Epidemiology of Nasopharyngeal Carriage by *Haemophilus influenzae* in Healthy Children

A Study in the Mediterranean Coast Region

María del Mar Ortiz-Romero, MD, María Pilar Espejo-García, MD,† Santiago Alfayate-Miguelez, PhD,‡ Francisco José Ruiz-López, MD,§ David Zapata-Hernandez, PhD,¶ and Ana Josefa Gonzalez-Pacanowska, PhD,‖ for the Collaborators of Study Group of Infectious Diseases in the Child in Cartagena*

Background: *Haemophilus influenzae*, a colonizer of the nasopharynx, in children causes mainly otitis and sinusitis. The primary objective of this study was to determine the prevalence of pharyngeal colonization by *H. influenzae*, and the secondary objectives were to identify risk factors associated with *H. influenzae* colonization and its antibiotic susceptibility.

Methods: A prospective, multicenter study of nasopharyngeal carriers of *H. influenzae* was conducted in the pediatric consulting rooms of 10 primary healthcare centers in Murcia (Spain). The study consisted of 404 healthy children less than 5 years of age and was carried out during winter (January–March) and summer (July–September) of 2015. A nasopharyngeal sample was collected from each child, and an epidemiologic survey was completed by a pediatrician.

Results: In total, 112 (27.7%) children had colonization by *H. influenzae*, with 73.2% of cases in winter and 26.8% of cases in summer ($P < 0.001$). The median (interquartile range) age in months of the colonized children (13 months, 12–47.5) was lower than that of the noncolonized children (46 months, 12–49) ($P < 0.001$). All *H. influenzae* found were nontypeable *H. influenzae* (NTHi). Among 112 isolates, 20% were ampicillin resistant, of which 10% produced β-lactamase, and 9% were ampicillin resistant and did not produce β-lactamase. A logistic regression analysis showed that young age (odds ratio: 0.98) and the winter period (odds ratio: 3.41; $P < 0.001$) were risk factors for colonization by NTHi.

Conclusions: Colonization by NTHi is high in this Mediterranean coast region with remarkable ampicillin resistant. Younger age and the winter period were facilitating factors.

Key Words: nasopharyngeal carriage, children, *Haemophilus influenzae*, resistant

(*Pediatr Infect Dis J* 2017;36:919–923)

Haemophilus influenzae is a Gram-negative coccobacillus, isolated exclusively in humans, predominantly from the respiratory tract. Nasopharyngeal colonization is the first step in a sequence of events that can lead to disease.[1,2] Since the introduction of vaccination for *H. influenzae* serotype b (Hib) in childhood immunization programs, there has been a global decline in *H. influenzae* colonization and invasive Hib disease in children, but the incidence of nonencapsulated strains infection, referred to as nontypeable *H. influenzae* (NTHi), has increased.[1,3,4] Approximately 20% of children are colonized by NTHi in the first year of life.[1] *H. influenzae* has been identified as an etiologic agent of otitis media and sinusitis, pathologies associated with a high consumption of antibiotics in children younger than 5 years of age.[1,2,5] The prevalence of *H. influenzae* in healthy children less than 5 years of age and the susceptibility to commonly used antibiotics are important for deciding appropriate antibiotic treatment.

METHODS

A multicenter, cross-sectional study of healthy children younger than 5 years of age in 10 primary care centers in the Cartagena area (Murcia, Spain) was performed.

Inclusion Criteria

Children younger than 5 years of age who attended optional appointments as part of the healthy child program were included: 10–15 and 42–54 months.

Exclusion Criteria

Children 54 months of age or over and children presenting with febrile or chronic disease, cystic fibrosis or immunodepressed were excluded from the study.

The study was conducted in 2015 in 2 phases: winter (January–March) and summer (July–September).

After providing a verbal explanation of the study, informed consent was obtained from parents of the children. An epidemiologic questionnaire was administered. The questionnaire included the following data: primary care center of provenance, date of collection, age in months, sex, systematic vaccinations (including *H. influenzae* and pneumococcal) and breastfeeding. Possible risk factors for colonization, such as attending school/day care, antibiotic treatment in the last month, smoking habits of the parents and the number of siblings attending school, were also recorded.

