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Evaluation of the impact of 13-valent pneumococcal conjugate vaccine immunization in children by surveillance of culture-confirmed pneumococcal disease: A prospective clinical microbiological study

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ABSTRACT

The study aimed to investigate the impact of 13-valent pneumococcal conjugate vaccine (PCV13) immunization on the overall pneumococcal disease in children in Taiwan by surveillance of culture-confirmed pneumococcal disease (CCPD). This study was conducted in a medical center from 2012 to 2016. Clinical isolates of *Streptococcus pneumoniae* were prospectively collected from pediatric patients. Serotyping, multi-locus sequence typing, and antimicrobial susceptibility testing were performed. A total of 473 patients with CCPD, including 58 with invasive pneumococcal disease (IPD), were identified. The incidence of CCPD per 10,000 admissions decreased from 71.7 in 2012 to 27.0 in 2016. The proportion of additional PCV13 serotypes significantly decreased from 52.0% in 2012 to 21.7% in 2015 but increased slightly to 26.7% because of serotype 19A in 2016 ($P < 0.0001$). The proportion of non-vaccine serotypes (NVTs) increased significantly from 18.4% in 2012 to 66.7% in 2016, but the increase of the incidence of CCPD caused by NVTs was not considered significant ($P = 0.0885$). Genotyping identified predominant clones, ST63^{15A}, ST83^{15B}, and ST166/338^{23A}, for major NVTs. The penicillin non-susceptibility of PCV13 serotypes was significantly higher than that of NVTs ($P < 0.0001$). Surveillance of CCPD appears superior to IPD alone for evaluation of the overall impact of pneumococcal immunization. Serotype replacement occurred quickly after the use of PCV13, while the incidence of NVT infection did not show a significant increase in children over the years. The gradual introduction of PCV13 into national immunization program is effective in reducing overall pneumococcal disease in children.

1. Introduction

Streptococcus pneumoniae is the leading cause of pneumonia, otitis media, bacteremia, and meningitis in children. *S. pneumoniae* causes more than 1.2 million infant deaths worldwide every year [1,2]. Heptavalent pneumococcal conjugate vaccine (PCV7) had significantly reduced the incidence of diseases caused by these vaccine serotypes after its licensure in 2000 [3]. However, non-vaccine serotypes (NVTs) have increased in a process called "serotype replacement" [4]. The increase in non-PCV7 serotypes, most notably caused by serotype 19A, was reported in several countries [5,6]. In Taiwan, 10-valent (PCV10) and 13-valent (PCV13) vaccines were licensed in 2009 and 2010, respectively, for broader serotype protection.

In Taiwan, different from other countries, children aged 2–4 years had the highest incidence of IPD [7,8]. PCV13 was introduced into Taiwan initially in the private sector. In 2011, the government provided PCV13 free of charge to children aged <5 years with underlying diseases, with low socioeconomic status, and who lived in areas with poor health care resources. In 2012, this was extended to those with middle socioeconomic status and those with muscular atrophy [9]. PCV13 catch-up immunization program was implemented to children aged 2–5 years in March 2013 and extended to children aged 1–5 years in January 2014. PCV13 immunization was integrated into the national

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immunization program in January 2015 with a three-dose schedule for infants at 2, 4, and 12 months of age.

The overall incidence of pneumococcal diseases was underestimated as most of the surveillance systems measured the incidence of IPD. Surveillance of culture-confirmed pneumococcal disease (CCPD) may provide a more comprehensive picture on the impact of PCV13 immunization than IPD. Therefore, the present study aimed to investigate the impact of such immunization program in Taiwan by prospective surveillance of pneumococcal serotypes, sequence types, and antibiotic susceptibility of pneumococcal isolates in children.

2. Methods

2.1. Study design

This study was approved by the institutional review board in Chang Gung Memorial Hospital (CGMH) (IRB number: 201801007B0). We collected all clinical isolates of *Streptococcus pneumoniae* from pediatric patients <18 years of age at CGMH, Linkou, Taoyuan from 2012 to 2016. CGMH in Taoyuan is a main referral hospital for cities in northern Taiwan, including branches in Taipei, New Taipei, and Taoyuan. The population in this region is approximately 7 million. The annual incidence of CCPD was calculated by dividing the number of cases by the admission number for that year. The numbers of annual pediatric admissions were as follows: 21,211 (2012), 21,163 (2013), 21,873 (2014), 21,740 (2015), and 22,205 (2016).

