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Original Article

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

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Background and Purpose: Surveillance data of colonization by *Haemophilus influenzae* in Taiwan are lacking. This study aimed to define the nasopharyngeal carriage rate of *H. influenzae* among children younger than 5 years in northern Taiwan, and to determine the antibiotic susceptibility, serotype and the clonal relationship of these isolates.

Methods: Nasopharyngeal specimens were obtained from 511 healthy children younger than 5 years. All *H. influenzae* isolates were serotyped. The minimal inhibitory concentrations for various antibiotics were determined. Pulsed-field gel electrophoresis (PFGE) was used for clonal analysis.

Results: Among 511 children, 269 (52.6%) had been vaccinated with at least one dose of *H. influenzae* type b (Hib) conjugate vaccine, 236 (46.2%) were unvaccinated and 6 (1.2%) had no vaccination records available. Twenty six *H. influenzae* strains were isolated. There were three Hib isolates and the others were nontypeable *H. influenzae* (NTHi). The carriage rate for Hib was 0.6% (3/511) and of NTHi was 5% (23/511). Three (1.27%) of the 236 unvaccinated children were carriers of Hib, whereas none of the 269 vaccinated children carried Hib. Two out of the three Hib isolates and 14 (60.9%) of 23 NTHi isolates were ampicillin-resistant. Multidrug resistance was found in 7 (26.9%) of the isolates. Among the isolates, 61.5% were beta-lactamase producers; there were no beta-lactamase-negative ampicillin-resistant isolates. The PFGE restriction patterns showed a wide diversity of genotypes.

Conclusions: There is very low nasopharyngeal carriage of Hib among children younger than 5 years in northern Taiwan. This may explain why the incidence of invasive Hib disease is also low in Taiwan. In addition, we found a high prevalence of beta-lactamase-positive ampicillin-resistant nasopharyngeal *H. influenzae* isolates.

Key words: Carrier state; Drug resistance, microbial; *Haemophilus influenzae*; *Haemophilus influenzae* type b; Nasopharynx; Vaccines, conjugate

Introduction

Haemophilus influenzae is a major bacterial pathogen and causes a variety of community-acquired infections, such as meningitis, septicemia, epiglottitis, cellulitis, septic arthritis, pneumonia, otitis media, sinusitis and a range of other respiratory tract infections among children [1]. *H. influenzae* can be divided into two groups based on the presence or absence of a capsule. Encapsulated *H. influenzae* is made up of six serotypes, a to f, which are defined by their structurally and serologically distinct capsular polysaccharides. The majority of invasive infections related to this species are caused by *H. influenzae* type b (Hib) [2]. Hib has been shown to be the leading cause of bacterial meningitis

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in children younger than 5 years old [3]. Previous surveillance data in Taiwan revealed a low incidence of invasive Hib diseases (1.6-1.9/100,000 population younger than 5 years of age) compared to western and most Asian countries [3-5]. The reason for the low incidence is still unclear. Although the incidence is very low, these diseases have a high rate of mortality (~10%) and neurological sequelae (~40%) [3], and this results in increased medical and social costs.

The most effective preventive method is vaccination against this disease. The conjugate Hib vaccine had been in use for over 10 years in western countries and has had a dramatic effect in terms of preventing invasive Hib diseases [6]. In Taiwan, this vaccine has been licensed since 1993 and is not provided free by the public health system. Up to the present, there are only limited data to support the hypothesis that this vaccine is acting to prevent invasive Hib diseases in Taiwan [7].

We conducted this study to define the prevalence of *H. influenzae* colonization among children younger than 5 years old in northern Taiwan, and its association with a low incidence of invasive Hib disease. In addition, antibiotic susceptibility, serotype and the clonal relationship of the isolates were determined. The results of this study will provide information on the treatment of *H. influenzae* infection and the appropriateness of universal Hib vaccination in Taiwan.

