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Original Article

Antimicrobial susceptibility and serotype replacement of *Streptococcus pneumoniae* in children before and after PCV13 introduction in Taiwan

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Antimicrobial susceptibility;
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Abstract *Background:* Since 2015, 13-valent pneumococcal conjugate vaccine (PCV13) was included in the national immunization program in Taiwan. Subsequently, the serotypes of the main circulating *Streptococcus pneumoniae* strains have changed. PCV administration is also associated with changes in the antimicrobial susceptibility of *S. pneumoniae* strains. Therefore, in this study, we analyzed the serotype distribution and antimicrobial susceptibility of *S. pneumoniae* in pediatric infections.
Methods: Children with *S. pneumoniae* infections, including invasive pneumococcal disease (IPD) and non-IPD, were enrolled from January 2010 to December 2020. The samples were collected from Mackay Memorial Hospital, MacKay Children's Hospital, and Hsinchu Mackay Hospital in Taiwan. We analyzed the epidemiology of sample collection site, infection diagnosis, and the serotype and antimicrobial susceptibility of *S. pneumoniae* strains. The study period was divided into time points before and after PCV13 administration.
Results: In total, 322 isolates were collected during the study period. The incidence of IPD declined annually, from 29.7% before 2015 to 7.3% after 2015 ($p < 0.001$). The prevalence of serotype 19 A had increased gradually since 2010 but declined rapidly after 2013. Serotypes 15 A and 23 A were the most common serotypes after 2015. The non-susceptibility of the *S. pneumoniae* isolates to penicillin, cefotaxime, and ceftriaxone decreased. Based on meningitis

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breakpoints, the non-susceptibility to cefotaxime and ceftriaxone gradually decreased, but increased in 2020.
Conclusion: PCV13 was considerably effective in reducing the incidence of IPD in children; however, the prevalence of serotypes 15 A and 23 A increased. The increase in antimicrobial non-susceptibility caused by non-vaccine serotypes must be continuously monitored.

Introduction

Streptococcus pneumoniae causes various diseases in children,[1] including pneumonia, otitis media, sinusitis, and even invasive pneumococcal disease (IPD), including sepsis, empyema, and meningitis. In 2000, *S. pneumoniae* infections caused 826,000 deaths in children aged <5 years worldwide, accounting for 11% of the deaths reported in this age group.[2]

Pneumococcal conjugate vaccines (PCVs), including 7-valent (PCV7), 10-valent (PCV10), and 13-valent (PCV13) PCV have been used to reduce the impact of *S. pneumoniae* on children.[3] By the end of 2015, PCV had been introduced in 129 countries worldwide, and deaths caused by *S. pneumoniae* infections had decreased.[4] PCV has effectively decreased the prevalence of disease, particularly IPD, in many countries, especially after the introduction of PCV13. However, the prevalence of diseases caused by non-vaccine serotypes has increased.[5–7] Continuous monitoring of serotype replacement plays an important role in the evolution of the vaccine policy.

PCVs were introduced in Taiwan in late 2005. In 2009, PCV7 was fully funded by the government for children aged <5 years who were considered high risk for certain medical conditions.[8] The administration of PCV7 was replaced with PCV10 in 2010. Special catch-up vaccination programs using PCV13 were provided to children

Aged 2—5 years with a single dose in March 2013 and this was then extended to children aged 1-<2 years with 2 doses in January 2014. In January 2015, all children aged <2 years were included in the publicly funded vaccination program. This program consists of two primary doses given at 2 and 4 months of age, followed by a booster between 12 and 15 months of age (2 + 1 program).[9,10] However, since implementing these policies, the serotypes of the main circulating *S. pneumoniae* strains have changed; between 2008 and 2012, the prevalence of PCV7 serotype IPD decreased significantly among children aged 2—4 years, from 65.8% in 2008 to 12.9% in 2012,[11] and serotype 19 A became the most prevalent serotype. A nationwide study in Taiwan showed that catch-up vaccination programs for children aged 2—5 years could also effectively reduce the incidence of IPD caused by serotype 19 A, with a rate of 32.6—44.3% per year. Serotype 15 has replaced serotype 19 as the most common serotype causing IPD in children aged 0—5 years.[9]

The use of PCV has resulted in changes in *S. pneumoniae* antimicrobial susceptibility. A study in New York, USA, showed that antimicrobial susceptibility has improved owing to the decline in serotype 19 A prevalence; however, since 2013, the emergence of new serotypes has been associated with a decrease in susceptibility to third-generation cephalosporins, fluoroquinolones, and carbapenems.[12] Certain emerging serotypes in Taiwan, including 15 A and 23 A, have also been found to be resistant to various β-lactam and non-β-lactam agents.[13]

Understanding the changes in serotype and antimicrobial susceptibility of *S. pneumoniae* is important for establishing future treatment and vaccine development. However, studies of *S. pneumoniae* infections, especially non-invasive pneumococcal disease (non-IPD) in Taiwanese children under the long-term pneumococcal vaccine policy, are limited. Therefore, we aimed to analyze the serotype distribution and antimicrobial susceptibility of *S. pneumoniae* infections in children aged <18 years in Taiwan.

Materials and methods

Study design and data collection

This retrospective study was conducted between January 2010 and December 2020 at Mackay Memorial Hospital (MMH), MacKay Children's Hospital, and Hsinchu Mackay Hospital (HCMMH), Taiwan. The study was approved by the Institutional Review Board (IRB) of MMH and the need for informed consent was exempted (IRB No. 21MMHIS124e). During these 11 years, *S. pneumoniae* was isolated from children aged <18 years who presented symptoms of *S. pneumoniae* infection. The samples were obtained from patients who were diagnosed with *S. pneumoniae* infection, from infection sites such as cerebrospinal fluid, eyes, ears, nasal cavity, sputum, blood, and pleural effusion. Only children who were confirmed to have a *S. pneumoniae* infection were included in this study. The clinical data were obtained via review of medical charts. Some patients provided multiple positive cultures from either sterile or nonsterile sites. As long as a positive culture was obtained from a sterile site, the patient was classified as having IPD. If multiple samples from a single patient were of the same serotype, they were regarded as the same strain. Patients were assigned to the following four groups based on age: (1) <1 year, (2) 1 to <2 years, (3) 2 to <5 years, and (4) 5 to <18 years. IPD was defined as an infection confirmed via isolation of *S. pneumoniae* from a normally sterile site (blood and cerebrospinal fluid, and pleural, joint, or peritoneal fluid, but not sputum). Non-IPD was defined by pneumococcus isolated from nonsterile sites plus clinical symptoms or signs suggestive of focal pneumococcal

infection. In addition, no other pathogens were identified or could support the full diagnosis. Cases without clinical symptoms or those who are accidently found to have pneumococcal colonization were excluded from this study. We analyzed the epidemiology of non-IPD and IPD cases in children, including sex, age at onset, season, sample collection site, the serotype of *S. pneumoniae* strain, diagnosis, and antimicrobial susceptibility. We divided the study period into time points before 2015 (Period 1) and 2015–2020 (Period 2) according to the full national immunization program using PCV13.

