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Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolated from children in Japan, 2023

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ABSTRACT

Background: The prevalence of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* was characterized among children thirteen years after the licensure of the pneumococcal conjugate vaccine (PCV) in Japan.
Methods: A total of 353 pneumococcal isolates were collected from Japanese children between March and July 2023. All the isolates were serotyped using genetic methods and tested for susceptibility to 14 antimicrobial agents.
Results: Among the 353 isolates, the prevalence rates of non-PCV13 and non-PCV20 serotypes were 96.9 % and 77.9 %, respectively, including the dominant non-PCV13/PCV20 serotypes 23A (16.1 %), 35B (15.3 %), 15A (10.5 %), 15C (9.3 %), and 34 (9.1 %), which accounted for 60.3 % of all isolates. The high non-susceptibility rates were observed for macrolides (erythromycin, azithromycin, and clarithromycin; ≥81.9 %) and tetracycline (80.7 %). Penicillin non-susceptibility and multidrug resistance (MDR) were detected in 49.9 % (6.8 % resistant and 43.1 % intermediate) and 68.3 % of the isolates, respectively. The three most common non-PCV13/PCV20 serotypes 15A, 23A, and 35B exhibited high prevalence rates of penicillin non-susceptibility (≥89.5 %) and MDR (≥81.5 %). Extensive drug resistance was observed in 10.5 % of all isolates belonging to six different serotypes (12F, 23A, 11A, 15A, 35B, and 15B) and in the non-encapsulated strains of *S. pneumoniae*.
Conclusions: Our study revealed a higher prevalence of non-susceptibility to penicillin with MDR in the three most common non-PCV13/PCV20 serotypes 15A, 23A, and 35B, in Japan, suggesting their persistence in the PCV13 era.

1. Introduction

Pneumococcal disease is a major global public health problem that can be prevented by vaccination [1]. Since the introduction of the pneumococcal conjugate vaccine (PCV; 7-, 10-, or 13-valent) for routine childhood immunization, the distribution of pneumococcal serotypes has changed. Surveillance by the US Centers for Disease Control and Prevention (CDC) reported that pneumococcal disease rates caused by vaccine serotypes have declined rapidly following the use of PCVs [2]. However, some increases in non-PCV serotypes, a process known as "serotype replacement", have been observed worldwide following the widespread introduction of PCV in other countries such as the USA [3–5], European countries [6,7], England [8], China [9], and Japan [10,11]. In addition, non-encapsulated *Streptococcus pneumoniae* (NESp), which lacks the *cps* locus, has emerged during the PCV era [12], and the selective pressure of the vaccine is thought to increase NESp [13]. Therefore, it is difficult to prevent pneumococcal infections due to the serotype replacement and the emergence of NESp that occurred as a result of the introduction of the vaccine. Accordingly, to address these global serotype changes, next-generation PCV15 and PCV20 were developed to cover all PCV13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) plus two or seven additional serotypes (22F and 33F for PCV15; 8, 10A, 11A, 12F, and 15B for PCV20). PCV15 and PCV20 have already been approved for routine vaccination of all children in the US in 2022 and 2023, respectively [3].

In Japan, PCV7 was introduced in 2010, followed by PCV13 in 2013.

Abbreviations: NESp, nonencapsulated *Streptococcus pneumoniae*; PCV, pneumococcal conjugate vaccine; MDR, multidrug resistance; XDR, extensive drug resistance; PRSP, penicillin-resistant *S. pneumoniae*.

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As for new PCVs, PCV15 was introduced in April 2023 for routine use in national childhood immunization programs. On the other hand, PCV20 is currently in the process of being routinely administered to Japanese children. During the use of PCVs, the investigation of serotype distribution and antimicrobial resistance provides valuable insights into the effectiveness of current vaccines and future vaccine development. Therefore, we conducted a cross-sectional study to investigate the trends in the serotype prevalence and antimicrobial resistance of *S. pneumoniae* isolated from children thirteen years after the licensure of PCV and prior to the introduction of PCV20 in Japan. The present results were compared with those of previous epidemiological surveillance studies conducted in Hokkaido, the northern main island of Japan, in 2011 [14] and 2016 [11].

