

Serotypes, antimicrobial susceptibility, and molecular epidemiology of invasive and non-invasive *Streptococcus pneumoniae* isolates in paediatric patients after the introduction of 13-valent conjugate vaccine in a nationwide surveillance study conducted in Japan in 2012–2014



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ABSTRACT

Pneumococcal infection in children is a major public health problem worldwide, including in Japan. The pneumococcal conjugate vaccine 7 (PCV7) was licensed for use in Japan in 2010 followed by PCV13 in 2013. This report includes the results of a nationwide surveillance of invasive pneumococcal disease (IPD) and non-IPD in paediatric patients from January 2012 to December 2014. We collected 343 isolates from 337 IPD patients and 286 isolates from 278 non-IPD patients. Of the IPD isolates, the most identified serotypes included 19A, 24F, and 15A. The prevalence of non-PCV13 serotype isolates increased significantly from 2012 to 2014 (51.6–71.4%, $p=0.004$). Serotypes 19A, 15A and 35B were highly non-susceptible to penicillin, and the rates of non-susceptible isolates from IPD patients to penicillin and cefotaxime significantly declined during the study period ($p=0.029$ and $p=0.013$, respectively). The non-susceptible rate to meropenem increased, particularly for serotype 15A. The IPD isolates comprised clonal complex (CC) 3111 (93.8% was serotype 19A) followed by CC2572 (81.5% was serotype 24F) and CC63 (97.1% was serotype 15A). CC3111, CC63 and CC156 (33.3% was serotype 23A, 28.6% was serotype 6B, and 14.3% was serotype 19A) were highly non-susceptible to penicillin. Of the non-IPD isolates, the most identified serotypes included 19A, 15A, and 3. In conclusion, the introduction of PCV7 and PCV13 resulted in increasing non-PCV13 serotypes and clones, including antimicrobial resistant serotypes 15A and CC63 (Sweden^{15A}-25 clone).

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1. Introduction

Streptococcus pneumoniae is a common pathogen of bacterial infections in children and is associated with high morbidity and mortality [1]. In 2000, an estimated 14.5 million serious infections and 826,000 deaths occurred in children younger than 5 years of age [2]. To protect children against invasive pneumococcal disease

(IPD), 7-, 10- and 13-valent pneumococcal conjugate vaccines were licensed for use in children worldwide. The widespread use of these vaccines has significantly decreased the incidence of serious IPD in children [3–9]. However, IPD continues to occur due to the emergence of non-vaccine serotypes [10–13]. Therefore, surveillance of pneumococcal infections including IPD is essential for monitoring the beneficial effects of pneumococcal conjugate vaccine (PCV) implementation and its potential impact on the structure of the pneumococcal population.

PCV7 was licensed in Japan in February 2010 and was used on a voluntary basis until April 2013. During this period, the estimated rates of PCV7 vaccination in children under 5 years of age increased

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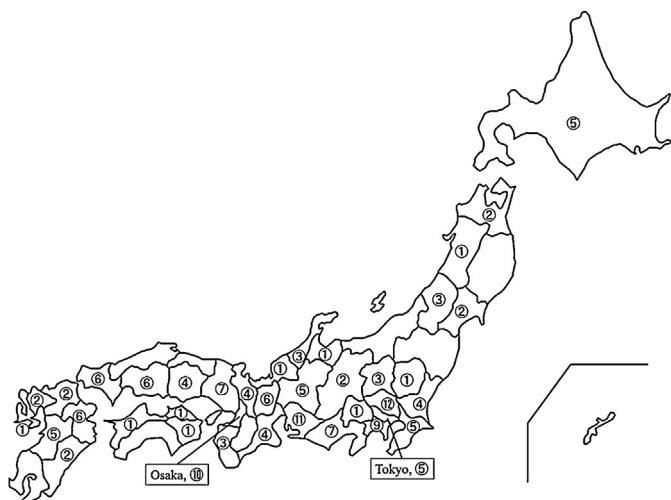


Fig. 1. Distribution of Japanese institutions participating in the present surveillance for invasive and non-invasive pneumococcal disease conducted from January 2012 to December 2014. The numbers in the figure shows the numbers of institutions in each prefecture.

from <10% in 2010 to 80–90% in 2012 [4]. In April 2013, PCV7 was approved as a routine vaccination in Japan, and the vaccine was switched to PCV13 in October 2013.

We conducted a nationwide pneumococcal surveillance programme of children to review the epidemiology of IPD and other pneumococcal infections, including bacterial pneumonia and acute otitis media (non-IPD), and the shift of serotype distribution from January 2012 to December 2014. Additionally, we clarified the distribution of the susceptibility and sequence types of the isolates.

2. Materials and methods

This study is a nationwide, ongoing, prospective surveillance of pneumococcal infections including IPD. This report presents data from a 3-year period (January 2012 through December 2014). The study was approved by the Ethics Committee of the National Hospital Organization Mie National Hospital (acceptance number, 23-8). Informed consent for collection and use of patient information and specimens was obtained from each parent/guardian by a primary physician.

2.1. Study population and case definition

During the study period, a total of 154 medical institutions, including 20 private clinics, actively participated in the surveillance (Fig. 1). All of the study participants who were diagnosed with IPD, pneumonia and/or otitis media were under 16 years and over 2 months of age. IPD was defined as the recovery of *S. pneumoniae* from a culture of a normal sterile body fluid, such as blood, cerebrospinal fluid (CSF), pleural effusion, and joint fluid, regardless of the identification of *S. pneumoniae* from other non-sterile samples. Basically, a single isolate was submitted for each IPD case; however, if at least two isolates with different serotypes were identified from at least one appropriate sample (e.g., blood and cerebrospinal fluid), all of the isolates were submitted as one IPD case. If at least two isolates with the same serotype were identified from sterile and non-sterile samples (e.g., cerebrospinal fluid and middle ear fluid), these were counted as one isolate, even though both infection sites (e.g., bacterial meningitis and otitis media) were submitted for the IPD diagnosis in Table 1. In cases of bacteraemia with other organ infection caused by isolates with the same serotype, the isolate from the original sterile infection site (e.g., cerebral

spinal fluid, joint fluid, and pleural fluid) was submitted. Pneumococcal pneumonia was diagnosed by clinical respiratory symptoms compatible with the presence of acute pneumonia with the recovery of *S. pneumoniae* from lower respiratory tract specimens. To obtain an appropriate sputum sample, we recommend performing a sputum induction with nebulized hypertonic saline or using a suction catheter. A routine chest X-ray was not needed at the time of pneumonia diagnosis. If *S. pneumoniae* was isolated from a sterile sample, such as blood, from a patient who was suspected of having pneumococcal pneumonia, the case was counted as only IPD. Pneumococcal otitis media was diagnosed by relevant clinical findings with the recovery of *S. pneumoniae* from middle ear fluid obtained by myringotomy incision. A case with both pneumococcal otitis media and pneumonia was counted as a pneumococcal pneumonia case to avoid duplication in non-IPD. Similar to the criteria of pneumococcal pneumonia with bacteraemia, if *S. pneumoniae* was isolated from a sterile sample, such as blood, from a patient who was suspected of having pneumococcal otitis media, the case was counted as only IPD.

