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Original Article

Longitudinal investigation of pathogenic bacterial colonization in early childhood: Emphasis on the determinants of *Moraxella catarrhalis* colonization

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KEYWORDS
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Abstract *Background: Moraxella catarrhalis* is a common, potential pathogen colonizing the respiratory tract in children. However, there is little information regarding the determinants of *M. catarrhalis* colonization and disease development.
Methods: A population-based cohort study was conducted to collect nasopharyngeal swabs from children aged 1, 2, 4, 6, 12, 18, 24, 36, and 60 months for the detection of four common respiratory tract pathogens, including *Staphylococcus aureus*, *M. catarrhalis*, *Streptococcus pneumoniae*, *and Haemophilus influenzae*. Questionnaires on breastfeeding status were administered during each visit.
Results: A total of 921 children were enrolled between 2012 and 2018. *S. aureus* was the most common pathogen, although the rates declined during the initial 18 months of life; in contrast, the other three pathogens increased during the first 5 years of life. *M. catarrhalis* was the second most common colonizing pathogen in all age groups, with prevalence ranging from 0.8% (7/842) at one month to 20.4% (33/162) at 60 months of age. Breastfed children (odds ratio [OR]:

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0.56; 95% confidence interval [CI]: 0.35–0.92; $P = 0.02$) had a lower potential for *M. catarrhalis* carriage; however, infants with a longer duration of exclusive breastfeeding (OR: 1.12; 95% CI: 1.01–1.25; $P = 0.04$), especially >12 months of age, had a higher rate of *M. catarrhalis* carriage.

Conclusion: Breastfeeding should be promoted because it may be correlated with a lower risk of *M. catarrhalis* carriage. However, an extended period of exclusive breastfeeding may be positively associated with *M. catarrhalis* colonization.

Introduction

Moraxella catarrhalis is an important pathogen in respiratory infections, including otitis media, acute bacterial rhinosinusitis, and pneumonia in children.[1] It is the third most common bacterial agent in pediatric respiratory tract infectious diseases, and is surpassed only by *Streptococcus pneumoniae* and *Haemophilus influenzae*.[1,2] A prospective cohort study from 2006 to 2016 revealed a change in the respiratory tract microbiome after the widespread use of pneumococcal vaccinations, which showed that the prevalence of *M. catarrhalis* infection increased during the past decade.[3]

M. catarrhalis is a member of the normal bacterial microflora commonly colonizing the nasopharynx of healthy children and *M. catarrhalis* colonization is thought to be a precursor to the development of respiratory infectious diseases.[4] The risk factors for *M. catarrhalis* colonization have previously been studied, and include sex, socioeconomic status, contact with children, and vaccination[5–8]; However, little is known about the dynamic changes and associated determinants between *M. catarrhalis* and other respiratory pathogens during early childhood, particularly during the era of the conditional use of the pneumococcal vaccine in Taiwan.

Therefore, we conducted a longitudinal birth cohort study to improve our understanding of *M. catarrhalis* colonization, including possible factors for colonization, the dynamics between *M. catarrhalis* and other pathogens, and infectious disease development in children during the conditional pneumococcal vaccination period in Taiwan.

Methods

Study population

This study was embedded in the Prediction of Allergen in Taiwanese Children (PATCH) study, a prospective population-based birth cohort study launched in 2012 to investigate bacterial colonization and factors related to the development of asthma and other allergic diseases. Women in their third trimester of pregnancy who were cared for at the Obstetrics Clinic of Keelung Chang Gung Memorial Hospital were invited to participate in the study. Infants were excluded from this study if they had severe congenital abnormalities, low birth weight (<2000 g), or required mechanical ventilation at any time after birth. This study was approved by the ethics committee of Keelung Chang Gung Memorial Hospital, and written informed consent was obtained from the parents of the infants.