2.2. S. pneumoniae culture and isolation

The isolates were cultured and identified using standard methods in the clinical microbiology laboratory [10]. IPD was defined as isolation of *S. pneumoniae* from normally sterile sites, while non-invasive pneumococcal disease (NIPD) was defined as isolation of *S. pneumoniae* from nonsterile sites. The diagnosis of sinusitis and otitis media was made according to the American Academy of Pediatrics guidelines with nasopharyngeal culture or otorrhea culture for *S. pneumoniae* [11,12]. The diagnosis of pneumonia was made based on the following criteria: radiographic lobar consolidation and positive sputum culture for *S. pneumoniae*. Some well-defined risk factors for pneumococcal diseases included immunocompromised conditions, functional or anatomic asplenia, cyanotic heart disease, chronic lung disease, chronic renal failure, nephrotic syndrome, cerebrospinal fluid leak or cochlear implant, malignancy, and transplantation [13]. After identification of *S. pneumoniae*, all isolates were subject to further antimicrobial susceptibility testing and molecular characterization.

2.3. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of penicillin were measured by E-test strips (bioMérieux, Marcy l'Etoile, France). The results were interpreted based on the 2013 Clinical and Laboratory Standard Institute guideline [14]. Antimicrobial susceptibility results were categorized as susceptible (S, $\leqq$0.06 mg/L), reduced-susceptible (RS, 0.12–1 mg/L), and non-susceptible (NS, $\geqq$2 mg/L).

2.4. Serotyping and genotyping

Serotypes of *S. pneumoniae* isolates were determined by commercialized antisera (Statens Serum Institut, Copenhagen, Denmark) and polymerase chain reaction (PCR) methods [15]. Serotypes were stratified as follows: PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F), additional PCV13 serotypes (1, 3, 5, 6A, 7F, and 19A), and NVTs. Multi-locus sequence typing (MLST) was determined by PCR-sequencing of a set of pneumococcal housekeeping genes (*aroE, gdh, gki, recP, spi, xpt and ddl*) [16]. The sequence data were compared with those in the *S. pneumoniae* MLST website (http://pubmlst.org/spneumoniae/) at Oxford University [17]. New alleles and allelic profiles were submitted to the database curator for the assignment of ST numbers.

2.5. Statistical analysis

MedCalc Statistical Software version 18.10.2 (MedCalc Software bvba, Ostend, Belgium) was used for statistical analysis. Chi-square test and Fisher's exact test were used to compare categorical variables. The Chi-square test for trend was used to evaluate the trend between variables. Mann-Whitney *U* test was used for continuous variables. A two-tailed p value of <0.05 was considered significant.

3. Results

3.1. Culture-confirmed pneumococcal disease

Initially 509 isolates of *S. pneumoniae* were identified from pediatric patients. Thirty-five isolates were considered as colonizing isolates as those patients had no clinical evidence of bacterial infections and hence were excluded. A total of 473 patients with CCPD, including 58 with IPD and 415 with NIPD, were further analyzed. The number of CCPD patients decreased from 152 in 2012 to 60 in 2016 (Fig. 1). The numbers of annual pediatric admissions were stable during 2012–2016. In this clinical setting, the incidence of CCPD per 10,000 admissions decreased from 71.7 in 2012 to 27.0 in 2016 ($P < 0.0001$). The incidence of IPD per 10,000 admissions decreased from 9.9 in 2012 to 2.3 in 2015 ($P = 0.0014$) but increased to 5.0 in 2016 ($P = 0.2104$). Among the 58 IPD patients, 20 had bacteremia, 20 had pulmonary empyema, 9 had bacteremic pneumonia, 8 had CNS infections, and 1 had septic arthritis. Of the 8 patients with CNS infections, 5 had meningitis, and the rest had meningoencephalitis, subdural empyema, and brain abscess. Among the 415 NIPD patients, 230 had sinusitis, 118 had otitis media, 59 had pneumonia, 4 had conjunctivitis, 3 had skin and soft tissue infections, and 1 had urinary tract

