less than 1 year old. Highly accurate determination of etiological agents in pediatric CAP therefore is an important need that is difficult to satisfy.

We constructed a real-time polymerase chain reaction (PCR) assay for rapid identification of CAP pathogens, complementing conventional culture methods [15, 16]. Agents responsible for CAP were assessed comprehensively based on the real-time PCR results for 6 bacteria and 11 viruses, bacterial cultures, and blood test results, as well as chest radiographic and clinical findings. All isolates in this study were determined from this evidence to be etiological agents of CAP in pediatric patients [5].

In this article, we describe correlation of susceptibility of oral and intravenous agents with genotypic (g) resistance based on identification of resistance genes by real-time PCR in *S. pneumoniae* and *H. influenzae*, and detection of a mutation in the sequence of the 23S rRNA gene in *M. pneumoniae*.

We also describe coverage rates of heptavalent pneumococcal conjugate vaccine (PCV7) and PCV13 based on serotype of *S. pneumoniae* shortly before approval of PCV7 for clinical use in Japan.

Patients and methods

Microorganisms

We identified *S. pneumoniae* ($n = 241$), *H. influenzae* ($n = 123$), and *M. pneumoniae* ($n = 54$) as etiological agents in pediatric inpatients with CAP treated at institutions belonging to the Acute Respiratory Diseases (ARD) study group. These agents were determined by real-time PCR results using nasopharyngeal swab samples, blood test results at admission, and clinical findings combined with chest radiography, as reported previously [5].

The ten institutions and affiliated pediatricians participating in the ARD study group between April 2008 and April 2009 included the National Hospital Organization Tokyo Medical Center (T. Okada, K. Matsubara, and S. Iwata), Asahikawa Kosei Hospital (H. Sakata), Tohoku Rosai Hospital (R. Takayanagi), Chiba University Hospital (N. Ishiwada), Health Insurance Society of General Ota Hospital, Fuji Heavy Industries Ltd. (Y. Sato), Niigata University Medical and Dental Hospital (T. Oishi), Hakujikai Memorial Hospital (T. Tajima), Kobe City Medical Center General Hospital (T. Haruta), Osaka Rosai Hospital (N. Kawamura), and Kawasaki Medical School (K. Ouchi).

Real-time PCR

Identification of resistance genes in *S. pneumoniae* and *H. influenzae* isolates by culture were performed by real-time PCR methods that we previously devised [8, 17].

To confirm that an isolate was *S. pneumoniae*, the *lytA* gene encoding the autolysin enzyme specific to this agent was amplified simultaneously during real-time PCR to amplify the three penicillin-binding protein (PBP) genes: the *pbp1a*, *pbp2x*, and *pbp2b* genes encoding PBP1A, PBP2X, and PBP2B enzymes, respectively. Positive reactions in the real-time PCR occurred only in susceptible strains possessing normal PBP genes because each probe and primer set was constructed to amplify part of a normal PBP gene. Additionally, *mef* (A) and *erm* (B) genes, which confer resistance to macrolide (ML) antibiotics, were identified. Resistance genotype (g) was represented as penicillin (PEN)-susceptible *S. pneumoniae* (gPSSP), PEN-intermediate *S. pneumoniae* (gPISP), and PEN-resistant *S. pneumoniae* (gPRSP).

For *H. influenzae*, a 16S rRNA gene specific to this organism and the *capB* gene encoding the serotype b capsule were identified by real-time PCR. Genes affecting antibiotic resistance were the *ftsI* gene encoding PBP3, where two amino acid substitutions, Asn526Lys and Ser385Thr, can cause β-lactam resistance; and a functioning bla_{TEM} gene, which encodes TEM-1 β-lactamase. On the basis of PCR results, genotypic resistance was classified as β-lactamase-nonproducing ampicillin (AMP)-susceptible (gBLNAS), β-lactamase-producing AMP resistance (gBLPAR), β-lactamase-nonproducing low-level AMP resistance (glow-BLNAR), β-lactamase-nonproducing AMP resistance (gBLNAR), or β-lactamase-producing amoxicillin (AMX)-clavulanic acid resistance (gBLPACR-I or gBLPACR-II).

Sequencing

The full length of the 23S rRNA gene of all *M. pneumoniae* strains was sequenced with an ABI Prism 3130/3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA, USA) by methods described previously [18]. ML-resistant *M. pneumoniae* (MRMP) possessing the A2063G, A2064G, or C2617A mutation was indicated as gMRMP.

Susceptibility testing

Antibiotic susceptibility testing of *S. pneumoniae* and *H. influenzae* was performed by an agar dilution method using Mueller–Hinton II agar (MH; Becton–Dickinson, Franklin Lakes, NJ, USA) as described previously [19, 20]. Susceptibility of *M. pneumoniae* isolates was determined by a microdilution method using pleuropneumonia-like organism (PPLO) broth [18].

The antibiotics tested were AMP, AMX, cefdinir (CDR), cefditoren (CDN), cefotaxime (CTX), tebipenem (TBM), meropenem (MEM), panipenem (PAM), tosufloxacin (TFX), erythromycin (ERY), clarithromycin (CLR),

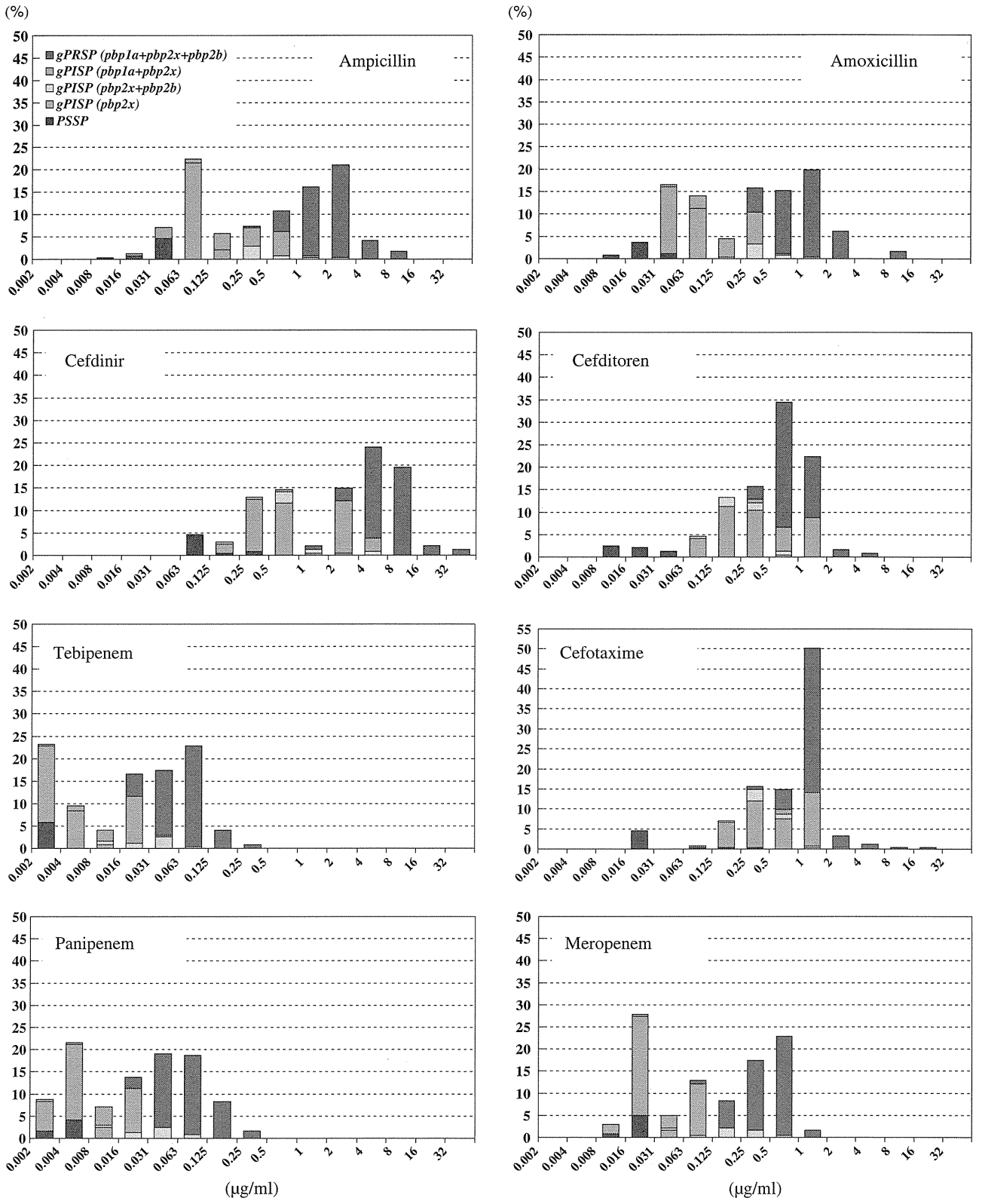

Fig. 1 Susceptibility distributions and genotypes of eight β-lactam agents for *Streptococcus pneumoniae* ($n = 241$)

azithromycin (AZM), telithromycin (TEL), and minocycline (MIN). Standard strains of *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and ATCC 49766, and *M. pneumoniae* M129 were used as quality control for susceptibility testing.

Serotyping

Serotype of *S. pneumoniae* strains was determined by the capsular swelling reaction using antiserum purchased from the Statens Serum Institute (Copenhagen, Denmark). Except for capsule type b, serotypes in *H. influenzae* were determined using antiserum (Denka Seiken, Tokyo, Japan).

Results

Streptococcus pneumoniae

Figure 1 shows minimum inhibitory concentration (MIC) distributions of eight β-lactam antibiotics for *S. pneumoniae* ($n = 241$). Genotype was based on real-time PCR results for the *pbp1a*, *pbp2x*, and *pbp2b* genes as already described. The resistance rate of gPRSP (*pbp1a* + *2x* + *2b*) was highest at 47.7 %, followed by 26.1 % for gPISP (*pbp2x*), 14.9 % for gPISP (*pbp1a* + *2x*), and 4.9 % for gPISP (*pbp2x* + *2b*). Occurrence of gPSSP having normal PBP genes was only 6.2 %.

Table 1 shows MIC_{90}s of antimicrobial agents in every resistant genotype together with comparison of the MIC_{90}s with that of gPSSP. MIC_{90}s of AMP, AMX, and TBM for gPISP (*pbp2x*) strains were influenced slightly by the *pbp2x* alterations, resulting in MIC_{90}s about 2 times greater than that for gPSSP. MIC_{90}s of the cephalosporin agents CDN, CDR, and CTX showed greater increases from the *pbp2x* alterations (4–32 times). No carbapenem agents (PAM, MEM, and TBM) were affected by this abnormal gene.