Bacterial isolates and clinical parameter collection

Mothers were asked to bring the enrolled participants at 1, 2, 4, 6, 12, 18, 24, 36, and 60 months of age to the Pediatric Clinic of Keelung Chang Gung Memorial Hospital for the collection of nasopharyngeal swabs and detection of pathogens, including *S. pneumoniae*, *Staphylococcus aureus*, *H. influenzae,* and *M. catarrhalis*. We chose these four bacteria for this study because they are important commensals or potential pathogens of the nasopharynx causing respiratory diseases.[9,10] Well-trained pediatricians collected nasopharyngeal swab samples for pathogen detection at each scheduled visit.

Questionnaire surveys were conducted at each scheduled visit to collect demographic data, breastfeeding conditions, living and housing conditions, socioeconomic status, history of respiratory tract infection, risk factors for colonization, and other clinical parameters. Upper respiratory tract infection (URI) included pharyngitis, croup, acute otitis media, or acute sinusitis, while lower respiratory tract infection (LRI) included bronchopneumonia, pneumonia, bronchitis, or bronchiolitis. Respiratory tract infections were recorded by parents if their children had been diagnosed with the aforementioned illnesses by physicians. We also encouraged the parents to bring their children to our clinic instead of the family doctors if any respiratory diseases occurred, and the diagnosis was made according to the definition of respiratory tract infections proposed by the pediatricians in our clinic. The breastfeeding types were defined as either exclusive or partial. Infants who were fed breast milk only without additional foods or drinks except water were considered exclusively breastfed, while those fed breast milk, formula, and other supplemental foods were considered partially breastfed.[11] To analyze the influence of exclusive breastfeeding duration on *M. catarrhalis* carriage, we further divided participants into several subgroups according to the length of exclusive breastfeeding periods: none, < 6 months, 6–11.9 months, and ≥12 months.

A cotton-tipped swab (Copan Swab Applicator; Copan Diagnostics Inc., Brescia, Italy) was used in our study, and nasopharyngeal swab collection was performed through the nose into the nasopharyngeal spaces to collect samples. After collection, the swabs were placed into transport medium for storage and transported to the microbiology laboratory within 2 h for bacterial culture using standard methods for identification.[12]

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) (version 22.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. Student's t-test was used to analyze the numerical data. If the data were not normally distributed, the Mann–Whitney U test was used for the analysis. The chi-square or Fisher's exact test, when appropriate, was used to analyze categorical data. To investigate factors associated with *M. catarrhalis* carriage by univariate analysis, each colonized participant was frequency-matched with non-colonized participants on variables of the year of study, season, age, and sex. Variables for which $P < 0.1$ were chosen for model selection in multivariate analysis. Statistical significance was set at $P < 0.05$.

Results

Distribution of bacterial colonization of four different pathogens at different time points during the first 5 years of life

From March 2012 to December 2018, we recruited 966 neonates, of whom 45 were excluded because of low birth weight (<2000 g, n = 40), ventilator use after birth (n = 4), and cerebral palsy (n = 1). Hence, 921 participants were enrolled, with the numbers of collected samples in different age groups being 841 (1 month), 357 (2 months), 358 (4 months), 766 (6 months), 708 (12 months), 503 (18 months), 498 (24 months), 405 (36 months), and 139 (60 months). Fig. 1 shows the detailed enrolment of this cohort. Nasopharyngeal cultures were used for the detection of *S. pneumoniae*, *S. aureus*, *H. influenzae,* and *M. catarrhalis*. Table 1 shows the bacterial colonization of the four different pathogens at the scheduled sampling time points during the first 60 months of age.

S. aureus was the most prevalent pathogen in all age groups, and the second was *M. catarrhalis*, followed by *S. pneumoniae* and *H. influenzae*. For the *S. aureus* colonization, the proportion of methicillin-resistant *S. aureus* (MRSA) carriers to the total *S. aureus* carriers was 67.2% (297/442) at 1 month, 62.8% (123/196) at 2 months, 68.9% (82/119) at 4 months, 62.7% (96/153) at 6 months, 58.2% (46/79) at 12 months, 67.3% (33/49) at 18 months, 59.7% (43/72) at 24 months, 51.7% (45/87) at 36 months, and 55.1% (27/49) at 60 months of age, respectively. The factors associated with MRSA and methicillin-sensitive *S. aureus* (MSSA) in participants at the age of 6 months were compared (Supplementary Table S1). Of the participants with *S. aureus* colonization, those delivered via normal spontaneous delivery were associated with an increased incidence of MRSA carriage (63.6% vs. 40.0%; odds ratio [OR] 2.76; 95% confidence interval [CI] 1.31–5.84; $P < 0.01$). In addition, pediatric intensive care unit admission at birth was negatively correlated with infants with MRSA carriage (OR 0.35; 95% CI 0.13–0.91; $P = 0.03$).