The study was approved by the Ethics Committee of our referral hospital.

Processing of Samples

Trained nurses collected all the nasopharyngeal samples using flexible nylon swabs (ESwab; CopanItalia spa, Brescia, Italy). Each sample was placed in a vial with transport media at room temperature and sent to the microbiology laboratory on the day of collection. In the laboratory, the samples were homogenized, and 10 µL were cultured in Columbia agar with colistin and nalidixic acid and chocolate agar PolyViteX (BioMérieux, Madrid, Spain),

Accepted for publication February 6, 2017.

From the *Servicio de Microbiología Hospital Universitario Santa Lucia, and †Centro de Salud Barrio Peral, Cartagena, Spain; ‡Servicio de Pediatría Hospital Universitario Virgen de la Arrixaca, and §Servicio de Neumología Hospital Universitario Virgen de la Arrixaca, Murcia, Spain; ¶Centro de Salud Mar Menor-El Algar, and ‖Centro de Salud Mazarrón, Cartagena, Spain.

The authors have no funding or conflicts of interest to disclose.

Address for correspondence: María del Mar Ortiz-Romero, MD, Hospital Universitario Santa Lucia, Calle Mezquita s/n, Paraje Los Arcos, Cartagena (Murcia), Spain, Postal code: 30202. E-mail: mmar.ortiz2@carm.es.

ISSN: 0891-3668/17/3610-0919
DOI: 10.1097/INF.0000000000001625

followed by incubation at 37°C in an atmosphere enriched with 5% Co_2. The plates were examined after 24 and 48 hours.

The identification of *H. influenzae* was performed according to standard laboratory procedures.[6] Detection of β-lactamase activity was determined using the chromogenic cephalosporin nitrocefin (BD BBL; DrySlide Nitrocefin, Sparks). Serotyping was performed by agglutination with the latex Phadebact Haemophilus Test (Bactus AB, Huddinge, Sweden).

The antibiotic sensitivity to *H. influenzae* was determined according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[7]

Standard laboratory procedures were performed to identify cocolonizers of *H. influenzae* (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Moraxella catarrhalis* and *Streptococcus pyogenes*).[6]

Statistical Procedures

The quantitative results were expressed as the median and interquartile range, and the qualitative data were expressed as percentages. The differences between qualitative variables were evaluated by a χ^2 test, and the differences between quantitative variables were examined with a Mann–Whitney *U* test. Significant differences were considered at a value of $P < 0.05$. A multivariate analysis was performed using multiple logistic regression. The probability of colonization by *H. influenzae* was the dependent variable. The analysis commenced with a model that included all variables that were significant in a bivariate analysis ($P < 0.05$), and all variables that were not significant were eliminated. This process was repeated until only the significant factors remained in the model.

RESULTS

In total, 404 samples were collected from 404 children, with a median age of 42 months (12–48). In the study, 196 (48.5%) of the children were between 10 and 15 months old, and 208 (51.5%) were between 42 and 54 months old. The samples were obtained from 227 males (56.2%) and 177 females (43.8%). From January to March 2015 (winter period), 214 samples were collected, and 190 samples were collected from July to September (summer period). In the winter and summer periods, 51.4% (n = 110) and 52.1% (n = 99) of the evaluated children, respectively, were attending school or day care, with no significant differences between the periods. Table 1 provides information on the association between the descriptive variables in the survey and colonization by *H. influenzae*.

All the children (100%) were vaccinated for *H. influenzae* type b (Hib), and 243 (60.1%) were vaccinated with pneumococcal conjugate vaccine (PCV) 13V.

In total, 112 (27.7%) children were colonized by *H. influenzae*, and all isolates were NTHi. Cocolonization (n = 73) was common rather than isolated NTHi colonization (n = 39). In 57 (50.9%) cases, cocolonization involved only 1 additional bacterial strain, whereas 2 or 3 additional bacterial strains were present in 16 (14.3%) cases ($P < 0.001$).

