Journal of Microbiology, Immunology and Infection (2019) 52, 75–80

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Original Article

Epidemiology and antimicrobial susceptibility of non-typeable *Haemophilus influenzae* in otitis media in Taiwanese children

Ying-Chun Cho [a,b], Nan-Chang Chiu [a,c,d], Fu-Yuan Huang [a], Daniel Tsung-Ning Huang [a,c], Lung Chang [e], Ching-Ying Huang [a], Yen-Hsin Kung [e], Kuo-Sheng Lee [f], Hsin Chi [a,c,d,*]

[a] *Department of Pediatrics, MacKay Children's Hospital, Taipei, Taiwan*
[b] *Department of Pediatrics, Taiwan Adventist Hospital, Taipei, Taiwan*
[c] *Department of Medicine, MacKay Medical College, New Taipei, Taiwan*
[d] *MacKay Junior College of Medicine, Nursing and Management, Taipei, Taiwan*
[e] *Department of Pediatrics, MacKay Memorial Hospital Tamshui, New Taipei, Taiwan*
[f] *Department of Otorhinolaryngology, MacKay Memorial Hospital, Taipei, Taiwan*

Received 24 April 2017; received in revised form 18 August 2017; accepted 28 August 2017
Available online 18 September 2017

KEYWORDS
Non-typeable *Haemophilus influenzae*;
Otitis media;
Children;
Recurrent otitis media;
Ventilation tube

Abstract *Background:* Concerns about non-typeable *Haemophilus influenzae* (NTHi) in otitis media (OM) have grown after the introduction of pneumococcal conjugate vaccine (PCV). We aim to better understand the clinical role of NTHi in pediatric OM.
Methods: Middle ear fluid samples from children <18 years with OM were obtained from 2010 to 2015. For culture-positive episodes (*Streptococcus pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes*), patients' demographic and clinical information were reviewed and analyzed.
Results: A total of 783 episodes were included with 31.8% of isolates as positive. *S. pneumoniae* was recovered in 69.4%, NTHi in 24.6%, *M. catarrhalis* in 5.6%, and *S. pyogenes* in 4.0% of culture-positive episodes. The proportion of pneumococcal OM has declined since 2012 (P for trend <0.005), but NTHi OM rose simultaneously (P for trend = 0.009). Factors associated with increased risk of NTHi infection included less spontaneous otorrhea (OR 0.15, 95% CI 0.06–0.39, $P < 0.001$), absence of fever (OR 0.30, 95% CI 0.14–0.66, $P = 0.003$), concurrent sinusitis (OR 2.91, 95% CI 1.36–6.20, $P = 0.006$), previous ventilation tube insertion (OR 12.02, 95% CI 3.15–45.92, $P < 0.001$) and recurrent OM (OR 3.43, 95% CI 1.01–11.71, $P = 0.049$). The susceptibility of NTHi to amoxicillin/clavulanate was 82.0%.

* Corresponding author. Department of Pediatrics, MacKay Children's Hospital, No. 92, Sec. 2, Zhongshan N. Road, Zhongshen Dist., Taipei, 10449, Taiwan. Fax: +886 2 2543 3642.
E-mail address: chi.4531@mmh.org.tw (H. Chi).

http://dx.doi.org/10.1016/j.jmii.2017.08.020

Conclusions: NTHi OM has trended upward in the post-PCV era. Concurrent sinusitis, previous ventilation tube insertion, and recurrent OM were associated with NTHi OM implicated a correlation between NTHi and complex OM. In consideration of NTHi infection, we suggest amoxicillin/clavulanate as the first-line therapy for OM among Taiwanese children.

