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Short Communication

Spread of multidrug resistance in non-PCV13/PCV20 serotypes of *Streptococcus pneumoniae*: A cross-sectional study ten years after the introduction of pneumococcal conjugate vaccine in Japan

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Abstract Ten years after the introduction of the pneumococcal conjugate vaccine (PCV) in Japan, the prevalence rates of non-PCV13 and non-PCV20 serotypes among pediatric pneumococcal isolates were 94.0% and 73.7%, respectively. The predominant non-PCV13/PCV20 serotypes (15A, 35B, and 23A) were mostly multidrug-resistant (≥80.5%), exhibiting non-susceptibility to penicillin.

Introduction

Diseases caused by *Streptococcus pneumoniae* (pneumococcus) are a global public health concern. The pneumococcal conjugate vaccine (PCV; 7-, 10-, or 13-valent) has been introduced into the infant immunization schedule in

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many countries worldwide since 2000. Although PCVs have provided a protective effect against pneumococcal diseases in children, several serotypes not included in PCV13 (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) with antimicrobial resistance, specifically 15A, 23A, and 35B, are increasing.[1,2] Furthermore, the emergence of nonencapsulated *S. pneumoniae* (NESp), which lacks the vaccine antigen capsule and is thus not preventable with the current PCVs, has also been reported.[3]

In Japan, PCV7 became available for voluntary vaccination of children in 2010. Thereafter, PCV7 was incorporated into the routine infant immunization program in April 2013 and was replaced by PCV13 in November 2013. Our previous surveillance studies demonstrated an evident reduction in pneumococcal infections caused by PCV serotypes following PCV7/PCV13 sequential implementation, which was associated with an increase in non-PCV13 serotypes/serogroup 15, 23A, 35B, and NESp with resistance to multiple antimicrobials, including penicillin.[4—6] Because β-lactam antibiotics are primarily used for the treatment of pneumococcal infections worldwide, antimicrobial resistance of pneumococcus remains a public health concern. Recently, new higher-valent PCVs, that is to say, PCV15 and PCV20 (PCV15, PCV13 serotypes plus 22F and 33F: PCV20, PCV15 serotypes plus 8, 10A, 11A, 12F, and 15 B), were developed and licensed for adults in the US in November 2021 (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/pcv.pdf) and are currently under clinical trials for US pediatric populations.[7] In such situations, surveillance studies are necessary to monitor non-vaccine serotypes of *S. pneumoniae* and NESp as well as isolates with multidrug resistance (MDR).

In the present study, we aimed to elucidate the serotype trends and antimicrobial resistance of noninvasive *S. pneumoniae* isolates from children ten years after the introduction of PCV in Japan. We also evaluated the presumptive serotype coverage rate of PCV20 before its introduction in Japan.

Methods

Settings and pneumococcal isolates

In this cross-sectional descriptive study, nonrepetitive 415 pneumococcal isolates were obtained from non-sterile sites, such as nasal discharge, sputum, and pharynx between July and December 2020. The clinical specimens were consecutively collected at medical facilities in Hokkaido prefecture, Japan, then they were transferred to the Sapporo Clinical Laboratory, Inc. for isolation and identification of pneumococcus. All isolates were collected from pediatric outpatients aged 0—15 years and were considered to be derived from colonization/noninvasive infections. The specimens were cultured on a blood agar base supplemented with 5% sheep blood (KYOKUTO Pharmaceutical Industrial Co., Ltd., Japan) and incubated overnight at 37 °C in 5% CO_2. *S. pneumoniae* was identified based on colony morphology, α-hemolysis, and optochin susceptibility. All isolates were stored in Microbank™ vials (Pro-Lab Diagnostics, Richmond Hill, ON, Canada) at −80 °C until further processing.

