Relationship between Nasopharyngeal Colonization and the Development of Otitis Media in Children

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Streptococcus pneumoniae, **nontypeable *Haemophilus influenzae,* and *Moraxella catarrhalis* are the predominant bacteria associated with otitis media. A cohort of 306 infants was followed from birth through 12 months to determine frequency and duration of colonization and risk of acute otitis media (AOM) and otitis media with effusion (OME). *M. catarrhalis* was the most common bacterium isolated. Infants colonized at ⩽3 months of age were at increased risk of AOM and OME. Early colonization with *M. catarrhalis* revealed the greatest risk (relative risk [RR] = 1.24), especially for OME (RR = 1.57). There was a strong relationship between the frequency of colonization and OM (r = .37, $P < .001$,) for each pathogen. Although *S. pneumoniae,* nontypeable *H. influenzae,* and *M. catarrhalis* are part of the normal nasopharyngeal flora during infancy, an increased rate of colonization may identify a subpopulation of children that is at increased risk of OM.**

Otitis media (OM) is the most common disease diagnosed among children [1]. The incidence of OM is greatest in the first 2 years of life [1–6]. *Streptococcus pneumoniae,* nontypeable *Haemophilus influenzae,* and *Moraxella catarrhalis* are major pathogens in OM and cause ~75% of the infections [7]. The development of OM begins in the nasopharynx. While the nasopharynx is normally colonized with avirulent bacteria, such as viridans streptococci, nonhemolytic streptococci, diphtheroids, and *Neisseria* species, potential middle ear pathogens may also be carried asymptomatically. The rates of colonization with *S. pneumoniae,* nontypeable *H. influenzae,* and *M. catarrhalis* vary widely in different reports [8–10]. Most studies agree that bacteria rapidly colonize the nasopharynx soon after birth and that the composition and proportional distribution of the bacteria are relatively consistent among healthy persons [8–10]. During periods of upper respiratory illness, and during episodes of OM in particular, colonization with middle ear pathogens increases significantly [9, 11]. This increase is especially prominent among children who are prone to recurrent episodes of OM [9, 12, 13].

The present study was designed to monitor nasopharyngeal flora in children from birth through 12 months of age and to relate colonization with frequency of OM. A total of 306 children were enrolled and prospectively followed, with nasopharyngeal cultures and ear examinations completed as part of scheduled visits; patterns of colonization with each of the major middle ear pathogens were characterized.

Methods

Study population. Eligible infants (n = 306) were consecutively enrolled at two large group practices in the suburban Buffalo area. Infants with craniofacial abnormalities, genetic disorders, and immune deficiencies were excluded from the study. Information gathered at entry included history of recurrent otitis media, allergies, and smoking history of parents. In addition, information regarding the number, age, history of recurrent middle ear disease, and day care attendance of siblings was obtained. Study infants were examined at regularly scheduled monthly visits (1–6 months) and at 8, 10, and 12 months of age. Data included mode of feeding, interim medical history (recent illnesses and antibiotic therapy), and middle ear examinations in study patients.

Physicians and trained nurse interviewers used standard criteria in evaluating clinical symptoms as well as in determining resolution or development of new episodes of OM. Ears were examined with pneumatic otoscopy and tympanometry by the same group of examiners for the duration of the study. Pneumatic otoscopy was used in every examination to determine tympanic membrane mobility; tympanometry was included for children over the age of 6 months. Given that physicians were not blinded to the nasopharyngeal culture results for ethical reasons, pneumatic otoscopy and tympanometry had to be in agreement to establish the presence or absence of a middle ear effusion.

Acute otitis media (AOM) episodes were defined by presence of fever, irritability, pulling at the ears, and tympanic membrane changes, including increased thickness, bulging, loss of landmarks, decreased mobility, and a flattened tympanogram, type b curve [12, 13]. De novo otitis media with effusion (OME) was diagnosed when fluid was visible in the middle ear in the absence of symptoms and was unrelated to a recent episode of AOM. The tympanic membrane was typically thin, exhibited decreased mobility, and produced a flattened tympanogram [12, 13]. Children were exam-

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Consent in accordance with Institutional Review Board Human Subject Guidelines was obtained from all parents or guardians at enrollment of infants into the study.