Introduction

Otitis media (OM) is one of the major causes for clinician visits and antibiotic prescriptions in children.[1] The most common bacteria responsible for OM in children globally are *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* (NTHi), followed by *Moraxella catarrhalis* and *Streptococcus pyogenes*.[2] The introduction of the pneumococcal conjugate vaccine (PCV) has reduced the rates of pneumococcal and overall OM[2,3] and is thought to have partly altered the bacteriology of acute otitis media (AOM) to some extent by reducing vaccine serotypes[3,4] and increasing the proportion of circulating serotypes that are not included in the vaccines, as well as of other pathogens, mainly NTHi.[2,5,6]

NTHi, a gram-negative microbe, is frequently carried in the human nasopharynx and oropharynx and is an important cause of OM in children and exacerbations of chronic obstructive pulmonary disease. Associations between NTHi and recurrent AOM (rAOM), chronic otitis media with effusion (COME), and AOM treatment failure have been established previously.[2,7–9] Preventing long-term complications, i.e., hearing loss and subsequent delay in language development, by averting complex OM episodes could have a large and positive effect on children and their families.

Clinical presentations may be associated with the etiologic agents of OM. Conjunctivitis-otitis syndrome is commonly associated with NTHi infection,[10,11] and the severity of AOM caused by NTHi typically is lower than that associated with pneumococcal AOM.[11,12] However, the overlap between different pathogens as well as a proportion of polymicrobial infection[2] make it difficult to distinguish clinical manifestations of one bacterial species from another.[13]

Understanding the role of NTHi in OM is currently of expanding interest in some regions, but recent data are scant in East Asia. Therefore, this study aims to better understand the various clinical aspects of OM caused by NTHi in comparison with *S. pneumoniae*, which has been reported as the predominant pathogen of OM in Taiwanese children.[14,15] We also studied the changing bacterial distribution and antimicrobial susceptibility following the introduction of PCVs in Taiwan.

Materials and methods

Study design

All middle ear fluid (MEF) samples from children <18 years diagnosed with AOM or COME from 2010 to 2015, in either outpatient or inpatient settings at MacKay Children's Hospital in Taiwan, were collected. The diagnosis was made by a pediatrician or an otorhinolaryngologist after otoscopic examination of the patient's eardrum. Culture specimens were obtained either by tympanocentesis or by collecting pus that drained from the middle ear during an episode of OM. The MEF sample was immediately applied onto a sterile swab preserved in a transport medium (Amies Agar Gel with Charcoal; COPAN Italia, Brescia, Italy) and was sent to a microbiology laboratory for analysis. The specimens were cultured in sheep blood agar and chocolate agar that were incubated aerobically at 35 °C for 48 h. Isolation of bacterial pathogens was carried out, and only the four highest-ranked otopathogens (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. pyogenes*) were considered true pathogens since the other isolated bacteria could be contamination.[16] For each culture-positive OM episode, patients' demographic and clinical information were retrospectively retrieved from the medical records regarding sex, age, date, clinical presentation, diagnosis, antibiotic susceptibility test reports, the method of MEF acquisition, and patients' history of OM and ventilation tube insertion (VTI). No change in policy with regard to tympanocentesis occurred during the study period. The Institutional Review Board of MacKay Children's Hospital approved this study.

Definitions

A new OM episode was defined by detection of different pathogens at any interval following a first instance or after an OM-free interval of 28 days between isolations of the same pathogen. If the same pathogen was isolated from both ears, one of the two isolates was randomly selected, and only one isolate was counted per episode. Recurrent AOM was defined as three or more well-documented and separate AOM episodes in the preceding six months or four or more episodes in the preceding 12 months with at least one episode in the past six months.[1] COME was defined as persistent MEF accumulation with minimal constitutional symptoms lasting longer than three months. A mixed infection was defined as ≥two pathogens found in the same specimen or different pathogens isolated from specimens obtained from different ears of the same patient during the same visit.

Antimicrobial susceptibility tests

The antimicrobial susceptibility test was performed in the microbiology laboratory in MacKay Memorial Hospital. The Clinical & Laboratory Standards Institute guidelines for the

antimicrobial susceptibility breakpoints of the minimum inhibitory concentration of antibiotics were adopted as criteria for interpreting drug susceptibility.[17] Breakpoints for penicillin and cefotaxime for non-meningitis were applied.