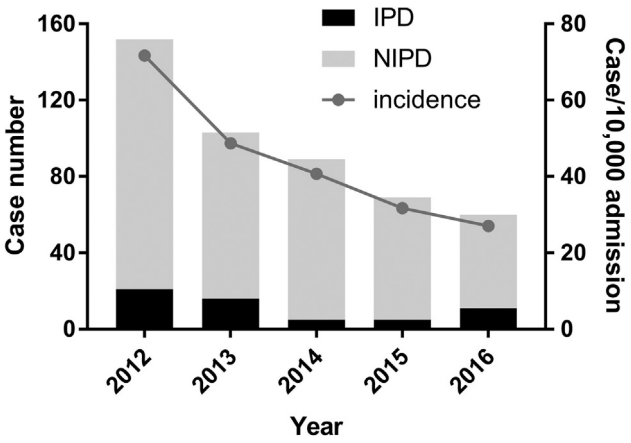

Fig. 1. Case number and incidence of culture-confirmed pneumococcal disease (CCPD) in 2012–2016. IPD, invasive pneumococcal disease. NIPD, non-invasive pneumococcal disease. NIPD includes sinusitis, otitis media, pneumonia, conjunctivitis, skin and soft tissue infection, and urinary tract infection. Incidence was defined as the ratio of CCPD case number to 10,000 admission number for that year. The numbers of annual pediatric admissions were as follows: 21,211 (2012), 21,163 (2013), 21,873 (2014), 21,740 (2015), and 22,205 (2016).

Table 1
Clinical characteristics of children with invasive pneumococcal diseases (IPD) cases and non-invasive pneumococcal diseases (NIPD).

Characteristics	IPD $n = 58$	NIPD $n = 415$	*P* value
Age (year)[a]	3.95 (0.3–17.2)	4.8 (0.3–17.6)	0.0632
Gender (Male/female)	34/24	243/172	0.9923
Risk factors[b]	11 (19.0%)	39 (9.4%)	0.0266
Admission	56 (96.6%)	110 (26.5%)	<0.0001
Death	3 (5.2%)	3 (0.7%)	0.0046
Serotype 19A	31 (53.4%)	108 (26.0%)	<0.0001

[a] Median (range).
[b] Risk factors include immunodeficiencies, steroid use, diabetes mellitus, asplenia, cyanotic heart disease, chronic lung disease, nephrotic syndrome, cerebrospinal fluid leak or cochlear implant, inflammatory bowel disease, and malignancy.

infection. Fifty (10.6%) children had risk factors for pneumococcal diseases. Six patients died within 30 days (Table 1). Of these patients, three died from pneumonia, two from bacteremia, and one from meningitis.

The age distribution of CCPD cases is shown in Fig. 2. CCPD was common among patients aged 3–6 years. IPD was common among patients aged 2–5 years. Age 1–6 years accounted for 79% of IPD cases. Only 17% of IPD occurred in children aged ≥6 years. NIPD was common among patients aged 3–7 and 1–2 years. There was no significant temporal shift in age distribution or diagnosis from 2012 to 2016 ($P > 0.05$).

The characteristics between children with IPD and those with NIPD were compared (Table 1). No significant difference was observed in age and gender between the two groups. Children with IPD had more risk factors than those with NIPD ($P = 0.0266$). Admission rate and mortality rate were significantly higher in children with IPD than in those with NIPD ($P < 0.0001$ and $P = 0.0046$, respectively). Serotype 19A was more common in children with IPD than in those with NIPD ($P < 0.0001$).

