High Rate of Transmission of Penicillin-Resistant *Streptococcus pneumoniae* between Parents and Children

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Transmission of *Streptococcus pneumoniae* between children and their parents was evaluated in 29 pairs from 25 families. The serotypes of 35 pneumococcal isolates from 18 (62.1%) of 29 child-parent pairs were identical. Of the 35 isolates, 23 showed intermediate resistance and 10 were fully resistant to penicillin G. PCR indicated that all 35 strains had at least one alteration in penicillin-binding protein genes *pbp1a*, *pbp2x*, and *pbp2b* and 33 strains had macrolide resistance genes *mef(A)* and/or *erm(B)*. As a result, the PCR patterns of 16 of 18 pairs were identical. Molecular typing by pulsed-field gel electrophoresis showed that 12 pairs were indistinguishable, 3 pairs were closely related, 2 pairs were possibly related, and only one pair was different. Our data indicate the presence of a high rate of transmission of penicillin-resistant *S. pneumoniae* between children and their parents.

Streptococcus pneumoniae, which can colonize the human nasopharynx, is a leading bacterial cause of pneumonia, as well as otitis media, sinusitis, septicemia, and meningitis (10). Especially, children are frequent carriers of pneumococci, which may lead to invasive pneumococcal disease (5, 6). Penicillin-resistant *S. pneumoniae* (PRSP) is widespread all over the world, and the resistance is broadening to include other antimicrobial agents (1, 9, 15). It has been reported that children could acquire PRSP at child care centers (13) or from their siblings (14). However, the problem of whether PRSP colonizing the upper respiratory tract can be transmitted between children and parents and cause invasive diseases is not clear. To address this issue, we conducted the following prospective study.

New patients with invasive infections (e.g., pneumonia, sinusitis, otitis media, etc.) caused by *S. pneumoniae* who visited Nagasaki University Hospital or the Sugita Otorhinolaryngologic Clinic from January 2000 to May 2001 were asked to bring their family as soon as possible for clinical examination and collection of biological specimens (e.g., sputum, nasopharynx, middle ear). To diagnose invasive disease caused by pneumococci, gram-stained smears and cultures of good-quality specimens, according to the criteria of Bartlett (2), that were obtained as recently as possible were performed by standard methods. Fifty-four *S. pneumoniae* isolates from 29 pairs of children (mean age, 1.7 years) and their parents (mean age, 31.5 years) in 25 families were evaluated. We collected two isolates from each of 21 families and three isolates from each of 4 families. Culture plates were incubated overnight in a 5% CO₂ incubator. Optochin sensitivity and bile solubility tests

were performed for confirmation of *S. pneumoniae*. The MICs of penicillin G (Meiji Seika Kaisha, Tokyo, Japan) for 54 *S. pneumoniae* isolates were determined by the broth microdilution method in accordance with the guidelines of the NCCLS (12). Pneumococci were serotyped on the basis of capsular swelling (Quellung reaction) observed microscopically after suspension in Pneumococcal Diagnostic Antiserum (Statens Serum Institut, Copenhagen, Denmark). PCR was performed for the 35 pneumococcal isolates whose serotypes were identical in the children and parents to detect the alteration of penicillin-binding protein genes *pbp1a*, *pbp2x*, and *pbp2b* and macrolide resistance genes *mef(A)* and *erm(B)* as described previously (11). Pulsed-field gel electrophoresis (PFGE) after digestion with *Sma*I (Takara Shuzo Co., Shiga, Japan) was also performed for the 35 pneumococci as described previously (17), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (16).

The serotypes of 35 strains of *S. pneumoniae* in 18 (62.1%) of 29 pairs from children and parents were identical. Those were classified into six different serotypes, and 19F, 23F, and 6A were predominant (Table 1). On the other hand, the serotypes of 19 strains of pneumococci that were not identical in children and their parents varied widely and those included three isolates each of serotypes 19F and 23A; two isolates each of serotypes 6A, 6B, and 23F; and one isolate each of serotypes 9V, 11A, 14, 18C, 28F, 29, and 34. Of the 35 isolates whose serotypes were identical in children and their parents, 23 (65.7%) showed intermediate resistance (MIC, 0.12 to 1.0 µg/ml) and 10 (28.6%) were fully resistant (MIC, ≥2.0 µg/ml) to penicillin G and only 2 isolates were susceptible (Table 1). Of the remaining 19 isolates, 15 showed intermediate resistance and 4 were susceptible to penicillin G. Thirty (85.7%) of 35 pneumococcal isolates had alterations in *pbp1a*, *-2x*, and *-2b*; 3 (8.6%) had alterations in *pbp2x* and *-b*; and 2 (5.7%) had alterations in *pbp2x*. Thirty-three strains (94.3%) had macro-