Statistical analysis

Statistical analysis was performed using Microsoft Excel (Redmond, WA, USA) and IBM SPSS Statistics for Macintosh version 23.0 (Armonk, NY, USA). The χ^2 test or Fisher's exact test were used for categorical variables, and Student's *t*-test was used for continuous variables. Variables implicated in the literature or statistically significant at the level of $P < 0.05$ in the univariate analyses were included in the multivariate logistic regression models. Odds ratios (OR) and 95% confidence intervals (CI) were used to measure the association between the various variables and AOM pathogens. A *P* value of <0.05 was considered statistically significant.

Results

From January 2010 to December 2015, MEF samples was collected from 783 children with OM who were <18 years old. The culture was positive for the defined pathogens in 31.8% (249/783) of cases, including 130 males (52.2%) and 119 females (47.8%), and the mean age was 3.6 years (standard deviation, 2.4 years). Among patients who had positive cultures, 75.1% (187/249) were <5 years old. The most commonly isolated pathogens were *S. pneumoniae* and NTHi, which were recovered in 172 (69.4%) and 61 (24.6%) episodes, followed by *M. catarrhalis* (14, 5.6%), *S. pyogenes* (10, 4.0%), and *H. influenzae* type b (1, 0.4%). Nine (3.6%) positive isolates were classified as mixed infections, from which more than one pathogen were identified. Patients' characteristics are shown in Table 1.

Fig. 1 displays the yearly changes in the proportion of the otopathogens detected with marks of different stages of PCV13 vaccination policy for children in Taiwan. *S. pneumoniae* maintained its predominance during the study years; however, the percentage has declined since 2012 (*P* for trend <0.005 during the period between 2012 and 2015), and remained similar in the following three years. In contrast, NTHi almost mirrored the change of pneumococcus with a rise since 2012 (*P* for trend = 0.009 during the period between 2012 and 2015), and the gap between the two narrowed in the second half of the study period.

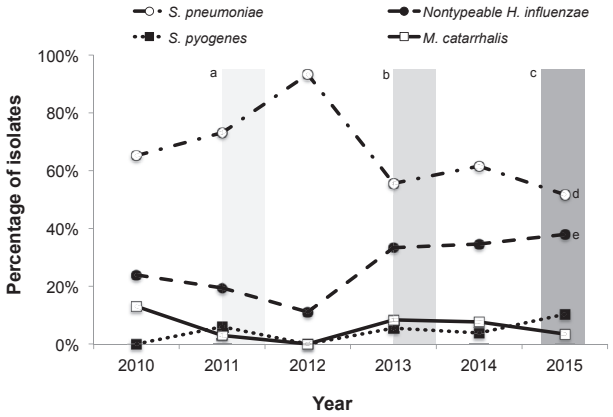

Figure 1. Percentage of isolates per year among isolated otopathogens from 2010 to 2015. [a]PCV13 was introduced in the private market in Taiwan in April 2011. [b]PCV13 catch-up program with partial reimbursement was started in Taiwan in March 2013. [c]PCV13 was integrated into national immunization program for infants in Taiwan in January 2015 with a 2 + 1 schedule of doses at 2, 4, and 12–15 months of age. [d]*P* for trend = 0.002 during 2010–2012, *P* for trend <0.05 during 2012–2015. [e]*P* for trend = 0.116 during 2010–2012, *P* for trend = 0.009 during 2012–2015.

Since NTHi and *S. pneumoniae* combined accounted for more than 90% of positive cultures, we compared NTHi with *S. pneumoniae* isolates and analyzed the isolates of single infections to single out the most relevant features. In terms

Table 1 Demographics and clinical information of patients with otitis media whose middle ear fluid yielded nontypeable *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, or *S. pyogenes*.