3.2. Serotypes

Approximately 121 (25.6%) were PCV7 serotypes, 173 (36.6%) were additional PCV13 serotypes, and 179 (37.8%) were NVTs. The proportion of PCV7 serotypes significantly decreased from 29.6% in 2012 to 6.7% in 2016 ($P < 0.0001$) (Fig. 3). The proportion of additional PCV13 serotypes significantly decreased from 52.0% in 2012 to 21.7% in 2015 ($P < 0.0001$) but slightly increased to

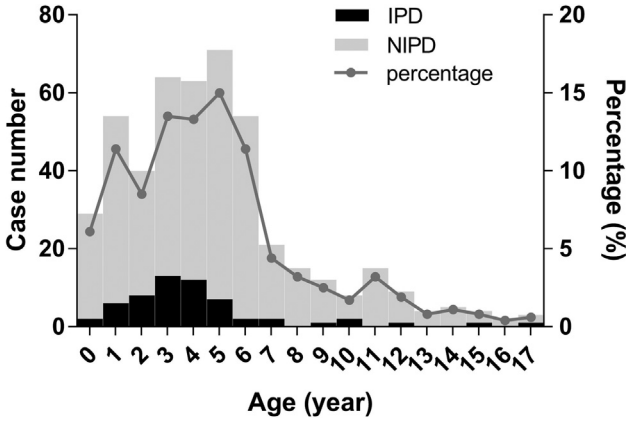

Fig. 2. Age distribution of culture-confirmed pneumococcal disease cases. IPD, invasive pneumococcal disease. NIPD, non-invasive pneumococcal disease. Age 0 means ≥0 month old and <12 months old, and age 1 means ≥12 months old and <24 months old, etc. NIPD includes sinusitis, otitis media, pneumonia, conjunctivitis, skin and soft tissue infection, and urinary tract infection. The IPD (or NIPD) percentage is the pneumococcal disease cases within the age range out of total IPD (or NIPD) cases.

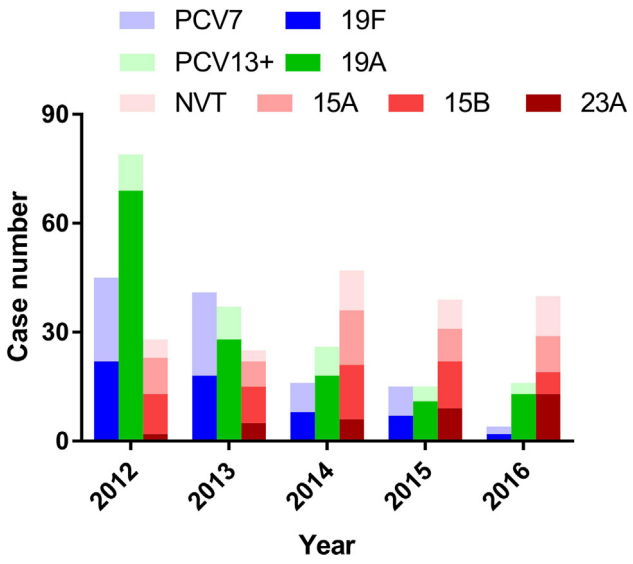

Fig. 3. The temporal trend of different serotypes causing pneumococcal diseases. PCV7, PCV7 serotypes. PCV13+, additional PCV13 serotypes. NVT, non-PCV13 serotypes.

26.7% in 2016 ($P = 0.5152$). The proportion of NVTs increased significantly from 18.4% in 2012 to 66.7% in 2016 ($P < 0.0001$). The case incidence of CCPD caused by NVTs per 10,000 admission increased from 13.2 in 2012 to 18.0 in 2016 but was not statistically significant ($P = 0.0885$). The four most common serotypes were 19A (29.4%), 19F (12.1%), 15B (11.6%), and 15A (10.8%). The proportion of serotype 19A decreased from 45.4% in 2012 to 15.9% in 2015 ($P < 0.0001$) but slightly increased to 21.7% in 2016 ($P = 0.4065$). The most common serotype in 2012–2014 was serotype 19A but shifted to 15B in 2015. Serotypes 19A and 23A were two most common serotypes in 2016. Among the 179 NVT isolates, 51 (28.5%) were serotypes 15A, 55 (30.7%) were serotype 15B, and 35 (19.5%) were serotype 23A.