The associations with the different types of cocolonizers and the number of cocolonizers that accompanied *H. influenzae* are described in Table 2 and Figure 1. No association was found between the presence of *H. influenzae* and the presence of the cocolonizers studied. In most cases, *H. influenzae* was present with a second bacterial strain, mainly *S. aureus* or *M. catarrhalis*, rather than present with 2 or 3 bacterial strains.

TABLE 1. Epidemiologic Survey of Parents and the Association of Variables With Colonization by *Haemophilus influenza*

Variable	Carriers, n = 112 (%)	Noncarriers, n = 292 (%)	*P*
Sex			
Female	47 (42)	130 (44.5)	
Male	65 (58)	162 (55.5)	n.s.
Season			
Summer	30 (26.8)	160 (54.8)*	
Winter	82 (73.2)*	132 (45.2)	<0.0001
Pneumococcal vaccination			
Yes	69 (61.6)	174 (59.6)	
No	43 (38.4)	118 (40.4)	n.s.
School/day care center			
Yes	45 (40.2)	164 (56.1)*	
No	67 (59.8)*	128 (43.9%)	<0.001
Siblings at school			
Yes	53 (47.3)	151(51.7)	
No	59 (52.7)	141 (48.3)	n.s.
Smoking habits of parents			
Yes	42 (37.5)	128 (55.9)	
No	70 (62.5)	164 (44.1)	n.s.
Antibiotic in previous month			
Yes	9 (0.1)	33 (11.3)	
No	103 (99.9)	259 (88.7)	n.s.
Age (mo)			
10–15	67 (59.8)*	129 (44.1)	
42–54	45 (40.2)	163 (55.9)*	<0.01
Breastfeeding (mo)			
No	25 (22.3)	65 (22.2)	
<3	22 (19.6)	84 (28.7)	
>3	65 (58.1)	147 (49.1)	n.s.

The percentages in parentheses refer to the total number of carrier or noncarrier children.
*More frequent association.
n.s. indicates not significant; *P*, degree of significance.

TABLE 2. Cocolonization by Other Bacteria in *Haemophilus influenza* Nasopharyngeal Carriers

Colonization and Cocolonization	n (%)
H. influenza	39 (34.8)
H. influenza + *Staphylococcus aureus*	25 (22.3)
H. influenza + *Moraxella catarrhalis*	22 (19.6)
H. influenza + *Streptococcus pneumoniae*	8 (7)
H. influenza + *Streptococcus pyogenes*	2 (1.7)
H. influenza + *M. catarrhalis* + *S. pneumoniae*	7 (6)
H. influenza + *S. aureus* + *M. catarrhalis*	5 (4)
H. influenza + *S. aureus* + *S. pneumoniae*	1 (0.9)
H. influenza + *S. aureus* + *M. catarrhalis* + *S. pneumoniae*	2 (1.7)
H. influenza + *S. aureus* + *S. pneumonia* + *S. pyogenes*	1 (1.7)

n indicates number of *H. influenza* carriers. The percentages in parentheses refer to the total number of *H. influenza* carriers.

The bivariate analysis revealed no association between colonization by *H. influenzae* and the sex of the colonized children [65 (28.6%) males vs. 47 (26.5%) females]. Children with *H. influenzae* colonization were younger than those not colonized [13 (12–47.5) vs. 46 (12–49) months; $P < 0.001$). When the samples were grouped according to the age of the children, colonization was more frequent among children 10–15 months old than among those 42–54 months old (Table 1).

Colonization by *H. influenzae* was more frequent in the winter period [n = 82 (73.2%)] than the summer period [n = 30 (26.8%); $P < 0.0001$].

Having a parent who smoked did not influence colonization by *H. influenzae*. Of 170 children with parents who smoked, 42 (24.7%) were colonized with *H. influenzae*. In contrast, 70 (28.8%) children of nonsmoking parents were colonized with *H. influenzae* (Table 1). The duration of breastfeeding did not influence carrier status, with infants colonized by *H. influenzae* being breastfed for a median of 4 months (1–10.5) and noncolonized infants being breastfed for a median of 3 months (1–8.5).