In contrast, MIC_{90}s of AMP, AMX, TBM, PAM, and MEM were affected slightly by *pbp2b* alterations. MIC_{90}s of all agents for gPRSP (*pbp1a* + *2x* + *2b*) generally were 32–128 fold that for gPSSP.

Table 1 MIC_{90} of β-lactam agents affected by *pbp* gene alterations in *Streptococcus pneumoniae*

	gPSSP ($n = 15$)	gPISP (*pbp2x*) ($n = 63$)	gPISP (*pbpla* + *2x*) ($n = 36$)	gPISP (*pbp2x* + *2b*) ($n = 12$)	gPRSP (*pbpla* + *2x* + *2b*) ($n = 115$)
Ampicillin	0.031	0.063 (×2[a])	0.5 (×16)	0.5 (×16)	4 (×128)
Amoxicillin	0.031	0.063 (×2)	0.25 (×8)	0.5 (×16)	2 (×64)
Cefdinir	0.125	0.5 (×4)	4 (×32)	4 (×32)	8 (×64)
Cefditoren	0.031	0.25 (×8)	1 (×32)	0.5 (×16)	1 (×32)
Cefotaxime	0.016	0.5 (×32)	1 (×64)	0.5 (×32)	2 (×128)
Tebipenem	0.002	0.004 (×2)	0.016 (×8)	0.031 (×16)	0.125 (×64)
Panipenem	0.004	0.004 (×1)	0.016 (×4)	0.063 (×16)	0.125 (×32)
Meropenem	0.016	0.016 (×1)	0.063 (×4)	0.25 (×16)	0.5 (×32)

gPSSP penicillin (PEN)-susceptible *S. pneumoniae*, *gPISP* PEN-intermediate *S. pneumoniae*, *gPRSP* PEN-resistant *S. pneumoniae*

[a] The × symbol throughout indicates multiple numbers compared to the minimum inhibitory concentration (MIC) of gPSSP

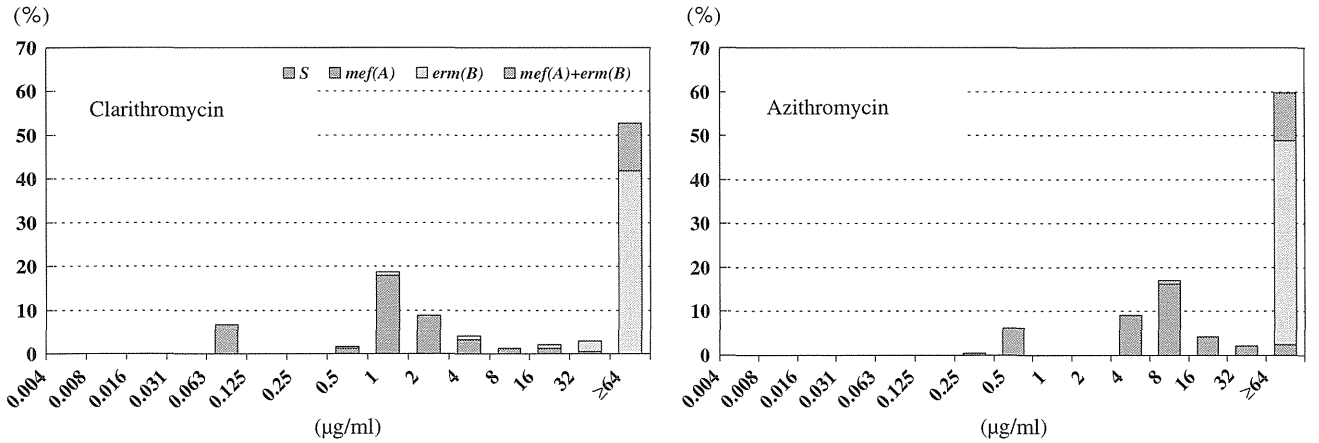

Fig. 2 Susceptibility distributions of clarithromycin (CLR) and azithromycin (AZM) for *Streptococcus pneumoniae* ($n = 241$)

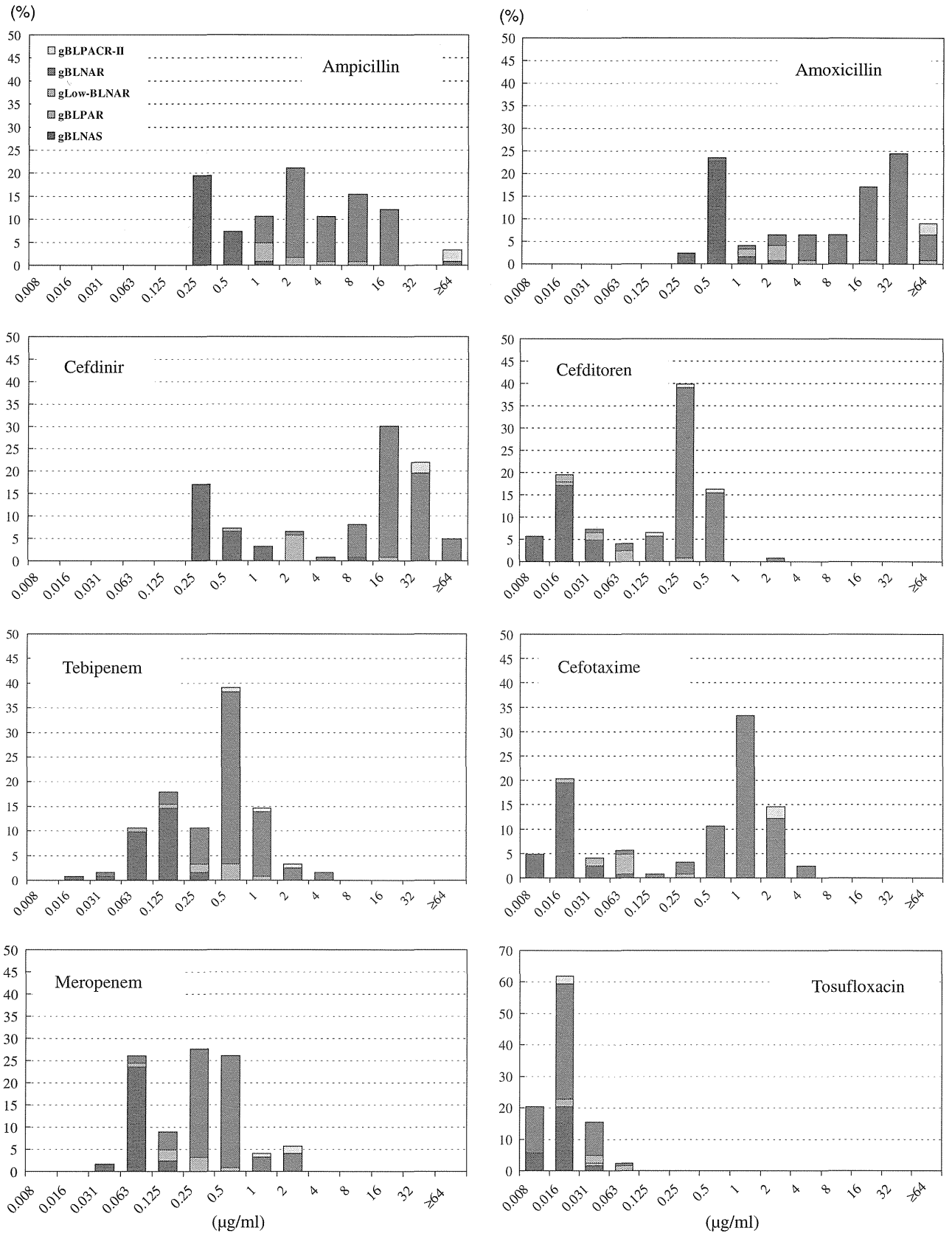

Fig. 3 Susceptibility distributions and genotypes of seven β-lactam agents and tosufloxacin (TFX) for *Haemophilus influenzae* ($n = 123$)

Two strains showing high MICs of 8 μg/ml or more for AMP, AMX, and CTX had two amino acid (aa) substitutions in a conserved motif of the *pbp2x* gene. This Ser-Thr-Met-Lys (STMK) motif was altered to become Ser-Ala-Phe-Lys (SAFK), although the first of these amino acid substitutions, from Thr to Ala, usually can be identified in most gPRSP [21]. Another two strains showing MICs of 8 μg/ml or more for AMP and AMX and 2 μg/ml for CTX had amino acid substitutions in the *pbp2b* gene.

Figure 2 shows MIC distributions of CLR and AZM for *S. pneumoniae*. ML-resistant strains were distinguished according to presence of *mef*(A), *erm*(B), or both genes, at respective frequencies of 34.4, 48.1, and 10.4 %. Susceptible strains possessing no ML resistance genes were less common (7.1 %). MIC_{90}s of CLR for strains with *mef*(A) or *erm*(B) were 4 μg/ml and ≥64 μg/ml, respectively; those of AZM were 16 μg/ml and ≥64 μg/ml.

Haemophilus influenzae

MIC distributions of eight antibiotics for *H. influenzae* are shown in Fig. 3. Resistance classes of 123 *H. influenzae* strains were distinguished according to real-time PCR results. Prevalence of each class among them was 27.6 % for gBLNAS, 0.8 % for gBLPAR, 6.5 % for glow-BLNAR, 62.6 % for gBLNAR, and 2.4 % for gBLPACR-II, respectively, showing predominance of gBLNAR among all isolates.

Table 2 shows MIC_{90}s and ranges of seven agents in every resistant genotype together with comparison with the gBLNAS MIC_{90}s. MIC_{90}s of seven β-lactam antibiotics for gBLNAR were affected in the following order: 64 μg/ml or grater (×64) for AMX and CDR, 2 μg/ml (×64) for CTX, 16 μg/ml (×32) for AMP, 0.5 μg/ml (×16) for CDN, and 1 μg/ml (×8) for TBM and MEM. Substitutions of Arg517His or Asn526Lys, plus Ser385Thr in PBP3, especially influenced MICs of penicillin and cephalosporin antibiotics [22, 23]. Influence of the substitutions on TBM and MEM MICs was less than for cephalosporins.

Table 2 MIC_{90} of β-lactam agents affected by *ftsI* gene mutations in *Haemophilus influenzae*

	gBLNAS ($n = 34$)	glow-BLNAR ($n = 8$)	gBLNAR ($n = 77$)
Ampicillin	0.5	2 (×4[a])	16 (×32)
Amoxicillin	1	4 (×4)	≥64 (×64)
Cefdinir	1	2 (×2)	≥64 (×64)
Cefditoren	0.031	0.063 (×2)	0.5 (×16)
Cefotaxime	0.031	0.063 (×2)	2 (×64)
Tebipenem	0.125	0.5 (×4)	1 (×8)
Meropenem	0.125	0.25 (×2)	1 (×8)

gBLNAS β-lactamase-nonproducing ampicillin (AMP)-susceptible, *glow-BLNAR* β-lactamase-nonproducing low-level AMP resistance, *gBLNAR* β-lactamase-nonproducing AMP resistance

[a] The × symbol throughout indicates multiple numbers compared to the MIC of gBLNAS

The only excellent MIC for gBLNAR was TFX, ranging from 0.008 to 0.063 μg/ml, with an MIC_{90} of 0.031 μg/ml.

