SHORT ARTICLE

Clonal spread of an invasive strain of *Haemophilus influenzae* type b among nursery contacts accompanied by a high carriage rate of non-disease-associated strains

MITSUMASA SAITO*†, KENJI OKADA†, KOUICHI TAKEMORI‡ and SHIN-ICHI YOSHIDA*

Departments of *Bacteriology and †Paediatrics, Graduate School of Medical Sciences, Kyushu University and ‡Department of Clinical Laboratory, Kyushu University Hospital, Fukuoka 812-8582, Japan

***Haemophilus influenzae* carriage was examined in unvaccinated nursery contacts of a patient with *H. influenzae* type b (Hib) meningitis and isolates were typed by pulsed-field gel electrophoresis (PFGE). Nasopharyngeal isolates were classified into eight PFGE patterns. Seven Hib carriers were found among 15 nursery contacts. The isolates from the carriers showed a PFGE pattern identical to that of the meningitis strain. The carrier rate of non-disease-associated strains was also high (47%, 7 of 15). This study suggests that the clonal spread of invasive (serotype b) *H. influenzae* strains is accompanied by a high carriage rate of non-disease-associated strains.**

Introduction

Haemophilus influenzae type b (Hib) is a common cause of meningitis and other serious infections in children. It has been reported that the Hib colonisation rate among children at day care centres during outbreaks of Hib disease increases significantly [1, 2]. It is conceivable that this increase is the result of clonal dissemination, a notion that needs to be confirmed by genetic typing of isolates. An earlier study found that pulsed-field gel electrophoresis (PFGE) analysis was useful in the epidemiological study of *H. influenzae* infection [3]. In October 1996, Hib meningitis occurred in a nursery in Saga City, Japan. The present study determined the rate of carriage of *H. influenzae* among the nursery contacts and analysed the isolates by PFGE. This is the first molecular epidemiological study of Hib carriers and carriers of non-disease-associated *H. influenzae* strains among unvaccinated nursery contacts.

Materials and methods

Patients

In October 1996, a 3-month-old Japanese female

infant was hospitalised because of fever and vomiting. She had been in a nursery in Saga City, Japan, since birth. *H. influenzae* was isolated from her cerebrospinal fluid (CSF) and she was diagnosed as having purulent meningitis. The isolate was serotyped as type b and biotyped as type I according to the methods described below. Antibiotic therapy led to a successful recovery.

A total of 16 children, including the patient with Hib meningitis, had lived together in the nursery. These children had left their parental care, for family reasons and received around the clock nursing care. None of the children had received Hib vaccine. Nasopharyngeal swabs were taken from all nursery contacts (15 children) just after the meningitis patient was hospitalised. Their ages ranged from 2 to 23 months (Table 1).

Microbiology

Swabs were streaked on the selective medium (*Haemophilus* chocolate agar; bioMérieux-Vitek, Lyon, France) which was supplemented with bacitracin to inhibit the growth of bacteria other than the *Haemophilus* group. For primary cultures, plates were incubated at 37°C overnight. Eight colonies were picked from primary cultures plates and subcultured on a fresh chocolate agar plate for further analysis. Serotyping, biotyping and PFGE typing were performed by methods described previously [3].

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Corresponding author: Dr M. Saito (email: msaito@bact.med.kyushu-u.ac.jp).

Table 1. Isolation of *H. influenzae* strains from a meningitis patient and her 15 nursery contacts at two different times

Subject	Age (months)		Sex	Length of stay in the nursery (months)	Isolation of <i>H. influenzae</i> (Feb. 1996)	Isolation of <i>H. influenzae</i> (Oct. 1996)	PFGE patterns (serotype) (Oct. 1996)
Patient		3	F	3	...	+	D (b)
Nursery contact	1	23	M	21	—	+	C (a), F (NT)
	2	23	F	19	—	—	...
	3	15	M	15	+	—	...
	4	20	M	14	—	+	D ₁ (b), D ₂ (b), E (NT)
	5	14	M	13	—	+	D ₁ (b)
	6	12	F	11	—	+	C (a), F (NT)
	7	20	M	10	—	+	F (NT)
	8	11	M	10	—	+	D ₁ (b)
	9	9	F	9	—	+	D ₁ (b), G (NT)
	10	9	F	8	—	+	D ₁ (b), G (NT)
	11	6	F	5	...	+	A (a), B (a)
	12	4	F	4	...	—	...
	13	4	M	3	...	+	D ₁ (b), D ₂ (b)
	14	9	M	3	...	+	D ₁ (b)
	15	2	F	1	...	—	...