Bacterial isolates and antimicrobial susceptibility tests

The samples collected from the patients were cultured on trypticase soy agar supplemented with 5% sheep blood and maintained at 35 °C with 5% carbon dioxide.[14] The cultivated isolates were identified using the VITEK® MS system (Biomérieux, Marcy-l'Étoile, France). In this study, 340 *S. pneumoniae* isolates were collected. Antimicrobial susceptibility was determined based on the minimum inhibitory concentration (MIC),[15] which was determined via the microdilution method using the VITEK 2 automated system (Biomérieux). The MIC results were interpreted for drug resistance according to the Clinical and Laboratory Standards Institute breakpoints.[16] Isolates with intermediate resistance were classified as non-susceptible. Susceptibility to β-lactams (amoxicillin, penicillin, cefotaxime, ceftriaxone, imipenem, ertapenem, and meropenem) and non-β-lactams (chloramphenicol, erythromycin, levofloxacin, moxifloxacin, linezolid, trimethoprim/sulfamethoxazole, and vancomycin) was tested. Multidrug resistance (MDR) was defined as resistance to at least one antibiotic in three or more antimicrobial classes.[17]

Serotyping

S. pneumoniae isolates were serotyped using the Quellung reaction,[18] with the SSI Diagnostica ImmuLex™ *S. pneumoniae* antisera (Statens Serum Institut, Copenhagen, Denmark). Serotyping was performed in accordance with the "Chessboard Method."[19] Serotypes were classified as vaccine serotypes (PCV13 VT: 1, 3, 4, 5, 6 A, 6 B, 7 F, 9 V, 14, 18C, 19 A, 19 F, and 23 F) and non-vaccine serotypes.

Statistical analysis

Data were analyzed using SPSS 24.0 (IBM, Armonk, NY, USA). Continuous variables are presented as the mean ± standard deviation. The distribution of categorical variables is presented in terms of numbers and percentages. The Chi-square test was used to determine the correlation of two variables, and Fisher's exact test was used for small sample sizes. Statistical significance was set at $p < 0.05$. We used the Chi-square test for trend (Mantel–Haenszel method) to analyze the relationship between the incidence observed and the year, for each year. Statistical significance was defined as p for trend <0.05.

Results

Totally, 340 isolates were collected from 322 patients during the study period. A total of 18 patients provided repeated positive cultures: 7 patients provided samples of cerebrospinal fluid and blood; 2 provided samples of blood and pleural effusion; 5 provided samples from the ear or nose or sputum, and blood; 1 provided samples from the ear and pleural effusion; 1 provided samples of sputum and from the nose; and 2 provided samples from the ear and nose. All repeated samples from the same patient were of the same serotype and were therefore regarded as the same strain.

Among the 322 valid isolates, 56 (17.4%) were obtained from patients with IPD and 266 (82.6%) from patients with non-IPD. The demographic characteristics of the patients are presented in Table 1. The mean age of the patients was 3.9 ± 2.9 years. Most of the children belonged to the 2- to <5-year group; however, this trend decreased over time (p for trend = 0.006), and the number of children belonging to the 5- to <18-year group increased annually (p for trend = 0.008) (Fig. 1A). A total of 2 deaths associated with IPD occurred during the study period (3.6%); the children were aged 2.4 and 5.4 years in 2013 and 2015, and the associated serotypes were 19 A and 19 F, respectively. Most isolates were collected in winter (30.7%) and spring (28.6%). The incidence of IPD declined annually, from 29.7% before 2015 to 7.3% after 2015 ($p < 0.001$) (Fig. 1B).

Of the 322 isolates, 32 could not be serotyped, while the remaining 290 isolates were successfully typed and analyzed. Serotypes 19 A (34.1%), 19 F (14.5%), 15 A (13.4%), 23 A (9.3%), and 3 (5.9%) were the five-most common serotypes. The distribution of the serotypes before and after vaccine administration is shown in Fig. 1D. A total of 11 serotypes were detected in Period 1 (2010–2014), and 20 serotypes were detected in Period 2 (2015–2020). The prevalence of the PCV7 serotype peaked in 2010 (55%) but declined annually. In 2011, PCV13-specific serotypes replaced PCV7 serotypes, and were dominant (79.6%) in 2012. In 2013, the prevalence of PCV13-specific serotypes gradually decreased, and until 2016, non-vaccine serotypes became dominant. Only one PCV10-specific serotype (7 F) was identified in 2016.

The serotype distributions in Periods 1 and 2 are shown in Table 2. The most common serotypes belonging to PCV13 serotypes were 19 A (34.1%), 19 F (14.5%), 3 (5.9%), and 6 B (4.1%) (Fig. 2A). The prevalence of serotype 19 A gradually increased since 2010 (35%) and reached a peak (75%) in 2012; however, it declined rapidly from 2013 to 2020 (4.3%) (p for trend <0.001). The prevalence of serotype 19 F increased from 2010 (20%) to 2015 (38.9%) and then decreased by 2020 (13%) annually (p for trend = 0.010). The prevalence of certain non-PCV13 serotypes gradually increased annually and replaced PCV13 serotypes (Fig. 2B). The prevalence of serotype 15 A significantly increased since 2015 (p for trend = 0.007), becoming the most common serotype in Period 2. We found the second-highest increase in prevalence for serotype 23 A, which accounted for 0% of all isolates in 2010 but 30.4% of isolates in 2020 (p for trend <0.001).

Among the IPD isolates, the most common serotypes during Period 1 were 19 A (58.1%) and 6 B (14%). In Period 2, 19 A (30.8%) and 19 F (30.8%) were the most common

Table 1 Baseline demographic and clinical characteristics of patients with pneumococcal disease.