Methods

Study population

Nasopharyngeal swab specimens were obtained from 511 children. The survey was conducted from January 2003 to December 2003. The study population comprised children attending day care centers and who had sought medical care for either a non-infectious disease or routine vaccination at a tertiary referral center in northern Taiwan. The study was approved by the institutional review board of Tri-Service General Hospital. Informed written consent for participation in the study was obtained from the children's parents or guardians. A questionnaire was completed for each participant providing information regarding the child's age, gender, family size, history of recent respiratory infection, recent antibiotic therapy and vaccine status (in particular, the Hib conjugate vaccine). Children aged less than 5 years were recruited. Patients with a history of respiratory tract infection and antibiotic use during the last month were excluded.

Sampling and microbiological methods

Nasopharyngeal swab specimens for culture were collected by a single investigator using a flexible calcium alginate-tipped metal swab (Copan Diagnostics Corona, CA, USA) placed 2 to 4 cm into the nasopharynx. The specimens were immediately inoculated onto chocolate agar. Cultures were incubated in 5% carbon dioxide at 37°C for 24-48 h. *H. influenzae* was identified by colony morphology, growth on chocolate agar, and requirement for X (hemin) and V (nicotinamide adenine dinucleotide) factors. Bacto *H. influenzae* anti-sera (Difco, Detroit, MI, USA) were used to separate typeable from non-typeable isolates. Typeable isolates were further serotyped by a slide agglutination test with type b antisera (Difco) to further separate them into type b and non-type b. The isolates were stored at –70°C in trypticase soy broth with 15% glycerol for further testing.

Antimicrobial susceptibility testing

Susceptibility tests for the isolates against 12 antibiotics were performed by Etest (AB Biodisk, Solna, Sweden) on HTM agar at 35°C under 5% carbon dioxide, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and the manufacturer's directions [8]. The antimicrobial agents tested were ampicillin, amoxicillin-clavulanate, cefaclor, cefuroxime, cefixime, ceftriaxone, trimethoprim-sulfamethoxazole (TMP-SMZ; cotrimoxazole), rifampin, chloramphenicol, tetracycline, ciprofloxacin and azithromycin. The minimal inhibitory concentration (MIC) was defined as the intercept of the zone of inhibition with the graded Etest strip. *H. influenzae* American Type Culture Collection (ATCC; Rockville, MD, USA) 49776 and ATCC 49247 were used as control strains for each set of tests. Strains were interpreted as sensitive, intermediate or resistant, according to the CLSI criteria [8].

Pulsed-field gel electrophoresis

Total DNA was prepared and pulsed-field gel electrophoresis (PFGE) was performed as described previously [5,9]. The DNA was digested using *Sma*I restriction enzyme (Biolabs, Beverly, MA, USA) at the manufacturer's suggested temperature. Restriction fragments were separated by PFGE on agarose 1% gels (Bio-Rad, Hercules, CA, USA) in 0.5 × TBE (tris-borate-ethylenediamine tetra-acetic acid) buffer using the BioRad CHEF Mapper apparatus (BioRad). Electrophoresis was performed for 31.5 h at 14°C at 6.0 V/cm

with a ramped pulse time of 1 to 30 sec. Gels were then stained with ethidium bromide and photographed under ultraviolet light.

The band patterns were compared visually and classified as indistinguishable (clonal), closely related (clonal variants, three or fewer band differences), possibly related (four to six band differences) and unrelated (over six band differences), according to previously described criteria [5,10]. Genetic similarities between strains were calculated by the unweighted pair-group method with arithmetic mean and were shown as a dendrogram. Similarity was adjusted using Jaccard's coefficients with a tolerance of 0.5%. The program for this calculation was supplied by BioRad.

Detection of beta-lactamase

All ampicillin-resistant isolates were tested for the production of beta (β)-lactamase with a nitrocefin-containing identification stick (BR66A; Oxoid, Cambridge, UK).