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ined monthly after the diagnosis of OM until resolution of middle ear disease was observed.

Nasopharyngeal cultures. Nasopharyngeal cultures were obtained with a small rayon swab. Swabs were transported to the laboratory in transport media, and specimens were cultured on trypticase soy agar with 5% sheep blood, chocolate agar, and MacConkey agar within 8 h of collection. Bacterial species were identified by standard laboratory procedures. Briefly, *S. pneumoniae* was identified by colonial morphology, Gram's-stained smear, optochin disk susceptibility, and bile solubility. Nontypeable *H. influenzae* was identified by exclusive growth on chocolate agar, Gram's-stained smear, requirement for X (hemin) and V (nicotinamide adenine dinucleotide) factors, and failure to group with standard haemophilus typing sera. *M. catarrhalis* was identified by colonial morphology, Gram's-stained smear, and biochemical reactions, including butyrate esterase.

Statistical methods. Descriptive summary statistics included percentages for categorical data and means ± standard deviations for interval data. Cumulative incidence of OM was calculated separately for AOM and OME. Colonization rates of specific pathogens were correlated with disease episodes. A life table analysis was performed and graphically summarized as time free of first episodes of OM (during the first 6 months) by age at pathogen colonization. Relative risks (RRs) and their respective 95% confidence intervals (CIs) were also calculated for each disease type by age at first colonization (≤3 months; >3 months). Risk estimates were calculated for cultures with 1 or more pathogens and for specific organisms, separately. Multivariate logistic regression analyses compared the relative importance of selected factors on risk of OM disease during the first year of life [14].

Results

The high compliance rate of the study cohort was notable. A high percentage (89%) of enrolled infants completed evaluation from birth to 12 months of age. Patients who failed to complete follow-up were similar to the compliant participants in most respects (demographic; clinical). The major reason for dropping out was moving to a new residential area.

Since the majority of nasopharyngeal cultures were collected during the first year of intensive follow-up, the study results presented here are limited to colonization patterns and early episodes of disease during the first 12 months of life. Shown in table 1, the vast majority of infants at the two practice sites were white. About 36% of the infants were in day care during the first year of life. Demographic and clinical characteristics have been more fully described elsewhere [12, 13].

Initial colonization by 1 or more of the 3 potential bacterial pathogens studied occurred as early as 1 month of age (mean ± SD = 4.3 ± 2.8 months). Colonization by *M. catarrhalis* occurred slightly earlier (4.8 ± 3.1 months) than colonization by *S. pneumoniae* (5.7 ± 2.9 months) or nontypeable *H. influenzae* (6.6 ± 3.2 months), but the differences were not statistically significant.

Cumulative acquisition rates with pathogens increased most rapidly in the first 6 months of life (figure 1). By 6 months, 68% of children still being followed (194/287) had been colonized with 1 or more of the 3 major pathogens. Colonization rates were highest for *M. catarrhalis* at 55%, followed by *S. pneumoniae* at 38% and nontypeable *H. influenzae* at 19%. By 1 year of age, the rates were 72%, 54%, and 33%, respectively.

Forty-two percent of children were colonized with a pathogen at 6 months; this prevalence rate persisted throughout the first year (not shown). The highest prevalence rates occurred at 6 months with *M. catarrhalis* at 26% and *S. pneumoniae* at 24%, followed by nontypeable *H. influenzae* at 9%.

We next examined the relationship between nasopharyngeal colonization and the development of OM. The number of episodes of OM was directly related to the frequency of colonization with middle ear pathogens ($r = .37$, $P < .001$) (figure 2). The relationship was higher for AOM than for OME (table 2). Among the specific pathogens, the association was stronger for *M. catarrhalis* than for the others.

Among colonized infants ($n = 194$ at 6 months), when age at first colonization was estimated (≤3 months; >3 months), life table survival curves for first episodes of AOM and OME are presented in figure 3. For infants colonized at ≤3 months, the survival curve (time free of disease) dropped at a significantly faster rate than did that of infants colonized at >3 months of age. Specifically, the risk of having a first episode of AOM by 6 months among subjects colonized at ≤3 months was nearly 2-fold higher (RR = 1.8, 95% CI = 1.07–2.67) than for infants >3 months of age at time of colonization. Infants with early colonization (≤3 months) were at even greater risk of first OME episode by 6 months (RR = 2.66, 95% CI = 1.12–3.45) compared with infants colonized at >3 months.