Serotype identification

All isolates were serotyped using conventional PCR serotype deduction protocols published by the Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/streplab/pneumococcus/resources.html), and a primer pair targeting the *cpsA* gene was used as a positive control for each reaction. After PCR, additional subtyping was performed using PCR-sequencing methods, as described in our previous studies.[4,5] Isolates that were negative for the *cpsA* gene were confirmed as nonencapsulated *S. pneumoniae* (NESp) using PCR as described previously.[3]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all isolates was performed by the broth microdilution method using the Dry Plate Eiken (Eiken, Tokyo, Japan), by measuring minimum inhibitory concentrations (MICs) within a limited concentration range. The following 14 antimicrobial agents were tested: β-lactams (penicillin, PEN; ceftriaxone, CRO; cefaclor, CEC; cefuroxime, CXM; imipenem, IPM; and meropenem, MEM) and non-β-lactams (erythromycin, ERY; azithromycin, AZM; clarithromycin, CAM; clindamycin, CLI; tetracycline, TET; levofloxacin, LVX; trimethoprim—sulfamethoxazole, SXT; and vancomycin, VAN). The MIC breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria (M100, 28th edition). For PEN, CLSI oral breakpoints (susceptible, $\leq$0.06 μg/mL; intermediate, 0.12—1.0 μg/mL; resistant, $\geq$2.0 μg/mL) were used. Isolates with intermediate resistance or resistance were defined as non-susceptible. According to previous studies,[5,8] MDR and extensive drug resistance (XDR) were defined as acquired resistance to $\geq$ 3 and $\geq$ 5 classes of antimicrobial agents, respectively.

Results

Prevalence of serotypes and NESp

Among the 415 pediatric pneumococcal isolates, the male-to-female ratio was 1.2. During the study period, 20 different serotypes and 14 NESp isolates (3.4%) were identified (Table 1 and Fig. 1). The prevalence rates of the non-PCV13 and non-PCV20 serotypes were 94.0% and 73.7%, respectively, with serotype 23A (13.0%) being dominant, followed by serotypes 34 (12.3%), 15A (11.1%), and 35B (9.9%). Among all isolates, the mucoid phenotype was identified in 28 isolates (5.5%, n = 28/415), which comprised serotypes 3 and 37 (12 and 16 isolates, respectively).

Antimicrobial susceptibility

The non-susceptibility/resistance rates to antimicrobials for each serotype are summarized in Table 1. High rates of non-susceptibility (77.1%—84.6%) were found for ERY, AZM, CAM, and TET. PEN-non-susceptible *S. pneumoniae* (PNSP) was identified in 37.8% of all isolates, including 2.4% of PEN-

Table 1 The non-susceptibility rates to 14 antimicrobial agents for each serotype.