M. catarrhalis colonization was found in 0.8% (7/842) of participants aged 1 month, followed by 1.9% (7/363) at 2 months, 3.0% (11/363) at 4 months, 6.0% (47/790) at 6 months, 6.2% (45/722) at 12 months, 7.1% (36/509) at 18 months, 13.2% (67/509) at 24 months, 15.4% (66/428) at 36 months, and 20.4% (33/162) at 60 months. Fig. 2 shows the trend of *M. catarrhalis* carriage at the scheduled sampling time points during the first 60 months of life.

Demographics and factors associated with *M. catarrhalis* colonization among the study population

We investigated the factors associated with *M. catarrhalis* colonization using a questionnaire-based survey at each planned visit. For the selection of non-*M. catarrhalis* carriers as the control group, each of the 319 *M. catarrhalis* carriers was frequency-matched with the non-carriers on variables including the year of study, season, age, and sex. Finally, 302 carriers and non-carriers were chosen for the investigation. The results are presented in Table 2. Three factors, namely, demographic characteristics, environmental features, and health conditions, were compared between *M. catarrhalis* carriers and non-carriers. There were no significant differences in the proportion of URI (51.7% vs. 36.0%; $P = 0.65$), LRI (22.6% vs. 23.9%; $P = 0.72$), hospitalization due to LRI (9.0% vs. 2.9%; $P = 0.15$), and antibiotic use (7.5% vs. 7.2%; $P = 0.90$) within 2 weeks between carriers and non-carriers. Breastfeeding since birth was negatively associated with *M. catarrhalis* carriage (84.4% vs. 90.1%; OR, 0.56; 95% CI 0.35–0.92; $P = 0.02$). However, *M. catarrhalis* colonization was associated with longer breastfeeding duration (median: 6 m vs. 4 m; $P = 0.03$), longer exclusive breastfeeding duration (median: 2 m vs. 1 m; $P < 0.01$), higher proportion of exclusive breastfeeding period ≧6 months of age (36.1% vs. 27.8%; OR 1.47; 95% CI 1.04–2.07; $P = 0.03$), higher proportion of exclusive breastfeeding period ≧12 months of age (20.9% vs. 12.6%; OR 1.83; 95% CI 1.18–2.84; $P < 0.01$), and day-care center attendance (11.6% vs. 3.8%; OR 3.28; 95% CI 1.03–10.44; $P = 0.04$). Furthermore, multiple logistic regression analysis revealed that *M. catarrhalis* carriage was borderline negatively associated with breastfeeding since birth (OR, 0.34; 95% CI 0.12–0.99; $P = 0.05$) and positively associated with a longer exclusive breastfeeding period (OR, 1.12; 95% CI 1.01–1.25; $P = 0.04$).

In Taiwan, a 13-valent pneumococcal conjugate vaccine (PCV13) was introduced as a routine immunization (2 + 1 dose schedule, immunization administered at 2, 4, and 12 months of age) for all infants in January 2015.[13] To analyze the association between *M. catarrhalis* colonization and the introduction of routine immunization with PCV13, the proportion of *M. catarrhalis* carriers in the total participants before and after the introduction of PCV13 were compared. The proportion of *M. catarrhalis* carriers was significantly lower during 2012–2014 than that during 2015–2018 (1.8% [44 of 2428] vs. 12.2% [275 of 2260], $P < 0.01$). Similar findings were also shown in the subgroups