Statistical analysis

Results were analyzed using the Statistical Package for the Social Sciences for Windows (Version 10.0; SPSS, Chicago, IL, USA) software package. Differences were evaluated using the Fisher's exact test or chi-squared test with Yate's correction. A p value of <0.05 was considered significant.

Results

From January 2003 to December 2003, 511 healthy children aged between 6 days and 4 years 11 months were enrolled in this study. Of the 511 children, 276 were male (54%) and 235 were female (46%). No significant difference in the *H. influenzae* carriage rate was found between males (5.43%) and females (4.68%). 269 children (52.6%) had been vaccinated with at least one dose Hib conjugate vaccine, 236 (46.2%) had not been vaccinated and 6 (1.2%) had no vaccination records available. Twenty six *H. influenzae* isolates were obtained from the 511 specimens. Among these strains, three isolates were Hib and the others were non-typeable *H. influenzae* (NTHi), giving a carriage rate for Hib of 0.6% and for NTHi of 5% (Table 1). The carriage rate for Hib was higher in children aged under 2 months (p=0.24), while the carriage rate for NTHi was higher in children aged between 2 and 5 years (p=0.07) [Fig. 1]. Three (1.27%) of the 236 unvaccinated children were carriers of Hib, whereas none of the 269 children who had received Hib conjugate vaccine carried Hib (p=0.12) [Table 2]. Ten (3.72%) of the 269 children who had received Hib conjugate vaccine showed NTHi carriage and eleven (4.66%) of the 236 unvaccinated children were carriers of NTHi (p=0.21).

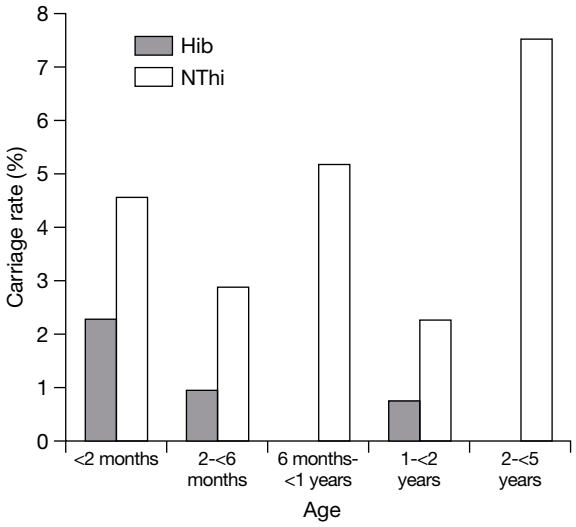

Fig. 1. Age distribution for the nasopharyngeal carriage rate of *Haemophilus influenzae*. Hib = *H. influenzae* type b; NTHi = non-typeable *H. influenzae*.

Antibiotic susceptibility analysis showed that two (66.7%) of the three Hib isolates were ampicillin resistant and one (33.3%) of Hib isolates was resistant

Table 1. Age distribution of the 26 *Haemophilus influenzae* isolates from Taiwanese children and the *H. influenzae* type b (Hib) conjugate vaccine status of these children

Age group	Number of children	Hib conjugate vaccine			Serotype of isolates	
		Yes	No	NA	Type b	Non-typeable
<2 months	44	0	44	0	1	2
2-<6 months	104	57	47	0	1	3
6 months-<1 year	97	72	24	1	0	5
1-<2 years	133	82	48	3	1	3
2-<5 years	133	58	73	2	0	10
Total	511	269	236	6	3	23

Table 2. Vaccination status versus nasopharyngeal colonization with *Haemophilus influenzae*

Hib vaccination	Hib carriage rate	Non-typeable *H. influenzae* carriage rate
Yes[a] (n = 269) [%]	0.00	3.72
No (n = 236) [%]	1.27	4.66
p	0.12	0.21

[a]At least one dose of *H. influenzae* type b (Hib) conjugate vaccine.

to TMP-SMZ, chloramphenicol and tetracycline. Amoxicillin-clavulanate, cefaclor, cefuroxime, cefixime and ceftriaxone were active against all the Hib isolates. In total, 14 of the NTHi isolates (60.9%) were resistant to ampicillin, 11 isolates (47.9%) were resistant to TMP-SMZ, 6 isolates (26.1%) were resistant to chloramphenicol, 6 (26.1%) were resistant to tetracycline, two isolates (8.7%) were resistant to azithromycin and one (4.3%) was resistant to rifampin (Table 3).