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total	*p-value* for trend
	(n = 20)	(n = 39)	(n = 44)	(n = 23)	(n = 19)	(n = 18)	(n = 47)	(n = 16)	(n = 33)	(n = 36)	(n = 27)	(n = 322)	
Demographics													
Male, n (%)	11 (55)	18 (46.2)	19 (43.2)	11 (47.8)	8 (42.1)	9 (50)	29 (61.7)	9 (56.3)	18 (54.5)	23 (63.9)	16 (59.3)	171 (53.1)	0.047[b]
Age cohort, n (%)													
<1 year	3 (15)	2 (5.1)	7 (15.9)	4 (17.4)	3 (15.8)	4 (22.2)	7 (14.9)	2 (12.5)	4 (12.1)	7 (19.4)	4 (14.8)	47 (14.6)	0.447
1 to <2 years	8 (40)	4 (10.3)	5 (11.4)	3 (13)	4 (21.1)	1 (5.6)	3 (6.4)	3 (18.8)	8 (24.2)	4 (11.1)	5 (18.5)	48 (14.9)	0.710
2 to <5 years	7 (35)	24 (61.5)	21 (47.7)	11 (47.8)	7 (36.8)	8 (44.4)	16 (34)	7 (43.8)	11 (33.3)	11 (30.6)	8 (29.6)	131 (40.7)	0.006[b]
5 to <18 years	2 (10)	9 (23.1)	11 (25)	5 (21.7)	5 (26.3)	5 (27.8)	21 (44.7)	4 (25)	10 (30.3)	14 (38.9)	10 (37)	96 (29.8)	0.008[b]
Mean age, yr. (SD)	2.5 (1.5)	4.2 (2.7)	3.6 (2)	3.4 (3)	3.4 (2.4)	3.5 (2)	5.4 (4.1)	3.2 (1.8)	3.9 (2.8)	4.4 (3.6)	3.6 (2.4)	3.9 (2.9)	—
Diagnosis													
IPD	6 (30)	9 (23.1)	12 (27.3)	10 (43.5)	6 (31.6)	6 (33.3)	3 (6.4)	2 (12.5)	1 (3)	1 (2.8)	0 (0)	56 (17.4)	<0.001[b]
Bacteremic pneumonia	4 (20)	4 (10.3)	3 (6.8)	5 (21.7)	3 (15.8)	1 (5.6)	1 (2.1)	1 (6.3)	1 (3)	1 (2.8)	0 (0)	24 (7.5)	0.001[b]
Bacteremia/Sepsis	6 (30)	7 (17.9)	9 (20.5)	9 (39.1)	3 (15.8)	3 (16.7)	2 (4.3)	2 (12.5)	1 (3)	1 (2.8)	0 (0)	43 (13.4)	<0.001[b]
Empyema/Pleural effusion	0 (0)	3 (7.7)	3 (6.8)	1 (4.3)	4 (21.1)	3 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (4.3)	0.020[b]
Encephalitis/Meningitis	7	0 (0)	0 (0)	2 (8.7)	0 (0)	1 (5.6)	2 (4.3)	0 (0)	0 (0)	0 (0)	0 (0)	9 (2.8)	0.020[b]
Non-IPD	14 (70)	30 (76.9)	32 (72.7)	13 (56.5)	13 (68.4)	12 (66.7)	44 (93.6)	14 (87.5)	32 (97)	35 (97.2)	27 (100)	266 (82.6)	<0.001[b]
Otitis media	15 (75)	32 (82.1)	35 (79.5)	15 (65.2)	15 (78.9)	13 (72.2)	23 (48.9)	8 (50)	22 (66.7)	22 (61.1)	16 (59.3)	216 (67.1)	0.002[b]
Sinusitis	3 (15)	7 (17.9)	9 (20.5)	3 (13)	2 (10.5)	4 (22.2)	28 (59.6)	8 (50)	25 (75.8)	22 (61.1)	17 (63)	128 (39.8)	<0.001[b]
Pneumonia	6 (30)	9 (23.1)	7 (15.9)	7 (30.4)	6 (31.6)	3 (16.7)	2 (4.3)	1 (6.3)	1 (3)	1 (2.8)	2 (7.4)	45 (14)	<0.001[b]
Tonsillitis	2 (10)	3 (7.7)	3 (6.8)	1 (4.3)	1 (5.3)	0 (0)	2 (4.3)	3 (18.8)	2 (6.1)	0 (0)	0 (0)	17 (5.3)	0.116
Mastoiditis	0 (0)	4 (10.3)	1 (2.3)	2 (8.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (2.2)	0.006[b]
Bronchopneumonia	0 (0)	1 (2.6)	5 (11.4)	2 (8.7)	0 (0)	2 (11.1)	0 (0)	1 (6.3)	1 (3)	1 (2.8)	0 (0)	13 (4)	0.210
Conjunctivitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (10.6)	3 (18.8)	1 (3)	4 (11.1)	1 (3.7)	14 (4.3)	0.003[b]
Nasolacrimal duct obstruction	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.3)	1 (6.3)	0 (0)	0 (0)	3 (11.1)	6 (1.9)	0.015[b]
Cellulitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	[a]
Others	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.8)	0 (0)	1 (0.3)	[a]
Culture Site													
Blood	2 (10)	7 (17.9)	9 (20.5)	7 (30.4)	3 (15.8)	2 (11.1)	1 (2.1)	2 (12.5)	1 (3)	1 (2.8)	0 (0)	35 (10.9)	<0.001[b]
CSF	4 (20)	0 (0)	0 (0)	2 (8.7)	0 (0)	1 (5.6)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	8 (2.5)	0.010[b]
Ear	14 (70)	30 (76.9)	32 (72.7)	13 (56.5)	14 (73.7)	12 (66.7)	7 (14.9)	2 (12.5)	4 (12.1)	6 (16.7)	4 (14.8)	138 (42.9)	<0.001[b]
Eye	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (10.6)	3 (18.8)	1 (3)	4 (11.1)	4 (14.8)	17 (5.3)	<0.001[b]
Nose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.6)	32 (68.1)	9 (56.3)	27 (81.8)	24 (66.7)	19 (70.4)	112 (34.8)	<0.001[b]
PLE	0 (0)	2 (5.1)	3 (6.8)	1 (4.3)	2 (10.5)	2 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (3.1)	0.034[b]
Pus/Wound	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.8)	0 (0)	1 (0.3)	[a]
Sputum	0 (0)	1 (2.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.9)	0 (0)	0 (0)	1 (3.6)	3 (0.9)	[a]
Throat	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	[a]
Patient source													

Inpatient	11 (55)	25 (64.1)	24 (54.5)	15 (65.2)	8 (42.1)	7 (38.9)	12 (25.5)	3 (18.8)	3 (9.1)	6 (16.7)	8 (29.6)	122 (37.9)	<0.001[b]
Outpatient department	9 (45)	14 (35.9)	20 (45.5)	8 (34.8)	11 (57.9)	11 (61.1)	35 (74.5)	13 (81.3)	30 (90.9)	30 (83.3)	19 (70.4)	200 (62.1)	<0.001[b]
Length of stay, mean days (SD)	16.3 (15.4)	13.2 (17.1)	11.2 (14.9)	10.3 (5.6)	12.1 (8.7)	8 (6.3)	5.9 (4.5)	14.3 (14.4)	5.7 (2.5)	6.5 (3.9)	11.6 (10.2)	11.1 (12.3)	–
ICU	3 (15)	3 (7.9)	2 (4.5)	4 (17.4)	1 (5.3)	2 (11.1)	1 (2.1)	1 (6.3)	0 (0)	0 (0)	2 (7.4)	19 (5.9)	0.029[b]
ICU days, mean days (SD)	10 (2)	22.7 (30.9)	6 (0)	4 (4.7)	2 (0)	3.5 (3.5)	7 (0)	23 (0)	0 (0)	0 (0)	9.5 (12)	9.7 (13.2)	–
Non-vaccine serotypes	1 (5)	0 (0)	3 (6.8)	5 (21.7)	3 (15.8)	4 (22.2)	32 (69.6)	9 (64.3)	15 (83.3)	22 (81.5)	16 (69.6)	110 (37.8)	<0.001[b]