Among the 58 IPD isolates, 11 (19.0%) were PCV7 serotypes, 36 (62.0%) were additional PCV13 serotypes, and 11 (19.0%) were NVTs (Fig. 4). The proportion of PCV7, additional PCV13 serotypes, and NVTs among IPD cases did not change significantly from 2012 to 2016 ($P > 0.5$). The most common serotype was 19A (53.4%) and the second most common was serotype 15A (8.6%). Serotype 19A was the most common vaccine serotype for IPD from 2012 to 2016. Vaccines serotypes that showed relatively higher potential

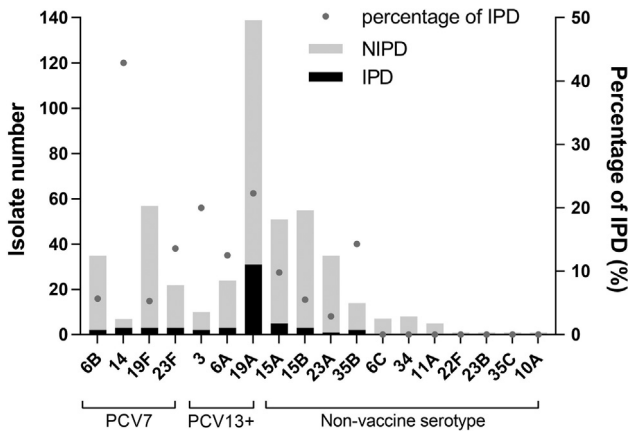

Fig. 4. Serotype distribution of pneumococcal isolates. PCV7, PCV7 serotypes. PCV13+, additional PCV13 serotypes. NIPD includes sinusitis, otitis media, pneumonia, conjunctivitis, skin and soft tissue infection, and urinary tract infection.

to cause IPD are 19A, 14, and 3; in contrast, among NVTs, 35B, though the case number was <15A, 15B, and 23A, showed relatively higher potential to cause IPD (Fig. 4).

3.3. Sequence types

A total of 74 sequence types were identified during the study period. Of all 473 isolates, sequence type 320 (ST320) was the most common sequence type and accounted for 27.9% of the isolates, followed by ST63 (10.8%), ST83 (9.5%), and ST81 (5.9%). Serotype 19A accounted for 128 (97.0%) of the 132 ST320 isolates. Serotype 15A, 15B, and 6A were the most common serotypes in ST63, ST83, and ST81, respectively. ST320, ST236 (a closely related sequence type), ST271, ST1464, and other 12 sequence types formed the largest clonal complex CC236/320 (n = 192, 40.6%). CC236/320 was mainly composed of serotype 19A (n = 136, 70.8%) and 19F (n = 55, 28.6%) isolates. The second largest clonal complex (CC81/83; n = 82, 17.3%) included most of the 15B (n = 47, 57.3%), 6A (n = 20, 24.4%), and 23F (n = 9, 11.0%) isolates. The third clonal complex (CC63; n = 54, 11.4%) mainly consisted of serotype 15A (n = 50, 92.6%). The major NVTs, serotype 15A, 15B, and 23A, contained a predominant clone, ST63^{15A}, ST83^{15B}, and ST166/338^{23A}, respectively.

3.4. Antimicrobial susceptibility

Among the 473 isolates, penicillin susceptibility results were available in 470 isolates. Seventeen (3.6%) isolates were S, 230 (48.9%) were RS, and 223 (47.4%) were NS (Supplementary Fig. S1). Among the 293 PCV13 serotype isolates, 36.2% were RS and 61.1% were NS. Among the 177 NVT isolates, 70.1% were RS and 24.9% were NS. The NS rate of PCV13 serotype isolates was higher than that of NVT isolates (P < 0.0001). Among the 58 isolates from IPD cases, only two (3.4%) isolates were S, 24 (41.4%) were RS, and 32 (55.2%) were NS. Among the 412 isolates from NIPD cases, 15 (3.6%) were S, 206 (50.0%) were RS, and 191 (46.4%) were NS. There was no significant difference in susceptibility rate between IPD and NIPD isolates.