	NTHi (n = 61)	*S. pneumoniae* (n = 172)	*M. catarrhalis* (n = 14)	*S. pyogenes* (n = 10)
Demographics				
Age cohort, n (%)[a]				
<12 mo	8 (25.0)	23 (71.9)	1 (3.1)	0 (0.0)
12–24 mo	15 (32.6)	29 (63.0)	1 (2.2)	1 (2.2)
2–3 y	6 (17.6)	26 (76.5)	2 (5.9)	0 (0.0)
3–4 y	6 (14.6)	32 (78.0)	1 (2.4)	2 (4.9)
4–5 y	11 (27.5)	24 (60.0)	4 (10.0)	1 (2.5)
5–18 y	15 (23.1)	38 (58.5)	5 (7.7)	6 (9.2)
Mean age ± SD (y)	3.42 ±2.26	3.39 ±2.16	5.68 ±3.14	6.32 ±2.90
Male, n (%)	36 (59.0)	86 (49.7)	9 (64.3)	4 (40.0)
Mixed infection, n (%)	7 (11.5)	7 (4.1)	4 (28.6)	0 (0.0)
Sample collection method, n (%)				
Tympanocentesis	44 (72.1)	87 (50.6)	4 (28.6)	0 (0.0)
Spontaneously draining pus	17 (27.9)	90 (52.3)	10 (71.4)	10 (100.0)

[a] The percentage was based on the number of each otopathogen over the total number of otopathogens isolated at a given age cohort.
NTHi, nontypeable *H. influenzae*.

of clinical manifestations, spontaneous otorrhea, otalgia, presence of fever, and complicated mastoiditis were more common in pneumococcal OM, while patients with NTHi OM were more often diagnosed with sinusitis in the same visit. There was no difference in concurrent conjunctivitis between the two groups. Epidemiologic factors that were significantly more frequent in NTHi OM in the univariate analyses, when compared with pneumococcal OM, were history of previous VTI and rAOM. No statistically significant difference was detected in the category of age, sex, and chronic OM (Table 2).

We preceded the binary logistic regression analysis using significant factors in the univariate analyses. The multivariate analysis identified concurrent sinusitis (OR 2.91, 95% CI 1.36–6.20, $P = 0.006$), previous ventilation tube insertion (OR 12.02, 95% CI 3.15–45.92, $P < 0.001$), and recurrent OM (OR 3.43, 95% CI 1.01–11.71, $P = 0.049$) as factors associated with increased risk of NTHi infections. The spontaneous otorrhea (OR 0.15, 95% CI 0.06–0.39, $P < 0.001$) and presence of fever (OR 0.30, 95% CI 0.14–0.66, $P = 0.003$) at diagnosis of OM were associated with decreased risk of OM caused by NTHi (Table 3).

The antimicrobial susceptibilities of the two most common pathogens were recorded (Table 4), including data from two earlier studies in our setting. The susceptibility of NTHi to ampicillin was low (19.7%), but it was higher for cefuroxime (77%) and amoxicillin/clavulanate (82.0%). Only 30.8% and 32.0% of *S. pneumoniae* were susceptible to amoxicillin and penicillin in our study. In contrast to the high susceptibilities of NTHi isolates to third-generation cephalosporins (98.4% for both cefotaxime and ceftriaxone), only 62.8% and 68.0% of pneumococcal isolates were susceptible to cefotaxime and ceftriaxone, respectively.

Table 2 Univariate analysis of the variables in patients with otitis media caused by nontypeable *H. influenzae* or *S. pneumoniae* as a single pathogen.

Variable	NTHi, n (%) (n = 54)	*S. pneumoniae*, n (%) (n = 165)	*P* value¶
Mean age ± SD, y	3.45 ±2.32	3.37 ±2.16	0.831§
Male	33 (60.0)	80 (48.5)	0.139
Clinical manifestation			
Spontaneous otorrhea	17 (30.9)	87 (52.7)	0.005
Otalgia	10 (18.2)	54 (32.7)	0.040
Presence of fever	23 (41.8)	127 (77.0)	<0.001
Concurrent diagnosis			
Sinusitis	27 (49.1)	38 (23.0)	<0.001
Conjunctivitis	3 (5.5)	3 (1.8)	0.152
Complicated mastoiditis	0 (0.0)	12 (7.3)	0.040
Previous ventilation tube insertion	15 (27.3)	6 (3.6)	<0.001
Recurrent OM	10 (18.2)	5 (3.0)	<0.001
Chronic OM	4 (7.3)	7 (4.2)	0.372

OM, otitis media; NTHi, nontypeable *H. influenzae*.
¶*P* value was found from χ^2 test.
§*P* value was found from Student's *t*-test.