2. Materials and methods

2.1. Pneumococcal isolates

Specimens were obtained from non-sterile sites of pediatric outpatients aged 0–15 years who had clinical symptoms by non-invasive infections and visited medical facilities in Hokkaido, Japan, between March and July 2023. Bacterial isolation and identification from the clinical specimens were both performed at Sapporo Clinical Laboratory, Inc. Only one pneumococcal isolate per patient was included in this study. When two or more isolates were obtained from a single patient, the earliest isolate was selected. Specimens were cultured on 5 % sheep blood agar plates and incubated at 37 °C under 5 % CO_2 for 18–20 h. Species identification was performed according by colony morphology, alpha-hemolysis, and optochin susceptibility. After confirmation, isolates were stored in Microbank vials (Pro-lab Diagnostics, Richmond Hill, Canada) at −80 °C until further testing.

2.2. Serotype assignment

Serotype detection was performed using conventional multiplex PCR protocols with serogroup/serotype primers provided by the CDC (listed at http://www.cdc.gov./streplab/pcr.html) [15]. A primer pair targeting the *cpsA* gene was used as a positive control for each reaction. After PCRs, additional subtyping for serogroups 6, and 15 and discrimination for serotypes 33F/33A/37 was performed using PCR-based sequencing methods as described in our previous studies [11,14]. The presence of the autolysin gene, a specific marker for pneumococcus, and *cpsA*--negative isolates were confirmed as NESp using PCR, as described previously [16].

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by the broth microdilution method using a Dry Plate (Eiken, Tokyo, Japan) by measuring the minimum inhibitory concentrations for all isolates within different concentration ranges. The following 14 antimicrobial agents were assayed: β-lactams (penicillin, cefaclor, cefuroxime, ceftriaxone, imipenem, and meropenem) and non-β-lactams (erythromycin, azithromycin, clarithromycin, clindamycin, tetracycline, levofloxacin, trimethoprim–sulfamethoxazole, and vancomycin). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria (M100, 28th edition). For penicillin, CLSI oral therapy breakpoints of susceptible (≤0.06 μg/mL), intermediate (0.12–1.0 μg/mL), and resistant (≥2.0 μg/mL) were used. Isolates with intermediate resistance or resistance were defined as non-susceptible. MDR and extensive drug resistance (XDR) were defined as resistance to at least three and five classes of antimicrobials, respectively.

3. Results

A total of 353 pediatric pneumococcal isolates (49.3 % males; 50.7 % females) were assessed. The majority of isolates were obtained from nasal discharge (53.6 %) or the nasal cavity (42.2 %), followed by pharynx and ear discharges. The detection rates of PCV and non-PCV serotypes among pediatric pneumococcal isolates in our previous studies in Hokkaido, Japan, from January to December 2011 (PCV7 voluntary immunization period; n = 998) [14] and from June to November 2016 (PCV13 routine immunization period; n = 678) [11], and the present study in 2023 (n = 353) are presented in Fig. 1. Compared to our previous data [14], PCV7 serotype decreased from 48.2 % in 2011 to 0.8 % in 2023. In contrast, non-PCV13 serotypes increased from 39.7 % in 2011 [14] to 87.6 % in 2016 [11], and to 96.9 % in 2023.

In the present study, the proportions of all isolates with serotypes covered by PCV13 and PCV20 were 3.1 % (1.1 %; <5 years, 11.9 %; 5–15 years) and 22.1 % (21.0 %; <5 years; 25.9 %; 5–15 years), respectively, and 77.9 % (79.0 %; <5 years; 73.1 %; 5–15 years) of isolates were non-PCV13/20 serotypes (Table 1). The five most common serotypes were non-PCV13/PCV20 serotypes 23A, 35B, 15A, 15C, and 34, these accounted for 60.3 % of all the isolates (n = 213/353). The serogroup 15 (15A/15B/15C) was more common in children under 5

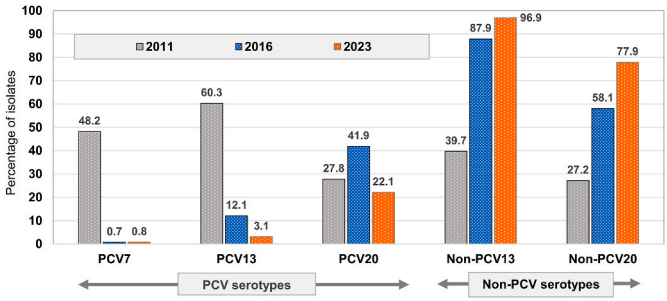

Fig. 1. Comparison of the PCV serotypes and non-PCV serotypes prevalence among pediatric isolates between our previous studies (2011 and 2016) and present study (2023).