Among all isolates, only 8.1 % could be serotyped: type b, 6.5 %, type e, 0.8 %, and type f, 0.8 %.The remainder were nontypeable (NT).

Mycoplasma pneumoniae

MIC distributions of six antibiotics for 54 *M. pneumoniae* isolates are shown in Fig. 4. ML-resistant strains possessing a mutation in domain V of the 23S rRNA were identified most frequently: A2063G (40.7 %), A2064G (1.9 %), or C2617A (1.9 %). Although MIC_{90}s of four ML for isolates without such mutations were excellent (0.0156 μg/ml or less), MRMP if possessing either an A2063G or an A2064G mutation showed high resistance to ERY, CLR, and AZM (MIC, 16 μg/ml or greater). A C2617 mutation was associated with a much smaller decrease in susceptibility to ML.

Although the MICs of MIN and TFX for *M. pneumoniae* were not excellent, these agents were equivalent in ML-susceptible and ML-resistant strains with MIC_{90}s of 1 and 0.5 μg/ml, respectively. No isolate showed resistance to both agents.

Serotype of *Streptococcus pneumoniae*

Figure 5 shows serotype distribution in relationship to genotypic resistance in 241 *S. pneumoniae* isolates. Serotype 6B (20.3 %) predominated in the isolates, followed, in order, by 19F (15.4 %), 14 (14.5 %), 23F (12.0 %), 19A (6.2 %), and 6C (5.4 %). Strains with the first four serotypes were almost all gPISP and gPRSP. The top four serotypes included no gPSSP.

Coverage of PCV7 and PCV13 for all isolates were calculated as 68.5 and 80.9 %, respectively; among gPRSP, coverage of both vaccines were high, calculated as 89.7 and 96.6 %, respectively. Notably, gPRSP strains were detected among non-vaccine types 19A, 6A, 35, and 15A.

Discussion

The three species *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Mycoplasma pneumoniae* are the main causative bacteria in patients with CAP [1–6]. Worldwide, the increase of resistant strains among these microorganisms poses problems in treatment for patients with CAP [7, 9, 11].

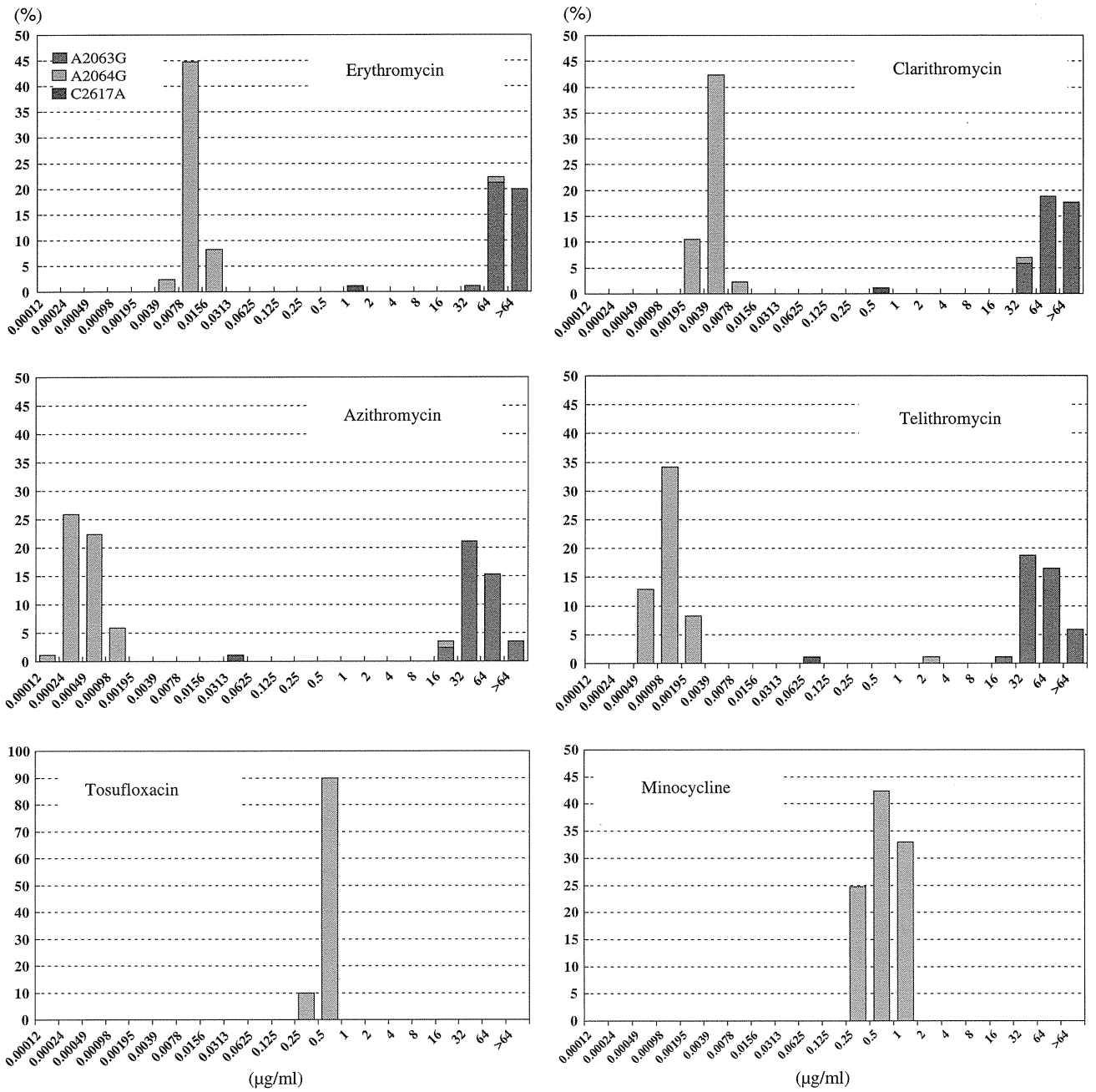

Fig. 4 Susceptibility distributions of macrolides, minocyclin (MIN), and TFX for *Mycoplasma pneumoniae* ($n = 54$)

In many countries, Hib conjugate vaccine and PCV7 have been incorporated into the pediatric vaccination schedule, with the aim of infection control. Presently, immunization with both Hib and PCV7 has been introduced in more than 100 countries worldwide.

Incidence of invasive pneumococcal disease and pneumonia causing vaccine-type *S. pneumoniae* has been reported to have decreased significantly after introduction of the vaccine [24, 25]. In Japan, PCV7 was approved in October 2009 by the government and marketed in February 2010. Although the pneumococcal isolates in this study were obtained just before marketing of PCV7, coverage for PCV 7 and PCV13 compared to our previous results in 2005 [21] has decreased somewhat, from 70.9 to 68.5 % for PCV7 and from 84.9 to 80.9 % for PCV13. Notably, in this study, gPRSP already were identified in non-vaccine types, specifically 19A, 6A, 35, and 15A.

Although reliable statistics are not yet available, the attainable vaccination rate for Japanese infants was considered to be about 60 % according to the 2011 official support provided by "Provisional Special Fund for the Urgent Promotion of Vaccination against Such Diseases as

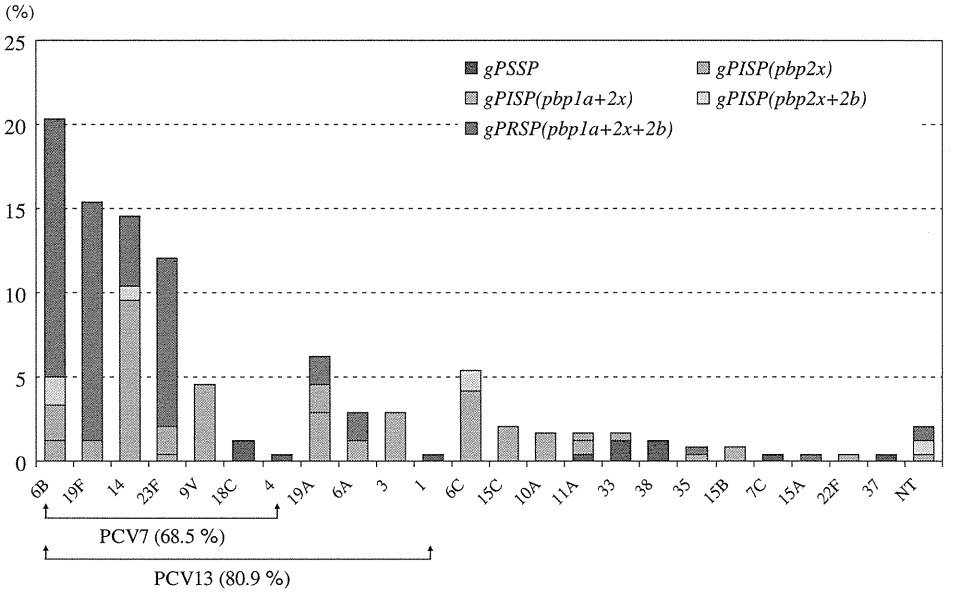

Fig. 5 Serotype distribution and resistance genes identified by polymerase chain reaction (PCR) in *Streptococcus pneumoniae* isolated from children ($n = 241$)

Cervical Cancer." The decrease in invasive pneumococcal diseases (IPD) from 333 cases in 2010 to 113 cases in 2011 (data not shown here) was attributed, in our nationwide surveillance, to the introduction of PCV7. However, the non-vaccine types 35 and 15A of gPRSP in addition to resistant 19A and 6A had increased significantly among IPD isolates in 2011, markedly limiting the efficacy of PCV7 to 46 %. Serotype changes favoring those not covered by PCV7 and PCV13 will become increasingly important clinical problems worldwide.

Nontypeable *H. influenzae* (NTHi) has attracted widespread attention for decreasing susceptibility to many oral and parenteral β-lactam antibiotics. In Japan, gBLNAR among Hib and NTHi isolated from patients with meningitis, pneumonia, and acute otitis media (AOM) has increased rapidly since 2000, to presently exceed 60 %, because the Hib vaccine was not marketed until late 2008 and also because oral cephalosporins were prescribed freely for respiratory diseases in children. Serum concentrations obtained using oral cephalosporins usually are low, exceeding the MIC of causative gBLNAR only on rare occasions.

