed them to early pathogen colonization from other children (26, 27).

In summary, although our study has not proved the beneficial effect of breastfeeding on nasopharyngeal colonization of *S. pneumoniae*, *H. influenzae,* and *M. catarrhalis* in children under 6 years old, considering the immunologic properties of human milk that may interfere with the attachment of bacterial pathogens to nasopharyngeal epithelial cells, it may play a role in the prevention of infection in other ways and additional study are warrant in this regard.

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Authors' Contributions

Mehdi Bakhshaee: Study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical, and material support, as well as study supervision. Mohsen Rajati Haghi: Study concept and design, critical revision of the manuscript for important intellectual content, drafting of the manuscript. Hamid Reza Naderi: Study concept and design, critical revision of the manuscript for important intellectual content. Mohammad Khomarian: Acquisition of data. Kiarash Ghazvini: Study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, administrative, technical, and material support, as well as study supervision.

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