2.2. Data collection

A standardized questionnaire was used to collect data on age, sex, current diseases, past pneumococcal vaccination history, past medical history and disease outcomes. Data were obtained by the primary physician of each patient and sent to the surveillance office.

2.3. Routine vaccination programme and vaccination rate

The Japanese routine vaccination programme recommends that all infants are given a primary series of PCV at 2, 4, and 6 months of age with a booster at 12–15 months of age. Children who fall behind are recommended for catch-up vaccinations through 59 months of age. This recommended vaccination programme is common among healthy and high-risk children. At the transition from PCV7 to PCV13, PCV13 was used to complete the series of PCV7. For children who had received a complete PCV7 schedule at the transition, a single supplemental dose of PCV13 was not recommended by the Japanese government.

Since the introduction of the Urgent Promotion of Vaccination incentive by the Japanese government in 2011, the vaccination rate in Japan has been increasing. According to previous reports, the rates were 50–60% in 2011 and 89.2% in 2014 [4,14].

2.4. Identification and serotyping

Pneumococcal identification was performed as described previously [15]. For serotyping, pneumococcal isolates were sent to one of two reference laboratories, LSI Medience Corporation and National Institute of Infectious Diseases. Serotyping was performed using pneumococcal typing antisera (Statens Serum Institut, Copenhagen, Denmark).

2.5. Susceptibility testing

Susceptibility testing for penicillin, erythromycin, cefotaxime, meropenem and levofloxacin was performed on isolates using the broth micro dilution method at LSI Medience Corporation. We opted to use the Clinical and Laboratory Standards Institute (CLSI) guidelines because these were the standards in use in Japan during the study period. In 2008, the CLSI changed the recommended breakpoints to those currently used to interpret minimum inhibitory concentration (MIC) values. For penicillin and cefotaxime, we used the 2008 CLSI recommended breakpoints for meningitis, and for erythromycin, meropenem and levofloxacin, we used the 2008 CLSI recommended breakpoints for all infection sites

Table 1
Characteristics of patients with IPD or non-IPD during the study period.

	IPD, n (%)				Non-IPD, n (%)			
	2012	2013	2014	Total	2012	2013	2014	Total
No. of isolates	64	153	126	343	70	134	82	286
No. of patients	64	148	125	337	70	126	82	278
Patient characteristics								
Age group								
<12 months	20(31.3)	30(20.3)	18(14.4)	68(20.2)	15(21.4)	28(22.2)	19(23.2)	62(22.3)
12–23 months	23(35.9)	71(48.0)	49(39.2)	143(42.4)	27(38.6)	61(48.4)	42(51.2)	130(46.8)
2–4 years	16(25.0)	36(24.3)	51(40.8)	103(30.6)	28(40.0)	37(29.4)	21(25.6)	86(30.9)
5–15 years	5(7.8)	11(7.4)	7(5.6)	23(6.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Male	31(48.4)	93(62.8)	75(60.0)	199(59.1)	40(57.1)	63(50.0)	48(58.5)	151(54.3)
Total no. of comorbidity								
Congenital heart disease	3(4.7)	6(4.1)	10(8.0)	19(5.6)	0(0.0)	1(0.8)	0(0.0)	1(0.4)
Chromosomal anomalies	2(3.1)	6(4.1)	4(3.2)	12(3.6)	0(0.0)	1(0.8)	0(0.0)	1(0.4)
Malignancy	3(4.7)	2(1.4)	2(1.6)	7(2.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Bronchial asthma	2(3.1)	2(1.4)	2(1.6)	6(1.8)	3(4.3)	0(0.0)	0(0.0)	3(1.1)
Others	1(1.6)	11(7.4)	10(8.0)	22(6.5)	2(2.9)	10(7.9)	3(3.7)	15(5.4)
Diagnosis								
Primary bacteraemia	43(67.2)	95(64.2)	79(63.2)	217(64.4)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Meningitis	12(18.7)	19 ^a (12.9)	18 ^c (14.4)	49(14.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Arthritis	1(1.6)	2(1.4)	0(0.0)	3(0.9)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Pleuritis	0(0.0)	1(0.7)	1(0.8)	2(0.6)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Peritonitis	0(0.0)	0(0.0)	1(0.8)	1(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Pneumonia ^f	5(7.8)	25 ^b (16.9)	18(14.4)	48(14.2)	19(27.1)	20(15.9)	5 ^e (6.1)	44(15.8)
Otitis media ^f	2(3.1)	7 ^{a,b} (4.7)	4 ^d (3.2)	13(3.9)	51(72.9)	106(84.1)	78 ^e (95.1)	235(84.5)
Other infection sites ^f	1(1.6)	4(2.7)	6 ^{c,d} (4.8)	11(3.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Outcomes								
Mortality	0(0.0)	2(1.4)	4(3.2)	6(1.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Sequelae	3(4.7)	2(1.4)	2(2.5)	7(2.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Absence of information	1(1.6)	6(4.1)	4(3.2)	11(3.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Completely cured	60(93.8)	138(93.2)	115(92.0)	313(92.9)	70(100.0)	126(100.0)	82(100.0)	278(100.0)

IPD, invasive pneumococcal disease; PCV, pneumococcal conjugate vaccine.

^a Among the nine cases of bacteraemia with meningitis, one case was complicated by otitis media.

^b Among the 25 cases of bacteraemia with pneumonia, four cases were complicated by otitis media.

^c Among the 10 cases of bacteraemia with meningitis, one case was complicated by infectious endocarditis.

^d Among the four cases of otitis media with bacteraemia, one case was complicated by bronchitis.

^e Among the five cases of pneumonia without bacteraemia, one case was complicated by otitis media.

^f All IPD cases with pneumonia, otitis media and other infection sites were complicated by bacteraemia.

[16]. Isolates identified as intermediate or resistant were grouped together as non-susceptible; for example, penicillin susceptible (S) and non-susceptible (NS) were defined as (in mg/l) ≤ 0.06 and > 0.06 , respectively. In the case of erythromycin, S and NS were defined as ≤ 0.25 and > 0.25 , respectively, and for cefotaxime, S and NS were defined as ≤ 0.5 and > 0.5 , respectively. In addition, for meropenem, S and NS were defined as ≤ 0.25 and > 0.25 , respectively, and for levofloxacin, S and NS were defined as ≤ 2 and > 2 , respectively. We used the same breakpoint criteria for IPD and non-IPD to facilitate their comparison with each other.

2.6. Multi-locus sequence typing (MLST)

MLST was performed as described previously [17], and sequence types (STs) were allocated using the MLST online database (www.pubmlst.org). We ran eBURST (<http://eburst.mlst.net>) with default settings, associating each sequence type (ST) with a clonal complex (CC) defined as a group of STs sharing six of seven loci on the entire MLST database and newly assigned STs within our database. Using the Pneumococcal Molecular Epidemiology Network (PMEN) database, we allocated the results of MLST for each PMEN clone.

2.7. Statistical analysis

Data were analyzed using Microsoft Excel and Stata, version 13.0 (Statcorp, TX, USA). We used the Cochran–Armitage trend test to assess significant differences in the serotype and susceptibility distribution during the epidemiological years of 2012 to 2014.