Many *H. influenzae* carriers did not attend day care or school (Table 1). There was no association between being a carrier of *H. influenzae* and having a sibling in school, with 59 (29.5%) carriers not having siblings in school and 53 (25.9%) having siblings in school (Table 1).

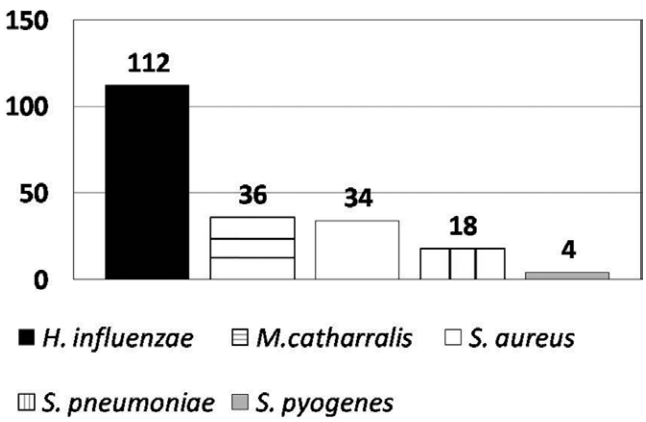

FIGURE 1. Cocolonization of bacteria in children colonized by *H. Influenzae*. Black bar, *H. influenzae*; bar with horizontal lines, *M. catarrhalis*; white bar, *S. aureus*; bar with vertical lines, *S. pneumonia*e and grey bar, *S. pyogenes*.

Pneumococcal vaccination did not influence NTHi carrier status. As shown in Table 1, 69 (28.4%) vaccinated children and 43 (26.7%) nonvaccinated children were colonized.

Only 9 children with *H. influenzae* colonization had received antibiotics in the previous month. Therefore, the influence of antibiotics on colonization could not be clinically assessed (Table 1).

The results of the multivariate analysis of the logistic regression of the association between *H. influenzae* colonization and age, day care or school attendance and the seasonal period are summarized in Table 3. The findings indicated that in the study region, a young age and the winter period were risk factors for colonization by *H. influenzae*.

Concerning antibiotic susceptibilities, 22 (19.6%) *H. influenzae* were resistant to ampicillin, of which 12 (10.7%) were β-lactamase producers and susceptible to amoxicillin-clavulanic acid and 10 (8.9%) were β-lactamase negative ampicillin-resistant (BLNAR). No *H. Influenza* β-lactamase positive amoxicillin-clavulanic resistant isolates were detected. All BLNAR *H. influenzae* isolates (100%) were detected in winter ($P < 0.05$).

Among the isolates, 100% were sensitive to nalidixic acid and therefore to fluoroquinolones, tetracyclines, chloramphenicol and cefixime. Twenty-two (19.6%) isolates were resistant to trimethoprim-sulfamethoxazole.

DISCUSSION

The introduction of the conjugated Hib vaccination has led to a drastic reduction in nasopharyngeal colonization by this serotype.[1,3,4,8–10] In Spain, the Hib conjugate vaccine has been incorporated into the national immunization plan since 1998. The present study did not detect any Hib isolates as a previous study in Spain,[8] possibly because all the children in the study have been correctly vaccinated.