Meningitis had decreased dramatically in 2011 because of official support as described above [26]. However, the Hib vaccine cannot contribute to the decrease in CAP caused by NTHi. Targeting other antigens in *H. influenzae* such as P6 membrane protein [27, 28] will be required to decrease NTHi diseases associated with resistance to β-lactam agents.

In this study, gMRMP was prevalent (44.4 %) and was associated with high ML resistance; a few patients initially received MIN. Subsequently, an outbreak of *M. pneumoniae* pneumonia occurred among children throughout Japan, beginning in early summer of 2011; 87.1 % of isolates were gMRMP [29]. Clinical findings of prolonged cough and fever characterized patients with gMRMP pneumonia; accordingly, the antibiotic was changed to MIN, doxycycline (DOX), or TFX for almost all patients. The clinical and bacteriological effectiveness of these three agents differed considerably, but only MIN is approved for treating *M. pneumoniae* infections in Japan. MIN or DOX was significantly more effective in achieving defervescence within 24 h and in decreasing numbers of gMRMP DNA copies 3 days after initiation than TFX ($p \leq 0.05$) [29]. Clinical trials of these agents are likely to gain approval for more agents in gMRMP-associated infections.

Finally, the rapid increase of resistant strains in these important causative pathogens contributes to narrowing the range of choice of oral antibiotics among pediatricians. To prevent increase of resistant strains, rapid identification of causative pathogens in CAP is needed, including both bacteria and viruses. Chest radiography and blood tests on admission also make important contributions. In likely bacterial infections, the most appropriate antibiotic must be chosen based on current information concerning antibiotic resistance and with reference to the Japanese Guidelines for the Management of Respiratory Infectious Diseases in Children 2007 [30].

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References

1. Don M, Canciani M, Korppi M. Community-acquired pneumonia in children: what's old? What's new? Acta Paediatr. 2010;99: 1602–8.
2. Honkinen M, Lahti E, Österback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. Clin Microbiol Infect. 2012;18: 300–7.
3. Juvén T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J. 2000;19:293–8.
4. MaIntosh K. Community-acquired pneumonia in children. N Engl J Med. 2002;346:429–37.
5. Okada T, Morozumi M, Sakata H, Takayanagi R, Ishiwada N, Sato Y, et al. A practical approach estimating etiologic agents using real-time PCR in pediatric inpatients with community-acquired pneumonia. J Infect Chemother. 2012;. doi:10.1007/s10156-012-0422.
6. Sinaniotis CA, Sinaniotis AC. Community-acquired pneumonia in children. Curr Opin Pulm Med. 2005;11:218–25.
7. Chetty K, Thomson AH. Management of community-acquired pneumonia in children. Paediatr Drugs. 2007;9:401–11.
8. Chiba N, Morozumi M, Ubukata K. Application of the real-time PCR method for genotypic identification of β-lactam resistance in isolates from invasive pneumococcal diseases. Microb Drug Resist. 2012;18:149–56.
9. Esposito S, Cohen R, Domingo JD, Pecurariu OF, Greenberg D, Heininger U, et al. Antibiotic therapy for pediatric community-acquired pneumonia: do we know when, what and for how long to treat? Pediatr Infect Dis J. 2012;31:78–85.
10. Kishii K, Chiba N, Morozumi M, Hamano-Hasegawa K, Kurokawa I, Masaki J, et al. Diverse mutations in the *ftsI* gene in ampicillin-resistant *Haemophilus influenzae* isolates from pediatric patients with acute otitis media. J Infect Chemother. 2010;16:87–93.
11. Morozumi M, Takahashi T, Ubukata K. Macrolide-resistant *Mycoplasma pneumoniae*: characteristics of isolates and clinical aspects of community-acquired pneumonia. J Infect Chemother. 2010;16:78–86.
12. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004;4:144–54.
13. Jourdain S, Smeesters PR, Denis O, Dramaix M, Sputael V, Malaviolle X, et al. Differences in nasopharyngeal bacterial carriage in preschool children from different socio-economic origins. Clin Microbiol Infect. 2011;17:907–14.
14. Murphy TF, Faden H, Bakaletz LO, Kyd JM, Forsgren A, Campos J, et al. Nontypeable *Haemophilus influenzae* as a pathogen in children. Pediatr Infect Dis J. 2009;28:43–8.
15. Hamano-Hasegawa K, Morozumi M, Nakayama E, Chiba N, Murayama SY, Takayanagi R, et al. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. J Infect Chemother. 2008;14: 424–32.
16. Morozumi M, Nakayama E, Iwata S, Aoki Y, Hasegawa K, Kobayashi R, et al. Simultaneous detection of pathogens in clinical samples from patients with community-acquired pneumonia by real-time PCR with pathogen-specific molecular beacon probes. J Clin Microbiol. 2006;44:1440–6.
17. Kishii K, Morozumi M, Chiba N, Ono A, Ubukata K. Direct detection by real-time PCR of *ftsI* gene mutations affecting MICs of β-lactam agents for *Haemophilus influenzae* isolates from meningitis. J Infect Chemother. 2011;17:671–7.
18. Morozumi M, Hasegawa K, Kobayashi R, Inoue N, Iwata S, Kuroki H, et al. Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. Antimicrob Agents Chemother. 2005;49:2302–6.
19. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 18th informational supplement. CLSI document M100-S18. Wayne, PA: CLSI; 2008
20. Ubukata K, Chiba N, Hasegawa K, Shibasaki Y, Sunakawa K, Nonoyama M, et al. Differentiation of beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae* from other *H. influenzae* strains by a disc method. J Infect Chemother. 2002;8:50–8.
21. Chiba N, Kobayashi R, Hasegawa K, Morozumi M, Nakayama E, Tajima T, et al. Antibiotic susceptibility according to genotype of penicillin-binding protein and macrolide resistance genes, and serotype of *Streptococcus pneumoniae* isolates from community-acquired pneumonia in children. J Antimicrob Chemother. 2005;56:756–60.
22. Ubukata K, Shibasaki Y, Yamamoto K, Chiba N, Hasegawa K, Takeuchi Y, Sunakawa K, Inoue M, Konno M. Association of amino acid substitutions in penicillin-binding protein 3 with beta-lactam resistance in beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae*. Antimicrob Agents Chemother. 2001;45:1693–9.
23. Witherden EA, Montgomery J, Henderson B, Tristram SG. Prevalence and genotypic characteristics of beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae* in Australia. J Antimicrob Chemother. 2011;66:1013–5.
24. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis. 2010;201: 32–41.
25. Isaacman DJ, McIntosh ED, Reinert RR. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. Int J Infect Dis. 2010;14:197–209.
26. Ubukata K, Chiba N, Morozumi M, Iwata S, Sunakawa K, and the Working Group of nationwide Surveillance for Bacterial Meningitis. Longitudinal surveillance of *Haemophilus influenzae* isolates from pediatric patients with meningitis throughout Japan, 2000 to 2011. J Infect Chemother. doi:10.1007/s10156-012-0448-x.
27. Chang A, Kaur R, Michel LV, Casey JR, Pichichero M. *Haemophilus influenzae* vaccine candidate outer membrane protein P6 is not conserved in all strains. Hum Vaccin. 2011;7:102–5.
28. Noda K, Kodama S, Umemoto S, Nomi N, Hirano T, Suzuki M. Th17 cells contribute to nontypeable *Haemophilus influenzae*-specific protective immunity induced by nasal vaccination with P6 outer membrane protein and α-galactosylceramide. Microbiol Immunol. 2011;55:574–81.
29. Okada T, Morozumi M, Tajima T, Hasegawa M, Sakata H, Ohnari S, et al. Rapid effectiveness of minocycline or doxycycline against macrolide-resistant *Mycoplasma pneumoniae* infection in a 2011 outbreak among Japanese children. Clin Infect Dis. doi:10.1093/cid/cis784.
30. Uehara S, Sunakawa K, Eguchi H, Ouchi K, Okada K, Kurosaki T, et al. Japanese guidelines for the management of respiratory infectious diseases in children 2007 with focus on pneumonia. Pediatr Int. 2011;53:264–76.

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Note

Changes in nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* among healthy children attending a day-care centre before and after official financial support for the 7-valent pneumococcal conjugate vaccine and *H. influenzae* type b vaccine in Japan

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Nasopharyngeal carriage

ABSTRACT

The 7-valent pneumococcal conjugate vaccine (PCV7) and *Haemophilus influenzae* type b (Hib) vaccine reduce nasopharyngeal carriage of vaccine-type bacteria, which may in turn influence the presence of other nasopharyngeal bacterial pathogens. To investigate this possibility, nasopharyngeal carriage of potential pathogens was examined before and after official financial support was provided to offer the PCV7 and Hib vaccines in healthy children attending a day care centre in Japan during 2011–2012. Despite a virtual disappearance of PCV7 serotypes over time, the overall pneumococcal carriage rate remained unchanged. Although others have reported an increase in PCV13 serotypes following PCV7 vaccination, only non-PCV13 serotypes were observed to have increased in this study. The majority of *H. influenzae* isolates were non-typeable and Hib was not found. Our data identified an unexpected pattern of pneumococcal serotype replacement following PCV7. Continuous monitoring of pneumococcal carriage is important for decisions regarding the future of national vaccination policy in Japan.

The 7-valent pneumococcal conjugate vaccine (PCV7) and *Haemophilus influenzae* type b (Hib) vaccine prevent nasopharyngeal acquisition and transmission of 7 serotypes of pneumococci and Hib in children, respectively [1–3]. Vaccinating children with both PCV7 and Hib vaccines offers effective protection against invasive disease due to PCV7 serotypes and Hib in all age groups [3–5]. However, in many countries, the nasopharyngeal flora of PCV7-vaccinated children is immediately occupied by non-PCV7 but PCV13 serotype pneumococci either due to true replacement, unmasking, or capsular switch, resulting in a similar overall pneumococcal carriage rate [1]. As a result, PCV13 vaccination is now a prevailing strategy to prevent against severe pneumococcal disease, including invasive pneumococcal disease (IPD), in the US and Europe. In Korean children, *Streptococcus pneumoniae* serotype 19A is increasingly recognized as a cause of IPD prior to the introduction of PCV7 [6]. In Japanese children, rates of invasive pneumococcal disease (IPD) due to 19A and non-PCV13 serotypes increased soon after the introduction of PCV7 [7].

In Japan, the PCV7 and Hib vaccines were not approved by the Japanese Ministry of Health, Labor and Welfare until January of 2007 and 2008, respectively. Therefore, it was not possible to have children voluntarily vaccinated against Hib and PCV7 until late 2008 and 2009, respectively. In November 2010, the Japanese Ministry of Labour Health and Welfare established a Provisional Special Fund and recommended vaccination of children with Hib vaccine and PCV7 for the Urgent Promotion of Vaccination. After

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this, Hib vaccine and PCV7 were formally added to the immunization schedule for Japanese infants in 2013.