3. Results

3.1. Patients and isolate collection

Between January 2012 (PCV7 era) and December 2014 (PCV13 era), we collected a total of 629 isolates (615 cases) from 154 medical institutions, including 20 private clinics in Japan. The participating institutions were distributed into 38 of 47 prefectures in Japan. Of these isolates, 343 isolates were derived from 337 IPD patients, and 286 isolates were obtained from 278 non-IPD (pneumococcal pneumonia and otitis media) patients (Table 1).

Of the IPD cases, 63 patients (18.7%) had comorbidity. The most prevalent comorbidities were congenital heart disease ($n = 19$) followed by chromosomal anomalies ($n = 12$), malignancy ($n = 7$), and bronchial asthma ($n = 6$). Six subjects (1.8%) died, and seven subjects (2.1%) had sequelae. In total, 313 subjects (92.9%) were cured completely, and we did not have information for 11 (3.3%) of these cases from a chief physician.

The immunization histories of PCV7 and PCV13 are described in Table 2. Of the IPD cases in 2012, 42 (65.6%) did not receive PCV7. The incomplete vaccination rate in IPD cases decreased over time to 57 cases (45.6%) in 2014.

3.2. Serotypes

We identified 27 and 21 different capsular types in IPD and non-IPD isolates, respectively (Fig. 2 and Table 3). Of the IPD isolates ($n = 343$), the most frequent isolates included serotype 19A ($n = 95$, 27.7%) followed by 24F ($n = 51$, 14.9%) and 15A ($n = 36$,

Table 2
Numbers of completed routine vaccine schedule of PCV7 or PCV13 in each age group.

Disease	Months of age	Total no. of cases			Complete PCV7 schedule, no.(%)			Complete PCV13 schedule, no.(%)			Incomplete PCV7 and 13 schedules, no. (%)		
		2012	2013	2014	2012	2013	2014	2012	2013	2014	2012	2013	2014
IPD	2–11	20	30 ^a	18	12(60.0)	26(86.7)	6(33.3)	0(0.0)	0(0.0)	6(33.3)	8(40.0)	3(10.0)	6(33.3)
	12–23	23	71 ^b	49	6(26.1)	28(39.4)	6(12.2)	0(0.0)	0(0.0)	16(32.7)	17(73.9)	39(54.9)	27(55.1)
	>23	21 ^c	47 ^d	58 ^e	3(14.3)	19(40.4)	28(48.3)	0(0.0)	0(0.0)	3(5.2)	17(81.0)	27(57.4)	24(41.4)
	Total	64	148	125	21(32.8)	73(49.3)	40(32.0)	0(0.0)	0(0.0)	25(20.0)	42(65.6)	69(46.6)	57(45.6)
Non-IPD	2–11	15	28	19 ^f	11(73.3)	17(60.7)	8(42.1)	0(0.0)	0(0.0)	5(26.3)	4(26.7)	11(39.3)	5(26.3)
	12–23	27	61 ^g	42 ^h	11(40.7)	18(29.5)	17(40.5)	0(0.0)	1(1.6)	9(21.4)	16(38.1)	40(65.6)	15(35.7)
	>23	28	37 ⁱ	21	4(14.3)	15(40.5)	9(42.9)	1(3.6)	0(0.0)	0(0.0)	23(82.1)	19(51.4)	12(57.1)
	Total	70	126	82	25(35.7)	48(38.1)	34(41.5)	1(1.4)	1(0.8)	14(17.1)	44(62.9)	72(57.1)	32(39.0)

For IPD and non-IPD, immunization information was absent in a total of 10 and 7 cases, respectively.

The complete course of PCV7 was defined by the numbers of PCV7 injections based on the Japanese schedule referring to the patient's age in months.

The complete course of PCV13 was defined by the number of PCV13 injections based on the Japanese schedule referring to the patient's age in months, including the transition from the PCV7 series to PCV13.

^{a,c,d,f,h}In one case, the vaccination information was missing.

^{e,i}In three cases, the vaccination information was missing.

^bIn four cases, the vaccination information was missing.

^gIn two cases, the vaccination information was missing.

10.5%). From 2012 (PCV7 era) to 2014 (PCV13 era), serotypes 19F, 6B and 6A decreased significantly ($p=0.002$, $p=0.003$, and $p=0.041$, respectively), and serotype 24F increased significantly ($p<0.001$). Of the non-IPD isolates ($n=286$), the most frequent isolates included serotypes 19A and 15A ($n=56$, 19.6%) followed by 3 ($n=33$, 11.5%) and serotype 15B/C ($n=28$, 9.8%). From 2012 to 2014, serotype 15B/C decreased significantly ($p=0.025$), and serotype 15A increased significantly ($p=0.003$). In total, the identification rate of serotypes 19F and 6B decreased significantly ($p=0.002$ and $p=0.005$, respectively). In addition, serotypes 15A, 22F, and 24F increased significantly ($p=0.028$, $p=0.041$, and $p<0.001$, respectively) from 2012 to 2014.

Year-to-year changes in the prevalence of PCV and non-PCV serotypes of *S. pneumoniae* are described in Table 3. The prevalence of non-PCV13 serotype isolates in IPD isolates and total isolates increased significantly between 2012 and 2014 ($p=0.004$ and $p=0.001$, respectively). The prevalence of PCV7 serotype in the IPD and total isolates decreased significantly from 2012 to 2014 ($p<0.001$ and $p<0.001$, respectively).

As regard to the relationship between PCV receipts and identified serotypes, 41.3% of IPD cases with a non-PCV13 serotype isolate completely received PCV7 or PCV13 based on the Japanese schedule, and 40.8% of the IPD cases with a PCV13 serotype isolate completely received PCV7 or PCV13 based on the Japanese schedule. With regard to 31 IPD patients who completely received PCV13, the numbers of each identified serotype were as follows: 11 cases of serotype 24F, five cases of serotypes 15A and 15B/C, three cases of serotype 24B, two cases of serotypes 19A and 22F, and one case each of serotypes 6A, 11A, and 38; thus, 90.3% of these serotypes were not included in the PCV13 serotypes.

3.3. Antimicrobial susceptibility

With regard to IPD isolates, non-IPD isolates, and total isolates, the proportions of non-susceptible isolates to the tested antimicrobials are summarized in Fig. 3. Of the 629 total isolates, the non-susceptible proportions were as follows: 46.1% to penicillin, 20.2% to cefotaxime, 20.5% to meropenem, 94.3% to erythromycin and 0.2% to levofloxacin. Of the 127 isolates that were non-susceptible to cefotaxime, 69 (54.3%) were non-susceptible to meropenem. Serotype 19A, 15A and 35B isolates were highly non-susceptible to penicillin (60.3%, 97.8% and 68.8%, respectively), whereas all serotype 22F and 24F isolates were susceptible to penicillin. The non-susceptible proportions to penicillin and cefotaxime

declined during our study period; however, the decline was statistically significant for only cefotaxime from 2012 to 2014 ($p=0.316$, penicillin; $p=0.030$, cefotaxime). However, if limited to only IPD isolates, the declines in non-susceptible isolates to penicillin and cefotaxime were significant ($p=0.029$, penicillin; $p=0.013$, cefotaxime). Additionally, significant increases were observed for the non-susceptible proportion to meropenem in the non-IPD and total isolates ($p<0.001$, non-IPD isolates; $p=0.007$, total isolates). Similarly, the analysis of serotype 15A in the total isolates revealed that the non-susceptible proportion to meropenem increased ($p=0.001$, serotype 15A).

