

# The Incidence of Pediatric Invasive Bacterial Diseases in Nippon Medical School Chiba Hokusoh Hospital before and after the Introduction of Conjugate Vaccines

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The introduction of the *Haemophilus influenzae* type b (Hib) vaccine and the 7-valent pneumococcal conjugate vaccine (PCV7) has led to dramatic reductions in cases of invasive *H. influenzae* disease and invasive pneumococcal disease (IPD). After the introduction of the PCV7 and the 13-valent pneumococcal conjugate vaccine (PCV13), the number of children with IPD markedly decreased in our hospital. However, since 2015, three children with IPD have been admitted to our hospital. We analyzed the serotype, multilocus sequence type, and antimicrobial susceptibility of *Streptococcus pneumoniae* strains isolated in these newly diagnosed cases. The strains were serotypes 7F and 12F. In addition, we analyzed the incidence of invasive bacterial disease before and after the introduction of conjugate vaccines and found no change in the incidences. We found that cases of IPD and invasive *H. influenzae* disease clearly decreased following the introduction of the PCV7, the PCV13, and the Hib vaccine, as well as disease caused by antibiotic-resistant strains. (J Nippon Med Sch 2018; 85: 34–38)

**Key words:** *Streptococcus pneumoniae*, *Haemophilus influenzae*, conjugate vaccine

## Introduction

*Streptococcus pneumoniae* is a major etiological pathogen of otitis media, pneumonia, bacteremia, and meningitis in children and causes substantial morbidity and mortality worldwide<sup>1</sup>. The introduction of the *Haemophilus influenzae* type b (Hib) vaccine and the 7-valent pneumococcal conjugate vaccine (PCV7) has led to dramatic reductions in cases of invasive *H. influenzae* disease and invasive pneumococcal disease (IPD) in the United States<sup>2</sup> as well as in Scandinavian countries<sup>3</sup>.

In Japan, the Hib vaccine and the PCV7 were licensed in 2008 and 2010, respectively, but were not included in the National Immunization Program (NIP) until 2013<sup>4</sup>. After the introduction of the PCV7 and the 13-valent pneumococcal conjugate vaccine (PCV13), the number of children with IPD markedly decreased in our hospital. However, since 2015, three children with IPD have been

admitted to our hospital. Here, we analyzed the serotype, multilocus sequence type, and antimicrobial susceptibility of *S. pneumoniae* strains isolated in these newly diagnosed cases. In addition, we analyzed the incidence of invasive bacterial disease before and after the introduction of conjugate vaccines.

## Introductory Cases

### Case 1

A 5-year and 4-month-old boy was admitted to our hospital because of fever and lethargy. Vaccination history included the diphtheria-pertussis-tetanus (DPT) vaccine (4×), the polio vaccine (4×), the measles-rubella (MR) vaccine (1×), the Japanese encephalitis (JE) vaccine (1×), the Hib vaccine (4×), and the PCV7 (4×). Neck stiffness, Kernig's sign, and Brudzinski's sign were positive. Laboratory examination showed a C-reactive protein (CRP)

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Table 1 *S. pneumoniae* serotype, sequence type, and antimicrobial susceptibility in our cases

Serotype								
Case 1	7F							
Case 2	12F							
Genotype	ST	<i>aroE</i>	<i>gdh</i>	<i>gki</i>	<i>recP</i>	<i>spi</i>	<i>xpt</i>	<i>ddl</i>
Case 1	191	8	9	2	1	6	1	17
Case 2	4846	12	32	111	1	13	48	6
Antibiotic MICs	PCG	CTX	MEPM	CDTR	EM	CLDM	VCM	
Case 1	≤0.03	≤0.12	≤0.12	≤0.06	≥2	≥1	0.5	
Case 2	0.06	≤0.12	≤0.12	≤0.06	≥2	≤0.12	0.25	
Case 3	≤0.03	≤0.12	≤0.12	≤0.06	≥2	≥1	0.5	

ST: sequence type

PCG: penicillin G, CTX: cefotaxime, MEPM: meropenem, CDTR: ceftidoren pivoxil, EM: erythromycin, CLDM: clindamycin, VCM: vancomycin.

level of 12.1 mg/dL and a white blood cell (WBC) count of 24,100/μL (neutrophils, 91%). Cerebrospinal fluid (CSF) examination showed a cell count of 1,048/μL (polynuclear cells, 94%) and a protein concentration of 99 mg/dL. CSF and blood cultures were positive for *S. pneumoniae*. We treated the patient with cefotaxime (CTX) and meropenem (MEPM) with dexamethasone and he was discharged 15 days after admission without any neurological sequelae.