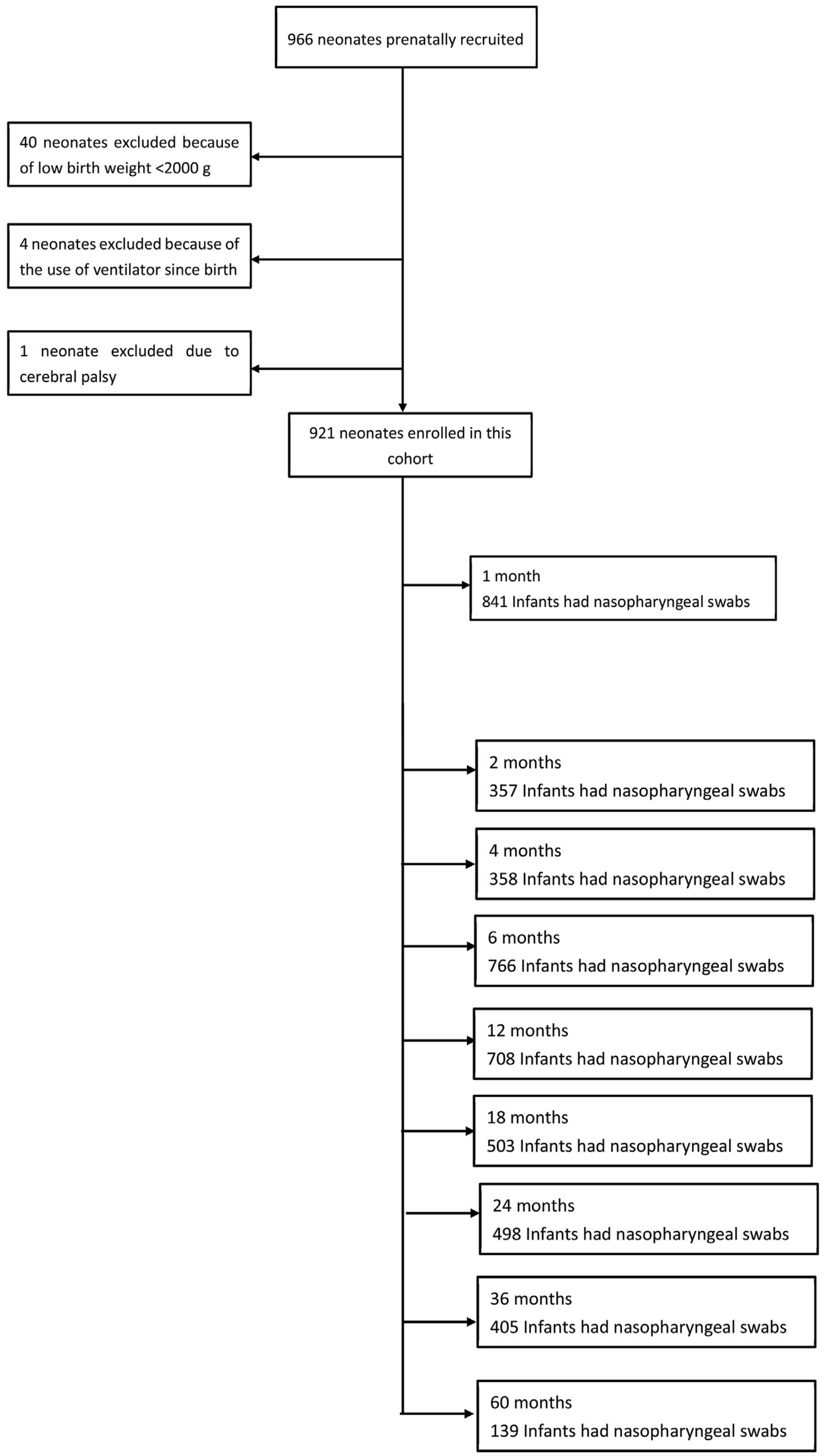

Fig. 1. Flowchart of enrollment during the study period.

Table 1 The distribution of bacterial colonization at the scheduled sampling time points during the study period.