3.4. MLST analysis

The distribution of STs of each serotype is described in Table 4. All 629 isolates were separated into 125 different STs. eBURST analyses clustered these STs into 51 CCs, including nine singletons. Of the 343 IPD isolates, 80 (23.3%) were CC3111, which was the main clone observed during the study period. Of these CC3111 isolates, 93.8% were serotype 19A, and one isolate (1.3%) was of each of the following serotypes: 10A, 15A, 24F, 34 and non-typeable. The second and third most common clones were CC2572 ($n=54$, 15.7%) and CC63 ($n=34$, 9.9%), respectively. Of the CC2572 isolates, 81.5% were serotype 24F, and of the CC63 isolates, 97.1% were serotype 15A (Sweden^{15A-25} clone). The proportions of CC2572 and CC433 (all of CC433 were serotype 22F) increased significantly ($p<0.001$, CC2572; $p=0.032$, CC433) from 2012 to 2014, whereas the proportion of CC156 decreased significantly ($p<0.001$) during this period. The CC156 isolates presented some serotype heterogeneity: 33.3% of serotype 23A, 28.6% of serotype 6B, and 14.3% of serotype 19A. The non-susceptible proportions to penicillin of CC3111, CC2572, CC63, CC433 and CC156 in the IPD isolates were 58.8%, 3.7%, 94.1%, 0.0% and 61.9%, respectively.

Of 286 non-IPD isolates, 55 (19.2%) were identified as CC63, which was the main clone observed in the study period. In addition, 96.4% of the CC63 isolates were serotype 15A, and there was one isolate (1.8%) each of serotype 11A and 23A. The second and third most commonly observed clones were CC3111 ($n=44$, 15.4%) and CC180 ($n=32$, 11.2%), respectively. In the CC180, 93.8% of the isolates were serotype 3 (Netherlands³⁻³¹ clone). CC63 increased significantly between 2012 and 2014 ($p<0.001$).

The total isolates resistant to meropenem primarily consisted of CC63 isolates followed by CC3111 and CC558 isolates. The non-susceptible proportions of CC63, CC3111 and CC558 isolates to meropenem were 69.7%, 16.1% and 90.9%, respectively.

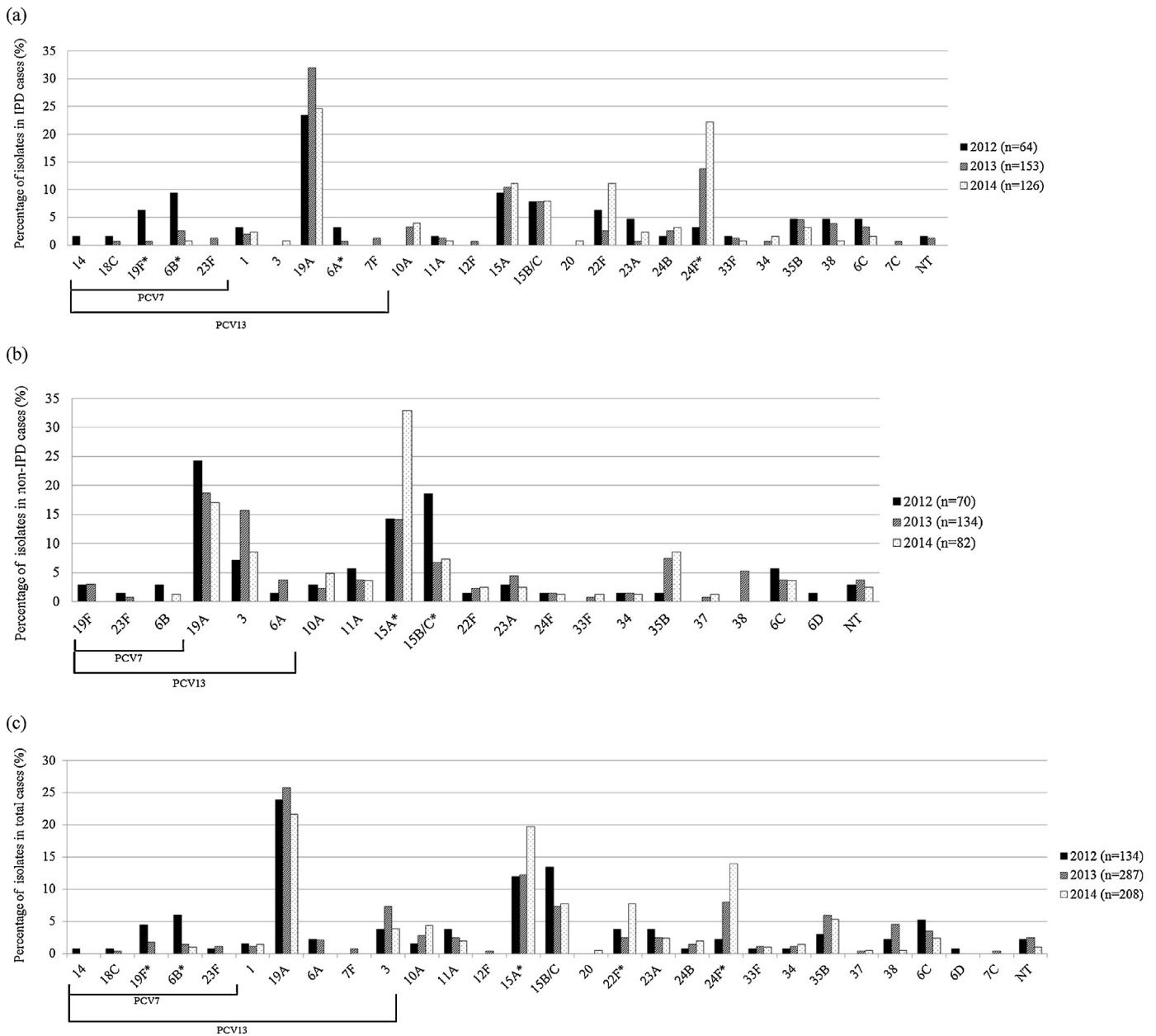


Fig. 2. Distribution of pneumococcal serotypes in Japan from 2012 (PCV7 era) to 2014 (PCV13 era). In Japan, PCV13 was approved as a routine vaccination in April 2013. NT, non-typeable. The asterisk (*) indicates statistically significant differences at $p < 0.05$, as determined through the Cochran–Armitage trend test. (a) IPD isolates. (b) Non-IPD isolates. (c) Total isolates.