Table 1. Demographic and clinical characteristics of infant cohort in study of nasopharyngeal colonization and OM.

Characteristic	No.	%
Sex		
Male	160	52.3
Female	146	47.7
Race		
White	302	98.7
African-American (and other minorities)	4	1.3
History of recurrent OM (>3 episodes)		
Maternal	93	30.5
Paternal	58	19.2
Siblings*	110	57.6
Current parental smoking		
Maternal	36	11.8
Paternal	54	17.8
Day care (public or private)		
Sibling(s)†	68	35.4
Infant	110	35.9

* % of infants for sibling history of recurrent OM is based on no. of infants with siblings with available data ($n = 191$).

† % of infants with siblings in day care is based on no. of infants with siblings with available data ($n = 192$).

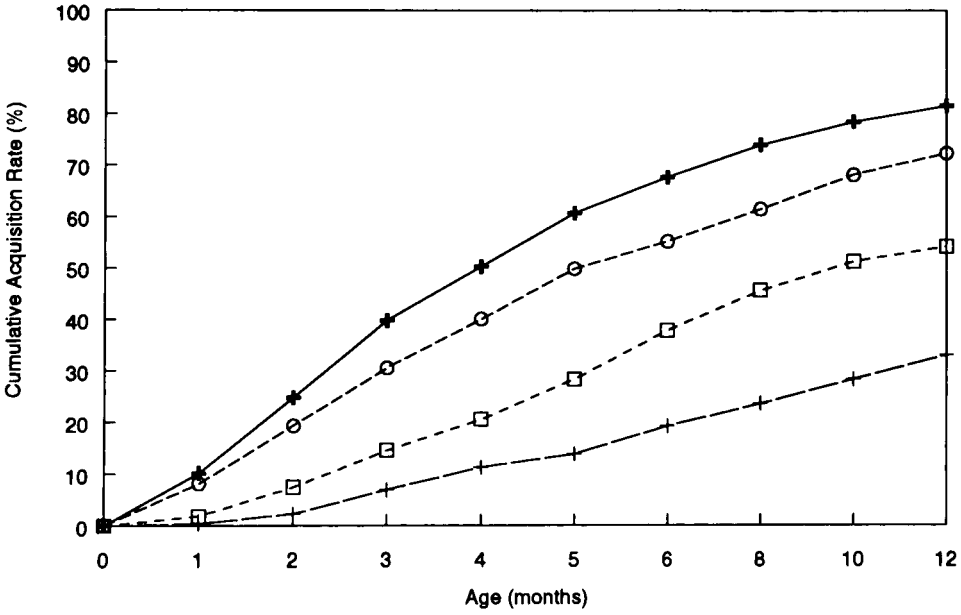

Figure 1. Cumulative acquisition rates of pathogens during first year. Top line: any pathogen; mean age ± SD, 4.3 ± 2.8 months; ○, *M. catarrhalis*; mean age, 4.8 ± 3.1 months; □, *S. pneumoniae*; mean age, 5.7 ± 2.9 months; +, nontypeable *H. influenzae*; mean age, 6.6 ± 3.2 months.

Colonization patterns were next examined relative to age. Summarized in table 3, infants first colonized at ⩽3 months of age [2] demonstrated an increased risk of first episode of OM (RR = 1.24, 95% CI = 1.06–1.44) compared with older infants. Earlier age of colonization (⩽3 months) had a higher risk of first OME (RR = 1.66, 95% CI = 1.17–2.37) than of first AOM (RR = 1.28, 95% CI = 1.03–1.60). Similarly, early colonization by *M. catarrhalis* revealed the greatest elevation in risk of first OM (RR = 1.24, 95% CI = 1.08–1.43) and was more evident for OME episodes (RR = 1.57, 95% CI = 1.14–2.17) than for AOM (RR = 1.34, 95% CI, 1.08–1.65). Risk of recurrent episodes of OM disease among colonized children experiencing at least one OM episode in year 1 (not shown) revealed a nearly 40% elevated risk in infants colonized with pathogenic bacteria at ⩽3 months of age (RR = 1.38, 95% CI = 1.07–1.77) compared with that in older infants. Notably, early colonization with *M. catarrhalis* (RR = 1.28, 95% CI = 1.00–1.64) and nontypeable *H. influenzae* (RR = 1.47, 95% CI = 1.12–1.93) increased the risk of recurrent OM. However, no increase in risk was noted for recurrent episodes of OME or AOM when analyzed separately.

