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**Abstract**

**Purpose.** An observational study was performed to investigate the carriage rate and serotypes of *Streptococcus pneumoniae* in the 13-valent pneumococcal conjugate vaccine (PCV13) era in Taiwan.

**Methodology.** From March 2014 to March 2015 a total of 500 healthy children and their households (631 adults) were enrolled from two large medical centres for nasopharyngeal carriage survey. Clinical isolates were prospectively collected from June 2012 to May 2015 at Chang Gung Memorial Hospital. We applied a multiplex polymerase chain reaction in addition to culture to detect *S. pneumoniae*.

**Results.** *S. pneumoniae* was isolated from 12.0 % of the children and 3.6 % of the households. In the children’s cohort only 23.3 % of the isolates could be assigned to PCV13 serotypes; non-vaccine serotypes were predominant (76.6 %) and the most frequently detected non-vaccine serotypes were 15A/F and 15B/C (both 13.3 %), followed by 23A (6.7 %). In the household cohort, 21.7 % belonged to PCV13 serotypes, and 78.3 % to non-vaccine serotypes. Clinical analysis of culture-confirmed pneumococcal infection showed that infection caused by PCV13 serotypes decreased by 47 % from 83 % in 2012–2013 to 44 % in 2014–2015, while infection caused by non-PCV13 serotypes increased from 17 to 56 %. Among the carriage isolates a significantly higher percentage belonged to serogroup 15 compared to serogroup 19 (26.6 vs 6.66 %, 2014–2015; \( P=0.003 \)). Therefore, clinical isolates belonging to serogroup 15 were more prevalent than those belonging to serogroup 19 (44.1 vs 32.3 %, 2014–2015; \( P=0.318 \)).

**Conclusion.** The isolation of non-vaccine serotypes and unknown serotypes after the introduction of PCV13 in children highlights the importance of continued surveillance for emerging serotypes.

**INTRODUCTION**

*Streptococcus pneumoniae* is the most common bacterial respiratory pathogen in humans worldwide [1]. More than 90 distinct serotypes of *S. pneumoniae* are known, the classification of which is based on the antibodies to capsular polysaccharide antigens. Serotype prevalence varies widely, and a limited number of serotypes account for a large proportion of pneumococcal infections. Nasopharynx of preschool children is the primary ecological niche of *S. pneumoniae* [1]. Asymptomatic nasopharyngeal colonization precedes invasive disease; the factors that permit pneumococci to spread beyond the nasopharynx vary depending on the virulence factors of the serotype, the host immune system and the existence of preceding respiratory viral infection [2].

The emergence of multidrug-resistant pneumococci has raised concern worldwide. The prevalence of antimicrobial resistance among *S. pneumoniae* colonizing in nasopharynx is an indicator of their prevalence in the community [3]. Routine immunization with the pneumococcal conjugate vaccine (PCV) decreased the incidence of invasive
Pneumococcal disease (IPD) in children [4]. 7-Valent PCV (PCV7) contains capsular polysaccharides corresponding to serotypes 4, 6B, 9V, 14, 18, 19F and 23F, while PCV13 additionally includes serotypes 1, 3, 5, 6A, 7F and 19A. Pneumococcal immunization also reduced nasopharyngeal colonization in the vaccinees’ contacts, thereby providing indirect immunity to the community [5, 6].

In Taiwan, 7-valent pneumococcal conjugate vaccine (PCV7) was first introduced in late 2005 and both PCV10 and PCV13 have now been licensed and are available [7–9]. Taiwan applies a step-by-step strategy to introduce PCV into the national immunization programme. PCV13 was introduced into Taiwan in 2011 in the private sector. In March 2013, a national catch-up immunization with PCV13 was launched for all children aged 2–5 years who have not received PCV13 [8, 9]. In 2014, the lower age for eligibility for the campaign was reduced to 1 year of age. By the end of 2014, the age-appropriate immunization rate had reached ~80 % according to a report from the Centers for Disease Control in Taiwan. Starting with risk groups and the catch-up programme, PCV13 has been included in the national immunization programme since January 2015. The epidemiology of IPD in the context of partial coverage of PCV showed some unique characteristics, with a rapid emergence of non-PCV13 serotypes in both paediatric and adult populations [9].