[a] Case number is too small.
[b] Statistically significant ($p < 0.05$).
CSF: Cerebrospinal fluid; PLE: Pleural effusion; ICU: Intensive care unit.

serotypes. The prevalence of serotype 6 B declined throughout the year (p for trend = 0.017). Among the non-IPD isolates, serotypes 19 A (58.8%) and 19 F (20.6%) were the most common serotypes during Period 1. During Period 2, 15 A (22%) and 23 A (18.9%) were the first- and second-most common serotypes, respectively, followed by 19 F (11.4%) and 15 B (8.3%). The prevalence of serotypes 19 A and 19 F significantly decreased (35.7%–4.3%, p for trend <0.001; and 28.6%–13.0%, p for trend = 0.012; respectively). The prevalence of serotype 15 A significantly increased each year (p for trend = 0.002). Serotype 23 A was detected in 2016 and significantly increased in prevalence (p for trend <0.001), becoming the most common serotype among non-IPD isolates in 2020.

Other pathogens, including *Mycoplasma pneumoniae*, respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, *Haemophilus influenzae* non-type b, and *Moraxella catarrhalis*, were detected in 82 patients (Table 3). The most common serotypes associated with coinfection were 15 A (20%), 23 A (20%), 19 A (16.92%), and 19 F (9.23%). Most of these samples were collected from patients with non-IPD.

Table 4 shows the antimicrobial susceptibility of the isolates obtained in the present study. MDR was detected in 275 isolates. The prevalence of MDR isolates decreased from 96.6% in Period 1–76.7% in Period 2 ($p < 0.001$) (Table 2). The prevalence of MDR isolates decreased during 2010–2018, however, it gradually increased after 2018 (p for trend <0.001). The serotypes 19 A (37.7% of 275 isolates), 19 F (16%), 15 A (14%), 23 A (7.4%), 3 (5.1%), and 6 B (4.3%) were identified among MDR isolates.

The trend in non-susceptibility of *S. pneumoniae* isolates to β-lactams and non-β-lactams during the study period is shown in Fig. 2C and D. Among the β-lactam antibiotics, non-susceptibility to penicillin, cefotaxime, and ceftriaxone decreased (p for trend <0.001). Using the meningitis breakpoints, the non-susceptibility to cefotaxime and ceftriaxone gradually decreased, but increased in 2020 (p for trend <0.001). Non-susceptibility of penicillin was persistently high; however, there was no significant difference. The non-susceptibility of amoxicillin decreased annually (p for trend <0.001); however, a non-susceptibility of approximately 50% was retained. Among non-β-lactam antibiotics, the non-susceptibility of erythromycin (p for trend = 0.002) and tetracycline (p for trend <0.001) significantly decreased but remained high. The non-susceptibility to trimethoprim/sulfamethoxazole also significantly decreased (p for trend <0.001).

Among the four-most common serotypes (Table 5), the non-susceptibility of serotype 19 A to penicillin and cefotaxime showed a significant decrease; however, it was persistently high based on meningitis breakpoints. Serotype 19 F also showed a high non-susceptibility to penicillin and cefotaxime based on meningitis breakpoints. The non-susceptibility of serotype 15 A to penicillin significantly decreased (p for trend = 0.002). Based on meningitis breakpoints, serotype 23 A was completely non-susceptible to penicillin, and the non-susceptibility of cefotaxime and ceftriaxone increased. The non-susceptibility to amoxicillin (p for trend = 0.013) and meropenem (p for trend = 0.003) showed an increasing trend.

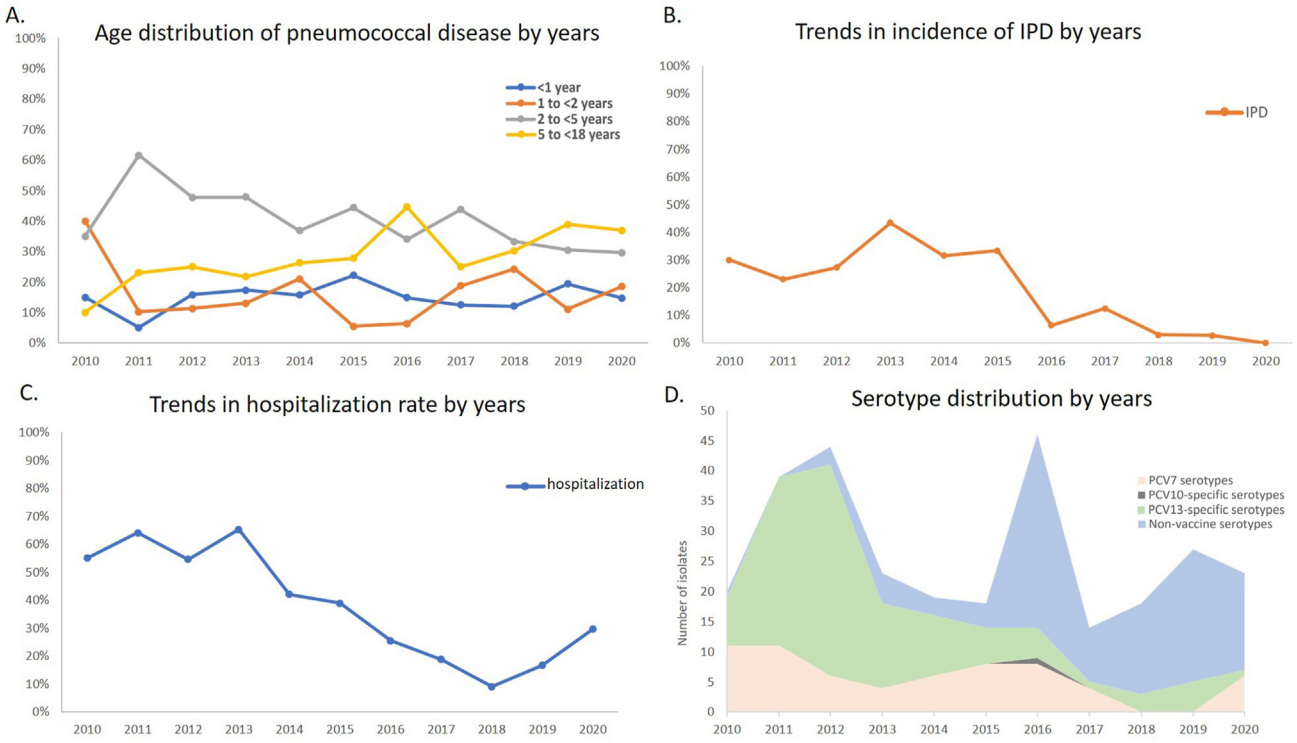

Figure 1. (A) Age distribution of pneumococcal disease by years (B) The trend in incidence of IPD by years. (C) The trend in hospitalization rate by years. (D) Serotype distribution of vaccine and non-vaccine serotypes by years. IPD: Invasive pneumococcal disease. a. PCV7 serotypes: 4, 6 B, 9 V, 14, 18C, 19 F, 23 F. b. PCV10-specific serotypes: 1, 5, 7 F. c. PCV13-specific serotypes: 3, 6 A, 19 A. d. Non-vaccine serotypes: Non PCV7/PCV10/PCV13 serotypes.