To clarify the relationship between time of culture and diagnosis of first OM episode, risk associations were also examined with and without a lag effect. For the lag analysis, the child's first OM episode was defined, and pathogens identified in the same month or previous month's culture results were included.

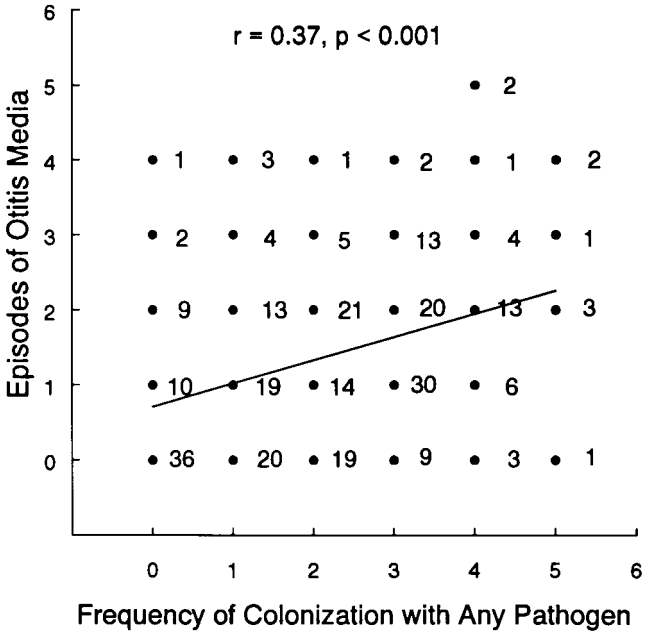

Figure 2. Relationship between frequency of colonization with any pathogen and number of episodes of otitis media. All children completing at least 6-month visit, $n = 287$.

Table 2. Correlation (r) between frequency of pathogen colonization and episodes of OM.

Pathogen colonization	OM r (P)	OME r (P)	AOM r (P)
S. pneumoniae	0.28 (<.001)	0.17 (.004)	0.22 (<.001)
Nontypeable *H. influenzae*	0.29 (<.001)	0.24 (<.001)	0.17 (.003)
M. catarrhalis	0.32 (<.001)	0.21 (<.001)	0.24 (<.001)
Any pathogen	0.37 (<.001)	0.20 (.001)	0.31 (<.001)

NOTE. All P values represent two-tailed level of significance. $n = 287$, all children completing at least 6th-month visit.