serotype/non-encapsuleted type (n, %)	PRSP[a]	Number of non-susceptible isolates (%)														
		β-lactams[b]						Non-β-lactams[b]								
		PEN	CEC	CXM	CRO	IPM	MEM	ERY	AZM	CAM	CLI	TET	LVX	SXT	MDR[c]	XDR[c]
PCV13 serotypes (25, 6.0%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**15 (60.0)**	**15 (60.0)**	**15 (60.0)**	**10 (40.0)**	**15 (60.0)**	**1 (4.0)**	**6 (24.0)**	**10 (40.0)**	**0**
3 (12, 2.9%)	0	0	0	0	0	0	0	10 (83.3)	10 (83.3)	10 (83.3)	9 (75.0)	10 (83.3)	0	0	8 (66.7)	0
23F (9, 2.2%)	0	0	0	0	0	0	0	1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)	1 (11.1)	0	3 (33.3)	1 (11.1)	0
19A (4, 1.0%)	0	0	0	0	0	0	0	4 (100)	4 (100)	4 (100)	0	4 (100)	1 (25.0)	3 (75.0)	1 (25.0)	0
PCV20 additional serotypes (84, 20.2%)	**0**	**10 (11.9)**	**13 (15.5)**	**7 (8.3)**	**1 (1.2)**	**1 (1.2)**	**4 (4.8)**	**63 (75.0)**	**61 (72.6)**	**59 (70.2)**	**51 (60.7)**	**69 (82.1)**	**0**	**15 (17.9)**	**54 (64.3)**	**0**
10A (35, 8.4%)	0	4 (11.4)	6 (17.1)	3 (8.6)	0	1 (2.9)	3 (2.9)	18 (51.4)	16 (45.7)	14 (40.0)	10 (28.6)	24 (68.6)	0	3 (8.6)	11 (31.4)	0
15B (27, 6.5%)	0	6 (22.2)	5 (18.5)	3 (11.1)	0	0	1 (3.7)	27 (100)	27 (100)	27 (100)	26 (96.3)	27 (100)	0	0	27 (100)	0
11A/11D (16, 3.9%)	0	0	2 (12.5)	1 (6.3)	1 (6.3)	0	0	16 (100)	16 (100)	16 (100)	14 (87.5)	16 (100)	0	12 (75.0)	15 (93.8)	0
22F (6, 1.4%)	0	0	0	0	0	0	0	2 (33.3)	2 (33.3)	2 (33.3)	1 (16.7)	2 (33.3)	0	0	1 (16.7)	0
Non-PCV20 serotypes (306, 73.7%)	**10 (3.3)**	**147 (48.0)**	**143 (46.7)**	**83 (27.1)**	**7 (2.3)**	**42 (13.7)**	**61 (19.9)**	**256 (83.7)**	**252 (82.4)**	**246 (80.4)**	**158 (51.6)**	**267 (87.3)**	**1 (0.3)**	**115 (37.6)**	**198 (64.7)**	**8 (2.6)**
23A (54, 13.0%)	3 (5.6)	46 (85.2)	43 (79.6)	8 (14.8)	3 (5.6)	5 (9.3)	6 (11.1)	47 (87.0)	46 (85.2)	43 (79.6)	41 (75.9)	49 (90.7)	0	15 (27.8)	45 (83.3)	5 (9.3)
34 (51, 12.3%)	0	3 (5.9)	3 (5.9)	3 (5.9)	0	1 (2.0)	2 (3.9)	45 (88.2)	44 (86.3)	43 (84.3)	3 (5.9)	45 (88.2)	0	10 (19.6)	5 (9.8)	0
15A (46, 11.1%)	4 (8.7)	44 (95.7)	44 (95.7)	31 (67.4)	3 (6.5)	21 (45.7)	18 (39.1)	46 (100)	46 (100)	46 (100)	45 (97.8)	45 (97.8)	0	34 (73.9)	45 (97.8)	2 (4.3)
35B (41, 9.9%)	0	36 (87.8)	36 (87.8)	31 (75.6)	0	8 (19.5)	29 (70.7)	39 (95.1)	37 (90.2)	36 (87.8)	8 (19.5)	35 (85.4)	0	20 (48.8)	33 (80.5)	0
15C (33, 8.0%)	0	8 (24.2)	4 (12.1)	1 (3.0)	0	1 (3.0)	1 (3.0)	32 (100)	32 (100)	32 (100)	31 (93.9)	32 (100)	0	4 (12.1)	32 (97.0)	0
21 (17, 4.1%)	0	1 (5.9)	1 (5.9)	0	0	0	0	2 (11.8)	1 (5.9)	2 (11.8)	1 (5.9)	16 (94.1)	0	0	1 (5.9)	0
37 (16, 3.9%)	1 (6.3)	1 (6.3)	2 (12.5)	1 (6.3)	0	1 (6.3)	0	14 (87.5)	14 (87.5)	14 (87.5)	13 (81.3)	13 (81.3)	0	1 (6.3)	13 (81.3)	1 (6.3)
23B (8, 1.9%)	0	0	0	0	0	0	0	0	1 (12.5)	0	0	0	0	0	0	0
24F (6, 1.4%)	0	1 (16.7)	1 (16.7)	1 (16.7)	0	0	0	6 (100)	6 (100)	6 (100)	5 (83.3)	5 (83.3)	0	5 (83.3)	5 (83.3)	0
6C (5, 1.2%)	0	1 (20.0)	1 (20.0)	1 (20.0)	0	1 (20.0)	1 (20.0)	5 (100)	5 (100)	5 (100)	3 (60.0)	5 (100)	0	5 (100)	5 (100)	0
16F (5, 1.2%)	0	0	2 (40.0)	2 (40.0)	0	0	0	3 (60.0)	3 (60.0)	3 (60.0)	1 (20.0)	4 (80.0)	0	2 (40.0)	3 (60.0)	0
31 (5, 1.2%)	0	0	0	0	0	0	0	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	1 (20.0)	0	5 (100)	1 (20.0)	0
7B/7C/40 (2, 0.5%)	0	0	0	0	0	0	0	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	0
Non-typeable (3, 0.7%)	0	1 (33.3)	1 (33.3)	0	0	0	0	3 (100)	3 (100)	3 (100)	2 (66.7)	3 (100)	0	2 (66.7)	2 (66.7)	0
NESp[d] (14, 3.4%)	2 (14.3)	5 (35.7)	5 (35.7)	4 (28.6)	1 (7.1)	4 (28.6)	4 (28.6)	11 (78.6)	11 (78.6)	10 (71.4)	2 (14.3)	12 (85.7)	1 (7.1)	10 (71.4)	6 (42.9)	0
Total (415, 100%)	**10 (2.4)**	**157 (37.8)**	**156 (37.6)**	**90 (21.7)**	**8 (1.9)**	**43 (10.4)**	**65 (15.7)**	**334 (80.5)**	**328 (79.0)**	**320 (77.1)**	**219 (52.8)**	**351 (84.6)**	**2 (0.5)**	**136 (32.8)**	**262 (63.1)**	**8 (1.9)**