In the present study of healthy children, more than one-quarter were carriers of NTHi. This rate was lower than that reported in a study of *H. influenzae* colonization in the Atlantic coast region of Spain, where the percentage of colonization was 42%.[8] However, the previous study was based only on samples collected in the winter.[8] The index of colonization in the present study was within the ranges published in other studies. The colonization rates were similar to those found in Brazil,[11] higher than those found in some areas of Italy[1] and lower than those found in France, the United States and some regions of Italy.[9,12,13]

A previous study by Bae et al[14] reported that *H. influenzae* was generally associated with cocolonizers. In accordance with that study, in the present study, *H. influenzae* colonization was mainly associated with cocolonization by *M. catarrhalis*, followed by *S. aureus* and *S. pneumoniae*. In this study, the presence of *S. aureus* did not preclude the presence of *H. influenzae*, probably because the cocolonization index with *S. aureus* was superior to the study by Bae et al.[14]

Several studies found a positive association of *H. influenzae* with *S. pneumoniae* and *M. catarrhalis*, but the frequencies of cocolonization varied from one study to another.[14–17] Numerous factors (eg, age, geographical area, socioeconomic level, immunization status of the children) may explain this finding.

TABLE 3. Results of the Multivariate Analysis and Logistic Regression

Variable	OR	*P*	95% CI
Season (winter)	3.41	0.001	2.10–5.54
Age (mo)	0.98	0.001	0.96–0.99

CI indicates confidence interval; OR, odds ratio; *P*, degree of significance.

As in other studies, neither sex[8,10,18] nor breastfeeding[18,19] showed a direct association with *H. influenzae* colonization in the present study.

A noteworthy fact of the current study was that younger children had a higher risk of *H. influenzae* colonization, which is in accordance with the findings of a study of children in Africa.[17] This finding may be explained by younger children having greater direct person-person contact, which is a necessary condition for the transmission and survival of the *H. influenzae* bacterium. However, some studies reported discordant results, finding that colonization by NTHi progressively increased with age and that maximum colonization occurred between 2 and 5 years.[10,19,20] Others concluded that age was not associated with NTHi colonization.[8,18] The discordance may be explained by distinct family relationships in different communities (eg, hygiene habits, prolonged close contact, etc.).

In the present study, children who did not attend day care or school were more likely to be colonized by NTHi. This observation, which may be correlated with the young age of non-school-going children, was in agreement with Schumacher et al,[19] who also found that children were colonized by the same NTHi strain carried by their primary caregivers. The results of the present study suggested that the family was the greatest source of bacterial transmission, a finding in accordance with that reported by Watanabe et al.[21] However, some researchers found that attending school or day care and living in an orphanage were associated with frequent colonization by NTHi.[22,23] Studies also suggested that the rate of colonization increased in accordance with the number of children per classroom.[8,18,24]

Some studies found that the number of siblings played a role in colonization by *H. influenzae*.[10,13,18,25] However, this was not the case in the present study, although half the children had at least 1 school-going sibling. This finding reinforces the idea that in the study setting, the family unit rather than attending school or having siblings at school influences colonization. The period in which the samples were obtained did not influence these results, as similar numbers of children attending school or day care were enrolled in the study in the winter and summer.

As in other studies,[17,19,26] the season, namely winter, was a risk factor for colonization. However, other studies reported conflicting findings, showing that the rainy season[17] and spring[16,23] were significantly associated with colonization.

The study region is located in the southeast of Spain, which is characterized by benign temperatures in winter and a rainy period after extremely dry summers. The higher rate of colonization in winter coincided with an increase in respiratory infections of the upper airway. If we consider that the temperature and humidity in the study region during the winter are similar to those of the spring in central European regions, the results obtained in studies carried out in Poland[23] and Milan, Italy,[16] are in agreement with those presented herein.

Regarding the smoking habits of the parents in the present study, as in other studies,[27] smoking did not influence NTHi colonization, although approximately 41% of the parents smoked.