In this study, the overall frequency of *H. influenzae* resistance to ampicillin was 61.5%, compared to 42.3% for TMP-SMZ, 26.9% for chloramphenicol, 26.9% for tetracycline, 7.7% for azithromycin and 3.8% for rifampin. Among the isolates that were ampicillin resistant (61.5%), all were β-lactamase producers. The rates of β-lactamase production were not significantly different among type b (2/3; 66.7%) and non-typeable isolates (14/23; 60.9%). The β-lactamase-positive isolates had higher resistance rates to TMP-SMZ (56.2%), tetracycline (43.7%) and chloramphenicol (43.7%) than the β-lactamase-negative strains (20%, 0% and 0%, respectively) [Table 4]. Multiply resistant strains of *H. influenzae* were also identified; seven (26.9%) of the 26 isolates were resistant to three drugs (ampicillin, chloramphenicol and tetracycline) and four (15.4%) were resistant to four drugs (ampicillin, chloramphenicol, tetracycline and TMP-SMZ). No β-lactamase-negative, ampicillin-resistant (BLNAR) strains were identified.

When analyzed by PFGE, the 23 NTHi isolates showed a heterogeneous pattern of DNA fingerprints. There were, altogether, 18 different pulsed field gel patterns among the 23 NTHi isolates, with only one set of strains (4/23, 17.4%) clustering with homology greater than 80% (Fig. 2A). Each of the three Hib isolates was unique in terms of its pulsed field gel pattern and therefore these strains were non-clonal (Fig. 2B). The dendrogram generated in this study shows a very extensive branching pattern, which suggests a wide genetic diversity among the *H. influenzae* isolates identified by this study.

Discussion

Colonization of the human nasopharynx is the first step in a sequence of events that may result in *H. influenzae* infection and disease [11]. Previous surveys have documented *H. influenzae* point prevalence carriage rates among various populations in the range 11% to 88% [12-14]. These rates are probably mainly affected by social and geographic factors, such as day care center attendance, living in a crowded area and having unvaccinated siblings in the family [11,15]. Carriage rates for Hib among young children, prior to the widespread use of the Hib conjugate vaccines,

Table 3. Minimal inhibitory concentration (MIC; μg/mL) parameters and sensitivity data for the 23 non-typeable *Haemophilus influenzae* isolates to 12 antimicrobial agents

Antimicrobial agent	MIC_{50}	MIC_{90}	MIC range	Number of isolates (%)		
				Sensitive	Intermediate	Resistant
Ampicillin	8.00	16.00	0.25-64.00	9 (39.1)	0 (0.0)	14 (60.9)
Amoxicillin-clavulanate	1.00	1.00	0.12-16.00	23 (100.0)	0 (0.0)	0 (0.0)
Cefaclor	2.00	4.00	0.25-8.00	23 (100.0)	0 (0.0)	0 (0.0)
Cefuroxime	0.50	2.00	0.06-4.00	23 (100.0)	0 (0.0)	0 (0.0)
Cefixime	0.03	0.06	0.03-0.25	23 (100.0)	0 (0.0)	0 (0.0)
Ceftriaxone	0.03	0.03	0.03-0.06	23 (100.0)	0 (0.0)	0 (0.0)
Trimethoprim-sulfamethoxazole	0.25	64.00	0.06-64.00	12 (56.2)	2 (8.7)	9 (39.2)
Rifampin	0.12	0.25	0.12-16.00	22 (95.7)	0 (0.0)	1 (4.3)
Chloramphenicol	0.50	8.00	0.12-4.00	17 (73.9)	1 (4.3)	5 (21.7)
Tetracycline	0.50	8.00	0.05	17 (73.9)	2 (8.7)	4 (17.4)
Ciprofloxacin	0.0150	0.03	0.00375-0.06	23 (100.0)	0 (0.0)	0 (0.0)
Azithromycin	2.00	4.00	0.25-8.00	21 (91.3)	0 (0.0)	2 (8.7)