Age (months)	1 (N = 842)	2 (N = 363)	4 (N = 363)	6 (N = 790)	12 (N = 722)	18 (N = 509)	24 (N = 509)	36 (N = 428)	60 (N = 162)	(N = 4688)
Number of colonization (%)	457 (54.3)	205 (56.5)	135 (37.2)	247 (31.3)	178 (24.7)	117 (23.0)	176 (34.6)	207 (48.4)	118 (72.8)	1840 (39.3)
Numbers of SA colonization (%)	442 (52.5)	196 (54.0)	119 (32.8)	153 (19.4)	79 (10.9)	49 (9.6)	72 (14.2)	87 (20.3)	49 (30.3)	1246 (26.6)
Numbers of MC colonization (%)	7 (0.8)	7 (1.9)	11 (3.0)	47 (6.0)	45 (6.2)	36 (7.1)	67 (13.2)	66 (15.4)	33 (20.4)	319 (6.8)
Numbers of SP colonization (%)	3 (0.4)	–	4 (1.1)	25 (3.2)	29 (4.0)	18 (3.5)	23 (4.5)	33 (7.7)	29 (17.9)	164 (3.5)
Numbers of HI colonization (%)	5 (0.6)	2 (0.6)	1 (0.3)	22 (2.8)	25 (3.5)	14 (2.8)	14 (2.8)	21 (4.9)	7 (4.3)	111 (2.4)

Number of colonization refers to any colonization with the 4 pathogens in different age groups.
SA indicates *Staphylococcus aureus*; MC, *Moraxella catarrhalis*; HI, *Haemophilus influenzae*; SP, *Streptococcus pneumoniae*.

with different ages under the age of 24 months except 1 month of age, which showed that no difference was noted between 2012-2014 and 2015—2018 (Supplementary Table S2). Furthermore, some children may receive an additional self-paid PCV13 shot between the ages of 6 and 12 months (i.e., a 3 + 1 dose schedule). To further investigate the influence of different PCV13 immunization schedules on the status of nasopharyngeal *M. catarrhalis* colonization, we compared the *M. catarrhalis* carriage status between participants with 2 + 1 and 3 + 1 dose schedules. We selected participants ≥18 months of age for analysis, and of these, 224 and 969 participants had 2 + 1 and 3 + 1 dose schedules, respectively. The results disclosed that there was no significant difference in the proportion of *M. catarrhalis* carriers (10.7% [24 of 224] vs. 14.4% [140 of 969], $P = 0.14$) between participants with 2 + 1 and 3 + 1 dose schedules. Besides, no difference was found in the proportion of *M. catarrhalis* carriers in the subgroups with different ages between participants with 2 + 1 and 3 + 1 dose schedules (Supplementary Table S3).

Association between maternal breastfeeding and *M. catarrhalis* colonization

A longer duration of exclusive breastfeeding may be correlated with a higher risk of *M. catarrhalis* colonization. Thus, we further divided the participants into several subgroups according to their exclusive breastfeeding period: none, < 6 months, 6—11.9 months, and ≥12 months. The results showed that the participants with an exclusive breastfeeding period ≥12 months had a greater risk of colonization than those without breastfeeding (OR 1.81, 95% CI 1.12—2.92), and no significant differences were found among the other subgroups.

Discussion

Our longitudinal birth cohort study showed that the prevalence of *M. catarrhalis* colonization gradually increased with age, which was also noted in other studies.[14] We found that *M. catarrhalis* was the second most common nasopharyngeal colonized pathogen and was more prevalent than *S. pneumoniae*. This is an interesting finding because, to our knowledge, the most common bacterial pathogen cultured from the nasopharynx of children during otitis media episodes or bacterial sinusitis is *S. pneumoniae*, followed by *H. influenzae*. The third most common pathogen is *M. catarrhalis*.[15] The increased colonization of *M. catarrhalis* may be related to the introduction of PCV. In Taiwan, the first PCV was the 7-valent PCV, which was introduced for private use in 2005, followed by the introduction of PCV10 in 2010 and PCV13 in 2011.[16,17] A national PCV13 vaccination program was initiated in March 2013 for children aged 2—5 years and has been included as a routine vaccination program for all infants since January 2015.[18] According to the results of other studies in the post PCV13 vaccination era, there was also a decrease in the carriage of *S. pneumoniae* with a simultaneous increase in the prevalence of *M. catarrhalis* as the causative pathogen of acute otitis media after the introduction of the pneumococcal conjugate vaccine.[19]