Since February 2011, Hib and PCV7 vaccines have been publically funded for children under 5 years old in Chiba Prefecture. However, because these vaccines were not yet widely accessible in Japan, vaccination rates among infants and younger children at risk were estimated to be 40–60% at the end of 2011. Therefore, the aim of this study was to investigate the nasopharyngeal carriage of bacteria in healthy children before and immediately after official financial support was provided for these vaccinations.

After obtaining written informed consent from at least one parent, a nasal swab was taken from the participating child and the parent was asked to complete a standardized short questionnaire. The study population consisted of 57 children.

Approval for this study was obtained from the Medical Ethical Committee of Chiba University (no.1120).

Children attending a day care centre at Chiba University Hospital were studied from February 2011 to October 2012 with nasopharyngeal swabs taken every 6 months. At least one of their parents worked at Chiba University Hospital. Parents of the participating children completed a brief survey about their PCV7 and Hib vaccination history. Samples of nasopharyngeal flora were obtained from the children with a nylon flocked flexible sterile Copan E-swab according to World Health Organization standard procedures [8]. After sampling, all swabs were directly inoculated in a liquid medium and plated within 1 h at the Microbiology Laboratory of Chiba University Hospital. All swabs were processed by the same laboratory and cultured to detect the presence of *S. pneumoniae, H. influenzae* and *Moraxella catarrhalis* according to standard bacteriological procedures for conventional cultures. One pneumococcal colony per plate was subcultured and serotyped by Quellung reaction using type-specific antisera from the Statens Serum Institute (Copenhagen, Denmark). To determine the sequence type (ST) of the isolates, multi-locus sequence typing (MLST) was performed as described previously [9]. STs were determined by an internet database search at http://spneumoniae.mlst.net/.

One *H. influenzae* colony per plate was subcultured and serotyped using a slide agglutination test using six monovalent antisera (serotypes a-f) manufactured by Remel (Remel Inc., Lenexa, KS, USA). Specimens were also inoculated on Hib antiserum agar prepared with Levinthal base and Hib antiserum as described previously [10].

SPSS statistics 18.0 software was used to examine differences in distribution between the studied populations. The crude odds ratio (OR) and Mantel–Haenszel OR stratified by age with 95% confidence intervals (CIs) were calculated using the χ^2 test. A P value of <0.05 was considered statistically significant.

Table 1 shows the baseline characteristics of the children who participated in the study. A total of 57 children aged from 5 months to 6 years were enrolled in the study. Twenty children participated once and 37 children participated more than once with 11, 22 and 4 children participating 2, 3 and 4 times, respectively. During the course of the study, no participants hospitalized with IPD or invasive Hib disease.

The number of non-immunized children and children vaccinated on a catch-up schedule gradually decreased, while the number of fully immunized children increased during this study.

S. pneumoniae, H. influenzae, M. catarrhalis and *Staphylococcus aureus* were isolated from nasopharyngeal culture as pathogenic bacterial species of interest. Because *S. aureus* was detected at a very low rate ($n = 6$), specific bacterial species refers to *S. pneumoniae, H. influenzae* and *M. catarrhalis* in this report. The distribution of carriage of each pathogen is shown in Table 2. Overall carriage rates of pathogenic bacteria were 47.6% ($n = 59$) for *S. pneumoniae*, 35.5% ($n = 44$) for *H. influenzae* and 58.1% ($n = 72$) for *M. catarrhalis*. No significant association was found between gender and colonization by specific bacterial species. The age-specific recovery of specific nasopharyngeal pathogens is shown in Fig. 1. *S. pneumoniae* and *M. catarrhalis* carriage rates were observed to decline with age, while *H. influenzae* carriage rates remained almost the same. Younger age (<24 months) was significantly associated with *S. pneumoniae* colonization (OR = 1.639, 95% CI 1.147–2.343, $P = 0.008$). Carriage of *H. influenzae* was not associated with age. *M. catarrhalis* declined with age and was significantly more prevalent among children

Table 1
Characteristics of the children participating in this study.

	Mar. 2011	Oct. 2011	Mar. 2012	Oct. 2012
Children	29	35	32	28
Male	18	20	22	16
Female	11	15	10	12
Age group				
<1 yr	2	4	3	0
1 yr	6	12	10	9
2 yr	9	8	8	9
3 yr	5	5	3	5
4 yr	2	2	4	4
>5 yr	5	4	4	1
PCV7 status				
Fully immunized (4 doses)	4	4	8	13
Catch up[a] (1–3 doses)	11	14	12	9
On going[b] (1–3 doses)	5	12	9	4
Not immunized[c]	9	5	3	2
Hib vaccine status				
Fully immunized (4 doses)	5	5	5	12
Catch up[a] (1–3 doses)	12	10	13	9
Ongoing[b] (1–3 doses)	8	16	12	7
Not immunized[c]	4	4	2	0

[a] Catch up: a child first vaccination started after 7 months old and finished with reduced doses.
[b] Ongoing: a child who has not completed his or her vaccination schedule.
[c] Not immunized: a child who has not been immunized.

Table 2
Characteristics of bacterial isolates.

	Mar. 2011	Oct. 2011	Mar. 2012	Oct. 2012	MLST (No. of isolates)
Total No. of *S. pneumoniae*	**16**	**15**	**14**	**14**	
PCV7 serotypes	7 (43.8%)	3 (20.0%)	1 (7.1%)	0 (0%)	
6B	4	2	0	0	ST902 (5)
					ST5233 (1)
19F	3	0	1	0	ST8454 (1)
					ST236 (3)
23F	0	1	0	0	ST242 (1)
Non-PCV13 serotypes	9 (56.2%)	12 (80.0%)	13 (92.9%)	14 (100%)	
23A	2	1	0	0	ST338 (3)
15A	1	2	1	2	ST63 (6)
15C	1	0	1	2	ST199 (4)
34	3	7	1	0	ST7388 (11)
35B	1	2	7	2	ST2755 (12)
37	1	0	0	0	ST447 (1)
15B	0	0	3	0	ST199 (3)
6C	0	0	0	3	ST2942 (1)
					ST5823 (2)
11A/E	0	0	0	3	ST8737 (3)
10A	0	0	0	1	ST5236 (1)
Non-typeable	0	0	0	1	ST4845 (1)
Total No. of *H. influenzae*	**5**	**4**	**24**	**11**	
Type d	0	0	0	1	
Type e	1	1	4	0	
Type f	1	0	0	0	
Nontypeable Hi	3	3	20	10	
Total No. of *M. catarrhalis*	**19**	**12**	**27**	**14**	

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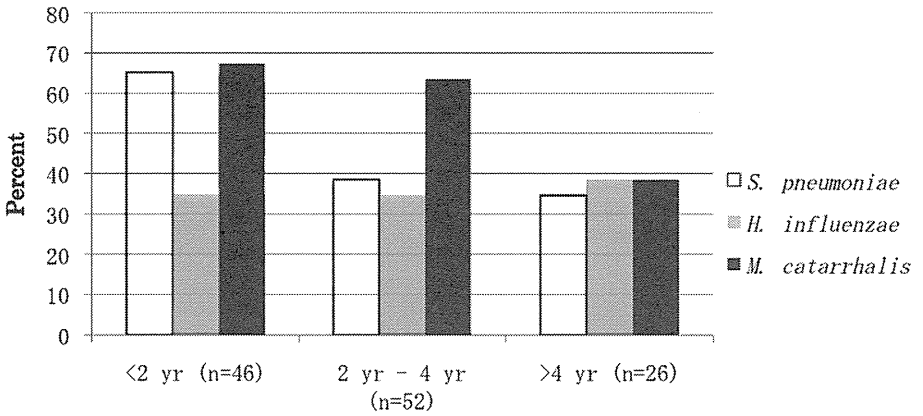

Fig. 1. Recovery of specific nasopharyngeal pathogens with age.

under 48 months (OR = 1.857, 95% CI 1.073–3.214 $P = 0.006$). Almost one third ($n = 45$, 36.3%) of the cases had only one respiratory pathogen. More than one pathogen was colonized in 56 cases (45.2%). Thirty-six cases (29.0%) had two respiratory pathogens and 20 children (16.1%) had three species. Even when the influence of age was eliminated using the Mantel–Haenszel test, a positive association was noted for co-colonization with *S. pneumoniae* and *M. catarrhalis* (OR 4.878, 95% CI 1.442–16.495, $P = 0.009$). No significant associations were observed between the presence of *H. influenzae* and colonization with the other two bacterial species.

We then analyzed the characteristics of 59 *S. pneumoniae* and 44 *H. influenzae* isolates. Near complete eradication of PCV7 serotype carriage was observed within 2 years of announcement of the Provisional Special Fund recommendation for PCV7 immunization. The 6B and 19F PCV7 serotypes were also effectively eliminated following vaccination (Table 2). Although previous studies have reported vaccination to produce an emergence of PCV13 serotypes 6A and 19A, only non-PCV13 serotypes were identified in this study. In PCV7 immunized children (including on going immunization schedule), 50 *S. pneumoniae* strains were isolated, whereas 9 *S. pneumoniae* strains were isolated from PCV7 non-immunized children. Forty-two non-PCV13-type strains and 8 PCV7-type strains were isolated from PCV7 immunized children. Six non-PCV13-type strains and 3 PCV7-type strains were isolated from unvaccinated participants. There was no significant association between the *S. pneumoniae* serotypes and PCV7 immunization status. There were 4 children who participated in this study 3 or 4 times, and carried a PCV7-type strain at the first culture. The PCV7 immunization status of all 4 children did not change during this study. Among them, one child acquired a non-PCV13-type strain and three did not carry any *S. pneumoniae* strains in the last culture. Furthermore, we performed MLST analysis and identified the sequence type (ST) of each serotype (Table 2). Most of isolates with the same serotype had one sequence type (ST).

Next, the capsular serotypes of 42 *H. influenzae* isolates were analyzed and 8 out of 42 (19%) of them were found to be capsulated, after which they were categorized as type d, e, or f (Table 2). No colony was identified on Hib antiserum agar.

Since bacterial colonization depends on numerous factors, including economic and environmental variables as well as host-related factors and vaccination effects, bacterial carriage rates vary widely among different studies and geographical sites [11]. The objective of the present study was to establish the prevalence and specific features of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* strains circulating amongst day care centre attendees in Japan.