The total number (%) of PCV13 serotypes, PCV20 additional serotypes, and non-PCV20 serotypes is shown in bold.

[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 VAN.

[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, nonencapsulated *S. pneumoniae*.

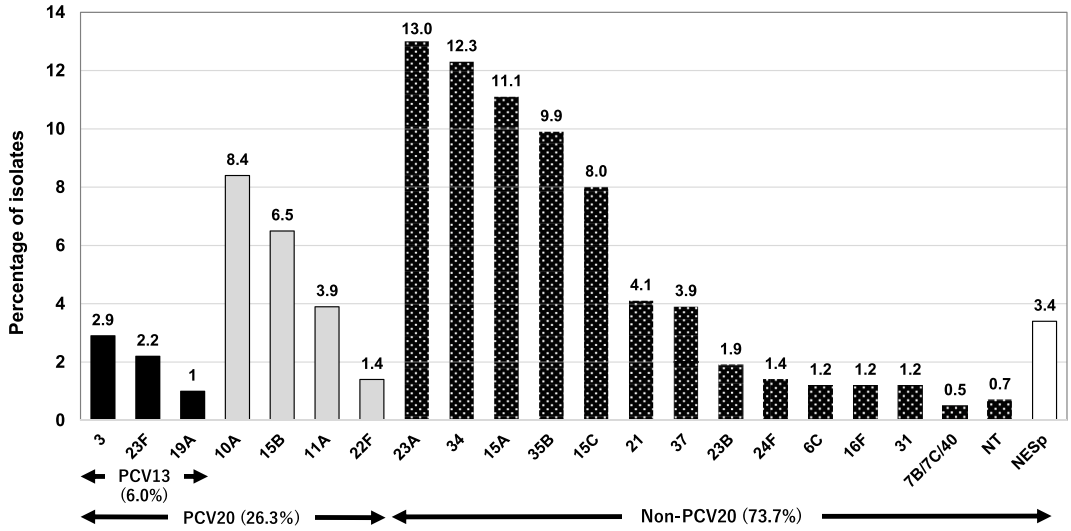

Figure 1. The prevalence rates of the PCV13/PCV20 and distribution of pneumococcal serotypes and nonencapsulated S. pneumoniae (NESp) among pediatric isolates.

resistant S. *pneumoniae* (PRSP). A high prevalence of PNSP was detected in non-PCV13/PCV20 serotypes 15A (95.7%), 35B (87.8%), and 23A (85.2%), and PRSP was also found in non-PCV13/PCV20 serotypes 15A, 23A, and 37. Among the 415 isolates, 262 (63.1%) and eight (1.9%) isolates exhibited MDR and XDR, respectively. MDR was identified in the non-PCV13/PCV20 serotypes 15A (97.8%), 15C (97.0%), and 23A (83.3%), while XDR was identified in serotypes 23A (9.3%), 15A (4.3%), and 37 (6.3%). In contrast, serotypes 3 and 37, which exhibited a mucoid phenotype, were mostly susceptible to β-lactams (96.4%, 27/28).