Comparing the susceptibility by individual serotype, serotype 19F had the highest penicillin NS rate (78.9%), followed by serotype 19A (76.3%) (Fig. S1). The following serotypes had an NS rate above 30%: 6A (58.3%), 11A (40%), 15B (47.3%), 19A (76.3%), 19F (78.9%), 23A (37.1%), and 23F (36.4%). Penicillin MIC_{50} of serotype 19F was 2 mg/L for ST271 isolates, 1.5 mg/L for ST236 isolates, 3 mg/L for ST1464 isolates, and 2 mg/L for other sequence types (Fig. S2). Penicillin NS rate of serotype 19F decreased significantly from 90.9% in 2012 to 61.5% in 2013–2014 (P = 0.0205), and then increased to 100% in 2015–2016 (P = 0.0300). Penicillin MIC_{50} of serotype 19A ST320 and non-ST320 were both 2 mg/L. Penicillin MIC_{90} of serotype 19A was 4 mg/L for ST320 isolates and 3 mg/L for non-ST320 isolates (Fig. S3). Penicillin NS rate of serotype 19A decreased significantly from 91.3% in 2012 to 55.6% in 2014 (P = 0.0001), and then slightly increased to 69.2% in 2016 (P = 0.4339).

4. Discussion

Methods to evaluate the impact of pneumococcal immunization on overall pneumococcal disease should incorporate available evidence from all pneumococcal diseases instead of focusing solely on IPD. Although IPD surveillance was still essential, NIPD was more prevalent in both inpatient and outpatient departments. Most of vaccine effectiveness studies only focused on IPD. Therefore, the overall cost-effectiveness and socioeconomic impact of pneumococcal immunization were underestimated. In this study, we surveillanced CCPD rather than IPD to assess the impact of a unique immunization program on overall pneumococcal disease in children in Taiwan. The incidence of CCPD cases decreased after the offer of PCV13 to high-risk children from late 2011 to 2012, and PCV13 catch-up immunization in 2013–2014, and further decreased after universal PCV13 immunization in 2015–2016. The incidence of CCPD cases decreased in all age children in 2012–2016. Therefore, the gradual implementation of PCV13 into national immunization program was effective in reducing IPD and NIPD according to the results of the current study.

The highest incidence of IPD occurred among children aged <2 years in most of countries [18,19]. In Taiwan, children aged 2–4 years had the highest incidence of IPD [7,8]. The current study showed that the highest incidence of IPD and NIPD occurred among children aged 2–5 years and 3–7 years, respectively. The cause of this unique age distribution is not fully understood. Firstly, previous epidemiologic studies revealed higher proportion of bacteremia and lower proportion of CNS infections in Taiwan than in western countries [7,18]. In the current study, bacteremia (including bacteremia and bacteremic pneumonia) constituted half of IPD and CNS infections constituted only about one seventh of IPD. Compared to other IPDs, bacteremia is relatively easy to be eliminated. In addition, the health care accessibility in Taiwan is great. Maybe most of bacteremia cases among infants were treated in clinics. Therefore, we speculated that the incidence of IPD among children aged <2 years were underestimated. Secondly, in Taiwan, most of children age <2 years are cared by relatives or nannies at home, and most of children aged 2–5 years attend day-care centers or kindergartens. Day-care center attendance is a risk factor for pneumococcal nasopharyngeal colonization and subsequent pneumococcal diseases [20,21]. Therefore, day-care center attendance may increase the risk for pneumococcal disease among children aged 2–5 years in Taiwan.