3.5. Mortality cases

During our surveillance period, six mortality cases were observed. These included three serotype 15A isolates and one case each of serotypes 22F, 23A and 10A; thus, all of these cases were caused by non-PCV13 serotype isolates. In addition, all six cases with observed mortality had bacteraemia, although one case also had pneumococcal meningitis, another case also had pneumococcal pneumonia and a third case also had necrotizing cellulitis. The other three cases were diagnosed as primary bacteraemia. Five out of the six cases had underlying disease: congenital heart disease (four cases) and cerebral palsy (one case). Four of the patients were 4 years of age, and the other two patients were 16 and 31 months of age, respectively. In addition, two of the patients were administered PCV7 completely on schedule.

4. Discussion

We conducted a nationwide surveillance programme of both IPD and non-IPD including patient characteristics, serotype prevalence, antibiotic susceptibility, and ST prevalence in Japan between 2012 (PCV7 era) and 2014 (PCV13 era). This report is the first in the literature describing antimicrobial susceptibilities, including meropenem and IPD patient characteristics, after the introduction of PCV13 in Japan.

Various studies have reported the serotype distribution shift to non-vaccine serotypes after the introduction of the pneumococcal conjugate vaccine [10–13]. In Japan, Chiba et al. reported a PCV7 coverage rate of 14.7% in paediatric IPD isolates in 2012 [4]. Our data indicated that the coverage rate in 2012 was 18.8%, which is similar to this report. Additionally, the significant decrease in IPD due

Table 3
Serotype distribution of *Streptococcus pneumoniae* isolates from children with invasive pneumococcal disease (IPD) and non-IPD in Japan during 2012–2014.

Serotype	Number (%) of IPD isolates				Number (%) of non-IPD isolates				Number (%) of total isolates			
	2012 (n = 64)	2013 (n = 153)	2014 (n = 126)	p Value	2012 (n = 70)	2013 (n = 134)	2014 (n = 82)	p Value	2012 (n = 134)	2013 (n = 287)	2014 (n = 208)	p Value
PCV7 serotypes												
14	1(1.6)	0(0.0)	0(0.0)	0.102	0(0.0)	0(0.0)	0(0.0)	NA	1(0.7)	0(0.0)	0(0.0)	0.124
18C	1(1.6)	1(0.7)	0(0.0)	0.181	0(0.0)	0(0.0)	0(0.0)	NA	1(0.7)	1(0.3)	0(0.0)	0.229
19F	4(6.3)	1(0.7)	0(0.0)	0.002	2(2.9)	4(3.0)	0(0.0)	0.202	6(4.5)	5(1.7)	0(0.0)	0.002
6B	6(9.4)	4(2.6)	1(0.8)	0.003	2(2.9)	0(0.0)	1(1.2)	0.369	8(6.0)	4(1.4)	2(1.0)	0.005
23F	0(0.0)	2(1.3)	0(0.0)	0.723	1(1.4)	1(0.7)	0(0.0)	0.291	1(0.7)	3(1.0)	0(0.0)	0.311
Total of PCV7 serotypes	12(18.8)	8(5.2)	1(0.8)	<0.001	5(7.1)	5(3.7)	1(1.2)	0.059	17(12.7)	13(4.5)	2(1.0)	<0.001
PCV13 serotypes												
1	2(3.1)	3(2.0)	3(2.4)	0.825	0(0.0)	0(0.0)	0(0.0)	NA	2(1.5)	3(1.0)	3(1.4)	0.977
19A	15(23.4)	49(32.0)	31(24.6)	0.845	17(24.3)	25(18.7)	14(17.1)	0.273	32(23.9)	74(25.8)	45(21.6)	0.541
6A	2(3.1)	1(0.7)	0(0.0)	0.041	1(1.4)	5(3.7)	0(0.0)	0.478	3(2.2)	6(2.1)	0(0.0)	0.061
7F	0(0.0)	2(1.3)	0(0.0)	0.723	0(0.0)	0(0.0)	0(0.0)	NA	0(0.0)	2(0.7)	0(0.0)	0.819
3	0(0.0)	0(0.0)	1(0.8)	0.256	5(7.1)	21(15.7)	7(8.5)	0.876	5(3.7)	21(7.3)	8(3.8)	0.809
Total of additional PCV13 serotypes	19(29.7)	55(35.9)	35(27.8)	0.552	23(32.9)	51(38.1)	21(25.6)	0.302	42(31.3)	106(36.9)	56(26.9)	0.242
Total of PCV13 serotypes	31(48.4)	63(41.2)	36(28.6)	0.004	28(40.0)	56(41.8)	22(26.8)	0.079	59(44.0)	119(41.5)	58(27.9)	0.001
Non-PCV13 serotypes												
10A	0(0.0)	5(3.3)	5(4.0)	0.156	2(2.9)	3(2.2)	4(4.9)	0.450	2(1.5)	8(2.8)	9(4.3)	0.127
11A	1(1.6)	2(1.3)	1(0.8)	0.615	4(5.7)	5(3.7)	3(3.7)	0.542	5(3.7)	7(2.4)	4(1.9)	0.326
12F	0(0.0)	1(0.7)	0(0.0)	0.802	0(0.0)	0(0.0)	0(0.0)	NA	0(0.0)	1(0.3)	0(0.0)	0.872
15A	6(9.4)	16(10.5)	14(11.1)	0.716	10(14.3)	19(14.2)	27(32.9)	0.003	16(11.9)	35(12.2)	41(19.7)	0.028
15B/C	5(7.8)	12(7.8)	10(7.9)	0.974	13(18.6)	9(6.7)	6(7.3)	0.025	18(13.4)	21(7.3)	16(7.7)	0.101
20	0(0.0)	0(0.0)	1(0.8)	0.256	0(0.0)	0(0.0)	0(0.0)	NA	0(0.0)	0(0.0)	1(0.5)	0.225
22F	4(6.3)	4(2.6)	14(11.1)	0.066	1(1.4)	3(2.2)	2(2.4)	0.671	5(3.7)	7(2.4)	16(7.7)	0.041
23A	3(4.7)	1(0.7)	3(2.4)	0.503	2(2.9)	6(1.5)	2(2.4)	0.853	5(3.7)	7(2.4)	5(2.4)	0.499
24B	1(1.6)	4(2.6)	4(3.2)	0.521	0(0.0)	0(0.0)	0(0.0)	NA	1(0.7)	4(1.4)	4(1.9)	0.371
24F	2(3.1)	21(13.7)	28(22.2)	<0.001	1(1.4)	2(1.5)	1(1.2)	0.908	3(2.2)	23(8.0)	29(13.9)	<0.001
33F	1(1.6)	2(1.3)	1(0.8)	0.615	0(0.0)	1(0.7)	1(1.2)	0.372	1(0.7)	3(1.0)	2(1.0)	0.868
34	0(0.0)	1(0.7)	2(1.6)	0.242	1(1.4)	2(1.5)	1(1.2)	0.908	1(0.7)	3(1.0)	3(1.4)	0.539
35B	3(4.7)	7(4.6)	4(3.2)	0.563	1(1.4)	10(7.5)	7(8.5)	0.079	4(3.0)	17(5.9)	11(5.3)	0.420
37	0(0.0)	0(0.0)	0(0.0)	NA	0(0.0)	1(0.7)	1(1.2)	0.372	0(0.0)	1(0.3)	1(0.5)	0.457
38	3(4.7)	6(3.9)	1(0.8)	0.091	0(0.0)	7(5.2)	0(0.0)	0.877	3(2.2)	13(4.5)	1(0.5)	0.177
6C	3(4.7)	5(3.3)	2(1.6)	0.212	4(5.7)	5(3.7)	3(3.7)	0.542	7(5.2)	10(3.5)	5(2.4)	0.171
6D	0(0.0)	0(0.0)	0(0.0)	NA	1(1.4)	0(0.0)	0(0.0)	0.152	1(0.7)	0(0.0)	0(0.0)	0.124
7C	0(0.0)	1(0.7)	0(0.0)	0.226	0(0.0)	0(0.0)	0(0.0)	NA	0(0.0)	1(0.3)	0(0.0)	0.872
NT	1(1.6)	2(1.3)	0(0.0)	0.215	2(2.9)	5(3.7)	2(2.4)	0.861	3(2.2)	7(2.4)	2(1.0)	0.334
Total of non-PCV13 serotypes	33(51.6)	90(58.8)	90(71.4)	0.004	42(60.0)	78(58.2)	60(73.2)	0.079	75(56.0)	168(58.5)	150(72.1)	0.001