Discussion

The incidence of IPD in children has decreased since the introduction of PCV7 and PCV13 in Taiwan.[9,20,21] During the study period, the incidence of IPD in children decreased annually, especially after 2015, indicating that PCV13 continues to be effective in reducing the incidence of IPD. Furthermore, the rates of IPD, MDR of isolates and high severity of infection (hospitalization and ICU care) significantly decreased after 2015. This highlights the benefits of universal PCV13 vaccination.

Similar to previous studies in Taiwan,[13,22,23] we found that the most common serotype was 19 A during the post-PCV7/pre-PCV13 period. After the administration of PCV13, the prevalence of PCV13 serotypes decreased annually, including 19 A, which was replaced by serotypes 15 and 23 A; this proved that vaccination altered the distribution of serotypes. The decrease in IPD prevalence is closely related to a decrease in the prevalence of serotype 19 A.[9,22,24] However, after 2015, although serotypes 15 A and 23 A increased in prevalence, serotypes 19 A and 19 F still represented the main causes of IPD, whereas 15 A and 23 A mainly caused non-IPD in the present study. A study on serotype and invasive capacity reported that serotype 19 A has a relatively high potential to cause IPD, whereas 15 A and 23 A are less invasive, and 19 F shows different results between studies.[25]

In the post-PCV13 period, the increasing prevalence of non-PCV13 serotypes varies in different countries and regions.[26] In Japan, the prevalence of serotypes 12 F and 23 A has increased.[27] Serotypes 8, 10 A, 12 F, and 22 F increased in prevalence in Europe,[28] while 35 B and 11A/D increased in prevalence in the United States.[29] To provide additional protection against *S. pneumoniae* infections caused by non-PCV13 serotypes, vaccines against additional serotypes, such as PCV15 (PCV13 serotypes + serotypes 22 F and 33 F) and PCV20 (PCV13 serotypes + serotypes 8, 10 A, 11 A, 12 F, 15 B, 22 F, and 33 F), have been produced. The U.S. FDA approved PCV15 (Vaxneuvance, Merck) and PCV20 (Prevenar 20, Pfizer) for PCV—naïve adults who are either aged ≥65 years, or 19—64 years with certain underlying conditions in October 2021.[30] PCV13 could be used against 58% of the isolates obtained in the present study, while PCV20 could be used against 63.2% of the isolates, mainly due to the addition of serotype 15 B. However, serotypes 15 A and 23 A are still not under vaccine coverage. The development of vaccines in the future must include additional serotypes, and suitable vaccines must be developed for different countries and regions.

The most common serotypes associated with coinfections in our study were 15 A and 23 A. To the best of our knowledge, no prior studies have clarified which serotypes are closely related to coinfections; however, Yan et al. reported that RSV infection might increase the adhesion of certain serotypes to the nasal epithelium.[31] Launes et al. found that certain viral infections may increase the invasiveness of serotypes that are not highly invasive.[32] Dunne et al. reported that co-colonization of *S. pneumoniae* with *H. influenzae* or *M. catarrhalis* increases the risk of otitis media,[33] which may explain why coinfection was relatively more common in non-IPD patients. Our findings suggest

Table 2 Comparison of serotype distribution, antimicrobial non-susceptibility and clinical characteristics before (period 1) and after (period 2) Full National Immunization Program using PCV 13.