In Taiwan, serotype 19A was the most common serotype causing pneumococcal disease among children in 2009–2010 [7]. Most of the 19A isolates belonged to ST320 [22]. After PCV7 introduction in Taiwan, we have observed the expansion and evolution of 19A ST320 in Taiwan, not only in colonization but also in overall incidence of pneumococcal infection, including IPD in children aged <5 years from 2008 to 2011 [22]. Most of the serotype 19A ST320 isolates were multidrug-resistant and were more resistant to cefuroxime, clindamycin, and trimethoprim/sulfamethoxazole than other STs [23]. A previous study of acute otitis media also found that serotype 19A ST320 isolates had higher rates of NS to penicillin and amoxicillin than non-19A ST320 [24]. The present study revealed that more than 90% of serotype 19A isolates were ST320 and belonged to the CC320, which is related to multidrug-resistant global clone Taiwan^{19F}-14 (ST236). Serotype 19A ST320 had higher penicillin MIC_{90} than serotype 19A non-ST320. Serotype 19A had the second highest penicillin NS rate, just next to serotype 19F. After the introduction of PCV13, not only the proportion of serotype 19A decreased gradually from 2012 to 2015, but also penicillin NS rate of serotype 19A decreased from 2012 to 2014. The slight increase in the proportion of serotype 19A from 2015 to 2016 was due to a significant decline in the case numbers of PCV7 serotypes. The cause of changing resistance prevalence of serotype 19F and 19A was not fully known but could be associated with the change of sequence types. Although serotype 19A remained the most common vaccine serotype causing pneumococcal disease in the post-PCV13 era, it can be expected that the burden of serotype 19A pneumococcal disease should continue to decrease in the near future.

This study confirmed a continued decrease in the incidence of NIPD with a slight increase in the incidence of non-PCV13 serotype infections. Reports from other countries also revealed a decline in the incidence of pneumococcal diseases caused by PCV13

serotypes after the introduction of PCV13, although the extent of replacement disease with non-PCV13 serotypes varied from country to country [25–28]. The distribution of emerging NVTs also differed. In the United Kingdom, a rapid increase in the incidence of NVT disease, predominantly IPD by serotypes 8, 12F and 9N, was observed [26]. In Japan, the occurrence of NVT IPD caused by serotypes 22F, 15A, and 23A occurred only second to infections caused by serotypes 3 and 19A in 2013–2015 [27]. In South Korea, serotypes 10A and 15C were the second and third most common serotypes for IPD in 2011–2013, respectively [28]. In the United States, there was no increase in the incidence of NVT IPD until 2016 [29]. In our study, NVTs have emerged to become the most common serotypes to cause CCPD in 2015 and 2016. Serotypes 15A, 15B, and 23A were the major NVTs in this period. Nonetheless, the incidence of CCPD caused by NVTs did not increase significantly over the years. Furthermore, the incidence of IPD significantly decreased in 2012–2015, and only few NVTs were identified from patients with IPD. Further, this prospective study showed that penicillin non-susceptibility rate of NVTs was significantly lower than that of vaccine types. Taken together, at least two years after the gradual introduction of PCV13 into national immunization program in Taiwan, we observed a positive impact on the clinical manifestations of pneumococcal diseases from the immunization.

The strength of our study lies in the longitudinal clinical microbiological surveillance after the gradual introduction of PCV13 into national immunization program. This entire process had gone through three periods: a conditional offer to certain high-risk participants, catch-up program, and universal childhood immunization with 2 + 1 schedule (2 months old, 4 months old, and 12–15 months old). Through these stages, a continued decline in the incidence of overall pneumococcal disease was observed. This study included both patients with IPD and NIPD. Apparently, majority of the benefits of PCV13 immunization actually occurred through the prevention of the large numbers of NIPD.

5. Conclusions

A unique immunization program was effective in reducing the incidence of CCPD in Taiwan. The decrease was mainly attributed to the reduction in the incidence of NIPD caused by additional PCV13 serotypes, especially 19A. A slight increase in the incidence of NVTs was observed in patients with CCPDs but that was not considered statistically significant. Emerging NVTs, such as serotypes 15B, 15A, and 23A, constituted the second, third, and fourth most common serotypes, respectively, to cause CCPD in children. Continuous surveillance of CCPD with NVTs, especially the predominant clones ($ST63^{15A}$, $ST83^{15B}$, and $ST166/338^{23A}$), rather than IPD alone, should be performed in the future.

Author contributions

CHC, LHS, TLW, and KCK facilitated data acquisition and interpretation, drafting, and revision of the manuscript; CHC, LHS, CCH, and CHC facilitated conception and design of the study, data acquisition and interpretation, and drafting and revision of the manuscript. HCL, MHH, and CLC carried out experiments and contributed to data acquisition and interpretation. All authors provided final approval of the manuscript and agree to be accountable for the accuracy and integrity of this work.

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Declaration of Competing Interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.07.073.

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