**Case 2**

A 7-year and 3-month-old boy was admitted to our hospital because of fever and neck pain. Vaccination history included the DPT vaccine (4×), the polio vaccine (4×), the MR vaccine (2×), the JE vaccine (3×), the Hib vaccine (2×), and the PCV7 (2×). Neck stiffness and Kernig's sign were negative. Laboratory examination showed a CRP level of 2.18 mg/dL and a WBC of 31,140/μL. CSF examination showed a cell count of 1/μL. Blood culture was positive for *S. pneumoniae*. We treated the patient with CTX and he was discharged 9 days after admission without any severe sequelae.

**Case 3**

A 1-year and 4-month-old boy was admitted to our hospital because of high-grade fever with prolonged febrile seizure. Vaccination history included the diphtheria-pertussis-tetanus-polio vaccine (3×), the MR vaccine (1×), the Hib vaccine (3×), and the PCV13 (3×). Laboratory examination showed a CRP level of 2.85 mg/dL and a WBC of 28,010/μL. CSF examination showed a cell count of 1.3/μL (mononuclear cells, 100%). Blood culture was positive for *S. pneumoniae*. We treated the patient with CTX and he was discharged 5 days after admission without any severe sequelae. Further examination of *S. pneumoniae* was not performed in this case because the

samples were unavailable.

**Materials and Methods**

IPD was defined as any disease in which *S. pneumoniae* was identified in normally sterile body fluids such as blood, CSF, bone aspirate, or synovial fluid. Invasive *H. influenzae* disease, or other invasive bacterial disease, was defined as any disease in which *H. influenzae* or other bacterial pathogens were identified in normally sterile body fluids. *S. pneumoniae* strains were serotyped by Quellung reaction using antisera purchased from the Statens Serum Institut (Copenhagen, Denmark). All collected strains, except case 3, were sent to the National Institute of Infectious Diseases (Tokyo, Japan), where serotypes were confirmed using the same methods. Multilocus sequence typing was performed and sequence type (ST) was determined according to allelic profile for *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*, as previously described<sup>5,6</sup>. The minimum inhibitory concentration (MIC) values of pneumococcal isolates were determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI M100-S18)<sup>7</sup>. The antibiotics tested were penicillin G, CTX, MEPM, ceftidoren pivoxil, erythromycin, clindamycin, and vancomycin. MIC breakpoints were defined according to the 2008 CLSI guidelines<sup>7</sup>. Statistical analyses were performed using the  $\chi^2$  or Fisher's exact test.

**Results*****S. pneumoniae* Serotype, Genotype, and Antimicrobial Susceptibility in Three Cases (Table 1)**

Characteristics of *S. pneumoniae* strains isolated in the three cases are shown in **Table 1**. Serotype 7F, in case 1, is included in the PCV13 but not the PCV7. Serotype 12F,

Table 2 MIC values for CTX in pediatric IPD cases

MIC	<0.06	<0.12	0.12	0.25	0.5	1	2
2000–2012	3*	0	1	2	5	2	2
2013–2016	0	3	0	0	0	0	0

A significant difference was observed in the susceptibility of *S. pneumoniae* strains to CTX between periods ( $p < 0.01$  statistical analyses were performed using the  $\chi^2$  test).

CTX: cefotaxime.

\*: number of patients

in case 2, is not included in either the PCV13 or the PCV7. We did not examine the serotype of case 3.

We also experienced two additional cases of pneumococcal-related bacterial pneumonia in a 1-year-old girl and a 7-year-old girl. In both cases, nasal discharge culture was positive for *S. pneumoniae*, urinary *S. pneumoniae* antigen test was positive, but blood cultures were negative. The serotype of the isolate from the 1-year-old girl was 15B, which is not included in the PCV13 or the PCV7. However, the serotype of the isolate from the 7-year-old girl was 7F, which is included in the PCV13 but not the PCV7. Vaccination history showed the former case received three doses of the PCV13 and the latter case received only two doses of the PCV7. Both patients quickly recovered after antibiotic administration. Clonal diversity of serotype showed a sequence type of 191, 4846.

#### Changes in Antimicrobial Susceptibility of IPD to CTX

The percentages of penicillin G-non susceptible strains did not differ significantly from before to after PCV7 introduction (data not shown). *S. pneumoniae* strains causing IPD isolated in 2013–2016 were more sensitive to CTX than those isolated in 2010–2012 ( $p < 0.01$ ) (Table 2).

#### Annual Change in the Incidence of Invasive Bacterial Disease

The annual change in the incidences of IPD, invasive *H. influenzae* disease, and other invasive bacterial diseases in children admitted to our hospital is shown in Table 3. The incidence of IPD clearly decreased after 2013 and increased in 2015. Invasive *H. influenzae* disease also decreased since the introduction of the Hib vaccine. However, the total incidence of invasive bacterial disease did not significantly decrease.

