Prevalence of Bacterial Respiratory Pathogens in the Nasopharynx in Breast-Fed versus Formula-Fed Infants

PHILLIP H. KALEIDA,1,2* DONNA G. NATIVIO,2,3 HAN-PU CHAO,1,2† AND SHARON N. COWDEN1

Department of Pediatrics, University of Pittsburgh School of Medicine,1 the Primary Health Care Nursing Graduate Program, University of Pittsburgh School of Nursing,2 and the Department of Pediatrics, Children's Hospital of Pittsburgh, 3705 Fifth Avenue,2 Pittsburgh, Pennsylvania 15213-2583

Received 9 April 1993/Returned for modification 3 June 1993/Accepted 2 July 1993

In several studies, breast-feeding has been associated with decreased frequency or duration of otitis media episodes. If a causal relationship exists, the mechanism of protection of breast-feeding has not been established. We hypothesized that infants who are breast-fed, compared with infants who are formula-fed, have a lower prevalence of nasopharyngeal colonization with the bacterial respiratory pathogens (Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes) commonly isolated from the middle ear effusions of children with acute otitis media. In two private pediatric practices, we obtained specimens from the nasopharynx for culture from 211 infants at 1 month of age and from 173 of these infants at 2 months of age. A swab was left in place in the nasopharynx for 45 s and was then immediately transferred onto appropriate culture media. Exclusively breast-fed (n = 84) and exclusively formula-fed (n = 76) infants were similar regarding the number of persons in the household, the number of children in the household, the number of siblings in day care, and the proportion with a recent upper respiratory tract infection. The two groups did not differ significantly in the proportions found to have one or more respiratory pathogens at 1 month of age (10.7 versus 18.4%; P = 0.12) or 2 months of age (34.8 versus 35.1%; P = 0.57). We conclude that during the first 2 months after birth, the exclusive receipt of breast milk appears to not substantially influence the prevalence of nasopharyngeal colonization with common bacterial respiratory pathogens.

Otitis media is a common problem in infants and children. Children who experience their first episode early in life often have multiple recurrences, which can be associated with complications and sequelae (7). Therefore, a method of prevention that is generally safe, inexpensive, easy to administer, and widely available would seem optimal. Breast-feeding meets these criteria, and in several studies (11, 22, 23, 25, 29) it has been associated with a decreased frequency or duration of otitis media episodes. In other studies (17, 21, 24, 28), however, this association has not been substantiated. The apparent disagreement in results of studies of the relationship between breast-feeding and otitis media may be due to difficulties inherent in such investigations. Confounding factors include inability to assign feeding methods and feeding groups that overlap and change over time (5, 15, 26).

If breast-feeding does have a protective effect, the precise mechanism of protection has not been described. Bacterial colonization of the oropharynx (19) and the nasopharynx (30) occurs soon after birth. Potential pathogens have been identified in the nasopharynx in both well and sick children (14, 32). In a group of infants and children with suppurative otitis media, Schwartz and colleagues (27) found that the same type of bacterial pathogen was isolated frequently from both the middle ear and the nasopharynx when direct plating and semiquantitative techniques were used. Other investigators (9, 12) studying infants and young children with otitis media found a high negative predictive value between the nasopharyngeal culture and particular isolates from the middle ear. In either case, the source of middle ear pathogens is the nasopharyngeal flora, even if there is not a one-to-one correlation between nasopharyngeal colonization and individual episodes of acute otitis media.

In a prospective study of rotavirus infection, Duffy and colleagues (8) reported an alteration of the enteric flora in breast-fed, but not bottle-fed, subjects. If one postulates that the presence of certain pathogens in the nasopharynx is associated with the occurrence of otitis media (20), then a potentially protective and analogous mechanism of action of breast-feeding might be an alteration of the nasopharyngeal flora. A reduction in the frequency or the quantity of bacterial pathogens in the nasopharynx might contribute to a reduction in the incidence of otitis media.