Table 1
Prevalence of pneumococcal serotypes in children by age group.

serotype/NESp	Number of isolates (%)		
	Overall (0–15 years)	<5 years	5–15 years
PCV13 serotypes	**11 (3.1)**	**3 (1.1)**	**8 (11.9)**
19A	6 (1.7)	2 (0.7)	4 (6.0)
3	3 (0.8)	0	3 (4.5)
23F	2 (0.6)	1 (0.3)	1 (1.5)
PCV20 additional serotypes	**67 (19.0)**	**57 (19.9)**	**10 (14.9)**
11A	22 (6.2)	17 (5.9)	5 (7.5)
10A	20 (5.7)	16 (5.6)	4 (6.0)
15B	16 (4.5)	16 (5.6)	0
22F	8 (2.3)	7 (2.4)	1 (1.5)
12F	1 (0.3)	1 (0.3)	0
Non-PCV serotypes	**275 (77.9)**	**226 (79.0)**	**49 (73.1)**
23A	57 (16.1)	47 (16.4)	10 (14.9)
35B	54 (15.3)	46 (16.1)	8 (11.9)
15A	37 (10.5)	34 (11.9)	3 (4.5)
15C	33 (9.3)	31 (10.8)	2 (3.0)
34	32 (9.1)	22 (7.7)	10 (14.9)
23B	23 (6.5)	18 (6.3)	5 (7.5)
37	13 (3.7)	10 (3.5)	3 (4.5)
21	7 (2.0)	4 (1.4)	3 (4.5)
7C	4 (1.1)	4 (1.4)	0
6C	2 (0.6)	1 (0.3)	1 (1.5)
16F	2 (0.6)	2 (0.7)	0
24F	2 (0.6)	0	2 (3.0)
31	2 (0.6)	1 (0.3)	1 (1.5)
NESp[a]	7 (2.0)	6 (2.1)	1 (1.5)
Total	353 (100)	286 (81.0)	67 (19.0)

[a] NESp, non-encapsulated *S. pneumoniae*.

Table 2
The non-susceptibility rates to 14 antimicrobial agents for each serotype of pneumococcal isolates in children.