The introduction of the PCV for children precipitated studies of bacterial interactions between *S. pneumoniae* and *H. influenzae* in the nasopharynx.[13] However, in the present study, vaccination with the PCV13 did not determine the carrier status of *H. influenzae*. However, as only 60% of the children were vaccinated, this may have influenced the results. Other studies found that vaccination with the conjugate PCV7/PCV13 vaccine was associated with colonization by *H. influenzae*.[10,13]

The resistance of *H. influenzae* to β-lactams varies according to the geographical area studied. In some cases, the resistance to ampicillin may exceed 60%,[20,28] which is a serious problem. In the present study, the percentage of resistance to ampicillin was not very high, similar to that found in other studies conducted in Spain,[8] France[9] and Italy.[10] In the Italian study,[10] the proportions of β-lactamase positive ampicillin-resistant and BLNAR strains were similar to those found in the current study. However, the percentages of BLNAR strains reported in other studies differed from those in the present study, with some reporting lower percentages[8,9,24] and others reporting higher percentages.[28,29] One study of the resistance of *H. influenzae* to β-lactams reported no BLNAR strains.[20]

In the current study, the antibiotic resistance of β-lactamases to ampicillin did not vary significantly according to the season, a finding in accordance with that reported by Marchisio et al.[16] However, the detection of BLNAR strains was significantly associated with winter in the present study.

In the study region, fluoroquinolones are widely used antibiotics in the general population but not in the pediatric population. However, resistance to fluoroquinolones was not observed in the present study. This finding is in accordance with the results of other studies.[20,29]

ACKNOWLEDGMENTS

Collaborators of Study Group of Infectious Diseases in the Child in Cartagena are as follows: Jimenez-Rejón S, Cáceres-Udina MJ, Fábrega-Valverde MT, Peñalver-Manrubia FJ, Mayordomo-Serna M, Ferrandez-García CM, Escudero-Rodriguez N, Alemán-Lorca MF and Conesa-Hernandez L.

REFERENCES

1. Agrawal A, Murphy TF. *Haemophilus influenzae* infections in the *H. influenzae* type b conjugate vaccine era. *J Clin Microbiol*. 2011;49:3728–3732.
2. Murphy TF, Faden H, Bakaletz LO, et al. Nontypeable *Haemophilus influenzae* as a pathogen in children. *Pediatr Infect Dis J*. 2009;28:43–48.
3. Madore DV. Impact of immunization on *Haemophilus influenzae* type b disease. *Infect Agents Dis*. 1996;5:8–20.
4. Takala AK, Eskola J, Leinonen M, et al. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. *J Infect Dis*. 1991;164:982–986.
5. Erwin AL, Smith AL. Nontypeable *Haemophilus influenzae*: understanding virulence and commensal behavior. *Trends Microbiol*. 2007;15:355–362.
6. Jorgensen J, Pfaller MA, eds. *Manual of Clinical Microbiology*. 11th ed. Washington, DC: American Society for Microbiology (ASM) Press; 2015.
7. EUCAST. European Committee on Antimicrobial Suceptibility Testing. Available at: http://www.eucast.org/clinical_breakpoints/. Accessed September 15, 2015.
8. Puig C, Marti S, Fleites A, et al. Oropharyngeal colonization by nontypeable *Haemophilus influenzae* among healthy children attending day care centers. *Microb Drug Resist*. 2014;20:450–455.
9. Dabernat H, Plisson-Sauné MA, Delmas C, et al. *Haemophilus influenzae* carriage in children attending French day care centers: a molecular epidemiological study. *J Clin Microbiol*. 2003;41:1664–1672.
10. Giufrè M, Daprai L, Cardines R, et al. Carriage of *Haemophilus influenzae* in the oropharynx of young children and molecular epidemiology of the isolates after fifteen years of *H. influenzae* type b vaccination in Italy. *Vaccine*. 2015;33:6227–6234.
11. de Carvalho CX, Kipnis A, Thörn L, et al. Carriage of *Haemophilus influenzae* among Brazilian children attending day care centers in the era of widespread Hib vaccination. *Vaccine*. 2011;29:1438–1442.
12. Farjo RS, Foxman B, Patel MJ, et al. Diversity and sharing of *Haemophilus influenzae* strains colonizing healthy children attending day-care centers. *Pediatr Infect Dis J*. 2004;23:41–46.
13. Camilli R, Vescio MF, Giufrè M, et al. Carriage of *Haemophilus influenzae* is associated with pneumococcal vaccination in Italian children. *Vaccine*. 2015;33:4559–4564.
14. Bae S, Yu JY, Lee K, et al. Nasal colonization by four potential respiratory bacteria in healthy children attending kindergarten or elementary school in Seoul, Korea. *J Med Microbiol*. 2012;61(pt 5):678–685.