Nasopharyngeal colonization of pneumococcus among children was also surveyed in Taiwan before and after the introduction of PCV7 [7, 10, 11]. Herein we conducted a prospective and multicentre study to assess the prevalence of nasopharyngeal S. pneumoniae carriage in Taiwanese children from 2014 to 2015. Nasopharyngeal colonization of the household contacts who were the major care givers for the pre-school children was surveyed. Culture-confirmed S. pneumoniae infection in children less than 5 years of age treated in a medical centre was also surveyed. The data obtained will facilitate further understanding of the impact of pneumococcal vaccination on nasopharyngeal carriage of S. pneumoniae.

METHODS
Study design
This was an epidemiological, observational study performed to investigate the carriage rate and serotypes of S. pneumoniae in children and their households in Taiwan. From March 2014 to March 2015 a total of 500 healthy children and up to 631 healthy adults were enrolled from two large medical centres – Chang Gung Memorial Hospital at Linkou and Taichung Veterans General Hospital. Informed consent and parental permission was obtained from the children and their parents/guardians after the study procedure was explained to them. Screening was performed on children attending paediatric clinics in hospitals, day care centres, or kindergartens, and it was also performed on their households. The exclusion criteria for children were moderate to severe disability, cerebral palsy, syndromes and neurological disorders affecting swallowing, confirmed or suspected immunodeficiency (congenital or acquired) and immunosuppressive therapy. Households referred to adults who were the major care givers for the children (i.e. parents, guardians, relatives, babysitters, etc.). No treatment was applied to the participants. A case report form (CRF) was designed to record all the information required by the study, such as the personal history of the subjects, the demographic data for each household and the risk factors in the S. pneumoniae carriage rate in children. The results for S. pneumoniae carriage in nasopharyngeal swabs were recorded. The study used stratified sampling based on age groups to ensure that the the sample was more representative of the whole population. As expected, the carriage increased with age. During each week, a proportion of samples were obtained from each of the age groups (e.g. <1 year, 1–2 years, 2–3 years, 3–4 years and 4–5 years).

After recruitment, information on the nasopharyngeal S. pneumoniae carriage status of the index child was collected and followed by an interview to record all the other information required by the study. When the index child was enrolled, his/her household (up to three people) was contacted and two swabs of nasopharyngeal samples were collected for each adult within 1 week, followed by an interview. A medical record review was performed when necessary. Clinical isolates of S. pneumoniae, one isolate per patient, were prospectively collected from June 2012 to May 2015 at Chang Gung Memorial Hospital. We defined IPD as isolation of S. pneumoniae from normally sterile sites such as blood, cerebrospinal fluid (CSF), or pleural fluid. For sputum culture, the quality of specimens was confirmed before the culture proceeded, and only the predominant micro-organisms were reported. Pus isolates were identified from ear discharge specimens of infants or pre-school children suffering acute otitis media with otorrhea [9]. All isolates were cultured and identified using standard methods [12].

The final protocol, any amendments and the informed consent documentation were reviewed and approved by the Institutional Review Board (IRB number: 102-2970A3) for each site participating in the study.

Serotyping of nasopharyngeal carriage and clinical isolates
Nasopharyngeal swab culture for S. pneumoniae was performed and pneumococci were cultured at 37 °C in 5 % CO2 on Mueller–Hinton agar (Difco Laboratories, Detroit, MI, USA) supplemented with 5 % lysed horse blood. Serotyping was performed by latex agglutination and confirmed by Quellung reaction (Statens Serum Institute, Copenhagen, Denmark). A multiplex PCR was also performed, as described previously [13, 14].

Statistical analysis
Data are expressed as numbers and percentages. The primary endpoint was the carriage rate of S. pneumoniae. The carriage rate was computed by dividing the number of positive samples by the total number of samples collected. The
levels of significance for the differences between the different groups were analysed using the chi-square test or Fisher’s exact test. A P-value of <0.05 was considered significant.

RESULTS
Demographic characteristics of the children’s and household cohorts
A total of 1131 subjects participated in this study, and among these 500 were children and 631 were households. In the children’s cohort, more males (55.5 %) participated in this study. The mean age was 2.4±1.4 years, with a comparable population distribution among the age groups. Of all the 500 analysed children, 141 (28.2 %) had attended day care centre for 2.4±1.1 years, and they spent 34.0±13.1 h per week in the day care centre on average. Most (85.6 %) of them had been vaccinated with the pneumococcal vaccines. A history of otitis media in the past year was found in 11.2 % of the children, while upper respiratory infection within the past 30 days was found in 38.9 % of them. Before joining the study, most children (93.4 %) had not taken antibiotics in the past 7 days, and none of the children had received immunosuppression therapy or systemic corticosteroid in the past year.