Discussion

This epidemiological surveillance study on the serotype prevalence and antimicrobial resistance of pneumococci isolated from children was conducted ten years after the introduction of PCV in Japan. Active Bacterial Core Surveillance (ABCs) from 1998 to 2018 in the US population by the Center for Diseases Control and Prevention (CDC) showed that invasive pneumococcal diseases due to PCV serotypes decreased after the introduction of the first PCV7, which was concurrent with the serotype replacement to non-PCV7/13 serotypes.[2] With the introduction of first PCV7 in Japan, we have been investigating the distribution of serotypes since 2011 under the same settings as the present study. Similarly, comparing the present results with our surveillance study in 2011,[4] the prevalence rate of non-PCV13 among clinical pneumococcal pediatric isolates increased from 39.7% in 2011 to 94.0% ($p < 0.001$) in the present study in 2020. We also observed that the prevalence of NESp, which lacks capsules and is unpreventable by PCV, increased from 1.6%–2.5% to 3.8%, compared to our previous longitudinal surveillance study (2011–2019)[6] in the present study. According to previous studies, immunization rate of PCV7/13 among Japanese children was estimated at 50%–60% in 2011, 80%–90% in 2012,[9] and >95.2% in 2013–2020 during the past ten years (The Ministry of Health, Labor and Welfare of Japan, https://www.mhlw.go.jp/topics/bcg/other/5.html). Hence, the persistent spread of non-PCV13 serotypes and the potential increase in NESp are of concern at the current stage of PCV13 use for routine childhood immunization.

The acquisition of antimicrobial resistance in pneumococci remains a major health burden worldwide. During the post-PCV era, surveillance reports detailed the high prevalence rates of resistance to antimicrobial drugs in non-PCV13 serotypes of S. *pneumoniae*.[1,2,10] In the CDC's ABCs, a higher prevalence of antimicrobial non-susceptibility was noted for the non-PCV13 serotypes 15A, 23A, and 35B during the post-PCV13 period.[2] Moreover, the greatest proportional increases in MDR were observed for serotypes 35B, 15B, and 23A among isolates from 42 US medical centers.[1] In the national surveillance of invasive pneumococcal disease (IPD) in South Korea from 2014 to 2019, the frequent non-PCV13 serotypes were 10A, 23A, and 34[10].

Similar to the above surveillance studies, in the present study, most isolates belonged to non-PCV13 serotypes, such as 23A, 34, 15A, and 35B (9.9%–13.0%), most of which exhibited MDR (80.5%–97.8%), except for serotype 34. In addition, serotypes with MDR, such as serotypes 23A, 15A, and 35B, exhibited a higher rate of penicillin non-susceptibility than other serotypes. Of these, five isolates of serotype 23A (9.3%) and two isolates of 15A (4.3%) exhibited XDR. Furthermore, all isolates of serotype 15B in this study had MDR traits, although they had a relatively moderate level of prevalence (6.5%). Serotypes 15A, 23A, and 35B are not included in either the current PCV13 or future PCV20, although serotype 15B is included in PCV20. In addition, a previous study on IPD reported that serotype 34 was associated with an increased risk of death compared with PCV13 serotypes.[9] Therefore, there is concern about the continued expansion of the non-PCV13/PCV20 serotypes, such as 15A, 23A, and 35B, associated with high MDR prevalence, and non-PCV13/PCV20 serotype 34 with an increased fatal risk.

