Possible High Rate of Transmission of Nontypeable *Haemophilus influenzae*, Including β -Lactamase-Negative Ampicillin-Resistant Strains, between Children and Their Parents

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The possible transmission of nontypeable *Haemophilus influenzae* between children and their parents was evaluated in 18 pairs of subjects from 15 families. Of the 33 isolates, 31 were found to be β -lactamase negative, including 10 β -lactamase-negative, ampicillin (AMP)-resistant (BLNAR) strains (AMP MIC, ≥ 1.0 $\mu\text{g/ml}$) and 2 were β -lactamase producing. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that 10 BLNAR isolates had 6 patterns, 23 non-BLNAR isolates had 13 patterns, and these patterns were different from each other, except for 1 pattern. As a result, the PFGE patterns in 14 of 18 pairs were indistinguishable and those in 4 pairs were different. These data indicate a possible high rate of intrafamilial transmission of nontypeable *H. influenzae*, including BLNAR strains, between children and their parents.

Nontypeable *Haemophilus influenzae* is a nonencapsulated, gram-negative, pleomorphic, rod-shaped bacterium that colonizes the human nasopharynx and can cause a variety of infections, including otitis media, sinusitis, bronchitis, and conjunctivitis (9). Children are frequent carriers of *H. influenzae*, and the rate of carriage is high in infancy. Their colonization may subsequently lead to the development of an invasive disease (3, 14). It has recently been reported that the prevalence of β -lactamase-negative, ampicillin (AMP)-resistant (BLNAR) strains (AMP MIC, ≥ 1.0 $\mu\text{g/ml}$) has increased in Japan (16) and Spain (8), although their global prevalence remains low (5, 6). It has already been reported that children can acquire *H. influenzae* at day care centers (12, 17, 20). However, it is not clear whether *H. influenzae*, when colonizing the upper respiratory tract, can be transmitted between children and their parents and cause invasive diseases. To address this issue, we conducted the following prospective study.

New patients with an invasive infection (e.g., pharyngitis, sinusitis, otitis media) caused by *H. influenzae* who visited the Sugita Otorhinolaryngologic Clinic from February to June 2001 were asked to bring their families as soon as possible for a clinical examination and collection of biological specimens (e.g., nasopharynx, middle ear, middle natus). Thirty-three *H. influenzae* strains that were collected from 18 pairs of children (mean age, 2.1 years) and parents (mean age, 33.1 years) in 15 families were evaluated. *H. influenzae* isolates were serotyped by the capsular-swelling technique with antisera purchased from Difco Laboratories (Detroit, Mich.), and β -lactamase production was detected by a disk impregnated with nitrocefin (Becton Dickinson, Sparks, Md.).

MICs were determined by the agar dilution method in ac-

cordance with the guidelines of the National Committee for Clinical Laboratory Standards (11). The MICs of the following four antibiotics for 33 *H. influenzae* isolates were tested: AMP (Meiji Seika Kaisha, Tokyo, Japan), amoxicillin-clavulanic acid (GlaxoSmithKline, Tokyo, Japan), cefaclor (Shionogi Co., Osaka, Japan), and cefditoren (Meiji Seika Kaisha). Pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I (Takara Shuzo Co., Shiga, Japan) was performed for the 33 *H. influenzae* isolates as described previously (20), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (18).

Thirty-three *H. influenzae* strains were isolated from the nasopharynx ($n = 19$), middle natus ($n = 12$), and middle ear ($n = 2$), and were all nontypeable. Twenty-five strains were detected in patients with invasive infections, and eight strains colonized the nasopharynx without causing any symptoms. Of the 33 isolates, 31 were β -lactamase negative, including 10 BLNAR strains (AMP MIC, ≥ 1.0 $\mu\text{g/ml}$), and 2 were β -lactamase producing (Table 1). Molecular typing of the 33 isolates by PFGE showed that 10 BLNAR isolates had 6 patterns, A, C, and E to H; 21 β -lactamase-negative, AMP-susceptible isolates (AMP MIC, ≤ 0.5 $\mu\text{g/ml}$) had 12 patterns, B, D, G, and I to Q; and 2 β -lactamase-producing isolates had pattern R. Of the 18 pairs from children and their parents, the PFGE patterns of 14 pairs (77.8%) were indistinguishable and those of 4 pairs (22.2%) were different (Table 1 and Fig. 1). Assuming that there are 18 types of PFGE patterns occurring randomly and independently with the same probability (1 in 18), the probability of finding 14 or more identical pairs out of 18 by chance is 8.16×10^{-15} . Considering that more types of PFGE patterns may exist, the 14 identical sets of PFGE patterns out of 18 pairs cannot be obtained by chance ($P < 0.001$).

Infants and young children tend to acquire *H. influenzae* in the upper respiratory tract because of their low immunity (20), and their colonization can become a risk factor for invasive diseases caused by *H. influenzae* (3, 14). In particular, nontype-

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TABLE 1. Epidemiological characteristics of nontypeable *H. influenzae* from children and their parents