Abbreviations: MIC_{50} = MIC inhibiting 50% of isolates; MIC_{90} = MIC inhibiting 90% of isolates

Table 4. Minimal inhibitory concentration (MIC; μg/mL) parameters and percentage susceptibilities for *Haemophilus influenzae* according to the presence of beta (β)-lactamase

Antimicrobial agent	β-Lactamase-negative [n = 10]				β-Lactamase-positive [n = 16]			
	MIC_{50}	MIC_{90}	Range	S (%)	MIC_{50}	MIC_{90}	Range	S (%)
Ampicillin	0.25	0.50	0.25-1.00	100.0	8.00	32.00	2.00-64.00	0.0
Amoxicillin-clavulanate	0.25	2.00	0.06-4.00	100.0	0.50	2.00	0.06-4.00	100.0
Cefaclor	1.00	8.00	0.25-8.00	100.0	2.00	4.00	1.00-8.00	100.0
Cefuroxime	0.50	1.00	0.12-1.00	100.0	1.00	2.00	0.50-4.00	100.0
Cefixime	0.03	0.12	0.03-0.25	100.0	0.06	0.06	0.03-0.12	100.0
Ceftriaxone	0.03	0.06	0.03-0.06	100.0	0.03	0.03	0.03-0.03	100.0
Trimethoprim-sulfamethoxazole	0.12	16.00	0.12-64.00	80.0	4.00	64.00	0.06-64.00	43.8
Rifampin	0.12	0.25	0.12-0.50	100.0	0.12	0.25	0.06-16.00	93.8
Chloramphenicol	0.50	0.50	0.12-0.50	100.0	0.50	8.00	0.12-32.00	56.3
Tetracycline	0.50	0.50	0.50-1.00	100.0	0.50	16.00	0.50-32.00	56.3
Ciprofloxacin	0.0075	0.0300	0.0038-0.0300	100.0	0.0150	0.0300	0.0038-0.06	100.0
Azithromycin	4.00	4.00	0.25-8.00	90.0	2.00	4.00	0.50-8.00	93.8

Abrreviations: MIC_{50} = MIC inhibiting 50% of isolates; MIC_{90} = MIC inhibiting 90% of isolates; S = susceptible

ranged from 3% to 5% [15,16]. Children aged less than 5 years are at highest risk of Hib disease and in most studies have the highest prevalence of carriage. Since the advent of Hib conjugate vaccine, the nasopharyngeal Hib carriage rate has decreased to <1% of vaccinated individuals [16]. There has been no previous surveillance of the nasopharyngeal colonization by *H. influenzae* in Taiwan. Also, it is not known how partial Hib vaccination would have impacted on nasopharyngeal carriage status in Taiwan. Our study showed that the carriage rates for both Hib and NTHi in Taiwanese children younger than 5 years old are significantly lower than those observed in populations in most parts of the world [12-16]. Our findings revealed a remarkably low nasopharyngeal carriage rate for Hib, which is comparable with the post-vaccination carriage rate in western countries [11,16]. A similarly low carriage rate for Hib has been reported in Hong Kong and Japan [17,18]. Many possible factors, including sampling or laboratory error, antibiotic overuse, vaccination and a low colonization rate, may contribute to this phenomenon [11,13-16]. Sampling or laboratory error is unlikely because of the experienced staff and the well-developed techniques used in this study.