The objective of the study described here was to determine whether breast-fed infants, compared with formula-fed infants, have a lower prevalence of nasopharyngeal colonization with bacterial respiratory pathogens commonly isolated from the middle ear effusions of children with acute otitis media.

(This report was presented in part at the Annual Meeting of the American Pediatric Society and the Society for Pediatric Research [poster symposium], New Orleans, La., 29 April 1991; the Fifth International Symposium on Recent Advances in Otitis Media, Ft. Lauderdale, Fla., 21 May 1991; and the 17th National Primary Care Nurse Practitioner Symposium, Keystone Resort, Colo., 11 July 1992.)

MATERIALS AND METHODS

Study population. Infants scheduled for well-child visits in two Pittsburgh private pediatric practices were enrolled in the study between January 1989 and October 1990. In order to be eligible for the study, infants had to be 1 month of age (23 to 37 days). In addition, infants had to have been fed by one of the following three methods: breast milk only (exclusively breast-fed), breast milk mainly (breast-fed with up to
four formula feedings per week, on average), and formula only (exclusively formula-fed). Breast-fed infants known to have received only one formula feeding ever were also classified in the breast milk-only group. At 2 months of age (50 to 70 days), there was an additional feeding group, the breast milk-partially formula feedings per 2-month visit. These children who had received antimicrobial therapy or who had potentially complicating or confounding conditions, e.g., a major congenital anomaly, stay in a special-care nursery, a postpartum hospital stay of longer than 7 days, or nasotracheal or endotracheal intubation, were excluded. The most common reason for exclusion, however, was inability to meet the feeding method entry criteria. We chose not to obtain a second nasopharyngeal specimen from the one child who was receiving antimicrobial therapy at the time of the 2-month visit.

At the time of entry into the study, we obtained written informed consent from each subject's parent(s) or legal guardian(s). The study was approved by the Human Rights Committee of the Children's Hospital of Pittsburgh.

Procurement of nasopharyngeal specimens and identification of bacteria. We obtained a nasopharyngeal specimen for culture at the time of entry into the study and again at 2 months of age. A curved calcium alginate swab was advanced along the floor of the nose into the posterior nasopharynx. In order to obtain a representative sample of the flora, we left the swab in place for 45 s (18). Each swab was then inoculated immediately and consecutively onto the following five media: sheep blood agar, chocolate agar, Columbia CNA agar, MacConkey agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.), and a Moraxella catarrhalis selective agar (31). The M. catarrhalis selective agar medium contained both acetalazolamide, which, under aerobic conditions (but not in 5% CO₂), reduces the growth of Neisseria species, and vancomycin, trimethoprim and amphotericin B, to inhibit the growth of normal flora. One of three designated medical technologists read each plate at 24 and 48 h. The bacteria were identified by standard laboratory methods (3). Typing of Haemophilus influenzae was not routinely performed. Using a modification of a previously described method (10), the technologists reported bacterial growth on a semiquantitative scale, where 0 represented no growth and 4+ represented heavy growth.

Analysis. The primary outcome measure in the present study was the proportion of subjects from whom one or more respiratory pathogens (Streptococcus pneumoniae, H. influenzae, M. catarrhalis, and Strepococcus pyogenes) were isolated from the nasopharynx in the breast milk-only and the formula-only groups. Throughout the text we use the term "pathogen" to refer to these bacteria, but we recognize that these (and other) bacteria may be present in the nasopharynx without producing clinical disease. Finally, although it was not an outcome measure, we also noted in each of the feeding groups the occurrence of presumed nonpathogens (coagulase-positive staphylococci, gram-negative rods, and other bacterial flora) in the nasopharynx.

When comparing the proportions of subjects from whom at least one pathogen was isolated from the nasopharynx at the 1-month or the 2-month visit, we computed significance levels using Fisher's exact test (one-sided). With the sample size in the present study, there were probabilities of 0.99 at the 1-month visit and 0.81 at the 2-month visit of detecting a 0.25 difference between the proportions of subjects from whom at least one pathogen was isolated from the nasopharynx in the breast milk-only and the formula-only groups.