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TABLE 1. Epidemiological characteristics of pneumococcal isolates with identical serotypes from children and their parents

Family	Strain no.	Age ^a	Specimen source	Infection	Date	MIC (PCG) ^f (μg/ml)	Serotype	PCR ^b result					PFGE patterns
								<i>pbp1a</i>	<i>pbp2x</i>	<i>pbp2b</i>	<i>mef(A)</i>	<i>erm(B)</i>	
A	1	2 (32)	Middle nutus	ABS ^c	3/10/2001	2	19F	+	+	+	+	—	Indistinguishable
	2	29	Nasopharynx	Pharyngitis	3/10/2001	2	19F	+	+	+	+	—	Indistinguishable
B	3	1 (18)	Nasopharynx	Pharyngitis	2/19/2001	1	19F	+	+	+	+	—	Possibly related/different
	4	32	Middle nutus	ABS	2/13/2001	2	19F	+	+	+	+	—	Possibly related/NA— ^e
	5	32	Nasopharynx	Pharyngitis	2/19/2001	1	19F	+	+	+	+	+	NA/different
C	6	1 (20)	Middle ear	AOM ^d	3/16/2001	1	19F	+	+	+	+	—	Indistinguishable
	7	32	Nasopharynx	Colonization	3/17/2001	1	19F	+	+	+	+	—	Indistinguishable
D	8	1 (18)	Middle nutus	ABS	2/24/2001	1	19F	+	+	+	+	—	Indistinguishable
	9	32	Middle nutus	ABS	3/2/2001	2	19F	+	+	+	+	—	Indistinguishable
E	10	1 (23)	Nasopharynx	ABS	3/6/2001	1	19F	+	+	+	+	—	Indistinguishable
	11	35	Nasopharynx	Colonization	3/6/2001	1	19F	+	+	+	+	—	Indistinguishable
F	12	0 (9)	Nasopharynx	AOM	5/10/2000	1	23F	+	+	+	—	+	Closely related
	13	32	Middle nutus	ABS	4/15/2000	0.5	23F	+	+	+	—	+	Closely related
G	14	1 (17)	Nasopharynx	ABS	3/6/2001	2	23F	+	+	+	—	+	Indistinguishable
	15	27	Nasopharynx	Pharyngitis	3/13/2001	1	23F	+	+	+	—	+	Indistinguishable
H	16	2 (34)	Nasopharynx	Colonization	2/27/2001	1	23F	+	+	+	—	+	Closely related
	17	36	Nasopharynx	ABS	2/27/2001	1	23F	+	+	+	—	+	Closely related
I	18	1 (20)	Middle nutus	ABS	2/28/2001	0.5	23F	+	+	+	—	+	Indistinguishable
	19	28	Nasopharynx	Pharyngitis	3/13/2001	0.5	23F	+	+	+	—	+	Indistinguishable
J	20	5 (61)	Nasopharynx	Pharyngitis	2/20/2001	2	23F	+	+	+	—	+	Possibly related
	21	35	Nasopharynx	Colonization	3/3/2001	0.125	23F	—	+	+	—	+	Possibly related
K	22	1 (22)	Middle nutus	ABS	3/10/2001	0.5	6A	+	+	+	+	—	Indistinguishable
	23	33	Nasopharynx	Pharyngitis	3/13/2001	0.5	6A	+	+	+	+	—	Indistinguishable
L	24	2 (34)	Nasopharynx	Pharyngitis	4/16/2001	2	6A	+	+	+	—	+	Indistinguishable
	25	30	Nasopharynx	ABS	4/7/2001	2	6A	+	+	+	—	+	Indistinguishable
M	26	3 (46)	Middle nutus	ABS	3/17/2001	1	6A	+	+	+	—	+	Indistinguishable
	27	38	Nasopharynx	Colonization	3/27/2001	2	6A	+	+	+	—	+	Indistinguishable
N	28	2 (29)	Nasopharynx	AOM	3/10/2001	1	6A	+	+	+	—	+	Indistinguishable
	29	27	Nasopharynx	Colonization	3/23/2001	2	6A	+	+	+	—	+	Indistinguishable
O	30	0 (8)	Nasopharynx	AOM	2/16/2001	0.5	6B	+	+	+	—	+	Indistinguishable
	31	30	Nasopharynx	Pharyngitis	2/27/2001	1	6B	+	+	+	—	+	Indistinguishable
P	32	0 (9)	Nasopharynx	AOM	2/17/2001	0.25	14	—	+	+	—	+	Indistinguishable
	33	30	Nasopharynx	ABS	2/17/2001	0.25	14	—	+	+	—	+	Indistinguishable
Q	34	4 (55)	Middle nutus	Colonization	5/14/2001	<0.016	34	—	+	—	—	—	Closely related
	35	32	Sputum	Acute pneumonia	5/8/2001	<0.016	34	—	+	—	—	—	Closely related