#### Discussion

After the inclusion of Hib in the NIP, reported cases of *H. influenzae* disease clearly decreased in our hospital. Although we did not discriminate *H. influenzae* type b from

*H. influenzae*, this decrease might be due to a decrease of *H. influenzae* type b infection. This result is in agreement with a nationwide survey in Japan<sup>8</sup> as well as in England<sup>9</sup>. This reduction may contribute to the ability of the Hib vaccine to reduce nasopharyngeal carriage of *H. influenzae* and induce herd immunity. However, recently, an increase in the incidence of Hib disease in England has been reported<sup>9</sup>. Therefore, it is important to maintain nationwide surveillance for invasive *H. influenzae* disease in Japan.

Conversely, IPD cases have re-emerged in recent years in our hospital. Case 1 was caused by the PCV13 serotype 7F, which is not covered by the PCV7. A nationwide population-based surveillance study showed serotypes 6B, 14, 23F, and 19F, which are included in the PCV7, significantly decreased after introduction of the PCV7 and the PCV13. However, serotype 19A, which is not included in the PCV7, but is included in the PCV13, significantly increased in number as well as in percentage of IPD<sup>2</sup>. Sequential introduction of the PCV7 followed by the PCV13 was performed in Israel<sup>10</sup>, as in Japan. During the PCV7 period, before introducing the PCV13, otitis media caused by *S. pneumoniae* serotype 7F did not decrease. However, after introduction of the PCV13, serotype 7F, as well as serotypes 1, 3, 5, and 19A, markedly decreased. We therefore consider additional vaccination with the PCV13 should be recommended in children previously vaccinated with the PCV7 only.

During the PCV13 vaccination program in Italy, a large diversity (33% of all IPD cases) of IPD caused by non-vaccine serotypes, which include serotypes 24F, 12F, 33F, 23B, 10A, and 25A, was observed<sup>11</sup>. Our IPD case, caused by serotype 12F (case 2), might have occurred by the same mechanism.

In our case, sequence type of *S. pneumoniae* showed serotype 7F with clonal type 191 and serotype 12F with clonal type 4846. Serotype 7F with clonal type 191 has been reported in many IPD cases in Spain, but serotype 12F with clonal type 4846 has not<sup>12</sup>.

Table 3 Incidence of invasive disease caused by *S. pneumoniae*, *H. influenzae*, or other bacteria in children in Nippon Medical School Chiba Hokusoh Hospital

year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
<i>Streptococcus pneumoniae</i> /Streptococcus pneumoniae PISP/Streptococcus pneumoniae PRSP	0	1	1	3	0	2	0	2	1	0	1	0	1	0	0	2	1
<i>Streptococcus agalactiae</i> (GroupB)	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haemophilus influenzae</i> /Haemophilus influenzae BLNAR	2	1	0	0	1	3	1	1	0	0	0	0	0	0	0	0	0
other gram positive coccus	0	1	1	0	1	0	2	4	0	2	1	1	1	0	1	0	0
<i>Staphylococcus aureus</i> /Staphylococcus aureus MRSA	5	1	1	2	1	6	4	4	0	3	3	2	0	0	8	2	2
<i>Cocci</i> negative staphylococcus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Micrococci and Related Genera/Enterococcus faecalis/Enterococcus faecium	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
other gram negative rod	0	1	0	0	2	0	0	0	0	2	0	0	0	0	2	0	1
<i>Escherichia coli</i> /Klebsiella pneumoniae/Salmonella species/Salmonella typhi	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Pseudomonas aeruginosa</i> /Acinetobacter Iwoffii	2	1	5	6	4	2	2	2	0	3	0	0	0	0	0	0	0
<i>Bacteroides uniformis</i> /Anaerobic Gram-positive rods/Propionibacterium acnes	0	1	1	0	0	0	1	0	0	1	1	1	3	0	3	1	2
<i>Bacillus species</i> /Gram-positive rod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Neisseria species</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Micrococci and Related Genera/Enterococcus faecalis/Enterococcus faecium	0	2	1	0	0	0	1	0	0	1	1	1	3	0	3	4	2
Total	9	10	11	11	9	13	11	13	1	12	7	6	8	0	17	12	9

It has long been recognized that PCVs are expected to be effective against IPD caused by drug-resistant strains<sup>2</sup>. In our case series, CTX susceptibility improved after introduction of the PCV7 and the PCV13, which is contrary to a previous nationwide survey<sup>2</sup>.

In conclusion, IPD and invasive *H. influenzae* disease clearly decreased following introduction of the PCV7, the PCV13, and the Hib vaccine, as well as disease caused by antibiotic-resistant strains. Because epidemiological changes are expected to occur in the following years, optimized and continuous surveillance is required to monitor the emergence of both vaccine and non-vaccine serotypes and to evaluate drug resistance in invasive bacterial infection in children.

**Conflict of Interest:** The authors declare that they have no conflict of interests.

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