to the PCV7 serotypes was observed in the following period in our study. Consequently, PCV7 serotype isolates disappeared almost completely in 2014 (PCV13 era). With regard to PCV13 serotypes, the serotype coverage rate in IPD decreased significantly from 2012 (PCV7 era) to 2014 (PCV13 era) ($p=0.004$). The coverage rate in 2014 was 28.6%, which is equal to or slightly reduced compared with previous reports from other countries [5,7,8,18]. Of note, in our study, the coverage rate of PCV13 was already low (48.4% in 2012) before the introduction of PCV13 in 2013. This rate was caused by the prevalence of serotypes 15A, 15B/C and 24F, which are not included in PCV 13 serotypes. A previous study in Japan also reported a low coverage rate of 39.1% in 2012 [4]. Regarding the year-to-year changes in serotypes of IPD isolates, serotypes 24F increased significantly during the study periods. A previous study from Japan also reported the increase of serotypes 15A, 15B/C, 24F, and 22F [4]; therefore, we presume that these serotypes will be prevalent in Japan in the PCV13 era. A trend in serotype shift similar to that found in Japan has also been observed in other countries. Recent studies conducted after the implementation of PCV13 have found the prevalence of serotypes 22F, 15B/C and 35B in the U.S. [19] and significant increases in serotypes 15A and 24F in England and Wales [20]. These studies also reported a significant decrease in serotype 19A, which is of great concern given its antimicrobial resistance. However, in our study, serotype 19A did not decrease in the PCV13 era, even though serotype 19A is included in PCV13.

This result is likely due to the short period since the introduction of PCV13. In our study, the most common serotype, i.e., serotype 19A, increased up to 2013 and then decreased in 2014, although this decrease was not significant, which is likely due to the short period of observation since the introduction of PCV13; in fact, the completely PCV13 immunization rate of IPD patients in 2014 was only 20.0%. Because 31 of the IPD cases that received a complete course of PCV13 in the present study were due to mostly non-PCV13 serotype isolates (90.3%), we expect a decrease in the proportion serotype 19A isolates in the future based on the prevalence of PCV13 in Japan.

The MLST analysis clarified a trend of prevalent genotypes of pneumococcal isolates in Japan. The most prevalent CC in total isolates in Japan was CC3111, which primarily included serotype 19A. Approximately 60% of isolates of this clone were not susceptible to penicillin. Serotype 19A CCs (199, 320/271, and 695) that are notably resistant in the United States were not prevalent in our region [19,21,22]. CC2572, which primarily included serotype 24F and was susceptible to penicillin, significantly spread between 2012 and 2014. Therefore, this clone is predicted to spread further in Japan because serotype 24F is not included in PCV13.

Previous studies reported that the introduction of PCV7 into the National Immunization Programme (NIP) was associated with a substantial decline in penicillin non-susceptible pneumococci prevalence [21,23,24]. This decline is due to the decrease in resistant clones and the increase in susceptible clones. In our study,