^a Ages are given in years for adults and in years (months) for children.

^b Penicillin binding protein genes *pbp1a*, *pbp2x*, and *pbp2a*: +, altered; —, not altered. Macrolide resistance genes: *mef(A)* and *erm(B)*: +, positive; —, negative.

^c ABS, acute bacterial sinusitis.

^d AOM, acute otitis media.

^e NA, not applicable.

^f PCG, penicillin G.

lide resistance genes *mef(A)* and/or *erm(B)*. As a result, the PCR patterns of 16 (88.9%) of 18 pairs were identical (Table 1). Of the 18 pairs, molecular typing by PFGE showed that 12 pairs (66.7%) were indistinguishable, 3 pairs (16.7%) were closely related, 2 pairs (11.1%) were possibly related, and only 1 pair (5.6%) was different (Table 1 and Fig. 1).

Infants and young children tend to acquire *S. pneumoniae* in

the upper respiratory tract because of their low immunity. This pneumococcal colonization can become a risk factor for invasive pneumococcal disease (5, 6), which is a major cause of morbidity and mortality in infants and young children worldwide (8). In our study, when patients with invasive infections caused by *S. pneumoniae* appeared, young children in the family were colonized by *S. pneumoniae* with and without infection

FIG. 1. PFGE patterns of *Sma*I-digested DNA of pneumococcal isolates from children and parents from 10 families (A to J). The patterns of pneumococcal isolates from children and their parents are indistinguishable in families A, C, D, E, G, and I; closely related in families F and H; possibly related in one pair each (strains 3 and 4) from families B and J; and different in the other pair (strains 3 and 5) from family B. Lanes M contained molecular size markers.

and the high rate of correlation between colonizations of children and parents was confirmed by serotyping, PCR, and PFGE. Moreover, most (94.3%) of the colonizations were by PRSP (MIC, >0.1 $\mu\text{g/ml}$) when the serotypes of pneumococci were identical in the children and their parents. It has been reported that immunization with pneumococcal polysaccharide vaccine was associated with poor results in children under the age of 2 years (3). On the other hand, pneumococcal conjugate vaccine seems to be effective for not only prevention of invasive pneumococcal disease but also reduction of nasopharyngeal carriage of vaccine serotypes in young children (4, 8, 18). In our study, the serotype coverage of such vaccine against *S. pneumoniae* was 71.4% (25 of 35) when the serotypes of pneumococci from children and their parents were identical, although we should note that invasive-disease-causing serotype 6A and 34 strains are not included in such a vaccine. Therefore, pneumococcal conjugate vaccine interrupts the transmission of pneumococci, including PRSP, and thus decreases the burden of antibiotic resistance in immunized children and their contacts (7).

In conclusion, our results demonstrated a high rate of transmission of PRSP between children and their parents. Therefore, we should consider the introduction of highly effective vaccines in young children for prevention of invasive pneumococcal disease.