M, male; F, female; NT, non-typable; ..., not tested.

Results

Immediately after the patient with Hib meningitis was hospitalised, the nasopharyngeal carriage of *H. influenzae* among the other nursery children was investigated. Eight months before this contact tracing (in Feb. 1996), nasopharyngeal samples had been taken from the nursery children for a different reason. The results of the investigations pre- and post-disease occurrence are shown in Table 1. Before identification of the index case, one (10%) of 10 children was found to be colonised with *H. influenzae*. However, on

contact screening, 11 (73%) of 15 children carried *H. influenzae*. The initial carrier (nursery contact 3) had become negative on this occasion.

Subtyping

Capsular type b strains were carried by the majority of *H. influenzae*-positive contacts (7 of 11). A total of eight PFGE patterns were distinguished among the *H. influenzae* strains examined in this study; these were labelled A–G (Fig. 1). As differences between patterns D₁ and D₂ consisted of only one extra band in pattern

Fig. 1. Eight PFGE patterns (A–G) of *H. influenzae* isolates. The serotypes and biotypes of each pattern are shown under each lane. NT, non-typable.

D₂, these isolates were considered to be genetically related [4]. The PFGE pattern of the patient's strain was pattern D₁ and the majority of isolates from nursery contact nos. 4, 5, 8, 9, 10, 13 and 14 had an indistinguishable pattern (Table 2). Moreover, contacts 1, 6, 7 and 11 harboured strains that were clearly different from the invasive strain of the index case. More than one PFGE pattern was identified among isolates from each of the contacts 1, 4, 6, 9, 10 and 11.

Discussion

Population-based studies have shown that children who attend day care centres are at a significantly higher risk for invasive Hib disease than children who do not [5–7]. One study estimated that up to 50% of all invasive Hib diseases may be attributable to day-care attendance [5].

Previous studies have shown that the carriage rates of Hib in children at day-care centres during Hib disease occurrence were 49% [1] and 23–56% [2], while disease-free periods are characterised by lower carriage rates of about 1% [1]. Other studies have reported that Hib is carried in the nasopharynx of 0.5–3% of healthy children aged <24 months [8–10]. The present finding that the rate of carriage of Hib in contacts of patients is *c.* 15–100 times higher than the rate in other children in the same age group is in close agreement with previous reports [1, 2].

The seven Hib carriers in this study had strains in their nasopharynges that showed the same PFGE pattern as that of the isolate from the CSF of the patient with Hib meningitis. This finding suggested that the invasive Hib strain had spread widely among the nursery contacts. It

was not possible to examine whether the nursery workers carried the patient's strain or not. However, it is possible that the staff transmitted Hib bacteria to susceptible children in the nursery.

In this study, the carriage rate of the non-disease-associated strains (PFGE patterns A, B, C, E, F and G) was 47% (7 of 15). It was noteworthy that not only the number of carriers of the invasive (serotype b) *H. influenzae* strains, but also that of the non-invasive strains had increased among the nursery contacts. It can be speculated that certain conditions favour the spread of *H. influenzae* regardless of their invasiveness. Further studies are necessary to elucidate such environmental and host factors.

Furthermore, PFGE analysis revealed that seven *H. influenzae* carriers had two or three strains with different PFGE patterns in their nasopharynx. This result suggests that more than one strain of *H. influenzae* often colonises the individual nasopharynx.

It can be concluded that clonal dissemination of more than one strain of *H. influenzae* precedes the occurrence of invasive disease in institutions where children were nursed in close proximity.

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Table 2. PFGE patterns and the distribution of the eight *H. influenzae* strains isolated from each nursery contact

Nursery contact no.	Numbers of <i>H. influenzae</i> colonies [†] showing PFGE patterns							
	A	B	C	D ₁ *	D ₂	E	F	G
1			5				3	
4				4	1	3		
5				8				
6			4				4	
7							8	
8				8				
9				7				1
10				6				2
11	1	7						
13				5	3			
14				8				

*The same pattern as the strain which caused meningitis.

[†]A total of eight colonies obtained from nasopharyngeal cultures and used for PFGE analysis.