Serotype, n (%)	IPD			Non-IPD			ALL		
	Period 1	Period 2	p-value	Period 1	Period 2	p-value	Period 1	Period 2	p-value
	(n = 43)	(n = 13)		(n = 102)	(n = 132)		(n = 145)	(n = 145)	
19 A	25 (58.1)	4 (30.8)	0.084	60 (58.8)	10 (7.6)	<0.001[a]	85 (58.6)	14 (9.7)	<0.001[a]
19 F	2 (4.7)	4 (30.8)	0.022[a]	21 (20.6)	15 (11.4)	0.052	23 (15.9)	19 (3.1)	0.504
15 A	3 (7.0)	2 (15.4)	0.580	5 (4.9)	29 (22.0)	<0.001[a]	8 (5.5)	31 (21.4)	<0.001[a]
23 A	0 (0.0)	2 (15.4)	0.051	0 (0.0)	25 (18.9)	<0.001[a]	0 (0.0)	27 (18.6)	<0.001[a]
3	2 (4.7)	1 (7.7)	0.555	8 (7.8)	6 (4.5)	0.292	10 (6.9)	7 (4.8)	0.453
6 B	6 (14.0)	0 (0.0)	0.318	1 (1.0)	5 (3.8)	0.236	7 (4.8)	5 (3.4)	0.555
15 B	1 (2.3)	0 (0.0)	0.999	0 (0.0)	11 (8.3)	0.003[a]	1 (0.7)	11 (7.6)	0.003[a]
35 B	–	–		2 (2.0)	7 (5.3)	0.306	2 (1.4)	7 (4.8)	0.173
14	3 (7.0)	0 (0.0)	0.999	2 (2.0)	1 (0.8)	0.582	5 (3.4)	1 (0.7)	0.214
15C	–	–		0 (0.0)	6 (4.5)	0.037[a]	0 (0.0)	6 (4.1)	0.030[a]
11 A	–	–		1 (1.0)	4 (3.0)	0.390	1 (0.7)	4 (2.8)	0.371
22 A	–	–		0 (0.0)	4 (3.0)	0.134	0 (0.0)	4 (2.8)	0.122
23 F	1 (2.3)	0 (0.0)	0.999	1 (1.0)	1 (0.8)	0.999	2 (1.4)	1 (0.7)	0.999
47 F	–	–		0 (0.0)	2 (1.5)	0.506	0 (0.0)	2 (1.4)	0.498
22 F	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
23 B	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
42	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
6C	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
7 A	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
7 F	–	–		0 (0.0)	1 (0.8)	0.999	0 (0.0)	1 (0.7)	0.999
9 V	–	–		1 (1.0)	0 (0.0)	0.436	1 (0.6)	0 (0.0)	0.999
Clinical Characteristics, n (%)	(n = 43)	(n = 13)		(n = 102)	(n = 164)		(n = 145)	(n = 177)	
MDR	40 (93.0)	12 (92.3)	0.999	100 (98.0)	123 (75.0)	<0.001[a]	140 (96.6)	135 (76.7)	<0.001[a]
Hospitalization	43 (100.0)	13 (100.0)	–	40 (39.2)	26 (15.9)	<0.001[a]	83 (57.2)	39 (22.0)	<0.001[a]
ICU	13 (30.2)	4 (30.8)	0.999	0 (0.0)	2 (1.22)	0.526	13 (9.03)	6 (3.39)	0.033[a]
Antimicrobial Agent, n (%)	(n = 43)	(n = 13)		(n = 102)	(n = 164)		(n = 145)	(n = 175)	
Penicillin (meningitis)	41 (95.3)	11 (91.7)	0.530	95 (93.1)	145 (91.2)	0.574	136 (93.8)	156 (91.2)	0.391
Penicillin (non-meningitis)	24 (63.2)	2 (16.7)	0.005[a]	70 (68.6)	39 (24.5)	<0.001[a]	94 (67.1)	41 (24)	<0.001[a]
Cefotaxime (meningitis)	34 (79.1)	4 (33.3)	0.005[a]	83 (81.4)	91 (61.9)	<0.001[a]	117 (80.7)	95 (59.7)	<0.001[a]
Cefotaxime (non-meningitis)	10 (26.3)	1 (8.3)	0.257	38 (37.3)	16 (10.9)	<0.001[a]	48 (34.3)	17 (10.7)	<0.001[a]
Ceftriaxone (meningitis)	32 (74.4)	4 (33.3)	0.015[a]	78 (76.5)	71 (48.3)	<0.001[a]	110 (75.9)	75 (47.2)	<0.001[a]
Ceftriaxone (non-meningitis)	8 (21.1)	1 (8.3)	0.425	33 (32.4)	11 (7.5)	<0.001[a]	41 (29.3)	12 (7.5)	<0.001[a]
Amoxicillin	11 (61.1)	4 (33.3)	0.136	42 (43.7)	55 (37.9)	<0.001[a]	53 (70.7)	59 (37.6)	<0.001[a]
Imipenem	33 (76.7)	0 (0.0)	–	84 (82.4)	2 (66.7)	0.454	117 (80.7)	2 (66.7)	0.483
Ertapenem	0 (0.0)	0 (0.0)	–	0 (0.0)	2 (1.4)	–	0 (0.0)	2 (1.3)	–
Meropenem	0 (0.0)	3 (25)	–	0 (0.0)	105 (72.9)	–	0 (0.0)	108 (69.2)	–
Chloramphenicol	6 (14)	2 (16.7)	0.999	6 (5.9)	28 (17.1)	0.008[a]	12 (8.3)	30 (17)	0.020[a]
Erythromycin	42 (97.7)	12 (100)	0.999	102 (100)	150 (91.5)	0.002[a]	144 (99.3)	162 (92)	0.002[a]
Levofloxacin	1 (2.3)	1 (8.3)	0.392	0 (0.0)	0 (0.0)	–	1 (0.7)	1 (0.6)	0.999
Moxifloxacin	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–
Linezolid	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–
Trimethoprim/sulfamethoxazole	37 (86)	9 (75)	0.392	87 (85.3)	97 (59.5)	<0.001[a]	124 (85.5)	106 (60.6)	<0.001[a]
Tetracycline	41 (95.3)	12 (100)	0.999	99 (97.1)	124 (84.4)	0.001[a]	140 (96.6)	136 (85.5)	<0.001[a]
Vancomycin	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–	0 (0.0)	0 (0.0)	–

[a] Statistically significant ($p < 0.05$).
IPD: Invasive pneumococcal disease; MDR: Multidrug-resistant; ICU: Intensive care unit.

that, because serotypes 15 A and 23 A usually cause non-IPD mucosal infection, they might be more easily co-detected alongside other pathogens. Further studies are required to elucidate the interactions between *S. pneumoniae* and other pathogens and the role of coinfection.

Serotypes 19 A and 19 F are highly non-susceptible serotypes.[13,22,34,35] Our results also showed that most MDR isolates belonged to serotypes 19 A and 19 F. Owing to the decrease in the number of serotypes 19 A and 19 F, the non-susceptibility to penicillin, cefotaxime, and ceftriaxone in