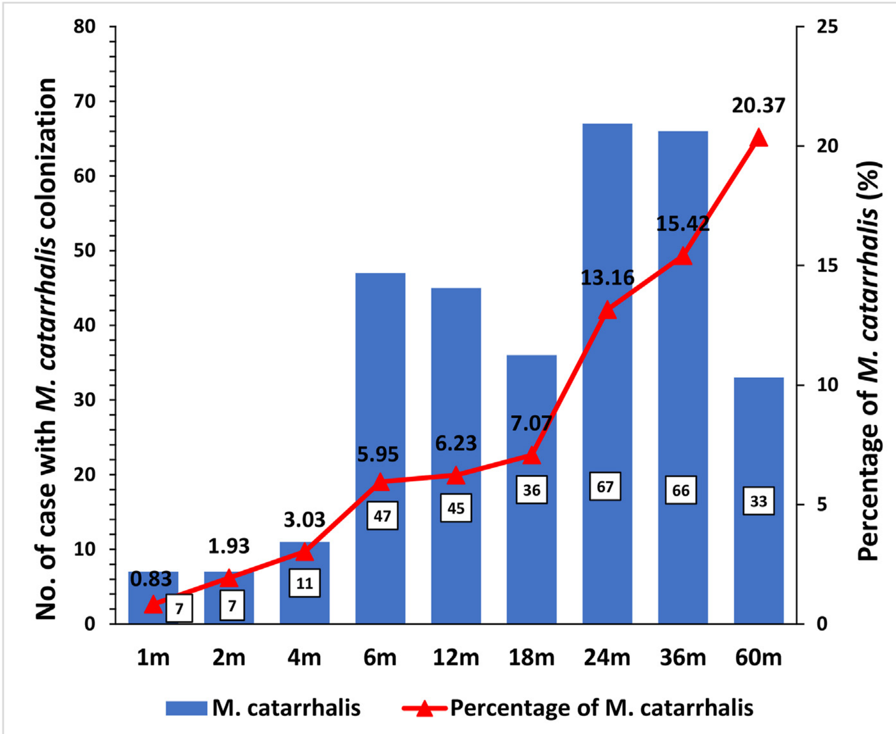

Fig. 2. The trend of *Moraxella catarrhalis* carriage at the scheduled sampling time points during the first 60 months of infant life.

S. aureus was the leading nasopharyngeal colonizing pathogen in this study. In the analysis of factors associated with MRSA colonization, we found an increased risk for infants born via vaginal delivery. Further studies, such as maternal-infant paired molecular genotype analyses, may be conducted to prove this hypothesis that the MRSA transmission route is more likely to be through vertical transmission rather than horizontal transmission. Of the environmental factors investigated in our study, only the presence of day-care attendance was a single predictor associated with the risk factors of *M. catarrhalis* colonization, which might be related to crowding and increased environmental exposure of children to facilitate *M. catarrhalis* colonization. Previous studies have revealed that day-care attendance and crowded gatherings are risk factors for *M. catarrhalis* colonization.[20,21] In our study, breastfeeding since birth was correlated with a lower risk of *M. catarrhalis* colonization, suggesting that breastfeeding is a protective factor against *M. catarrhalis* colonization and infection. A possible mechanism may be that breastfeeding stimulates the immune response of infants, and a higher serum antibody level or specific protein (i.e., natural resistance-associated macrophage protein 1) is produced against pathogens.[22,23] However, even though breastfeeding is recognized as the best source of nutrition for almost all infants and has health benefits for their mothers, the World Health Organization recommends exclusive breastfeeding for approximately the first six months, and continued breastfeeding should be complemented by the introduction of solid food.[24] After 6 months of age, the volume of human milk ingested by exclusively breastfed infants generally becomes insufficient to meet the infant's requirement for energy, protein, iron, zinc, and some fat-soluble vitamins.[25] To increase understanding the association between *M. catarrhalis* carriage and nutrient supplement, further studies may be performed for analyzing the relationship between complementary feeding, particularly iron/Vitamin D supplement, and the proportion of *M. catarrhalis* carriage among exclusively breastfed infants $\geq$6 months of age in the future. In addition to nutritional and energy supplements, complementary feeding plays an important role in the development of the gut microbiome, which in turn influences infant immune development.[26] Kamenju et al. reported that low dietary diversity and complementary feeding are likely associated with an increased risk of acute respiratory infection among exclusively breastfed human immunodeficiency virus (HIV)-exposed infants in Tanzania.[27] Furthermore, in our study, we divided the participants into several subgroups according to their exclusive breastfeeding period to analyze the association between the breastfeeding period and *M. catarrhalis* carriage. The results showed that the subgroup with an exclusive breastfeeding period of $\geq$12 months had a greater risk of colonization than those without, but no similar results were found among the other subgroups. Thus, we believe that exclusive breastfeeding, especially at $>$12 months of age, may not provide sufficient nutrition and immunity to children older than 12 months of age, resulting in an increase in the proportion of *M. catarrhalis* carriers. In our previous study, exclusive breastfeeding periods were also compared between patients with and without pneumococcal colonization, but no significant difference was found between the two groups.[13] Further studies are required to investigate the mechanism underlying the