Table 3 Multivariate regression analysis of the factors associated with nontypeable *H. influenzae* versus *S. pneumoniae* as a single pathogen in middle ear fluid cultures in children with otitis media.

Variable	NTHi single infection OR (95% CI)	*P* value
Spontaneous otorrhea	0.15 (0.06–0.39)	<0.001
Otalgia	0.56 (0.23–1.36)	0.202
Presence of fever	0.30 (0.14–0.66)	0.003
Concurrent sinusitis	2.91 (1.36–6.20)	0.006
Previous ventilation tube insertion	12.02 (3.15–45.92)	<0.001
Recurrent OM	3.43 (1.01–11.71)	0.049

OM, otitis media; NTHi, nontypeable *H. influenzae*.
OR, odds ratio; CI, confidence interval.

Discussion

In this six-year study of OM characteristics in children, NTHi was the second most prevalent pathogen, which presented a growing proportion during the PCV policy evolution in Taiwan. Our results also allowed us to attempt to differentiate NTHi OM from *S. pneumoniae* OM regarding clinical features and risk factors.

Several studied have reported rAOM in association with NTHi and our results supported the relevance.[2,7,9] Kilpi et al.[7] and Barkai et al.[9] have further speculated that *H. influenzae*, primarily NTHi, may secondarily predispose infants to rAOM after episodes of pneumococcal AOM, which has an important interplay with early and first episodes of OM in children. On the other hand, VTI is usually performed for various complicated or chronic conditions, including persistent COME or rAOM with effusion.[18] Our study suggested that the history of VTI could be an independent risk factor for OM caused by NTHi. Some studies have suggested that NTHi biofilms increase during OM and that its high biofilm formation capacity relates to treatment failures in chronic OM and rAOM in children.[19–21] These risk factors and the biofilm formation ability together might imply the complexity of OM caused by NTHi.

NTHi also predominates in children with acute sinusitis.[22] We found that NTHi OM coexists with sinusitis more commonly than pneumococcal OM. Nevertheless, there was no difference in concurrent conjunctivitis despite the well-documented conjunctivitis-otitis media syndrome in relation to NTHi. It may be that if a pediatric patient had signs of OM concurrently with an intact tympanic membrane and conjunctivitis, the parents were more likely to allow clinicians to obtain a culture of eye discharge instead of performing tympanocentesis to culture ear pus. Our findings of the absence of fever and the lack of initial presentation of otorrhea were correlated with NTHi OM, which were compatible with previous reports.[11,12] This may indicate a milder clinical severity and acuteness of NTHi OM compared to pneumococcal OM.

Seven-valent PCV (PCV7) was licensed in Taiwan in October 2005 and was replaced by 13-valent PCV (PCV13) in the private market since April 2011. PCV13 has been reimbursed by the government since March 2013 as part of a

Table 4 Antimicrobial susceptibility of nontypeable *H. influenzae* and *S. pneumoniae* isolates from middle ear fluid samples from pediatric patients with otitis media in the same setting in the past 17 years.

	NTHi (%)				*S. pneumoniae* (%)			
	1999–2003[14,a]	2004–2008[14,a]	2009–2011[15,b]	2010–2015	1999–2003[14,a]	2004–2008[14,a]	2009–2011[15,b]	2010–2015
Ampicillin	24.7	33.3		19.7				N/A
Amoxicillin				N/A				30.8
Amoxicillin/clavulanate	79.5	81.8	62.5	82.0				N/A
Chloramphenicol	61.8	75.8	68.8	73.8	69.8	83.9	94.9	93.0
Cefuroxime	87.1	81.8	75.0	77.0				N/A
Cefotaxime*	90.5	90.9	100	98.4	100.0	84.6	53.8	62.8
Ceftriaxone			100	98.4			53.8	68.0
Levofloxacin				N/A			97.4	100.0
Moxifloxacin			93.8	100.0			100.0	100.0
Erythromycin				N/A	8.3	5.0	0.0	0.6
Penicillin**				N/A	15.2	10.6	28.2	32.0
Vancomycin				N/A			100.0	100.0
TMP/SMX	35.5	30.3	43.8	31.1	20.3	12.6	12.8	12.8
Imipenem				N/A			15.4	13.5