### RESULTS

**Subject characteristics.** The study was designed to compare the nasopharyngeal bacterial flora of breast-fed infants with those of formula-fed infants. A total of 211 infants from two private pediatric practices were enrolled in the study. One practice contributed 129 subjects (61.1%), and the other contributed 82 subjects (38.9%). Table 1 provides the pertinent demographic and clinical characteristics of the subjects by feeding method at the time of entry into the study. None of the differences between the feeding groups was statistically significant at \( P \leq 0.05 \). The mean birth weight was 3.4 kg.

**Trends in feeding methods.** One hundred seventy-three infants (82.0%) had a 2-month study visit. The most common reason for infants not being seen at 2 months of age was
TABLE 2. Proportions of subjects from whom one or more pathogens were isolated from the nasopharynx by feeding method and age*  

<table>
<thead>
<tr>
<th>Feeding method at time of visit</th>
<th>% Positive (n) by ageb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mo</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
</tr>
<tr>
<td>Breast milk only</td>
<td>10.7 (84)</td>
</tr>
<tr>
<td></td>
<td>34.8 (46)</td>
</tr>
<tr>
<td>Breast milk mainly</td>
<td>17.6 (51)</td>
</tr>
<tr>
<td></td>
<td>26.2 (42)</td>
</tr>
<tr>
<td>Breast milk partially</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>32.1 (28)</td>
</tr>
<tr>
<td>Formula only</td>
<td>18.4 (76)d</td>
</tr>
<tr>
<td></td>
<td>35.1 (57)d</td>
</tr>
<tr>
<td>Total</td>
<td>15.2 (211)</td>
</tr>
<tr>
<td></td>
<td>32.4 (173)</td>
</tr>
</tbody>
</table>

* See text for definitions of pathogens and feeding methods.

Scheduling difficulties. Of 117 such infants who were receiving either breast milk only (59.8%) or breast milk mainly (40.2%) at the 1-month visit, all but one were still receiving breast milk (breast milk only, 39.3%; breast milk mainly, 35.0%; breast milk partially, 25.9%) at the 2-month visit. All 56 infants in the formula-only group at the 1-month visit continued to be exclusively formula-fed at the 2-month visit.

Presence of nasopharyngeal pathogens. Table 2 shows the proportions of subjects from whom one or more pathogens were isolated from the nasopharynx by feeding method and age. At 1 month of age, the difference in these proportions between the breast milk-only and the formula-only groups was not statistically significant (P = 0.12).

When comparing the breast milk-only group with the formula-only group at 2 months of age, we again found that the difference in the proportions of subjects from whom one or more pathogens were isolated from the nasopharynx was not statistically significant (P = 0.57). A cohort analysis comparing infants in the breast milk-only group at both visits with those in the formula-only group at both visits yielded similar results.

We also evaluated the overall acquisition and loss of pathogens between the 1-month and the 2-month visits. In order to eliminate the potential influence of different feeding methods at each visit, we limited this analysis to the 112 infants who had no change in feeding method between visits. Of 92 such infants from whom no pathogen was isolated at the 1-month visit, no pathogen was isolated at the 2-month visit from 68 infants (73.9%). Of the remaining 24 such infants from whom one or more pathogens were isolated at the 1-month visit, one or more pathogens were isolated from 15 infants (75.0%) at the 2-month visit.

TABLE 3. Proportions of subjects from whom specific pathogens were isolated from the nasopharynx by feeding method and age

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Breast milk only</th>
<th>Breast milk mainly</th>
<th>Breast milk partially</th>
<th>Formula only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mo (n = 84)</td>
<td>2 mo (n = 46)</td>
<td>1 mo (n = 51)</td>
<td>2 mo (n = 42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 mo (n = 28)d</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 mo (n = 76)</td>
<td>2 mo (n = 57)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 mo (n = 211)</td>
<td>2 mo (n = 173)</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>3.6</td>
<td>8.7</td>
<td>5.9</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>H. influenzae</td>
<td>1.2</td>
<td>6.5</td>
<td>5.9</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>7.1</td>
<td>26.1</td>
<td>9.8</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

* See text for definitions of feeding methods.