serotype/NESp (n, %)	PRSP[a]	Number of non-susceptible isolates (%)													MDR[c]	XDR[c]
		β-lactams[b]						Non-β-lactams[b]								
		PEN	CEC	CXM	CRO	IPM	MEM	ERY	AZM	CAM	CLI	TET	LVX	SXT		
PCV13 serotypes (11, 3.1 %)	**0**	**3 (27.3)**	**3 (27.3)**	**1 (9.1)**	**0**	**0**	**0**	**8 (72.7)**	**10 (90.9)**	**8 (72.7)**	**6 (54.5)**	**9 (81.8)**	**0**	**1 (9.1)**	**6 (54.5)**	**0**
19A (6, 1.7 %)	0	2 (33.3)	2 (33.3)	1 (16.7)	0	0	0	4 (66.7)	6 (100)	4 (66.7)	2 (33.3)	5 (83.3)	0	1 (16.7)	2 (33.3)	0
3 (3, 0.8 %)	0	0	0	0	0	0	0	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	2 (66.7)	0	0	2 (66.7)	0
23F (2, 0.6 %)	0	1 (50.0)	1 (50.0)	0	0	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	0	2 (100)	0
PCV20 additional serotypes (67, 19.0 %)	**6 (9.1)**	**15 (22.7)**	**15 (22.7)**	**11 (16.4)**	**2 (3.0)**	**6 (9.1)**	**8 (11.9)**	**54 (81.8)**	**53 (80.3)**	**55 (83.3)**	**45 (68.2)**	**54 (81.8)**	**0**	**13 (19.4)**	**48 (71.6)**	**7 (10.3)**
11A (22, 6.2 %)	5 (22.7)	5 (22.7)	5 (22.7)	5 (22.7)	1 (4.5)	5 (22.7)	5 (22.7)	22 (100)	22 (100)	22 (100)	18 (81.8)	20 (90.9)	0	10 (45.5)	18 (81.8)	5 (22.7)
10A (20, 5.7 %)	0	3 (15.0)	3 (15.0)	2 (10.0)	0	0	1 (5.0)	12 (60.0)	12 (60.0)	13 (65.0)	10 (50.0)	15 (75.0)	0	0	12 (60.0)	0
15B (16, 4.5 %)	0	4 (25.0)	4 (25.0)	2 (12.5)	0	0	1 (6.3)	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)	0	1 (6.3)	16 (100)	1 (6.3)
22F (8, 2.3 %)	0	2 (25.0)	2 (25.0)	1 (12.5)	0	0	0	3 (37.5)	2 (25.0)	3 (37.5)	0	2 (25.0)	0	1 (12.5)	1 (12.5)	0
12F (1, 0.3 %)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)
Non-PCV serotypes (275, 77.9 %)	**18 (6.5)**	**158 (57.2)**	**142 (51.6)**	**92 (33.5)**	**12 (4.4)**	**55 (20.0)**	**86 (31.3)**	**227 (82.2)**	**230 (83.3)**	**226 (81.9)**	**142 (51.4)**	**222 (80.4)**	**6 (2.2)**	**80 (29.1)**	**187 (68.0)**	**30 (10.9)**
23A (57, 16.1 %)	10 (17.5)	51 (89.5)	49 (86.0)	22 (38.6)	5 (8.8)	16 (28.1)	18 (31.6)	52 (91.2)	52 (91.2)	51 (89.5)	40 (70.2)	53 (93.0)	4 (7.0)	20 (35.1)	49 (86.0)	17 (29.8)
35B (54, 15.3 %)	2 (3.7)	49 (90.7)	47 (87.0)	45 (83.3)	3 (5.6)	18 (33.3)	43 (79.6)	52 (96.3)	53 (98.1)	50 (92.6)	11 (20.4)	41 (75.9)	0	23 (42.6)	44 (81.5)	5 (9.3)
15A (37, 10.5 %)	5 (13.5)	36 (97.3)	35 (94.6)	20 (54.1)	2 (5.4)	18 (48.6)	22 (59.5)	37 (100)	37 (100)	37 (100)	35 (94.6)	37 (100)	1 (2.7)	14 (37.8)	36 (97.3)	7 (18.9)
15C (33, 9.3 %)	0	10 (30.3)	3 (9.1)	0	1 (3.0)	0	0	33 (100)	33 (100)	33 (100)	32 (97.0)	33 (100)	0	1 (3.0)	33 (100)	0
34 (32, 9.1 %)	0	3 (9.4)	2 (6.3)	1 (3.1)	0	0	0	26 (81.3)	26 (81.3)	26 (81.3)	3 (9.3)	24 (75.0)	0	6 (18.8)	5 (15.6)	0
23B (23, 6.5 %)	0	1 (4.3)	1 (4.3)	1 (4.3)	0	0	0	0	1 (4.3)	1 (4.3)	0	0	0	2 (8.7)	0	0
37 (13, 3.7 %)	0	2 (15.4)	0	0	0	0	0	12 (92.3)	12 (92.3)	12 (92.3)	10 (76.9)	10 (76.9)	0	0	6 (46.2)	0
21 (7, 2.0 %)	0	0	0	0	0	0	0	0	0	1 (14.3)	0	7 (100)	0	0	0	0
7C (4, 1.1 %)	0	0	0	0	0	0	0	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	0	3 (75.0)	4 (100)	0
6C (2, 0.6 %)	0	0	0	0	0	0	0	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	0	2 (100)	2 (100)	0
16F (2, 0.6 %)	0	1 (50.0)	1 (50.0)	1 (50.0)	1 (50.0)	0	0	1 (50.0)	1 (50.0)	1 (50.0)	0	1 (50.0)	0	1 (50.0)	1 (50.0)	0
24F (2, 0.6 %)	0	0	0	0	0	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	0
31 (2, 0.6 %)	0	1 (50.0)	0	0	0	0	0	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	0	1 (50.0)	1 (50.0)	0
NESp[d] (7, 2.0 %)	1 (14.3)	4 (57.1)	4 (57.1)	2 (28.6)	0	3 (42.9)	3 (42.9)	4 (57.1)	5 (71.4)	4 (57.1)	3 (42.9)	6 (85.7)	1 (14.3)	5 (71.4)	4 (57.1)	1 (14.3)
Total (353, 100 %)	**24 (6.8)**	**176 (49.9)**	**160 (45.3)**	**104 (29.5)**	**14 (3.7)**	**61 (17.3)**	**94 (26.6)**	**289 (81.9)**	**293 (83.0)**	**289 (81.9)**	**193 (54.7)**	**285 (80.7)**	**6 (1.7)**	**94 (26.6)**	**241 (68.3)**	**37 (10.5)**