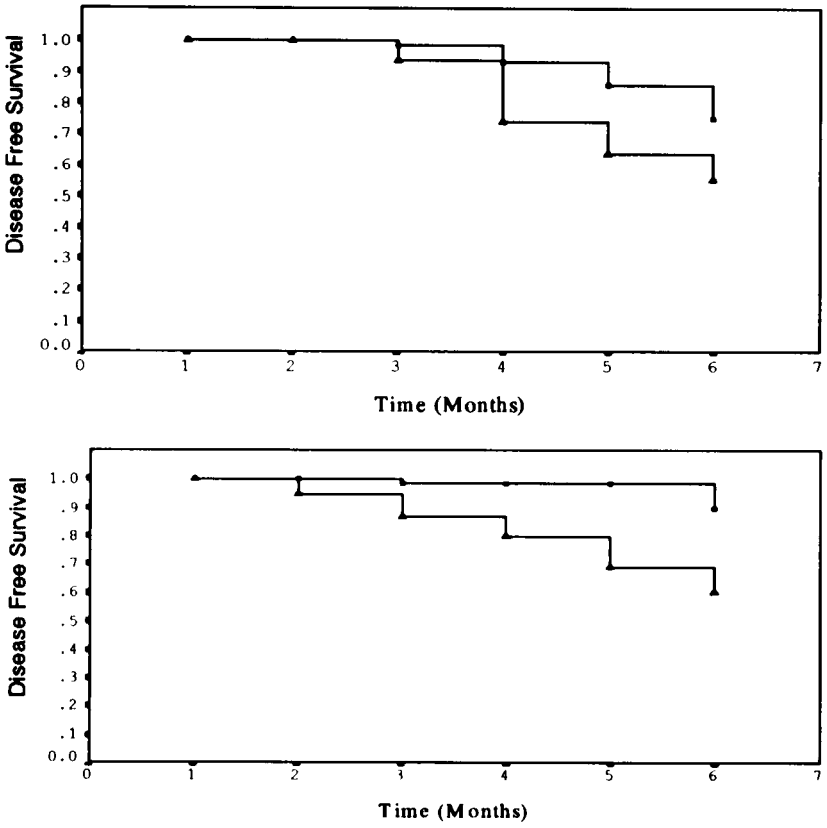

Figure 3. Top, Onset of first acute otitis media (children whose first episode was otitis media with effusion [n = 45] were excluded from analysis). ■, >3 months (n = 71); ▲, ⩽3 months (n = 78). Bottom, Onset of first otitis media with effusion (children whose first episode was acute otitis media (n = 60) were excluded from analysis). ■, >3 months (n = 59); ▲, ⩽3 months (n = 75). Disease-free survival refers to fraction of children free of any episodes of otitis media.

Infants having no OM episodes in the first 12 months were used as the reference and were dichotomized by whether or not any pathogens were identified. First episodes of OM were only marginally elevated in infants colonized by pathogenic bacteria compared with noncolonized children when only colonization during the current month was considered (RR = 1.09, 95% CI = 0.92–1.30) (not shown). However, when the lag effect of the carriage of a pathogen in the previous month was included in the estimate, a substantial increase in the risk of first episodes was observed (RR = 1.32, 95% CI = 1.08–1.60). A nearly 50% increase in OME-specific risk (RR = 1.51, 95% CI = 1.00–2.29) and AOM-specific risk (RR = 1.44, 95% CI = 1.09–1.91) was apparent when the lag effect of the previous month was considered in addition to pathogen carriage in the current month. Duration and recurrence of OM episodes, however, were not shown to be substantially different whether or not the lag effect of carriage in the previous month was considered in the calculation. This finding needs to be interpreted cautiously, because of the small number of infants with prolonged or recurrent episodes of OM in this infant cohort.

Table 3. Effect of age at pathogen colonization (⩽3, >3 months) and risk of first episode of OM during first 12 months.

Pathogen colonization*	OM†	OME‡	AOM‡
S. pneumoniae	0.97 (0.82–1.15)	0.92 (0.61–1.38)	0.97 (0.75–1.25)
Nontypeable *H. influenzae*	1.05§ (0.83–1.31)	1.24§ (0.75–2.07)	1.01§ (0.70–1.46)
M. catarrhalis	**1.24 (1.08–1.43)**	**1.57 (1.14–2.17)**	**1.34 (1.08–1.65)**
Any pathogen	**1.24 (1.06–1.44)**	**1.66 (1.17–2.37)**	**1.28 (1.03–1.60)**

NOTE. Children not colonized during first year were excluded from analysis. Bolded values indicate statistically significant results. Data are relative risk (95% confidence interval).

* Pathogen colonization was dichotomously compared, ⩽3 and >3 months.

† 1st episode of either OME or AOM versus reference group of no OM of any type.

‡ 1st episode versus reference group of no OM of any type.

§ 25% or 1 of cell's expected frequency is <5.

Entry into day care, smoking history of parents, and breast-feeding are important covariables that may influence colonization patterns and risk of developing first episodes of OM disease in this cohort. Table 4 summarizes the relative importance of age at colonization in the presence of selected covariables. The significance of early colonization (⩽3 months) on OM episodes during the first year remained an important determinant of disease in the presence of the independent risk factors considered in the multivariate analysis (β =.86, 95% CI = 0.06–1.66).