Family and strain no.	Age ^a	Specimen	Infection(s)	Date	β-Lactamase	MIC (μg/ml)				PFGE pattern
						AMP	AMC ^e	CEC ^f	CDN ^g	
a										
1	4 (52)	Nasopharynx	ABS ^b	4/7/2001	—	2	2	16	1	A
2	28	Nasopharynx	AP ^c	4/17/2001	—	2	2	16	1	A
b										
3	3 (45)	Middle nutus	ABS, AOM ^d	3/19/2001	—	0.125	0.25	0.5	0.004	B
4	38	Nasopharynx	Colonization	3/19/2001	—	1	0.5	16	0.25	C
c										
5	0 (4)	Nasopharynx	AOM	6/15/2001	—	0.5	0.25	16	0.016	D
6	4 (49)	Middle nutus	ABS	6/15/2001	—	0.5	1	16	0.125	D
7	34	Nasopharynx	Colonization	6/16/2001	—	0.5	1	16	0.016	D
d										
8	1 (19)	Middle nutus	ABS	6/13/2001	—	2	2	64	0.5	E
9	30	Nasopharynx	AP	5/31/2001	—	2	2	64	0.5	E
10	32	Middle nutus	ABS	6/2/2001	—	2	2	64	0.5	E
e										
11	6 (78)	Middle nutus	ABS	2/27/2001	—	1	0.5	8	0.016	F
12	36	Nasopharynx	Colonization	3/10/2001	—	1	0.5	8	0.016	F
f										
13	2 (26)	Middle nutus	ABS	5/31/2001	—	1	1	16	0.125	G
14	31	Nasopharynx	AP	5/31/2001	—	0.5	1	16	0.125	G
g										
15	4 (59)	Nasopharynx	ABS	2/27/2001	—	0.125	0.125	0.5	0.004	B
16	41	Nasopharynx	AP	2/27/2001	—	2	2	64	0.5	H
h										
17	1 (22)	Middle nutus	ABS	5/25/2001	—	0.25	0.25	4	0.016	I
18	32	Nasopharynx	Colonization	6/7/2001	—	0.25	0.25	4	0.016	I
i										
19	3 (46)	Middle nutus	ABS	3/17/2001	—	0.125	0.125	0.5	0.016	J
20	38	Nasopharynx	Colonization	3/27/2001	—	0.125	0.25	0.5	0.016	K
j										
21	0 (11)	Nasopharynx	AOM and AP	3/10/2001	—	0.125	0.125	0.5	0.016	L
22	33	Nasopharynx	Colonization	3/17/2001	—	0.5	0.5	4	0.016	M
k										
23	2 (26)	Nasopharynx	Colonization	6/6/2001	—	0.25	0.25	0.5	0.032	N
24	4 (51)	Nasopharynx	Colonization	6/6/2001	—	0.25	0.25	0.5	0.032	N
25	30	Nasopharynx	AP	6/6/2001	—	0.25	0.25	0.5	0.032	N
l										
26	0 (11)	Middle nutus	ABS, AOM	6/16/2001	—	0.25	0.25	1	0.016	O
27	27	Middle nutus	ABS	6/18/2001	—	0.25	0.25	1	0.016	O
m										
28	1 (14)	Middle ear	AOM	3/12/2001	—	0.25	0.25	1	0.032	P
29	35	Nasopharynx	AP	3/7/2001	—	0.5	0.25	1	0.063	P
n										
30	1 (12)	Middle nutus	ABS	3/23/2001	—	0.125	0.125	0.5	0.016	Q
31	35	Nasopharynx	AP	3/16/2001	—	0.125	0.25	0.5	0.016	Q
o										
32	0 (11)	Middle ear	AOM	6/8/2001	+	64	0.5	0.5	0.032	R
33	30	Middle nutus	ABS	6/8/2001	+	64	0.5	0.5	0.032	R

^a Ages are given in years for adults and in years (months) for children.^b ABS, acute bacterial sinusitis.^c AP, acute pharyngitis.^d AOM, acute otitis media.^e AMC, amoxicillin-clavulanic acid.^f CEC, cefaclor.^g CDN, cefditoren.

FIG. 1. (A) PFGE patterns of *Sma*I-digested DNAs of *H. influenzae* isolates from children and their parents in seven families (a to g). The patterns of *H. influenzae* isolates in children and their parents are indistinguishable in families a, c, d, e, and f and different in families b and g. Lanes M contained a molecular size marker. (B) PFGE patterns of *Sma*I-digested DNAs of *H. influenzae* isolates from children and their parents in eight families (h to o). The patterns of *H. influenzae* isolates from children and their parents are indistinguishable in families h, k, l, m, n, and o and different in families i and j.

able *H. influenzae* often causes otitis media, which leads to loss of hearing as a complication at a critical stage in the development of speech, language, and cognitive abilities in children (2, 4). In our study, when patients with invasive infections caused by nontypeable *H. influenzae* appeared, infants and young children in the family colonized by nontypeable *H. influenzae* with and without infection and the possible high rate of correlation between children and their parents were confirmed by PFGE. In addition, some investigators have reported the presence of multiple strains in the same child's throat or nasopharynx isolates (15, 19). In this respect, the mismatched one might be tested instead of the matching one, even in discordant pairs. Therefore, our data reveal that infants and young children can acquire nontypeable *H. influenzae* not only at day care centers (12, 17, 20) but also at home. Moreover, 10 (30.3%) were BLNAR strains, which had six PFGE patterns and five of the six patterns were different from those of non-BLNAR isolates. In Japan, BLNAR strains are increasing rapidly in prevalence and are frequently detected in pretreated patients (16). Our data also provide evidence that at least some types of BLNAR strains are spreading. Pneumococcal and *H. influenzae* type b conjugate vaccine appears to be effective not only for the prevention of invasive disease but also in the reduction of nasopharyngeal carriage in young children (1, 7, 13). On the other hand, a vaccine to prevent infections caused by nontypeable *H. influenzae* is not available, although several laboratories have active programs with the goal of developing such a vaccine (2, 10).

In conclusion, our results demonstrated a possible high rate of intrafamilial transmission of nontypeable *H. influenzae*, including BLNAR strains, between children and their parents. Therefore, the development of highly effective vaccines for young children is important for the prevention of invasive diseases caused by nontypeable *H. influenzae*.

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