Semiquantitative growth of pathogens. We compared semiquantitative growth of organisms from the subjects from whom a pathogen was isolated from the nasopharynx. When more than one pathogen was present, we counted the isolate with the heaviest growth. At the 1-month visit, the proportions of such subjects whose pathogens had 3+ or 4+ growth were 6 of 9 in the breast milk-only group and 10 of 14 in the formula-only group. At the 2-month visit, the corresponding proportions were 11 of 16 and 16 of 20.

Specific nasopharyngeal bacteria. Table 3 displays the proportions of subjects from whom specific pathogens were isolated from the nasopharynx by feeding method and age. For each pathogen, the proportions of subjects with positive cultures increased during the interval from 1 month to 2 months of age. At 1 month of age, the carriage rates of all pathogens were low, as follows: S. pneumoniae, 3.8%; H. influenzae, 3.3%; M. catarrhalis, 10.4%; and S. pyogenes, 0.5%. At 2 months of age, the carriage rates of all pathogens increased, as follows: S. pneumoniae, 9.2%; H. influenzae, 11.0%; M. catarrhalis, 22.0%; and S. pyogenes, 1.2%. Except for the breast milk-partially group at 2 months of age, M. catarrhalis was the pathogen that was most frequently isolated from infants in all other feeding groups at both visits. Our recovery of pathogens was greater in the winter and spring.

Selective medium for M. catarrhalis. Use of the selective medium increased the overall recovery of M. catarrhalis. Of 60 M. catarrhalis isolates in pure or mixed culture at both time points, 8 grew only on the selective medium, 4 grew only on the standard media, and 48 grew on both types of media. In addition, the laboratory technologists reported that the organism was easier to identify when the selective medium was used because colonies were usually isolated, and therefore, subculturing was required less frequently.

Presumed nonpathogens. We determined the proportions of subjects from whom presumed nonpathogens were isolated from the nasopharynx by feeding method and age. A high rate of recovery of coagulase-positive staphylococci was noted, particularly in the breast milk-only group at both 1 month (42.9%) and 2 months (50.0%) of age. The proportions were 23.5 and 23.8%, respectively, in the breast milk-mainly group and 34.2 and 28.1%, respectively, in the formula-only group. The rate of recovery of gram-negative rods was highest at 1 month of age in the formula-only group (22.4%) and at 2 months of age in the breast milk-mainly group (16.7%). Other bacterial flora (e.g., coagulase-negative staphylococci and non-group A streptococci) were recovered frequently in all feeding groups at both 1 month of age (90.5%) and 2 months of age (91.3%).
DISCUSSION

It has been postulated that, in many children, eustachian tube dysfunction plays a major role in the pathogenesis of otitis media. It is also thought that common bacterial respiratory pathogens may then be aspirated, insufflated, or refluxed from the nasopharynx into the middle ear, resulting in infection (6). In the present study we examined one possible mechanism whereby breast-feeding might provide protection against otitis media by altering the nasopharyngeal flora.

Even though the peak incidence of otitis media is known to occur later, we chose to enroll infants at 1 month of age because the prevalence of exclusive breast-feeding is highest in infants in this age group (compared with that in older infants and children) and because nasopharyngeal colonization occurs early in infancy. In part, for these reasons, we limited our follow-up period to the first 2 months after birth. Furthermore, prevention of an early first episode of otitis media may be part of the protection offered by breast-feeding. Early episodes may set the stage for frequent and persistent recurrences of otitis media.

Most of our subjects were white, and almost all of the families had private health care coverage. The feeding groups were comparable regarding variables that might affect the prevalence of nasopharyngeal pathogens. When we compared the breast milk-only and the formula-only groups, we found no statistically significant differences in the proportions of subjects from whom at least one bacterial respiratory pathogen was isolated from the nasopharynx at both 1 month and 2 months of age. Our findings and conclusions may be limited to populations that are similar to the one that we studied.