In contrast, all the isolates of serotypes 3 and 37 identified in the present study exhibited a mucoid phenotype with higher susceptibility to β-lactams. A previous study reported that all mucoid serotype 3 isolates are susceptible to β-lactams and resistant to macrolides.[11] These findings suggest that β-lactams are the best first-line treatment, especially for mucoid-phenotype pneumococci.

In conclusion, the present study demonstrated the spread of non-PCV13/PCV20 serotypes and their drug resistance trends ten years after the introduction of PCV in Japan. The implementation of PCV in childhood immunization programs is considered to be related to serotype replacement and an increase in NESp associated with changes in antimicrobial resistance. Therefore, further consistent surveillance is important during the post-PCV era, especially in the introduction of a new version of PCV.

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Ethical statement

In this molecular epidemiology surveillance-based study, no human participants were directly involved. Hence, clearance of human ethics is not required. No sample was newly collected for this study, and we used isolates from regular clinical examination without personal information.

Conflict of interest

The authors declare no competing interests.

References

1. Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Riahi F, Doern GV. Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-valent conjugate vaccine in the United States. *Antimicrob Agents Chemother* 2014;**58**:6484–9.
2. Bajema KL, Gierke R, Farley MM, Schaffner W, Thomas A, Reingold AL, et al. Impact of pneumococcal conjugate vaccines on antibiotic-nonsusceptible invasive pneumococcal disease in the United States. *J Infect Dis* 2022;**226**:342–51.
3. Park IH, Kim KH, Andrade AL, Briles DE, McDaniel LS, Nahm MH. Nontypeable pneumococci can be divided into multiple cps types, including one type expressing the novel gene pspK. *mBio* 2012;**3**.
4. Kawaguchiya M, Urushibara N, Ghosh S, Kuwahara O, Morimoto S, Ito M, et al. Serotype distribution and susceptibility to penicillin and erythromycin among noninvasive or colonization isolates of *Streptococcus pneumoniae* in northern Japan: a cross-sectional study in the pre-PCV7 routine immunization period. *Microb Drug Resist* 2014;**20**:456–65.
5. Kawaguchiya M, Urushibara N, Aung MS, Ito M, Takahashi A, Habadera S, et al. High prevalence of antimicrobial resistance in non-vaccine serotypes of non-invasive/colonization isolates of *Streptococcus pneumoniae*: a cross-sectional study eight years after the licensure of conjugate vaccine in Japan. *J Infect Public Health* 2020;**13**:1094–100.
6. Kawaguchiya M, Urushibara N, Aung MS, Kudo K, Ito M, Sumi A, et al. Clonal lineages and antimicrobial resistance of nonencapsulated *Streptococcus pneumoniae* in the post-pneumococcal conjugate vaccine era in Japan. *Int J Infect Dis* 2021;**105**:695–701.
7. Senders S, Klein NP, Lamberth E, Thompson A, Drozd J, Trammel J, et al. Safety and immunogenicity of a 20-valent pneumococcal conjugate vaccine in healthy infants in the United States. *Pediatr Infect Dis J* 2021;**40**:944–51.
8. Golden AR, Baxter M, Adam HJ, Martin I, Demczuk W, Mulvey MR, et al. PCV-15 and PPSV-23 coverage of invasive and respiratory tract *Streptococcus pneumoniae*, including MDR and XDR isolates: canward 2007-20. *J Antimicrob Chemother* 2022;**77**:1444–51.
9. Ubukata K, Takata M, Morozumi M, Chiba N, Wajima T, Hanada S, et al. Effects of pneumococcal conjugate vaccine on genotypic penicillin resistance and serotype changes, Japan, 2010-2017. *Emerg Infect Dis* 2018;**24**:2010–20.
10. Jung YH, Choe YJ, Lee CY, Jung SO, Lee DH, Yoo JI. Impact of national pneumococcal vaccination program on invasive pneumococcal diseases in South Korea. *Sci Rep* 2022;**12**:15833.
11. Yang Y, Hua CZ, Fang C, Xie YP, Li W, Fu Y, et al. Properties of mucoid serotype 3 *Streptococcus pneumoniae* from children in China. *Front Cell Infect Microbiol* 2021;**11**:648040.