In our study, a majority of children (81.5%) harbored at least one potential respiratory pathogen. Rates of Hib and PCV7 vaccination were high among subjects even before public funding became available. This might be attributable to greater interest among parents regarding their children's health, since at least one parent of each child was working at Chiba University Hospital.

This study shows that the risk of being colonized by *S. pneumoniae* is not associated with colonization by *H. influenzae* but positively associated with colonization by *M. catarrhalis*. The risk of being colonized by *H. influenzae* was not associated with colonization by *M. catarrhalis*, which is consistent with the findings of a previous report [12].

After the introduction of PCV7 vaccination, the prevalence of PCV13 serotypes 6A and 19A has been reported to increase, while PCV7 serotypes are known to become less dominant. PCV13, including serotypes 6A and 19A, replaced PCV7 in vaccination schedules in the US in 2010. Presently, PCV7 is being gradually replaced with PCV13 worldwide. In addition, an increase in non-PCV13 serotypes 15A and 22F has been reported in the US [13]. In our study, carriage of vaccine type strains decreased significantly after PCV7 vaccination became publically funded. Unlike the findings reported in the US and elsewhere, an increase in non-PCV13 serotypes, including serotypes 6C, 15A, 15C, 35B and 11A/E, was observed as opposed to PCV13 serotypes. A similar prevalence of pathogens has been reported in Japanese pediatric patients with IPD [7]. Spread of microorganisms is commonplace in the era of globalization. As such, replacement of the PCV7 vaccine with a PCV13 vaccine may have little efficacy, even in those areas that are currently observing emergence of PCV13 serotypes in the setting of PCV7 vaccination. Prevention and control of pneumococcal infections in young children will require the development of new vaccination strategies aimed at targeting additional serotypes or other antigens.

Introduction of Hib vaccination led to a significant reduction of Hib disease and carriage in both vaccinated and unvaccinated children due to a herd immune effect [14]. More than 80% of children in our study were vaccinated against Hib, and no Hib strain was recovered in any child. Kuroki et al. reported a Hib carriage rate of 0.84% among healthy Japanese children prior to introduction of the Hib vaccine [10]. Specific data regarding the prevalence of Hib carriage prior to introduction of the Hib vaccination are not available in this setting and the absence of Hib isolates is likely to be the result of a very low prevalence of Hib carriage alone. *H. influenzae* capsular type d, e and f were present in small amounts but detected every time. Invasive disease due to *H. influenzae* type d, e or f is rare, but needs to be considered as a possibility. Although a randomized controlled study reported that no changes in carriage rate with *H. influenzae* and *M. catarrhalis* were observed after vaccination with 2 or 3 of doses PCV7 [15], the carriage rate of *H. influenzae* and

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M. catarrhalis in this study seems to have increased after official financial support for the PCV7 and Hib vaccine was introduced. Higher PCV pressure following a 4 doses schedule and nationwide introduction in the routine infant vaccination schedule may induce bacterial shifts. We should monitor the colonization status of immunized children to evaluate this potential phenomenon.

Continuous surveillance of carriage of invasive disease pathogens will allow us to establish the effect of conjugate vaccine use on *S. pneumoniae* and *H. influenzae* serotype distribution.

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Conflict of interest

None.

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References

[1] Kayhty H, Auranen K, Nohynek H, Dagan R, Makela H. Nasopharyngeal colonization: a target for pneumococcal vaccination. Expert Rev Vaccines 2006;5:651–67.

[2] Spijkerman J, van Gils EJ, Veenhoven RH, Hak E, Yzerman EP, van der Ende A, et al. Carriage of *Streptococcus pneumoniae* 3 years after start of vaccination program, the Netherlands. Emerg Infect Dis 2011;17:584–91.

[3] McVernon J, Howard AJ, Slack MP, Ramsay ME. Long-term impact of vaccination on *Haemophilus influenzae* type b (Hib) carriage in the United Kingdom. Epidemiol Infect 2004;132:765–7.

[4] Grijalva CG, Griffin MR. Population-based impact of routine infant immunization with pneumococcal conjugate vaccine in the USA. Expert Rev Vaccines 2008;7:83–95.

[5] Rose M, Zielen S. Impact of infant immunization programs with pneumococcal conjugate vaccine in Europe. Expert Rev Vaccines 2009;8:1351–64.

[6] Choi EH, Kim SH, Eun BW, Kim SJ, Kim NH, Lee J, et al. *Streptococcus pneumoniae* serotype 19A in children, South Korea. Emerg Infect Dis 2008;14:275–81.

[7] Chiba N, Morozumi M, Shouji M, Wajima T, Iwata S, Sunakawa K, et al. Rapid decrease of 7-valent conjugate vaccine coverage for invasive pneumococcal diseases in pediatric patients in Japan. Microb Drug Resist 2013;19:308–15.

[8] O'Brien KL, Nohynek H. Report from a WHO Working Group: standard method for detecting upper respiratory carriage of *Streptococcus pneumoniae*. Pediatr Infect Dis J 2003;22:e1–11.

[9] Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. Microbiology 1998;144:3049–60.

[10] Kuroki H, Ishikawa N, Uehara S, Himi K, Sonobe T, Niimi H. Nasopharyngeal colonization with *Haemophilus influenzae* type b among infants and children in Japan. Acta Paediatr Jpn 1997;39:541–5.

[11] Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother 2002;50:59–73.

[12] Dahlblom V, Soderstrom M. Bacterial interactions in the nasopharynx – effects of host factors in children attending day-care centers. J Infect Public Health 2012;5:133–9.

[13] Gertz Jr RE, Li Z, Pimenta FC, Jackson D, Juni BA, Lynfield R, et al. Increased penicillin nonsusceptibility of nonvaccine-serotype invasive pneumococci other than serotypes 19A and 6A in post-7-valent conjugate vaccine era. J Infect Dis 2010;201:770–5.

[14] Heath PT, McVernon J. The UK Hib vaccine experience. Arch Dis Child 2002;86:396–9.

[15] van Gils EJ, Veenhoven RH, Rodenburg GD, Hak E, Sanders EA. Effect of 7-valent pneumococcal conjugate vaccine on nasopharyngeal carriage with *Haemophilus influenzae* and *Moraxella catarrhalis* in a randomized controlled trial. Vaccine 2011;44:7595–8.

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第8回　日本小児耳鼻咽喉科学会

シンポジウム4

予防接種のインパクト

細菌性髄膜炎予防ワクチン定期接種化のインパクトを考える

小児耳鼻咽喉科領域感染症への影響も含めて

石和田　稔　彦

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小児に細菌性髄膜炎を惹起する2大原因菌は，インフルエンザ菌b型（Hib）と肺炎球菌である。また，Hibは急性喉頭蓋炎の主要な原因菌でもある。Hib，肺炎球菌による感染症を予防するワクチンは，世界中の国々で使用され劇的な効果をあげている。一方，日本においては2008年12月からHibワクチンが，2010年2月から7価肺炎球菌結合型ワクチン（PCV7）が使用可能となった。Hibワクチン，PCV7は，当初任意接種ワクチンとして導入されたが接種率が低く十分な予防効果が認められていなかった。しかし，2011年になり，全国的に公費助成が受けられる体制が出来たことから接種率が上昇し，髄膜炎をはじめとする重症感染症が全国的に減少している。今年両ワクチンが定期接種化されることで，更なる効果が期待される。今後ワクチンの有効性を正しく評価するためには，正確な罹患率調査と分離菌の莢膜型別解析が重要な課題である。

キーワード：髄膜炎，インフルエンザ菌b型ワクチン，7価肺炎球菌結合型ワクチン，莢膜型，小児

はじめに

細菌性髄膜炎は急激に進行する予後不良な疾患である。小児では典型的な臨床症状を呈さず，初期診断が困難なことが多い。また，適切な治療を行っても，永続的な後遺症を残すことや不幸にして亡くなることも多い。小児に細菌性髄膜炎を惹起する2大原因菌は，インフルエンザ菌と肺炎球菌であり，2007年～2008年の日本での小児細菌性髄膜炎に関する全国アンケート調査では，インフルエンザ菌が髄膜炎原因菌全体の57%，肺炎球菌が19%を占めていた[1]。これら2大原因菌に対する予防ワクチンである，インフルエンザ菌b型（Hib）ワクチンと，7価肺炎球菌結合型ワクチン（PCV7）が開発され，日本でも広く使用されるようになり，2013年4月から定期接種化された。本講演では，細菌性髄膜炎予防ワクチンの導入効果

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と今後の課題を中心に，小児耳鼻咽喉科領域感染症への影響も含め概説したい。

インフルエンザ菌感染症

インフルエンザ菌は6種類の莢膜型（a, b, c, d, e, f）と無莢膜株に分けられる。Hibは主に乳幼児に髄膜炎，喉頭蓋炎などの侵襲性感染症（血液や髄液から細菌が分離される感染症）を惹起する。Hib以外の莢膜型も侵襲性感染症を惹起するがその頻度は低い。一方，無莢膜株は小児及び成人の気管支炎，肺炎，中耳炎，副鼻腔炎，結膜炎などの局所感染症の主たる原因菌である。分離されたインフルエンザ菌がHibであるかどうかの判定は，熟練した細菌検査技師であればコロニーの性状から判別可能であるが，一般的には抗血清による凝集法もしくはPCR法を用いた判定が必要となる。しかし，莢膜型別は一般の医療機関では行われていない。私たちは，Hibワクチン導入前の小児インフルエンザ菌侵襲性感染症調査と分離株に対するPCR法を用いた莢膜型解析を行った[2]。その結果，小児インフルエンザ菌侵襲性感染症の中で髄膜炎は最も頻度が高く，その他の病型としては，菌血症，関節炎，喉頭蓋炎，肺炎が認められた。莢膜型の解析においては，Hibが侵襲性感染症の88.8%，髄膜炎の95.1%を占めており，日本においてもHibは髄膜炎を主体とするインフルエンザ菌侵襲性感染症の主体と考えられた。急性喉頭蓋炎は，耳鼻咽喉科との連携により診断，治療にあたる必要のある重要な小児救急疾患のひとつであるが，日本で原因菌に関するまとまった報告は少ない。2000年～2010年にかけて，東京都と千葉県の6施設で調査したところ，5歳以下の小児急性喉頭蓋炎の80%がHib菌血症を伴っていた[3]（図1）。この他，眼窩周囲蜂窩織炎もHibによるものが多いとされる。私たちは1985年から経年的に千葉県におけるインフルエンザ菌侵襲性感染症の罹患率調査を実施しているが，1985年5歳未満人口10万人あたり1.2であった罹患率は徐々に増加し，2005年には16.5となった[4]。