15. Dunne EM, Manning J, Russell FM, et al. Effect of pneumococcal vaccination on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* in Fijian children. *J Clin Microbiol*. 2012;50:1034–1038.
16. Marchisio P, Gironi S, Esposito S, et al; Ascanius Project Collaborative Group. Seasonal variations in nasopharyngeal carriage of respiratory pathogens in healthy Italian children attending day-care centres or schools. *J Med Microbiol*. 2001;50:1095–1099.
17. Abdullahi O, Nyiro J, Lewa P, et al. The descriptive epidemiology of *Streptococcus pneumoniae* and *Haemophilus influenzae* nasopharyngeal carriage in children and adults in Kilifi district, Kenya. *Pediatr Infect Dis J*. 2008;27:59–64.
18. Barbosa-Cesnik C, Farjo RS, Patel M, et al. Predictors for *Haemophilus influenzae* colonization, antibiotic resistance and for sharing an identical isolate among children attending 16 licensed day-care centers in Michigan. *Pediatr Infect Dis J*. 2006;25:219–223.
19. Schumacher SK, Marchant CD, Loughlin AM, et al. Prevalence and genetic diversity of nontypeable *Haemophilus influenzae* in the respiratory tract of infants and primary caregivers. *Pediatr Infect Dis J*. 2012;31:145–149.
20. Wang SR, Lo WT, Chou CY, et al. Low rate of nasopharyngeal carriage and high rate of ampicillin resistance for *Haemophilus influenzae* among healthy children younger than 5 years old in northern Taiwan. *J Microbiol Immunol Infect*. 2008;41:32–40.
21. Watanabe H, Hoshino K, Sugita R, et al. Possible high rate of transmission of nontypeable *Haemophilus influenzae*, including beta-lactamase-negative ampicillin-resistant strains, between children and their parents. *J Clin Microbiol*. 2004;42:362–365.
22. Peerbooms PG, Engelen MN, Stokman DA, et al. Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. *J Clin Microbiol*. 2002;40:2832–2836.
23. Sulikowska A, Grzesiowski P, Sadowy E, et al. Characteristics of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolated from the nasopharynges of asymptomatic children and molecular analysis of *S. pneumoniae* and *H. influenzae* strain replacement in the nasopharynx. *J Clin Microbiol*. 2004;42:3942–3949.
24. Talon D, Leroy J, Dupont MJ, et al. Antibiotic susceptibility and genotypic characterization of *Haemophilus influenzae* strains isolated from nasopharyngeal specimens from children in day-care centers in eastern France. *Clin Microbiol Infect*. 2000;6:519–524.
25. Munsawaengsub C, Pitikultang S. Factors associated with oropharyngeal carrier of Haemophilus influenzae and antimicrobial resistance in healthy children attending day-care center of a health promotion hospital. *J Public Health*. 2010;40:281–290.
26. Hashida K, Shiomori T, Hohchi N, et al. Nasopharyngeal *Haemophilus influenzae* carriage in Japanese children attending day-care centers. *J Clin Microbiol*. 2008;46:876–881.
27. Greenberg D, Givon-Lavi N, Broides A, et al. The contribution of smoking and exposure to tobacco smoke to *Streptococcus pneumoniae* and *Haemophilus influenzae* carriage in children and their mothers. *Clin Infect Dis*. 2006;42:897–903.
28. Bae SM, Lee JH, Lee SK, et al. High prevalence of nasal carriage of β-lactamase-negative ampicillin-resistant *Haemophilus influenzae* in healthy children in Korea. *Epidemiol Infect*. 2013;141:481–489.
29. Mzilem S, Ksiaa S, Smaoui H, et al. Haemophilus influenzae strains in children: increasing resistance to beta-lactam antibiotics. *Int J Microbiol Immunol Res*. 2015;3:084–089.