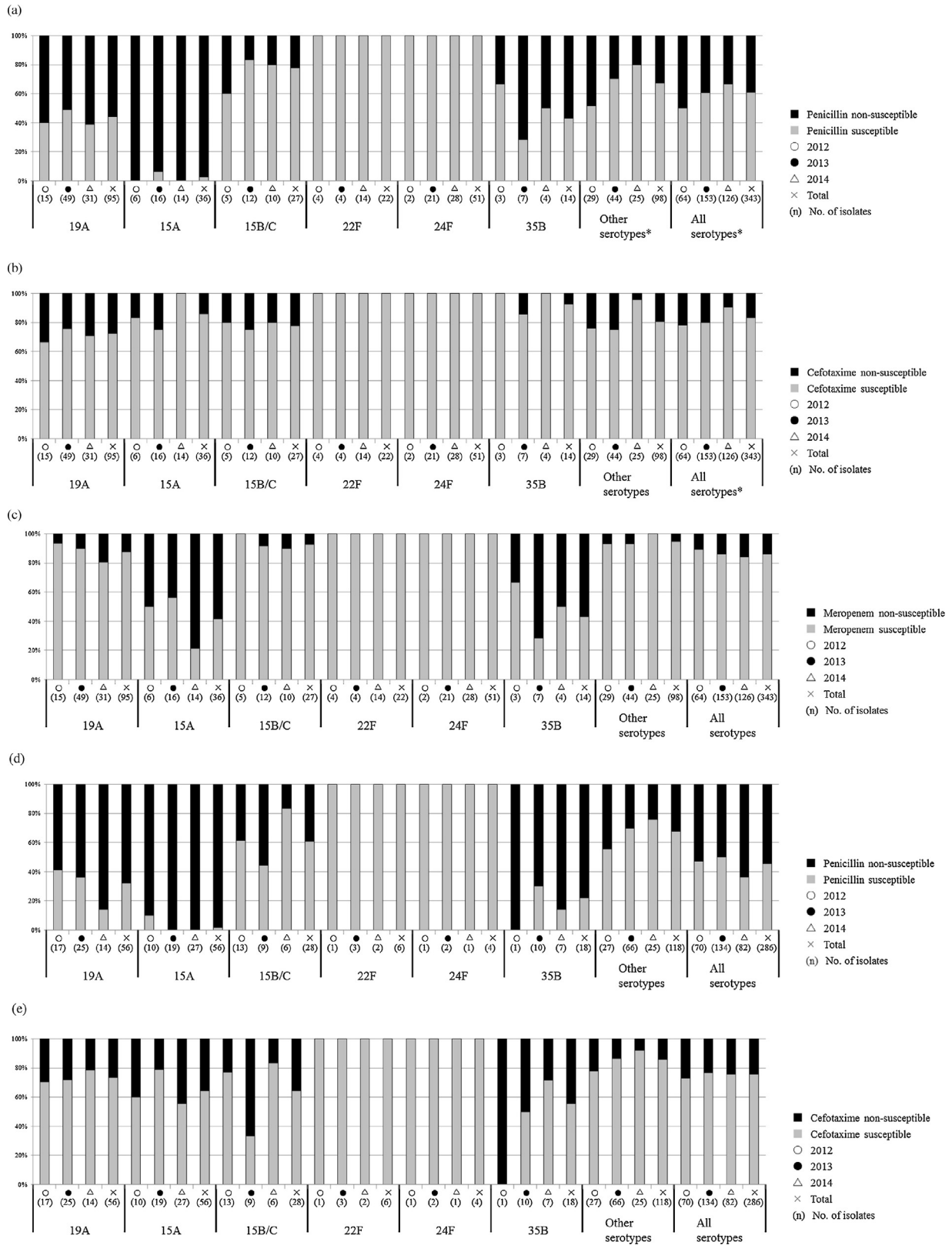


Fig. 3. Year-to-year changes in the antimicrobial resistance rates to penicillin, cefotaxime, and meropenem of the major serotypes in IPD isolates and total isolates in Japan from 2012 (PCV7 era) to 2014 (PCV13 era). In Japan, PCV13 was approved as a routine vaccination in April 2013. The asterisk (*) indicates statistically significant differences at $p < 0.05$, as determined through the Cochran–Armitage trend test. (a) Proportion of penicillin-non-susceptible IPD isolates. (b) Proportion of cefotaxime-non-susceptible IPD isolates. (c) Proportion of meropenem-non-susceptible IPD isolates. (d) Proportion of penicillin-non-susceptible non-IPD isolates. (e) Proportion of cefotaxime-non-susceptible non-IPD isolates. (f) Proportion of meropenem-non-susceptible non-IPD isolates. (g) Proportion of penicillin-non-susceptible total isolates. (h) Proportion of cefotaxime-non-susceptible total isolates. (i) Proportion of meropenem-non-susceptible total isolates.

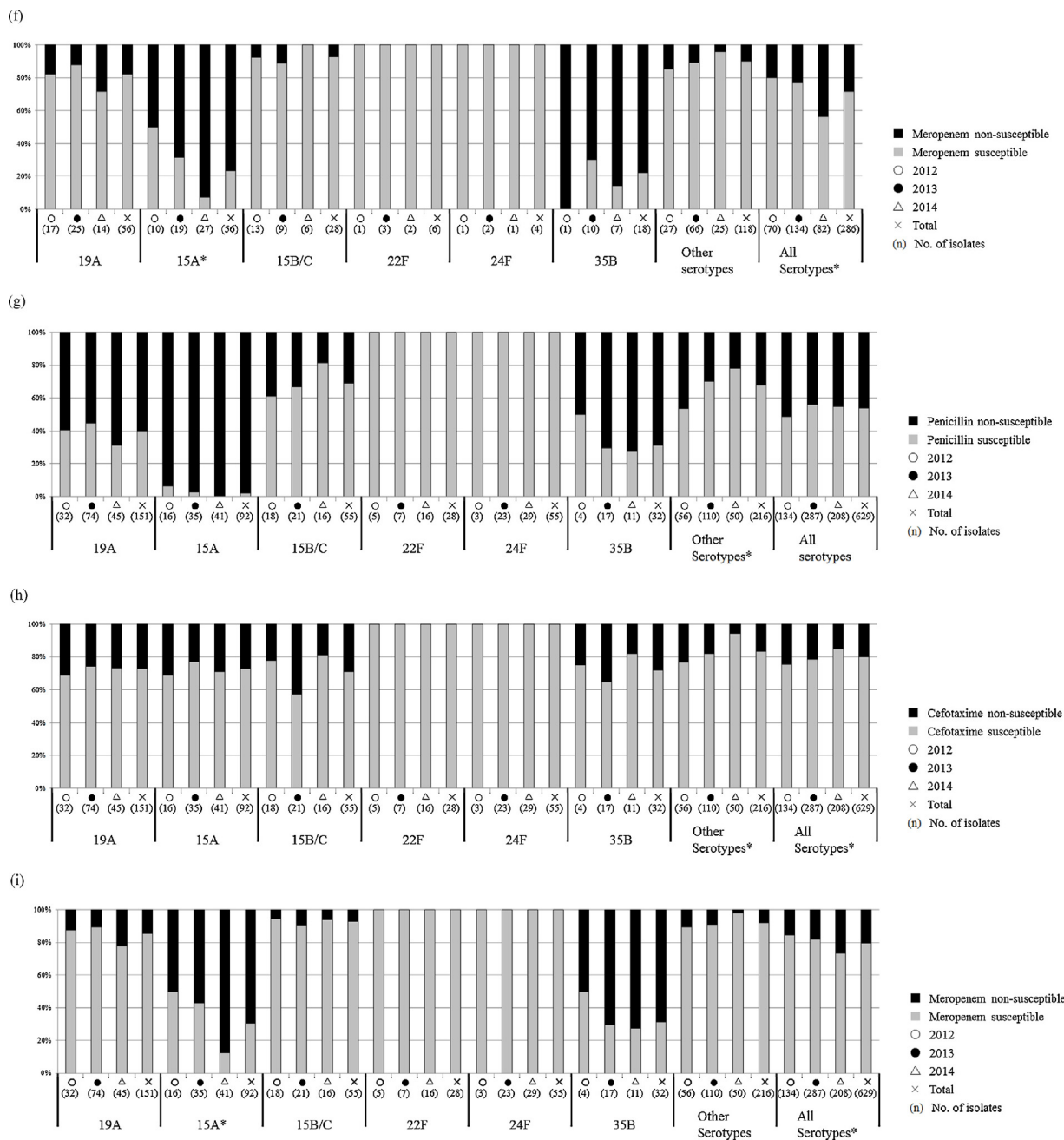


Fig. 3. (Continued).

PCV7 serotypes including serotypes 6B, 19F and 23F, which were not susceptible to penicillin, disappeared. In contrast, CC2572 serotype 24F, which is a non-PCV13 serotype and is susceptible to penicillin, increased during the study period. Additionally, our study revealed that CC63 serotype 15A (Sweden^{15A}-25 clone) and CC 558 serotype 35B, which are not susceptible to penicillin, appeared to spread in Japan. CC3111 serotype 19A, a portion of which exhibited a penicillin-resistant prevalence, did not decrease during our study period. As a result, the proportions of IPD isolates that were non-susceptible to penicillin declined significantly during the study period.

Notably, a significant increase in the proportion of isolates non-susceptible to meropenem was observed in our study. Previous studies reported the proportion of *S. pneumoniae* non-susceptible to carbapenems in other countries [25–28]. According to these reports, the resistance rate appears to be higher in Asia compared

with other regions. Few reports have referred to the correlation between meropenem resistance and its genetic feature in the post-PCV13 era. In our study, a high proportion of meropenem resistance was observed in serotype 15A CC63 (Sweden^{15A}-25 clone); therefore, we assumed that the increase in the meropenem resistance rate was caused by the spread of specific resistant CC63 with serotype 15A (Sweden^{15A}-25 clone) isolates. Additionally, our study found a high proportion of CC558 serotype 35B isolates were non-susceptible to meropenem, which is similar to the finding observed in the United States [19].