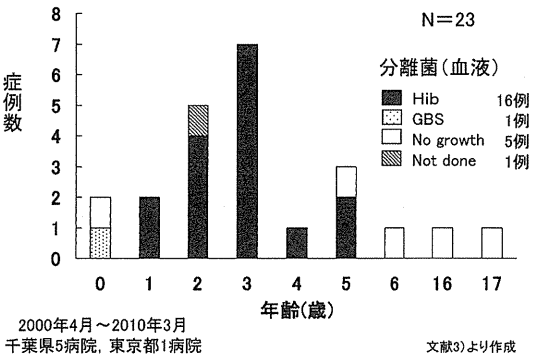

図1　急性喉頭蓋炎の原因菌と年齢分布

Hibワクチン

Hibワクチンは，Hibの病原性の主体となる莢膜多糖体（PRP）に破傷風菌のトキソイドを結合させ，乳児にも十分な免疫をつけることが出来るように工夫されたワクチンである。1987年に米国ではHibワクチンが導入され，患者数が減少したと報告されている[5]。また，フィンランドにおいては，Hibワクチン導入後，髄膜炎と共に急性喉頭蓋炎も激減したことが報告されている[6]。現在Hibワクチンは世界100か国以上で認可されている。このような状況の下，日本においてもHibワクチンの接種が2008年12月から可能となった。Hibワクチンの効能・効果はHibによる感染症予防であり，Hib以外の莢膜型と無莢膜株に対しての予防効果はない。日本におけるHibワクチンの接種対象者は，生後2カ月以上5歳未満で，推奨される接種開始時期は，生後2カ月～6カ月である。初回免疫を4～8週間隔（医師が必要と認めた場合には3週間隔でも可能）で3回行い，おおむね7～13カ月後に追加接種を1回行うことが標準的な接種スケジュールとなっている（図2）。Hibワクチン導入後の状況であるが，接種開始当初は，品不足や任意接種であったこともあり，接種率が伸びず明らかな予防効果は認められなかった。しかし，2011年から「子宮頸がん等ワクチン接種緊急促進臨時特例交付金制度」により，全国的に公費助成制度

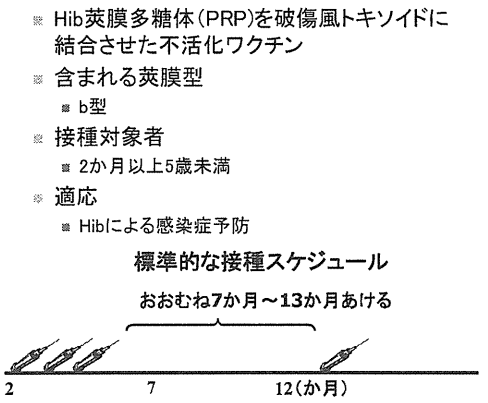

図2　インフルエンザ菌ｂ型（Hib）ワクチン

が導入され大多数の市町村で5歳未満の小児に対して，Hibワクチンが無料で接種可能となった。ワクチンの出荷状況や公費助成制度導入後の市町村での推定接種率などから総合的に判断すると，Hibワクチンの接種率は公費助成制度導入後上昇していると考えられ，それに伴い髄膜炎をはじめとするインフルエンザ菌侵襲性感染症は，明らかに減少してきている。千葉県ではインフルエンザ菌による髄膜炎は，2010年30例発生していたが，2011年7例，2012年4例，2013年6月現在0例である。

肺炎球菌感染症

Hibワクチン，PCV7普及前の日本において小児細菌性髄膜炎の原因菌として，インフルエンザ菌に次いで多かったのが肺炎球菌である[1]。インフルエンザ菌と異なりほとんど全ての肺炎球菌は莢膜を有しており，莢膜型は現在93種類に分類される。莢膜型別は，インフルエンザ菌と同様一般の医療機関では実施されていない。肺炎球菌は，小児から成人に至るまで幅広い年齢層に侵襲性感染症も局所感染症も共に惹起するが，侵襲性感染症で最も頻度の高いものは菌血症ついで肺炎，髄膜炎の順となる。急性乳突洞炎も耳鼻咽喉科医との連携が必要となる重要な肺炎球菌関連疾患である。PCV7導入前の千葉県では2007年39例，2008年61例，2009年76例と年々肺炎球菌侵襲性感染症による入院例は増加傾向を認め，5歳未満人口10万人あたりの罹患率は2009年26.1に達した[7]。肺炎球菌菌血症は，外来症例を中心に検討するとより多いとする報告があり，西村らは菌血症の5歳未満人口10万人あたりの罹患率は328と推計している[8]。なお，肺炎球菌性髄膜炎に関しては，2007年～2010年における千葉県内の調査において，19例のうち，7例（29.0%）が後遺症を残し1例（3.1%）が死亡しており，予後不良例が多かった。

7価肺炎球菌結合型ワクチン（PCV7）

PCV7は4, 6B, 9V, 14, 18C, 19F, 23Fの7つの血清型の莢膜多糖体をジフテリア毒素の変異蛋白（ジフテリア CRM_{197}）に結合させたものであり，乳児にも十分な免疫が誘導できる。海外におけるPCV7導入前の小児侵襲性感染症症例から分離される肺炎球菌のPCV7カバー率は70%を超えており，実際米国では2000年にPCV7を導入した後，PCV7に含まれる莢膜型の肺炎球菌による侵襲性感染症は激減した[9]。日本におけるPCV7の効能・効果は，PCV7に含まれる血清型による肺炎球菌侵襲性感染症予防で，接種対象は生後2カ月～10歳未満であり，推奨される接種開始時期は生後2カ月～6カ月で，初回免疫を4～8週間隔で3回行い，生後12～15カ月に追加接種を行うというスケジュールになっている（図3）。PCV7導入後の状況に関して，PCV7導入前の国内の肺炎球菌に関する疫学調査結果からみるとPCV7に含まれる血清型別のカバー率は肺炎球菌全体の約70%となっており[10]，日本においてもPCV7を導入した場合，充分な効果が得られることが予想されていた。しかし，2010年2月からPCV7は使用可能となったもののHibワクチンと同様，任意接種の段階では接種率が伸びず，患者数は減少しなかった。公費助成が認められるようになってから，患者数は減り，千葉県では2010年肺炎球菌髄膜炎は10例発生していたが，2011年2例，2012例3例，2013年6月現在1例となっている。

- 7つの肺炎球菌莢膜多糖体をジフテリア毒素の変異蛋白(ジフテリアCRM$_{197}$)に結合させた不活化ワクチン
- 含まれる莢膜型
  - 4, 6B, 9V, 14, 18C, 19F, 23F
- 接種対象者
  - 2か月以上10歳未満
- 適応
  - PCV7に含まれる血清型による侵襲性肺炎球菌感染症予防

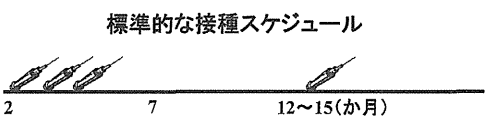

図3　7価肺炎球菌結合型ワクチン（PCV7）

PCV7の肺炎，中耳炎，保菌に対する影響

PCV7は導入後海外において，2歳未満の市中肺炎入院例が減少したこと[11]や肺炎球菌による中耳炎の罹患率を低下させたという報告がなされている[12]。日本では侵襲性感染症予防の適応しかないが，海外においては肺炎や中耳炎を予防適応疾患としている国も多く認められる。我々は，PCV7導入前の2008年と導入後の2012年，千葉市において小児市中肺炎の罹患率に関する調査を行ったところ，喀痰から肺炎球菌が有意に分離され，肺炎球菌性肺炎と考えられる入院例が減少し，分離された菌株をみると2012年は2008年に比べてPCV7に含まれる莢膜型が有意に減少していた。詳細な分析が必要ではあるが，PCV7が日本の小児市中肺炎の疫学に影響を与えていると考えている。中耳炎に関しては，PCV7導入前の小児急性化膿性中耳炎患者の中耳貯留液での分離菌の32％が肺炎球菌であり，分離された肺炎球菌のうち，62.7％がPCV7に含まれる莢膜型ということが報告されている[13]。今後PCV7導入の小児中耳炎に与える影響に関する検討の結果が待たれる。私たちは，PCV7が肺炎球菌保菌に与える影響について千葉大学医学部附属病院に併設されている保育園において保護者の同意のもと，園児の保菌調査を定期的に実施した（2011年2月，10月，2012年2月）。検討時期における園児のPCV7接種率は62.5％，86.9％，94.0％と上昇し，それに伴い，分離される肺炎球菌の中でPCV7に含まれる莢膜型の比率が，44.4％，20.0％，7.1％と減少した。ちなみにHibに関しては，初回調査時すでにHibワクチン接種率は84.4％に達しており，3回の保菌調査においてHibは1株も分離されなかった[14]。

表1　全国10道県の小児期侵襲性細菌感染症罹患率の推移

	2008～2010	2011	2012	減少率（％）2008～2010年と2012年の比較
Hib 髄膜炎	7.7	3.3	0.6	92
Hib 非髄膜炎	5.1	3.0	0.9	82
肺炎球菌髄膜炎	2.8	2.1	0.8	71
肺炎球菌非髄膜炎	22.2	18.1	10.6	52
GBS 髄膜炎	1.3	1.3	1.5	−15
GBS 非髄膜炎	1.2	1.1	1.2	0

罹患率：5歳未満人口10万人あたり
厚生労働科学研究費補助金　新しく開発されたHib，肺炎球菌，ロタウイルス，HPV等の各ワクチンの有効性，安全性並びにその投与方法に関する基礎的・臨床的研究　平成22～24年度　総合研究報告書P. 16から作成

今後の課題

千葉県を含む全国10道県において，厚生労働省の研究班（研究代表者：国立三重病院　庵原俊昭先生）によりHibワクチン，PCV7導入前から，インフルエンザ菌，肺炎球菌侵襲性感染症の疫学調査が行われている。この調査においても，千葉県と同様Hib，肺炎球菌の侵襲性感染症罹患率は，2011年になり減少傾向が認められており，公費助成によるワクチン接種率の上昇が大きく影響していると考えられる。一方，ワクチンのないB群レンサ球菌（GBS）侵襲性感染症の罹患率は変化が認められていない（表1）。今後の課題としてはワクチンの効果を正しく評価するための体制整備があげられる。幸い2013年4月1日より，感染症法施行規則改正に伴い，インフルエンザ菌侵襲性感染症および肺炎球菌侵襲性感染症は成人も含め5類全数届け出疾患となった。今後は全数調査の

徹底により，全国の罹患状況が明らかになることが期待される。発生届には，ワクチン接種歴を記載する項目が設けられており，ワクチン接種の有無を確認することが重要である。同時に，分離された菌株の血清型解析を行っていくことも大切である。