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REFERENCES

- Appelbaum, P. C. 1992. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. *Clin. Infect. Dis.* **15**:77–83.
- Bartlett, R. C. 1974. Medical microbiology: quality, cost and clinical relevance, p. 24–31. John Wiley & Sons, Inc., New York, N.Y.
- Borgono, J. M., A. A. McLean, P. P. Vella, A. F. Woodhour, I. Canepa, W. L. Davidson, and M. R. Hilleman. 1978. Vaccination and revaccination with polyvalent pneumococcal polysaccharide vaccines in adults and infants. *Proc. Soc. Exp. Biol. Med.* **157**:148–154.
- Butler, J. C., R. F. Breiman, H. B. Lipman, J. Hofmann, and R. R. Facklam. 1995. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States, 1978–1994: implications for development of a conjugate vaccine. *J. Infect. Dis.* **171**: 885–889.
- Dejsirilert, S., K. Overweg, M. Sluif, L. Saengsuk, M. Gratten, T. Ezaki, and P. W. Hermans. 1999. Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* among children with acute respiratory tract infections in Thailand: a molecular epidemiological survey. *J. Clin. Microbiol.* **37**:1832–1838.
- Faden, H., L. Duffy, R. Wasielewski, J. Wolf, D. Krystofik, and Y. Tung. 1997. Relationship between nasopharyngeal colonization and the development of otitis media in children. *J. Infect. Dis.* **175**:1440–1445.
- Klugman, K. P. 2001. Efficacy of pneumococcal conjugate vaccines and their effect on carriage and antimicrobial resistance. *Lancet Infect. Dis.* **1**:85–91.
- Kyaw, M. H., I. G. Jones, and H. Campbell. 2001. Prevention of pneumococcal disease in children. Pneumococcal conjugate vaccines: their use globally could have a major impact on public health. *Acta Paediatr.* **90**:473–476.
- Linares, J., R. Pallares, T. Alonso, J. L. Perez, J. Ayats, F. Gudiol, P. F. Viladrich, and R. Martin. 1992. Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979–1990). *Clin. Infect. Dis.* **15**:99–105.
- Musher, D. M. 1992. Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. *Clin. Infect. Dis.* **14**: 801–807.
- Nagai, K., Y. Shibasaki, K. Hasegawa, T. A. Davies, M. R. Jacobs, K. Ubukata, and P. C. Appelbaum. 2001. Evaluation of PCR primers to screen for *Streptococcus pneumoniae* isolates and beta-lactam resistance, and to detect common macrolide resistance determinants. *J. Antimicrob. Chemother.* **48**:915–918.
- National Committee for Clinical Laboratory Standards. 1998. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Pons, J. L., M. N. Mandement, E. Martin, C. Lemort, M. Nouyellon, E. Mallet, and J. F. Lemeland. 1996. Clonal and temporal patterns of nasopharyngeal penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae* strains in children attending a day care center. *J. Clin. Microbiol.* **34**:3218–3222.
- Shimada, J., N. Yamanaka, M. Hotomi, M. Suzumoto, A. Sakai, K. Ubukata, T. Mitsuda, S. Yokota, and H. Faden. 2002. Household transmission of *Streptococcus pneumoniae* among siblings with acute otitis media. *J. Clin. Microbiol.* **40**:1851–1853.
- Song, J. H., N. Y. Lee, S. Ichihara, R. Yoshida, Y. Hirakata, W. Fu, A. Chongthaleong, N. Aswapokee, C. H. Chiu, M. K. Lalitha, K. Thomas, J. Perera, T. T. Yee, F. Jamal, U. C. Warsa, B. X. Vinh, M. R. Jacobs, P. C. Appelbaum, and C. H. Pai. 1999. Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Clin. Infect. Dis.* **28**:1206–1211.
- Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
- Yano, H., M. Suetake, A. Kuga, K. Irinoda, R. Okamoto, T. Kobayashi, and M. Inoue. 2000. Pulsed-field gel electrophoresis analysis of nasopharyngeal flora in children attending a day care center. *J. Clin. Microbiol.* **38**:625–629.
- Zimmerman, R. K. 2001. Pneumococcal conjugate vaccine for young children. *Am. Fam. Phys.* **63**:1991–1998.