Previous hospital-based surveys in Taiwan have revealed an annual incidence of invasive Hib disease ranging from 1.6 to 1.9 per 100,000 children younger than 5 years old, which is much lower than in western and most other Asian countries [3,4]. Invasive *H. influenzae* diseases have been a notifiable infectious diseases since 2000. According to the statistical data from the National Notifiable Disease Surveillance System of Centers for Disease Control, Taiwan, confirmed Hib cases under 5 years old decreased progressively between 2001 and 2005, with 35, 28, 12, 11 and 8 cases in each sequential year, respectively. The annual incidences of Hib disease between 2001 and 2005 also declined (2.45, 2.07, 0.91, 0.88 and 0.70 cases per 100,000 population younger than 5 years old, respectively) [19]. Therefore, the low Hib nasopharyngeal carriage rate appears to reflect the morbidity rates. We also found no nasopharyngeal Hib carriers among the vaccinated group, whereas there were three Hib carriers (1.27%) in the unvaccinated group. Hib vaccination thus seems to reduce colonization, although not that significantly, as it probably depends on multiple factors. We have identified at least 2 reasons for this. First, the pre-existing low carriage rate for Hib in our population; however, we have no surveillance data on Hib colonization prior to introduction of the Hib vaccine. Second, it is known that the widespread use of Hib conjugate vaccine is able to dramatically decrease Hib colonization and transmission in industrialized countries [11,16]. The Hib vaccination coverage rate was 52.6% in our Taiwanese community; this had not eliminated carriage but had probably contributed to a lower carriage prevalence. The more than 50% vaccine coverage may have induced effective herd immunity. Further large-scale surveillance in Taiwan is necessary to elucidate the relationship between Hib conjugate vaccination and carriage status.

The prevalence of invasive Hib disease was two-fold higher in the male population during the period 2001 to 2005 [19], but we found no gender differences in terms of colonization by *H. influenzae*. The carriage