[a] Reference: Chiu NC et al. *J Formos Med Assoc* 2012;**111**:53–541.
[b] Reference: Kung YH et al. *J Microbiol Immunol Infect* 2014;**47**:239–244.
NTHi, nontypeable *H. influenzae*: TMP/SMX, trimethoprim/sulfamethoxazole; N/A, not available.
*,** Non-meningitis breakpoint.

catch-up program targeting children aged 2–5 years, which was then expanded to cover children aged 1–5 years between January 2014 and December 2014. The government has integrated PCV13 into the universal routine immunization of infants since January 2015 with a 2 + 1 schedule of doses at 2, 4, and 12–15 months of age.[23]

An initial growth in the recovery of *S. pneumoniae* from the MEF of OM children followed by a decline after the PCV13 catch-up program in Taiwan was revealed in our study. Meanwhile, NTHi stayed in the second place throughout the study years, but the rise in proportion has brought it closer to *S. pneumoniae*. In comparison with our previous 10-year data during 1999–2008,[14] the proportion of NTHi isolates in positive OM culture was higher in our current study (25.0% vs. 19.2%). The trends were in line with the findings of two reviews in 2016 that PCV7 has resulted in more frequent isolation of NTHi from MEF specimen in OM patients.[2,24] In a systematic review, Dagan et al. have concluded that reduction of early AOM episodes, which were more commonly caused by *S. pneumoniae*, following the introduction of PCVs can decrease the number of subsequent events associated with less invasive or nonvaccine-type *S. pneumoniae* and NTHi. The pathogen dynamics required longer monitoring after the nationwide PCV13 immunization program deployment in Taiwan in 2015.

The susceptibility of *H. influenzae* to ampicillin and *S. pneumoniae* to penicillin and amoxicillin were low in our setting. According to several drug susceptibility studies in Taiwan, around half of *H. influenzae* isolates were resistant to ampicillin and one-third of *S. pneumoniae* were resistant to penicillin.[25–27] The nationwide surveillance of *S. pneumoniae* resistance by Tsai et al.[27] during 2006–2010 also revealed the low resistance of pneumococcus to amoxicillin/clavulanate (16.1%) but high resistance to cefuroxime (82.8%). Therefore, our report supported ampicillin/clavulanate as the first-line drug of choice for children with OM in Taiwan, together with heightened concerns about NTHi.

This study has certain limitations. First, we collected only spontaneous otorrhea specimens in some patients, so we could have missed a portion of pathogens present in the MEF.[28] Additionally, we excluded other bacteria in the analysis, such as *Streptococcus aureus* or *Pseudomonas* species, which were likely to be contamination, particularly those grown from spontaneously draining pus; however, in some cases they may have been causative organisms. Moreover, we had limited access to patients' histories of antibiotic use prior to enrollment, so the effect of previous antibiotic exposure, which could possibly stimulate biofilm formation by NTHi[29] and was described as an additional risk factor for the increased occurrence of NTHi AOM,[9] could not be evaluated. Finally, our study has involved data from a single tertiary hospital, which might introduce referral bias and higher antimicrobial susceptibilities should be interpreted with caution.

In conclusion, a relative increase of NTHi necessitates monitoring changes in the bacteriology of OM in children in the post-PCV era. Our study emphasizes that NTHi plays a certain role in OM recurrence and complexity in children despite its milder clinical picture. Moreover, the presence of concurrent sinusitis may be helpful in diagnosing NTHi OM. Establishing the contribution of NTHi to OM may be helpful in the development of a new vaccine against NTHi that outweighs the cost of long-term healthcare in terms of repeated episodes and sequelae.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Disclosure

The authors declare no conflicts of interest or sources of funding.

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