In the breast milk-only infants that we studied at 2 months of age, the prevalences of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in the nasopharynx were 8.7, 6.5, and 26.1%, respectively. In a cross-sectional study, Aniasson and colleagues (1) found corresponding culture-positive rates of 11, 2, and 18%, respectively, in infants aged 6 weeks to 3 months. Most (84%) of their 57 infants were exclusively breast-fed. The authors reported a significantly lower frequency of *S. pneumoniae* and *H. influenzae* in the exclusively breast-fed group compared with that in the “mixed-feeding” group. However, the number of infants in the mixed-feeding category in that pilot study was small. In an abstract published later (2), Aniasson and coworkers found “no overall difference in nasopharyngeal carriage between breast-fed and non-breast-fed children.”

In the present study, *M. catarrhalis* was the most frequently isolated pathogen. A selective medium was used, which did contribute in part to the increased identification rate of this organism. In a group of children with acute otitis media, Kovatch and colleagues (16) found that *M. catarrhalis* occurred at a younger age, relative to the age of occurrence of *S. pneumoniae* and *H. influenzae*.

We found that 50.0% of the 2-month-old infants in the breast milk-only group had positive cultures for coagulase-positive staphylococci. These data are similar to those of Aniasson and colleagues (1), who reported that 54% of cultures of specimens from infants aged 6 weeks to 3 months were positive for *Staphylococcus aureus*.

In a longitudinal study of oropharyngeal colonization with gram-negative rods in the first 6 months after birth, Baltimore and colleagues (4) found, during the period from 1 to 3 weeks of age, a prevalence of 29% in both the breast-fed and the formula-fed groups. During the period from >3 weeks to 2.5 months of age, the prevalence of such bacteria increased but was lower in the breast-fed group (40%) than in the non-breast-fed group (64%). These prevalences of gram-negative rods were higher than those that we found, particularly at 2 months of age. Baltimore and coworkers (4) used a different definition of breast-feeding (one or more breast-feedings per day at the time of specimen collection) than we used. In addition, the investigators sampled the oropharynx rather than the nasopharynx.

There are several possible explanations for our study findings. Howie and colleagues (13) reported that infants who were breast-fed for 13 weeks or longer had significantly reduced rates of gastrointestinal illness when compared with bottle-fed infants, whereas infants who were breast-fed for a shorter period of time had rates of illness that were similar to those in the bottle-fed group. Therefore, it is conceivable that breast-feeding for longer than 2 months could alter the nasopharyngeal flora in those in an older age group. Another possible explanation for our findings is that an alternative mechanism of action of breast milk exists, e.g., protection against viral pathogens with an associated decrease in the incidence and severity of the mucosal inflammation that leads to eustachian tube dysfunction.

We conclude that during the first 2 months after birth, the exclusive receipt of breast milk appears not to substantially influence the prevalence of nasopharyngeal colonization with common bacterial respiratory pathogens.

ACKNOWLEDGMENTS

We are grateful to the Children’s Hospital of Pittsburgh Microbiology Laboratory staff and to the following individuals: Lee W. Bass, Jane M. Breck, Lorraine M. Chesek, Paul F. Dubner, Cindy L. Feidt, Ruth G. Gee, David C. Kazimer, Marcia Kurs-Lasky, Lillian M. Martin, Howard A. Mermelstein (posthumously), Bernard I. Michaels, H. Richard Paul, Dedra A. Stevenson, Robert M. Wadowsky, Diane C. Weidner, and Jerome H. Wolfson. We express our particular appreciation to Karen A. Boltey and Brian T. Clista for assistance in specimen collection and to Ellen R. Wald for support of the project and for helpful suggestions regarding the manuscript.

We also acknowledge funding from the Biomedical Research Support Grant Program (BRS grant SO7 RR050726 from the Division of Research Resources, National Institutes of Health) and the New Horizons Research Fund, awarded by the Research Advisory Committee, Children’s Hospital of Pittsburgh.

REFERENCES