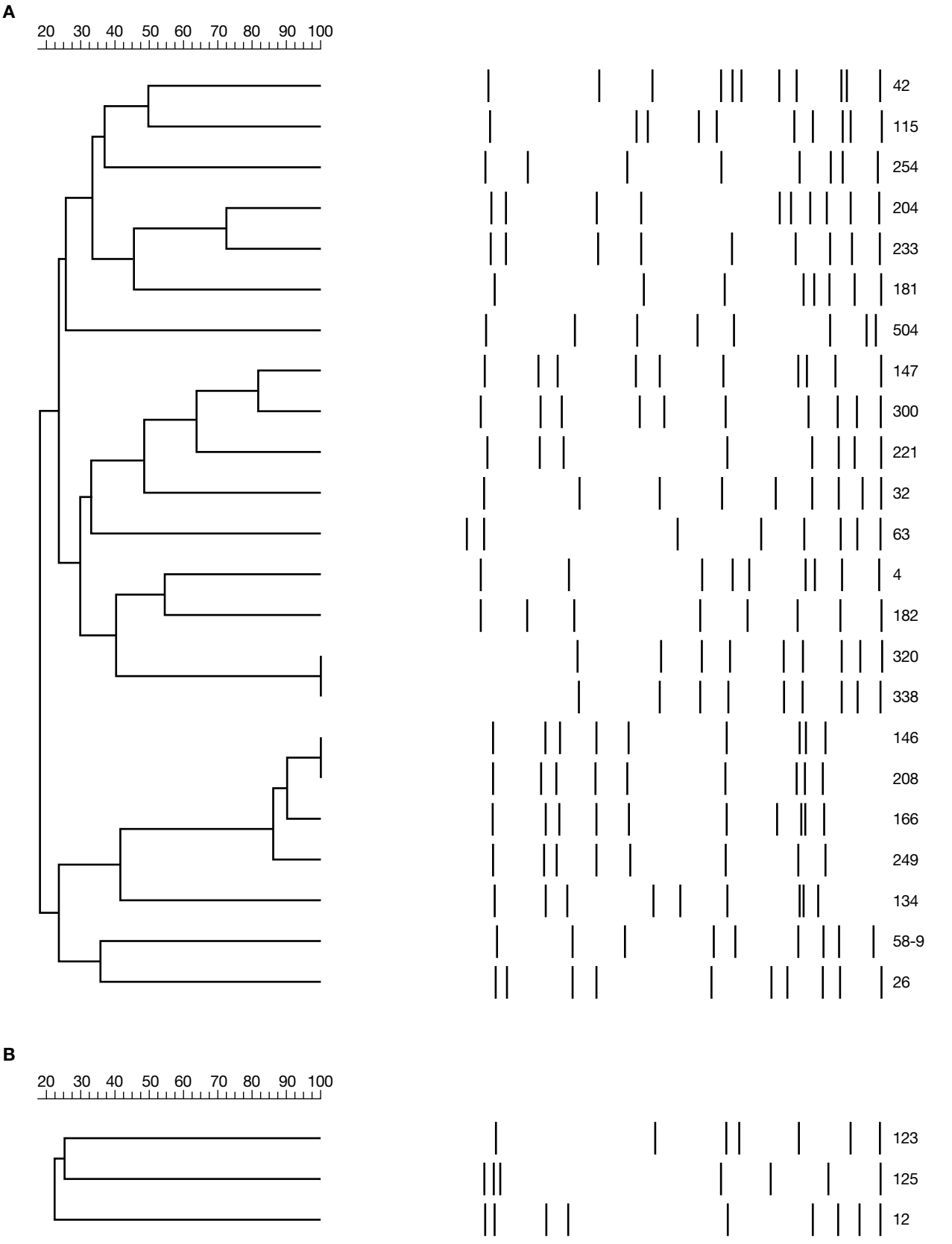

Fig. 2. Pulsed-field gel electrophoresis analysis dendrogram showing the genetic relationships among the 23 strains of non-typeable *Haemophilus influenzae* (A) and 3 strains of *H. influenzae* type b (B) isolated from children <5 years old.

rate for NTHi was marginally significantly higher in children aged between 2 and 5 years old (p=0.07) in our study. The increased carriage rates are probably due to increased exposure resulting from crowding in day care centers; this effect has been previously suggested to explain the higher carriage rates in children with more siblings [14]. Capsule replacement in *H. influenzae* disease has been reported in at least two countries (Brazil and Portugal) after extensive use of the Hib vaccine [20,21]. We did not identify any serotype replacement with other non-type b *H. influenzae*.

Since ampicillin-resistant *H. influenzae* due to β-lactamase production was first reported in 1974, emerging resistance to tetracycline, chloramphenicol, TMP-SMZ, erythromycin, cefaclor and rifampin have subsequently been reported [22]. Previous studies have shown a high prevalence of ampicillin resistance among clinical *H. influenzae* strains in Taiwan (58.1% between 1994 and 1995, 59.5% between 1996 and 1998, 58% in the Study for Monitoring Antimicrobial Resistance Trends [SMART] 1998-1999 and 70% in the SMART study 2002) [22-24]. The Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin (PROTEKT) study reported ampicillin-resistant *H. influenzae* rates of 64.7% in South Korea, 17.1% in Hong Kong and 8.5% in Japan [25]. The highest prevalence of ampicillin-resistant isolates of *H. influenzae* in North America was 27.9% in the SENTRY Antimicrobial Surveillance Program [26]. In our study, the colonizing *H. influenzae* isolates also showed a high rate of resistance to ampicillin (61.5%), and this was comparable to previous clinical surveys in Taiwan [22-25]. Chloramphenicol-resistant *H. influenzae* isolates are still rare in most parts of the world, accounting for 0.2% of isolates in North America and 4.7% in Turkey [26]. In Taiwan, varying frequencies of chloramphenicol-resistant *H. influenzae* have been reported and range between 20.6% and 51% [22,24]. We found a 26.9% rate of chloramphenicol resistance among the *H. influenzae* in the nasopharynx, but these possessed lower resistance than clinical isolates.