インフルエンザ菌も肺炎球菌も前述したように，一般の医療機関では莢膜型別検査は実施されていないため，菌株を保管しておき，研究機関で精査することが望ましい。実際，PCV7既接種者の肺炎球菌侵襲性感染症の原因菌はPCV7に含まれない莢膜型が主体となっている。また，PCV7導入後PCV7に含まれない莢膜型が相対的に増加しており，その中でも特に莢膜型19Aが問題となっている[15]。PCV7に6つの莢膜型（1, 3, 5, 6A, 7F, 19A）を加えた13価肺炎球菌結合型ワクチン（PCV13）が，PCV7に代わり世界中で使用されるようになっている。日本においても今年度中にPCV7に代わりPCV13が導入される予定となっており，今後PCV7からPCV13への切り替え方法を早急に検討していく必要がある。

おわりに

小児感染症は治療の時代から積極的な予防の時代に入った。今後，耳鼻咽喉科と小児科でより一層連携した予防対策をとっていく必要があるであろう。

文　献

1) 砂川慶介，酒井文宜，平尾百合子，他：本邦における小児細菌性髄膜炎の動向（2007～2008）．2010; 84(1): 33-41.
2) Ishiwada N, Cao LD, Kohno Y: PCR-based capsular serotype determination of *Haemophilus influenzae* strains recovered from Japanese paediatric patients with invasive infection. Clin Microbiol Infect. 2004; 10(10): 895-898.
3) 及川純子，太田節雄，秋山　類，他：小児急性喉頭蓋炎23症例の検討．小児感染免疫 2011; 23(3): 281-282.
4) 石和田稔彦，黒崎知道，寺嶋　周，他：インフルエンザ菌による小児全身感染症罹患状況　日児誌 2007; 111(12): 1568-1572.
5) Adams WG, Deaver KA, Cochi SL, et al.: Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. JAMA 1993; 269(2): 221-226.
6) Peltola H.: *Haemophilus influenzae* type b disease and vaccination in Europe: lessons learned. Pediatr Infect Dis J 1998; 17(9): S126-132.
7) 石和田稔彦，荻田純子，菱木はるか，他：2007年から2009年のインフルエンザ菌・肺炎球菌全身感染症罹患状況　日児誌 2011; 115(1): 50-55.
8) 西村龍夫：小児医療の大変動を予測する　Hib・肺炎球菌ワクチンの時代を前にして　Hib・肺炎球菌ワクチンが必要な訳　開業医が経験するoccult bacteremiaとHib髄膜炎．日小医会報 2008; 36(10): 9-14.
9) Whitney CG, Farley MM, Hadler J, et al.: Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 2003; 348(18): 1737-1746.
10) Chiba N, Morozumi M, Sunaoshi K, et al.: Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. Epidemiol Infect 2010; 138(1): 61-68.
11) Griffin MR, Zhu Y, Moore MR, et al.: U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. N Engl J Med. 2013; 369(2): 155-163.
12) Whitney CG, Grijalva CG., Nuorti JP, et al.: Decline in pneumonia admissions after routine childhood immunization with pneumococcal conjugate vaccine in the USA: a time-series analysis. Lancet. 2007; 369(9568): 1179-1186.
13) Eskola J, Kilpi T, Palmu A, et al.: Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001; 344(6): 403-409.
14) 神谷　齊，加藤達夫，富樫武弘，他：小児急性化膿性中耳炎における肺炎球菌血清型に関する疫学調査．感染症誌 2007; 81(1): 59-66.
15) 及川純子，石和田稔彦，菱木はるか，他：保育園児における，肺炎球菌とインフルエンザ菌の保菌に対する小児用肺炎球菌ワクチンとヒブワクチンの公費助成導入効果の検討試験．小児感染免疫2013; 25(1): 82.
16) Chiba N, Morozumi M, Shouji M, et al.: Rapid decrease of 7-Valent conjugate vaccine coverage for invasive pneumococcal diseases in pediatric patients in Japan. Microb Drug Resist 2013: Epub Ahead of Print.

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Impact of vaccination

Impact of *Haemophilus influenzae* type b and 7-valent pneumococcal conjugate vaccine in Japan

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Key words: meningitis, *Haemophilus influenzae* type b vaccine, 7-valent pneumococcal conjugate vaccine, capsular typing, child

Key words
7価肺炎球菌結合型ワクチン
13価肺炎球菌結合型ワクチン
侵襲性肺炎球菌感染症
定期接種
5類感染症

特集 予防接種法改正 予防接種とワクチンの現状 16
○定期接種A

小児肺炎球菌

石和田 稔彦（いしわだ なるひこ）*

要旨

7価肺炎球菌結合型ワクチン（PCV7）は，侵襲性肺炎球菌感染症（IPD）予防を目的に，2010年2月日本に導入され，公費助成制度適用により普及し，IPDは全国的に減少してきている．PCV7は予防接種法の改正により，2013年4月から定期接種ワクチンとなった．一方，血清型19Aを主体とする非PCV7含有株によるIPDが増加してきており，PCV7は13価肺炎球菌結合型ワクチンに切り替わる予定となっている．また，2013年4月からIPDは感染症法の5類全数届け出疾患となった．ワクチンの有効性をはかるため，IPDから分離された菌株の血清型解析の実施体制を整えていく必要がある．

はじめに

小児の肺炎球菌感染症は，髄膜炎や菌血症などの侵襲性感染症と気管支炎，中耳炎などの非侵襲性感染症，両者の主要な原因菌である．7価肺炎球菌結合型ワクチン（7-valent pneumococcal conjugate vaccine：PCV7）は，ワクチンに含まれる血清型の侵襲性肺炎球菌感染症（invasive pneumococcal disease：IPD）予防を目的に，2010年2月から日本でも使用可能となり，予防接種法の改正により，2013年4月から定期接種ワクチンとなった．また，IPDは感染症法の5類全数届け出疾患となった．本稿では，日本における小児肺炎球菌感染症のPCV7導入後の状況と，予防接種法改正により何が変わったのかという点を中心に概説する．なお，PCV7は今年度中に13価肺炎球菌結合型ワクチン（13-valent pneumococcal conjugate vaccine：PCV13）に切り替わる予定であり，この点に関しても触れたい．

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I 肺炎球菌と肺炎球菌感染症

肺炎球菌は，グラム陽性の双球菌で，その大多数は外殻に莢膜多糖体を有している．莢膜は，好中球やマクロファージなどの貪食細胞が貪食をする際に抵抗性を示すことから，病原性の主体となる．莢膜型は，血清型ともよばれ，現在93種類に分類される．肺炎球菌は，小児から成人に至るまで幅広い年齢層に感染する．肺炎球菌感染症は，ヒトの鼻咽腔に定着（保菌）した後，血中に入り全身に散布される侵襲性感染症と，直接上気道や下気道に侵入し感染を惹起する非侵襲性感染症の2つのタイプに分類される．IPDの代表的な疾患として，菌血症，肺炎，髄膜炎があり，非侵襲性感染症の代表的な疾患として，副鼻腔炎，中耳炎，気管支炎がある．肺炎球菌はウイルス感染症の二次感染の主要な原因菌でもあり，症状の重症化や遷延化に関与する．そのため，菌血症を伴わない肺炎の

重要な原因菌でもある．

PCV7導入前，千葉県ではIPDによる入院例は年々増加し，2009年には5歳未満人口10万人あたりのIPD罹患率は26.1に達した[1]．西村は肺炎球菌菌血症を外来症例を中心に検討し，5歳未満人口10万人あたりの罹患率は328と推計しており[2]，この結果から考えると実際には入院例の10倍以上発症していることとなる．なお，肺炎球菌性髄膜炎に関しては，2007～2010年における千葉県内の調査において，19例のうち7例（29.0%）が後遺症を残し，1例（3.1%）が死亡しており，現在でも予後不良な感染症である．また，肺炎球菌は薬剤耐性化が問題となっており，ペニシリン結合蛋白（penicillin binding proteins：PBPs）に何らかの変異をきたした菌株が，本邦ではIPD分離株の80%程度となっている[3]．PBPs変異を有する肺炎球菌は，ペニシリン系薬のみならずセフェム系薬にも感受性の低下を認める．また，マクロライド系薬に対しても，マクロライド結合部位の変異あるいは薬剤排出機構により耐性を示す肺炎球菌が臨床分離株の主体となっている．

Ⅱ 7価肺炎球菌結合型ワクチン

日本で1980年代から使用可能であった23価肺炎球菌多糖体ワクチンは，莢膜多糖体のみで構成されており，多糖体はT細胞非依存性抗原であるため，B細胞の発達が未熟な2歳未満の乳幼児では十分な免疫が誘導できず予防効果は得られなかった．そこで，4，6B，9V，14，18C，19F，23Fの7つの血清型の莢膜多糖体に，T細胞依存性抗原であるジフテリア毒素の変異蛋白（ジフテリアCRM_{197}）を結合させたワクチンが開発され，乳児にも十分な免疫を誘導できるようになった．PCV7は，2000年から米国で接種が開始され，その後世界中で使われるようになり，2010年2月からは日本でも使用可能となった．PCV7は，メモリーB細胞を誘導できるため，複数回接種によるブースター効果も期待できる．接種対象者は，生後2カ月～9歳のすべての小児であり，標準的な接種スケジュールは，生後2～6カ月齢で接種を開始し，初回免疫3回接種の後，生後12～15カ月時に1回追加免疫を行うという方法である．IPDの確実な予防のためには，生後2カ月からの接種開始と追加免疫の徹底が重要である．

海外ではPCV7導入後，5歳未満小児のPCV7に含まれる血清型によるIPDが激減したと報告されている[4]．PCV7導入前の日本における国内の肺炎球菌に関する疫学調査によるとPCV7に含まれる血清型は，小児IPD分離株の75.4%とされており[3]，この割合は欧米諸国のPCV7導入前の比率と大きな違いはない．PCV7の副反応の主体は接種部位の腫脹，発赤，硬結といった局所反応である．全身的な副反応として発熱，易刺激性，傾眠なども認められるが，米国の市販後有害事象調査では，その頻度は他のワクチンと同程度と報告されている[5]．

日本では2011年2月末～3月にかけて，PCV7を含めたワクチン同時接種を行った小児4例が接種後3日以内に死亡するという報告がなされた．この報告を受け，厚生労働省は2011年3月4日からPCV7の接種を一時見合わせたが，ワクチンと死因との直接的な因果関係は証明されないという結論により，2011年4月1日から接種が再開されている．現在も継続的に厚生労働省において安全性に関する検討会が行われているが，新たな問題は発生していない．PCV7は任意接種の形で導入されたが，標準接種回数は4回であり保護者への費用負担は大きいものであった．2011年になり，ワクチン接種緊急促進事業により一部の市町村を除き，全国的にPCV7に対する公費助成が決定されたことにより，PCV7の接種率は上昇した．