[a] PRSP, PEN-resistant *S. pneumoniae*.
[b] Abbreviations: PEN, penicillin; CEC, cefaclor; CXM, cefuroxime; CRO, ceftriaxone; IPM, imipenem; MEM, meropenem, ERY, erythromycin; AZM, azithromycin, CAM, clarithromycin; CLI, clindamycin; TET, tetracycline; LVX, levofloxacin; SXT, trimethoprim-sulfamethoxazole. All isolates were susceptible to vancomycin (data not shown).
[c] Multidrug resistance (MDR) and extensive drug resistance (XDR) were defined as acquired resistance to >3 and > 5 antimicrobial classes (PEN-resistant, MIC >2 mg/L), respectively.
[d] NESp, non-encapsulated *S. pneumoniae*.

years than in 5–15 years. In contrast, serotype 34 was more prevalent in 5–15 years group. Seven isolates (2.0 %) were identified as NESp. All isolates of serotypes 3 (0.8 %) and 37 (3.7 %) and one isolate of serotypes 21 (0.3 %) and 24F (0.3 %) showed a mucoid phenotype (5.1 %, n = 18/353).

The rates of antimicrobial non-susceptibility to the 14 antimicrobials for each serotype of all isolates are summarized in Table 2. All isolates were susceptible to vancomycin (data not shown). Isolates exhibited high prevalence rates of non-susceptibility to macrolides (erythromycin, azithromycin, and clarithromycin; ≥81.9 %) and tetracycline (80.7 %). In contrast, only a small number of isolates were non-susceptible to levofloxacin (0.6 %). The penicillin non-susceptibility rate was 49.9 % (6.8 % resistance and 43.1 % intermediate resistance). The high prevalence rates of non-susceptibility rates to penicillin (89.5%–97.3 %) were observed in the three most common non-PCV13/PCV20 serotypes 15A, 35B, and 23A. The penicillin-resistant *S. pneumoniae* (PRSP) was detected in serotypes 11A (22.7 %), 23A (17.5 %),15A (13.5 %), 35B (3.7 %), and NESp (14.3 %). The dominant serotypes 35B and 15A showed a high prevalence of non-susceptibility to meropenem. In contrast, most isolates with a mucoid phenotype (88.9 %, n = 16/18) were susceptible to penicillin (including other β-lactams), except for two isolates of serotype 37. Overall, MDR and XDR rates were 68.3 % and 10.5 %, respectively. A high prevalence of MDR and XDR (% of isolates with MDR/XDR) was identified in the prevalent serotypes 15A (97.3/18.9 %), 23A (86.0/29.8 %) and 35B (81.5/9.3 %).