Table 2 Univariate and multivariate analysis of factors associated with *Moraxella catarrhalis* colonization among the study population.

Factors associated with *Moraxella catarrhalis* colonization among the study population by univariate logistic regression analysis

Variables	Value		Odds ratio (95% confidence interval)	*p*-value
	Cases with *M. catarrhalis* colonization N = 302	Cases without *M. catarrhalis* colonization N = 302		
Demographics				
Male (%)	163/302 (54.0)	163/302 (54.0)	1.00 (0.73–1.38)	1.00
Age (mo)	23.1 ± 16.6	23.1 ± 16.6	1.00 (0.99–1.01)	1.00
Breast feeding[a]				
Breastfeeding (%) since birth	255/302 (84.4)	272/302 (90.1)	0.56 (0.35–0.92)	0.02
Breastfeeding period (mo) median (interquartile range)	6.0 (1.0–12.0)	4.0 (1.0–8.5)	1.03 (1.00–1.05)	0.03
Exclusive breastfeeding period (mo) median (interquartile range)	2.0 (0.0–9.0)	1.0 (0.0–6.0)	1.04 (1.01–1.06)	<0.01
Exclusive breastfeeding period ≧6 mo (%)	109/302 (36.1)	84/302 (27.8)	1.47 (1.04–2.07)	0.03
Exclusive Breastfeeding period ≧12 mo (%)	63/302 (20.9)	38/302 (12.6)	1.83 (1.18–2.84)	<0.01
Pneumococcal vaccine				
Vaccinated infants (%)	81/92 (88.0)	146/172 (84.9)	1.31 (0.62–2.79)	0.48
Age of initial vaccination (mo)	3.2 ± 3.8	2.9 ± 3.3	1.00 (0.98–1.02)	0.97
No. of injected vaccine	2.4 ± 1.3	2.4 ± 1.4	0.98 (0.82–1.19)	0.86
Environmental				
Passive smoking (%)	46/90 (51.1)	73/170 (42.9)	1.39 (0.83–2.32)	0.21
No. of the family members median (interquartile range)	4.0 (4.0–6.0)	5.0 (4.0–6.0)	1.01 (0.88–1.17)	0.85
No. of children in the family median (interquartile range)	2.0 (2.0–2.3)	2.0 (1.0–2.0)	1.36 (0.96–1.92)	0.08
Day-care center attendance (%)	8/69 (11.6)	5/130 (3.8)	3.28 (1.03–10.44)	0.04
Health conditions				
PROM[b] at birth (%)	4/92 (4.3)	7/172 (4.1)	1.07 (0.31–3.76)	0.91
Preterm birth (%)	48/302 (15.9)	42/302 (13.9)	1.17 (0.75–1.83)	0.49
Delivery via NSD[c] (%)	196/302 (64.9)	177/302 (58.6%)	0.77 (0.55–1.06)	0.11
PICU[c] admission at birth (%)	70/302 (23.2)	67/302 (22.2)	1.06 (0.72–1.53)	0.77
Vitamin D deficiency[d] (%)				
Maternal Vitamin D deficiency	30/40 (75.0)	52/68 (76.5)	1.08 (0.43–2.69)	0.86
Infantile Vitamin D deficiency	27/32 (84.4)	55/70 (78.6)	0.68 (0.22–2.06)	0.49
URI[e] within 2 weeks (%)	143/296 (51.7)	105/292 (36.0)	1.11 (0.72–1.69)	0.65
LRI[e] within 2 weeks (%)	67/296 (22.6)	70/293 (23.9)	0.93 (0.64–1.37)	0.72
Antibiotics usage within 2 weeks[f] (%)	22/295 (7.5)	21/292 (7.2)	1.04 (0.56–1.94)	0.90
Hospitalization due to LRI within 2 weeks (%)	6/67 (9.0)	2/70 (2.9)	3.34 (0.65–17.19)	0.15