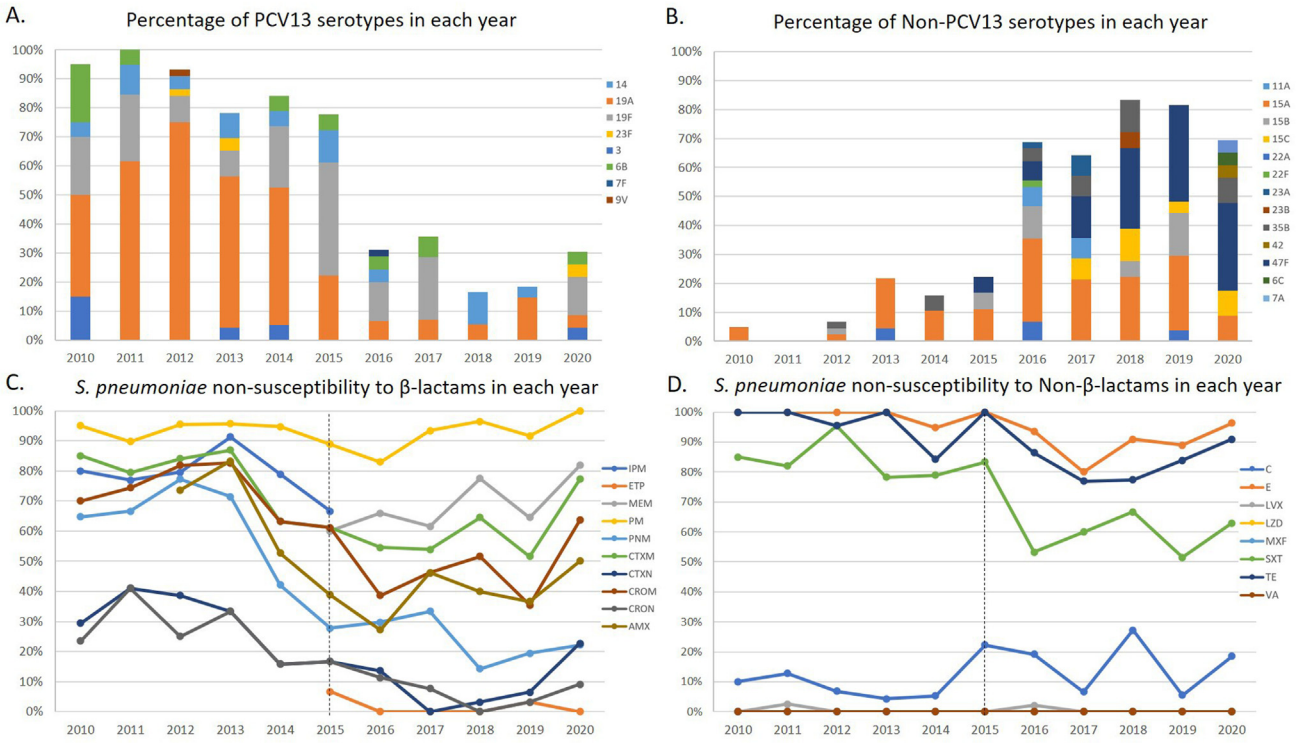

Figure 2. The annual trend in the proportion of (A) PCV13 serotypes (B) Non-PCV13 serotypes (C) non-susceptibility to β-lactams (D) non-susceptibility to non-β-lactams among *S. pneumoniae* isolates during 2010—2020. IPM: Imipenem; ETP: Ertapenem; MEM: Meropenem; PM: Penicillin (meningitis); PNM: Penicillin (non-meningitis); CTXM: Cefotaxime (meningitis); CTXN: Cefotaxime (non-meningitis); CROM: Ceftriaxone (meningitis); CRON: Ceftriaxone(non-meningitis); AMX: Amoxicillin; C: Chloramphenicol; E: Erythromycin; LVX: Levofloxacin; MXF: Moxifloxacin; LZD: Linezolid; SXT: Trimethoprim/sulfamethoxazole; TE: Tetracycline; VA: Vancomycin. a. Amoxicillin was tested after 2012. b. Imipenem was only tested during 2010—2015. c. Ertapenem, meropenem, and linezolid were tested since 2015. d. All of the isolates are susceptible to vancomycin, linezolid, and moxifloxacin.

all isolates in this study also decreased accordingly. In addition, the non-susceptibility of serotype 19 A to these three antibiotics decreased significantly after the administration of PCV13. This further contributed to the decrease in overall non-susceptibility. Based on the meningitis breakpoints, although serotypes 19 A and 19 F remained highly non-susceptible to cefotaxime and ceftriaxone, the overall non-susceptibility was still reduced based on the reduction in the prevalence of these serotypes.

Table 3 Co-detection of other pathogens in the study patients.

Other pathogens (n = 82)	IPD (n = 11)	Non-IPD (n = 71)
Mycoplasma pneumoniae	5	4
Mycoplasma pneumoniae + *Moraxella catarrhalis*	0	2
Respiratory syncytial virus	2	5
Respiratory syncytial virus + *Haemophilus influenzae* non-type b	0	1
Respiratory syncytial virus + *Moraxella catarrhalis*	0	2
Influenza	3	1
Influenza + *Moraxella catarrhalis*	0	1
Haemophilus influenzae non-type b	0	26
Haemophilus influenzae non-type b + *Moraxella catarrhalis*	0	10
Moraxella catarrhalis	0	18
Parainfluenza	1	1

IPD: Invasive pneumococcal disease.

Serotypes 15 A and 23 A were the third- and fourth-most common serotypes among the MDR isolates. This may be associated with an increase in the prevalence of MDR isolates after 2018. In addition, using the meningitis breakpoints, the non-susceptibility of serotype 23 A to cefotaxime and ceftriaxone gradually increased, which led to an increase in the overall non-susceptibility of the isolates to cefotaxime and ceftriaxone after 2018. Previous studies have found that increasingly non-susceptibility clones are associated with serotypes 15 A-ST63 and 23 A-ST166.[13,23,36] Wu et al. found that the number of isolates of serotypes 15 A-ST63 and 23 A-ST166 has increased in Taiwan in the post-PCV13 period, resulting in increased meropenem non-susceptibility.[13] Notably, we also observed an increase in the increasing non-susceptibility of serotype 23 A to meropenem.

Penicillin and other β-lactams are still used in the first-line treatment for non-meningitis *S. pneumoniae* infections, while amoxicillin is more commonly used orally because of its ease of absorption and relatively long half-life.[37] We found that the isolates showed that β-lactams, including penicillin, cefotaxime, and ceftriaxone, had a significant decrease in

Table 4 Antimicrobial resistance patterns among *Streptococcus pneumoniae* strains isolated from children.

Antimicrobial agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	S%	I + R%
Penicillin (meningitis)[a]	0.06–8	2	4	7.59	92.41
Penicillin (non-meningitis)[b]	0.06–8	2	4	56.59	43.41
Cefotaxime (meningitis)[c]	0.06–4	1	2	30.26	69.74
Cefotaxime (non-meningitis)[d]	0.06–4	1	2	78.26	21.74
Ceftriaxone (meningitis)[e]	0.06–4	1	2	39.14	60.86
Ceftriaxone(non-meningitis)[f]	0.06–4	1	2	82.27	17.73
Amoxicillin[g]	0.06–8	2	8	51.72	48.28
Imipenem[h]	0.03–1	0.25	0.5	19.59	80.41
Ertapenem[i]	0.5–2	0.5	1	98.72	1.28
Meropenem[j]	0.06–1	0.5	1	30.77	69.23
Chloramphenicol[k]	2–32	2	16	86.92	13.08
Erythromycin[l]	0.25–1	1	1	4.67	95.33
Levofloxacin[m]	0.5–8	0.5	1	99.38	0.62
Moxifloxacin[n]	0.25–2	0.25	0.25	100.00	0.00
Linezolid[o]	2–2	2	2	100.00	0.00
Trimethoprim/sulfamethoxazole[p]	10–320	80	160	28.13	71.88
Tetracycline[q]	1–16	16	16	9.21	90.79
Vancomycin[r]	1–1	1	1	100.00	0.00

[a] S: ≦0.06 μg/ml; R: ≧0.12 μg/ml.
[b] S: ≦2 μg/ml; I: 4 μg/ml; R: ≧8 μg/ml.
[c] S: ≦0.5 μg/ml; I: 1 μg/ml; R: ≧2 μg/ml.
[d] S: ≦1 μg/ml; I: 2 μg/ml; R: ≧4 μg/ml.
[e] S: ≦0.5 μg/ml; I: 1 μg/ml; R: ≧2 μg/ml.
[f] S: ≦1 μg/ml; I: 2 μg/ml; R: ≧4 μg/ml.
[g] S: ≦2 μg/ml; I: 4 μg/ml; R: ≧8 μg/ml.
[h] S: ≦0.12 μg/ml; I: 0.25–0.5 μg/ml; R: ≧1 μg/ml.
[i] S: ≦1 μg/ml; I: 2 μg/ml; R: ≧4 μg/ml.
[j] S: ≦0.25 μg/ml; I: 0.5 μg/ml; R: ≧1 μg/ml.
[k] S: ≦4 μg/ml; R: ≧8 μg/ml.
[l] S: ≦0.25 μg/ml; I: 0.5 μg/ml; R: ≧1 μg/ml.
[m] S: ≦2 μg/ml; I: 4 μg/ml; R: ≧8 μg/ml.
[n] S: ≦1 μg/ml; I: 2 μg/ml; R: ≧4 μg/ml.
[o] S: ≦2 μg/ml.
[p] S: ≦0.5/9.5 μg/ml; I: 1/29–2/38 μg/ml; R: ≧4/76 μg/ml.
[q] S: ≦1 μg/ml; I: 2 μg/ml; R: ≧4 μg/ml.
[r] S: ≦1 μg/ml.
MIC: Minimum inhibitory concentration; S: susceptible; I: intermediate; R: resistant.
MIC breakpoint for susceptibility by CLSI criteria.