TMP-SMZ-resistant *H. influenzae* has been reported in Taiwan at frequencies between 33.8% and 63% [22-24]. TMP-SMZ resistance is most widespread in Brazil (70.0%), Mexico (56.3%) and South Korea (51.5%) [25]. The rate of resistance to TMP-SMZ in our study (42.3%) was higher than reported in North America and could be a consequence of the widespread use of TMP-SMZ for the treatment of upper respiratory tract infections, including otitis media and sinusitis [22]. An increasing prevalence of clinical *H. influenzae* isolates resistant to azithromycin in Taiwan has been reported, up from 6.7% in 1994-1995 to 31% in 1998-1999, which is higher than most regions of the world [22,23,25]. Overall, two of our isolates (7.7%) showed resistance to azithromycin. Probably of greatest concern, however, is the increasing prevalence of strains with intermediate resistance to clarithromycin (31-32%), as shown by the subtle shift in the MIC distribution [23-25]. Emergence of azithromycin-resistant strains may have important implications for other macrolide antibacterials, as such isolates may also show resistance to other macrolides. This pattern of cross-resistance has been observed for *S. pneumoniae*, although the mechanism that confers macrolide resistance in *H. influenzae* remains to be elucidated [23,24]. Our findings highlight the need for physicians to be aware of local resistance patterns to ensure judicious use of these macrolides. The impact of the government's policy to restrict antibiotic prescriptions for acute upper respiratory tract infections without evidence of bacterial infection has been implemented since 2001 and further surveys on antibiotic resistance in *H. influenzae* are warranted.

No BLNAR strains were identified among the nasopharyngeal isolates. However, the increasing prevalence of BLNAR isolates among invasive strains of *H. influenzae* from 1.7% to 3.4% is alarming, although the rate is still low [23,24]. The BLNAR strain involves a decreasing affinity of penicillin-binding proteins to β-lactams, caused by conformational changes with genetic mutations, and this non-β-lactamase-mediated resistance usually increases the MICs for amoxicillin-clavulanate and third-generation cephalosporins [24,27].

In this study, we documented a high prevalence of multiple drug-resistant strains of *H. influenzae* among the nasopharyngeal isolates from northern Taiwan. β-Lactamase-positive strains are often resistant to multiple drugs [25]. The prevalence of β-lactamase-producing strains is also higher than in most parts of the world [23-25]. Production of plasmid-mediated TEM-1 β-lactamase is the primary mechanism of resistance to ampicillin and ROB-1 β-lactamase-producing strains remain rare in Taiwan [27].

Clonal analysis by PFGE revealed a heterogeneous profile for our nasopharyngeal isolates, and this supports the hypothesis that no endemically colonized strain of either Hib or NTHi has spread across our

community. Previous epidemiological studies of clinical *H. influenzae* isolates have also concluded that there is possible spread of invasive *H. influenzae* type b, but no clonal spread of non-type b *H. influenzae* in Taiwan [5]. An analysis using a longitudinal study carried out in children attending day care centers in three distinct geographic areas has shown that colonization by *H. influenzae* appears to be a dynamic process involving a high degree of genomic heterogeneity among the non-typeable colonizing strains in any given geographic area [13].

Our data indicate a remarkably low nasopharyngeal carriage rate of *H. influenzae* among children younger than 5 years in northern Taiwan. This may explain why the incidence of invasive Hib disease was also low in Taiwan. However, the results of this study cannot be generalized, as it was a cross-sectional study involving a selected community in a particular area of Taiwan. A more comprehensive longitudinal study involving a larger population and distinct geographic areas should be conducted in order to represent the Taiwanese population.

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