Factors associated with *Moraxella catarrhalis* colonization among the study population by multiple logistic regression analysis

Variables	*M. catarrhalis* carriers vs. non- *M. catarrhalis* carriers Odds ratio (95% confidence interval)	*p*-value
Breastfeeding (%) since birth	0.34 (0.12–0.99)	0.05
Breastfeeding period (mo)	0.91 (0.81–1.02)	0.10
Exclusive breastfeeding period (mo)	1.12 (1.01–1.25)	0.04
No. of children in the family	1.32 (0.93–1.89)	0.12

[a] Infant with breastfeeding means that infant had a history of breast feeding including exclusive breast feeding for at least 4 weeks.
[b] PROM indicates pre-labor rupture of membrane. It is defined as rupture of the membrane of the amniotic sac and chorion ≧1 h before the onset of labor.
[c] NSD indicates normal spontaneous delivery; PICU indicates pediatric intensive care unit.
[d] Vitamin D deficiency denotes serum 25-hydroxyvitamin D level <20 ng/mL.
[e] URI indicates upper respiratory tract infection, including pharyngitis, croup, acute otitis media and acute sinusitis; LRI indicates lower respiratory tract infection, including bronchopneumonia, pneumonia, bronchitis and bronchiolitis.
[f] Of the 43 cases (22 in carriers; 21 in non-carriers) with antibiotics usage within 2 weeks, 11 received clinical care in the local clinics and 32 were brought to our clinic for treatment.

association between prolonged exclusive breastfeeding and increased *M. catarrhalis* carriage.

The limitation of this study was that it is an ongoing prospective study, but the period of this report was limited during 2012–2018. Thus, some of the 921 cases were less than 60 months of age and could not all complete the first 60-month study. Due to the above reason, the selection bias might occur. However, we considered that a prospective cohort-designed study may reduce the possibility of selection bias because in a cohort study, the outcome is not known at case enrollment when exposure status is established. Nevertheless, selection bias may occur in a retrospective study since the outcome has already occurred at the time of selection. Besides, the clinical information was mainly obtained from the questionnaire, and some data (i.e., antibiotics prescription) may be lost if the parents of the children did not fully understand the diagnosis of respiratory diseases or completely receive the records of medical prescription from the local clinics.

M. catarrhalis was the second most common nasopharyngeal colonized pathogen, and colonization was related to day-care center attendance and prolonged exclusive breastfeeding. Therefore, breastfeeding should be promoted because it may protect against the risk of *M. catarrhalis* carriage; however, exclusive prolonged breastfeeding, especially exceeding 12 months of age, may increase the risk of *M. catarrhalis* carriage. Compared with exclusive prolonged breastfeeding at $>$12 months of age, partial breastfeeding with optimal complementary food introduced after 6 months of age may be recommended to decrease the potential risk of children carrying *M. catarrhalis*.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2022.08.020.