non-susceptibility, which further supports our choice of using these antibiotics as first-line antibiotics. Owing to the increasing non-susceptibility to amoxicillin in serotype 23 A, the overall non-susceptibility to amoxicillin was still 52% in 2020. However, this problem can be overcome using higher doses of antibiotics.[38,39] The susceptibility to vancomycin and linezolid was 100% in the present study. Based on meningitis breakpoints, the non-susceptibility to penicillin was always high, whereas cefotaxime and ceftriaxone showed a decreasing trend initially, but increased in recent years. Therefore, we suggest that cefotaxime or ceftriaxone, along with vancomycin should be used to treat meningitis caused by *S. pneumoniae* in Taiwan, which is consistent with guidelines.[40]

Our study had a few limitations. First, the number of cases in our study was small, and certain data might not be significant. Second, this was a two-center study conducted in northern Taiwan, which might not accurately represent the overall population. Thirdly, not all cases were tested for additional pathogens, therefore, the incidence of co-infection might be underestimated. However, our study confirmed the changes in serotypes and drug resistance in strains affecting children after long-term vaccination, providing a good reference for future vaccine development and antibiotic use.

In conclusion, PCV13 was considerably effective in reducing the incidence of IPD in children; however, there was an increasing trend of non-vaccine serotypes, especially serotypes 15 A and 23 A. In addition, the increasing trend in antimicrobial non-susceptibility caused by non-vaccine serotypes must be continuously monitored. The development of next-generation pneumococcal vaccines and the use of antibiotics should consider local serotype dynamics and changes in antimicrobial susceptibility.

Table 5 Comparison of antimicrobial non-susceptibility between isolates of serotypes 19 A, 19 F, 15 A, and 23 A.

19 A	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total	*P*-value
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	for trend
	(n = 7)	(n = 24)	(n = 33)	(n = 12)	(n = 9)	(n = 4)	(n = 3)	(n = 1)	(n = 1)	(n = 4)	(n = 1)	(n = 99)	
Penicillin (non-meningitis)	5 (75.0)	17 (72.0)	29 (88.6)	10 (84.6)	5 (55.6)	2 (40.0)	2 (66.7)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	71 (71.7)	<0.001[d]
Cefotaxime (non-meningitis)	2 (37.5)	10 (44.0)	14 (42.9)	4 (38.5)	0 (0.0)	1 (20.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	32 (32.3)	0.021[d]
Ceftriaxone (non-meningitis)	1 (25.0)	10 (44.0)	9 (28.6)	4 (38.5)	0 (0.0)	1 (20.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	26 (26.3)	0.066
Amoxicillin[a]	—	—	26 (86.7)[c]	11 (100.0)[c]	6 (66.7)	3 (80.0)[c]	2 (66.7)	0 (0.0)	1 (100.0)	3 (75.0)	1 (100.0)	52 (82.5)[c]	0.250
19 F	**(n = 4)**	**(n = 9)**	**(n = 4)**	**(n = 2)**	**(n = 4)**	**(n = 7)**	**(n = 6)**	**(n = 3)**	**(n = 0)**	**(n = 0)**	**(n = 3)**	**(n = 42)**	
Penicillin (non-meningitis)	4 (100.0)	8 (90.0)	3 (75.0)	2 (100.0)	3 (75.0)	3 (50.0)	6 (100.0)	2 (100.0)[c]	0 (0.0)	0 (0.0)	1 (33.3)	32 (78.0)[c]	0.077
Cefotaxime (non-meningitis)	3 (75.0)	6 (60.0)	2 (50.0)	2 (100.0)	3 (75.0)	2 (37.5)	3 (50.0)	0 (33.3)[c]	0 (0.0)	0 (0.0)	2 (66.7)	23 (56.1)[c]	0.246
Ceftriaxone (non-meningitis)	3 (75.0)	6 (60.0)	2 (50.0)	2 (100.0)	3 (75.0)	2 (37.5)	4 (66.7)	1 (66.7)[c]	0 (0.0)	0 (0.0)	1 (33.3)	24 (58.5)[c]	0.210
Amoxicillin[a]	—	—	3 (75.0)	2 (100.0)	4 (100.0)	3 (50.0)	5 (83.3)	2 (100.0)[c]	0 (0.0)	0 (0.0)	2 (66.7)	21 (75.0)[c]	0.734
15 A	(n = 1)	(n = 0)	(n = 1)	(n = 4)	(n = 2)	(n = 2)	(n = 13)	(n = 3)	(n = 4)	(n = 7)	(n = 2)	(n = 39)	
Penicillin (non-meningitis)	1 (100.0)	0 (0.0)	1 (100.0)	3 (75.0)	0 (0.0)	0 (0.0)	1 (7.1)	1 (33.3)	1 (25.0)	0 (0.0)	0 (0.0)	8 (20.5)	0.002[d]
Cefotaxime (non-meningitis)	0 (0.0)	0 (0.0)	1 (100.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.3)	[b]
Ceftriaxone (non-meningitis)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.6)	[b]
Amoxicillin[a]	—	—	0 (0.0)	1 (50.0)[c]	0 (0.0)	0 (0.0)	2 (15.4)	2 (66.7)	1 (25.0)	1 (14.3)	0 (0.0)	7 (20.6)[c]	0.693
23 A	(n = 0)	(n = 0)	(n = 0)	(n = 0)	(n = 0)	(n = 1)	(n = 3)	(n = 2)	(n = 5)	(n = 9)	(n = 7)	(n = 27)	
Penicillin (non-meningitis)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	5 (55.6)	2 (25.0)	8 (30.8)	0.341
Cefotaxime (non-meningitis)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)[c]	0 (0.0)	1 (5.0)[c]	[b]
Ceftriaxone (non-meningitis)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	—
Amoxicillin[a]	—	—	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)[c]	4 (80.0)[c]	3 (80.0)[c]	9 (50.0)[c]	0.013[d]

[a] Amoxicillin was tested after 2012.
[b] Case number is too small.
[c] Sample may variable to missing data.
[d] Statistically significant ($p < 0.05$).

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Declaration of competing interest

None.

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