4. Discussion

The present study documented the relationship between serotype prevalence and antimicrobial susceptibility of *S. pneumoniae* isolated from children thirteen years after the introduction of PCV in Japan. The present results and our previous studies of isolates from children in Hokkaido, northern Japan, revealed that the prevalence rate of non-PCV13 serotypes in children increased from 39.7 % in 2011, one year after the PCV7 introduction [14], to 87.9 % in 2016 during the PCV13 era [11], and 96.9 % in the present study in 2023. In addition, in this study 2023, the distribution of PCV13 serotypes among those under 5 years of age was 1.1 %, which was less than among those 5–15 years of age, indicating a benefit of PCV13 vaccination. This trend, serotype replacement from PCV serotypes to non-PCV serotypes in pneumococcal disease, has occurred in countries where PCVs have been introduced since the routine use of PCVs in children [4–10].

Following the widespread introduction of PCVs, non-PCV13 serotypes, specifically 15A, 23A, and 35B, which are not included in the new PCV20, have emerged and spread worldwide [4,5,9,14]. In this study, the three most common serotypes, 23A, 35B, and 15A, were non-PCV13/PCV20 serotypes and were mostly associated with MDR with non-susceptibility to penicillin (≥81.5 %). A similar trend was observed in China for serotypes 23A and 15A in 2021–2022 [9] and in Japan for serotypes 35B and 15A in 2016 [10,11]. Pneumococcal isolates collected from the U.S. children between 2011 and 2021, common non-susceptible serotypes were 15A, 23A, and 35B and the majority of serotypes 15A (86.7 %) and 35B (87.9 %) were non-susceptible to penicillin [21]. These findings strongly suggest that MDR/non-susceptible non-PCV serotypes may have contributed to their persistence during the PCV13 era.

The currently licensed PCV20, which was developed to address pneumococcal infections caused by increased non-vaccine serotypes, was approved and recommended for use in children in the U.S. in 2023 [3], while the PCV20 is currently under the application for approval process for use in Japanese children. However, novel PCV20 does not prevent the predominant non-PCV13 MDR serotypes 15A, 23A, and 35B. In the present study, 77.9 % of the isolates belonged to non-PCV20 serotypes, which was similar to findings for pneumococcal isolates collected from Japanese children (79.6 %) [10], suggesting that the potential effect of PCV20 in children may be low in Japan. In addition, seven isolates in the present study were identified as NESp, which are not preventable by PCVs. Compared to our previous study on NESp during 2011–2019 [13], the prevalence rate remained at a relatively low level over the 13 years.

In the present study and our previous study [11], all serotype 3 and 37 isolates had a mucoid colony phenotype with a thick capsule. A precious study investigating mucoid-type *S. pneumoniae* demonstrated that 99.5 % of mucoid phenotype isolates belonged to either serotypes 3 or 37 [17]. However, since the introduction of PCV13 in the U.S., an increasing trend of serotype 3 has been observed, even though PCV13 contains serotype 3 [18]. According to a previous study, effective protection with PCV13 was more limited for serotype 3 than for the other serotypes in Portugal [19]. Yang et al. reported that the thick capsule of serotype 3 may be an important factor in bacterial cell evasion [20]. More importantly, in this study, we observed a mucoid phenotype in serotypes 21 and 24F, which are not targeted by either PCV13 or PCV20. To the best of our knowledge, there are no reports of the mucoid phenotype in pneumococcal serotypes 21 and 24F. A recent study has suggested that the change from non-mucoid to mucoid may occur via capsular switching in response to changes induced by the introduction of vaccines [17]. Therefore, it is concerning that serotypes that have acquired the mucoid phenotype with thick capsule may continue to emerge among *S. pneumoniae* during the PCV era.

In conclusion, this study provides valuable insights into the serotype prevalence and antimicrobial susceptibility of *S. pneumoniae* during the use of PCV13, particularly when developing next-generation of PCV formulations. Therefore, consistent monitoring of serotype trends is required for during the PCV era.

CRediT authorship contribution statement

Mitsuyo Kawaguchiya: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis. **Noriko Urushibara:** Investigation. **Meiji Soe Aung:** Investigation. **Nobuhide Ohashi:** Investigation. **Sho Tsutida:** Resources. **Kayo Kurashita:** Resources. **Masahiko Ito:** Resources. **Nobumichi Kobayashi:** Writing – review & editing, Methodology.

Competing interests

None declared.

Ethical statement

Not required.

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Declaration of competing interest

The author declares no conflict of interest.

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