We did not detect a relationship between serotypes and mortality or sequelae of IPD in the present study because the numbers of mortality and sequelae cases were very low (Table 1). Easy access to hospitals in Japan given the national insurance system may have led to this outcome. Of note, most of mortality cases investigated in the present study had underlying disease. Although the

Table 4
Distribution of sequence types (STs) and clonal complex (CC) in IPD and non-IPD isolates.

Serotype (n)	CC	ST (n: PMEN clone)
6B (14)	156	90 (2: Spain ^{6B} -2), 2983 (2), 5497 (1), 9333 (1)
	3787	2756 (1), 3787 (1)
	66	902 (2), 2923 (1)
	242	242 (1)
	2224	9335 (1)
	2924	1437 (1)
19F (11)	320	236 (7: Taiwan ^{19F} -14), 926 (1)
	115	115 (1)
	156	280 (1)
	393	393 (1)
23F (4)	2924	1437 (3)
	242	242 (1: Taiwan ^{23F} -15)
18C (2)	3594	3594 (2)
14 (1)	230	5240 (1)
19A (151)	3111	3111 (111), 7988 (1), 8339 (1), 9016 (1), 9334 (1), 10257 (1), new (1)
	2331	2331 (11), 10252 (3), 5842 (2), 9647 (2), 5237 (1), 10260 (1)
	156	338 (1), 3109 (1), 5242 (1), 5246 (1)
	320	320 (4)
	6429	6429 (2)
	99	7811 (1)
	199	199 (1)
	558	558 (1)
	2572	5496 (1)
3 (34)	180	180 (25: Netherlands ³ -31), 5234 (3), 4054 (1), 10246 (1), 10258 (1)
	448	7494 (1)
	1106	7803 (1)
	Singleton	9336 (1)
6A (9)	3787	2756 (3), 3113 (2)
	81	81 (1)
	180	180 (1)
	5832	5832 (1)
	6429	6429 (1)
1 (8)	306	306 (8: Sweden ¹ -28)
7F (2)	191	191 (2: Netherlands ^{7F} -39)
15A (92)	63	63 (52: Sweden ^{15A} -25), 10245 (11), 9084 (6), 7874 (3), 9646 (3), 10244 (2), 1621 (1), 2105 (1), 4532 (1), 4899 (1), 9617 (1), 9644 (1), 10256 (1), 10259 (1), new (2)
	Singleton	10249 (1)
	156	5246 (1)
	292	292 (1)
	2572	5496 (1)
	3111	3111 (1)
15B/C (55)	199	199 (33: Netherlands ^{15B} -37), 10250 (5), 1531 (2), 7793 (2), 419 (1), 2704 (1), 3934 (1)
	156	5242 (3), 9643 (1)
	81	83 (3)
	Singleton	9622 (1), 9648 (1), 9649 (1)
24F (55)	2572	2572 (30), 5496 (15), 7984 (1), 10022 (1), 10248 (1)
	4982	4982 (4)
	156	5241 (1)
	3111	3111 (1)
	Singleton	9621 (1)
35B (32)	558	558 (19), 10255 (1)
	1816	2755 (10)
	3111	3111 (1)
	7786	7502 (1)
22F (28)	433	433 (20), 819 (1), 9288 (1), 10023 (1), 10026 (1), new (1)
	113	5236 (2), 7158 (1)
6C (22)	2924	2924 (4), 6183 (2), 10243 (1)
	5832	5832 (6), 5025 (1)
	156	5241 (3)
	66	2923 (2)
	63	63 (1)
	242	242 (1)
	Singleton	9336 (1)

Table 4 (Continued)

Serotype (n)	CC	ST (n: PMEN clone)
38 (17)	6429	6429 (8)
	393	393 (4)
	115	115 (1)
	180	180 (1)
	199	199 (1)
	7786	7502 (1)
	Singleton	9336 (1)
23A (17)	156	338 (9), 5242 (4), 5246 (2)
	63	63 (1)
	180	180 (1)
10A (19)	113	5236 (9), 10247 (1)
	156	7810 (1), 10254 (1)
	558	558 (1)
	3111	3111 (1)
	Singleton	10021 (1)
11A (16)	99	99 (12)
	62	62 (2)
	63	63 (1)
	393	393 (1)
24B (9)	2572	2572 (4), 5496 (4)
	2754	2754 (1)
34 (7)	1439	7388 (3)
	3116	3116 (3)
	3111	3111 (1)
33F (6)	717	717 (6)
37 (2)	447	447 (2)
12F (1)	1527	4846 (1)
6D (1)	81	282 (1)
7C (1)	2758	10251 (1)
Non-typeable (12)	3111	3111 (2)
	66	9642 (1)
	113	5236 (1)
	199	199 (1)
	230	4845 (1), 10253 (1)
	3116	3116 (2)
	6429	6429 (1)
	7834	7834 (1)
	Singleton	7019 (1)

number of mortality cases was very small, we should pay attention to such patients with severe underlying disease for the prevention and treatment of IPD.

The limitations of this study should be noted. Our study period of surveillance during the PCV 13 era was approximately 1 year, which may not be sufficient time to observe the long-term impacts of the introduction of PCV13. However, the present results showed decreases in the proportion of PCV13 serotype isolates from 2013 to 2014 in IPD, which must be partly due to PCV13. Second, our surveillance was not a population-based study but rather an actively participating surveillance. The implement of our surveillance was not well known throughout all of the institutions in Japan in 2012, the first year of the surveillance; therefore, the number of IPD isolates collected in 2012 was less than those collected in the other years. A previous surveillance in Japan reported a PCV7 coverage rate of 14.7% in paediatric IPD isolates in 2012 [4], which is similar to that found in our study (18.8%). We evenly collected isolates from nationwide institutions throughout the surveillance period; thus, we believe that the data obtained from even the small number of isolates collected in 2012 are reliable in terms of serotype proportions and susceptibility distribution.

In conclusion, we conducted a nationwide surveillance programme of IPD and non-IPD cases from 2012 (PCV7 era) to 2014 (PCV13 era). This surveillance revealed that IPD isolates were mainly serotypes 19A, 24F, and 15A, and the serotype distribution was shifting to non-PCV13 serotypes continuously during the PCV7 era and after the introduction of PCV13. The proportion of IPD isolates non-susceptible to penicillin and cefotaxime declined significantly during the study period, and the

proportion of total isolates non-susceptible to meropenem increased significantly. The MLST analysis clarified that CC3111, CC2572 and CC63 were the main clones in IPD isolates and that CC63 with serotype 15A (Sweden^{15A}-25 clone) was primarily associated with meropenem resistance. To control and prevent pneumococcal infections, a sustained surveillance and a multifaceted study to identify complicated trends of pneumococci are needed.

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Authors contribution

Satoshi Nakano was responsible for the study design, data collection, and writing of the manuscript. Yutaka Ito was responsible for the study design and writing of the manuscript. Takano Fujisawa, Shigeru Suga and Toshiaki Ihara were responsible for the study design and data collection. Bin Chang and Makoto Ohnishi were responsible for the data collection. Taro Noguchi, Yasufumi Matsumura, Masaki Yamamoto, Miki Nagao, Shunji Takakura, and Satoshi Ichiyama reviewed the manuscript.

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