Worth noting, exclusive breast-feeding or any breast-feeding for 6 months did not substantially diminish the importance of early colonization with pathogens on first OM episodes occurring in the first 12 months. Additionally, infant attendance

Table 4. Multivariate logistic regression of risk factors and first episodes of OM during first 12 months.

Factor	Regression coefficient ± SE	95% Confidence interval
Intercept	−0.06 ± 0.39	−0.82, 0.71
Sex	0.37 ± 0.40	−0.42, 1.15
Age at first pathogen colonization*	**0.79 ± 0.40**	**0.01, 1.58**
Day care	**0.87 ± 0.44**	**0.00, 1.73**
Maternal smoking	1.31 ± 0.80	−0.26, 2.88
Feeding†	0.25 ± 0.40	−0.54, 1.05
Intercept	−0.35 ± 0.48	−1.29, 0.59
Sex	0.30 ± 0.46	−0.61, 1.21
Age at first pathogen colonization*	0.64 ± 0.47	−0.29, 1.57
Day care	**1.04 ± 0.53**	**0.00, 2.08**
Maternal smoking	1.78 ± 1.07	−0.35, 3.90
Feeding‡	0.77 ± 0.47	−0.17, 1.71
Intercept	−0.22 ± 0.38	−0.96, 0.52
Sex	0.43 ± 0.39	−0.34, 1.21
Age at first pathogen colonization*	**0.86 ± 0.40**	**0.06, 1.66**
Day care	0.60 ± 0.42	−0.23, 1.43
Maternal smoking	1.84 ± 1.06	−0.26, 3.95
Feeding§	0.61 ± 0.40	−0.17, 1.39

NOTE. Values in bold indicate significant results.
* ⩽3, >3 months.
† Exclusive breast-feeding duration of 3 months.
‡ Exclusive breast-feeding duration of 6 months.
§ Combined breast and formula feeding at 6 months compared with full formula feeding at 6 months.

at day care appeared to be an important determinant of first episodes of OM occurring during the first year of life.

Discussion

The bacterial flora of the nasopharynx is in a constant state of flux. Bacteria are acquired, eliminated, and reacquired many times over the life of a person [12, 13]. Factors controlling the trafficking of bacteria into and out of the nasopharynx are poorly understood. The present study details the colonization patterns of a cohort of infants followed prospectively from birth through 1 year of age. The results demonstrate that *M. catarrhalis* was the first respiratory pathogen to colonize infants in this cohort and the most prevalent pathogen throughout infancy. *S. pneumoniae* was next in frequency, followed by nontypeable *H. influenzae,* which appeared about one-half as often as *M. catarrhalis.*

Many factors may affect colonization rates. For example, season, siblings, day care, and respiratory illness are all known to increase colonization with middle ear pathogens [8, 9, 11, 15, 16]. Recent data from our laboratory suggest that the mucosal immune response plays a role in eliminating and possibly preventing colonization with pathogens [12, 17]. Regardless of circumstances leading to colonization, data from the present study demonstrate a direct relationship between frequency of colonization and episodes of OM. Furthermore, early colonization may be a risk factor for first infection and recurrent disease as well. Three months of age was selected as the period to be examined, since colonization with pathogens prior to this age is relatively uncommon in the general population [9].

Results from previous studies that detected a direct relationship between early first infection and persistent or recurrent disease may have actually reflected the effects of colonization on OM [18–22]. It is unclear whether acquisition of a new pathogen was associated with the development of otitis media in the present report, as suggested previously [21]. The present study provides further evidence to support the notion that early intervention to reduce colonization may interrupt the cycle of recurrent OM episodes. Hypothetically, prevention of early and recurrent colonization would substantially reduce development of OM. Modes of intervention could include antibiotic administration to eradicate or prevent colonization [23], active colonization of the nasopharynx with a probiotic or protective bacterium (such as viridans streptococci [24]), immunization with middle ear pathogens (preferably by the mucosal route [25, 26]), or procedures yet to be defined.

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