For the household cohort, almost two-thirds of the subjects were female (69.9 %). The mean age was 35.7±7.0 years. Most of them spent more than 8 h per day taking care of their children (8–12 h/day: 55.3 %, 12–24 h/day: 42.0 %). Smokers and ex-smokers only accounted for 12.2 and 2.4 % of the households, respectively. Ex-smokers had quit smoking for 4.0±3.1 years. In other words, more than 85.4 % of the households were non-smokers when looking after their children. Of the 631 households, none of them had ever received pneumococcal vaccination, and nor had they ever suffered from chronic obstructive pulmonary disease. A history of asthma and influenza within the past 30 days was only found in 1.7 and 0.2 % of the households, respectively.

Carriage rates of S. pneumoniae in the children’s and household cohorts
A subject was considered to be an S. pneumoniae carrier when S. pneumoniae was identified using either approach. Of the 1131 subjects analysed, S. pneumoniae was isolated from 60 of the 500 children and 23 of the 631 households, representing a corresponding carriage rate of 12.0 and 3.6 %. Serotyping was performed on all the S. pneumoniae-positive specimens. The detailed serotypes are displayed in Table 1. In the children’s cohort, only seven of the 13 capsular serotypes included in PCV13 could be isolated. Fourteen (23.3 %) of the 60 S. pneumoniae isolates were assigned to PCV13 serotypes; among these, the most frequently found pneumococcal serotypes were 6A/B/C/D (6.7 %), 14 and 19A (both 5 %). Four vaccine serotypes were only identified in one child each, including serotypes 3, 18A/B/C/D, 19F and 23F (all 1.7 %). Non-vaccine serotypes were predominant (76.6 %, 46 of 60) in children; the most frequently found non-vaccine serotypes were 15A/F and 15B/C (both 13.3 %), followed by 23A (6.7 %). Three non-vaccine serotypes were only identified in one child each, including serotypes 18A/B/C/D, 22F and 35A/C/42 (all 1.7 %). Serotypes isolated from 40.0 % carriers (24 of the 60 isolates) were unknown. Seven of the PCV13 serotypes were found in vaccinated children, and only serotype 14 (one isolate) was found in unvaccinated children. Serogroup 15 was present in both vaccinated (27.0 %) and unvaccinated children (25.0 %). Among the 60 children with pneumococcal carriage, there was no significant difference in the carriage rate of vaccine or non-vaccine serotypes between those with and without a history of immunization (P>0.05) (Table 1). There was also no significant difference in the carriage rate of vaccine serotypes or non-vaccine serotypes between children and adults with pneumococcal colonization in the nasopharynx (Table 1).

In the household cohort, only four of the 13 serotypes included in PCV13 could be identified (Table 1). Five (21.7 %) of the 23 S. pneumoniae isolates were assigned to PCV13 serotypes; the most prevalent vaccine serotype was 19A (8.7 %), followed by 3, 6A/B/C/D and 19F (4.3 %). Non-vaccine serotypes were also predominant (18 of 23) in household subjects. The most frequently found non-vaccine serotypes were 23A (8.7 %) and 22F (4.3 %). Serotypes isolated from 15 of the 23 household carriers (65.2 %) were unknown. Vaccine serotypes 14, 18A/B/C/D and 23F, and other serotypes, including 15A/F, 15B/C and 35A/C/42, were only found in children.

In this study, a total of 45 pairs of enrolled children were found to be siblings. Among these siblings, three pairs of them (6.7 %) were both identified as S. pneumoniae carriers. However, only one pair of siblings was infected with the same non-vaccine serotype 15A/F. We also observed that the serotype distribution of pneumococcal serotypes differed between children and adults. None of them were infected with the same S. pneumoniae serotypes.

Serotype distribution of isolates from children receiving different doses of pneumococcal vaccination
No association was found between the detection rate for the vaccine serotypes and the number of vaccinations (Table S1, available in the online Supplementary Material). The highest proportion of PCV13 serotypes was observed in children who had been vaccinated four times (40.9 %, including serogroup 6, 14, 19A and 23F), followed by children who had been vaccinated once (18.2 %, including 14 and 19A). Interestingly, the non-vaccine serotype 23A and 15 detection rate was higher after four vaccinations. Serotype 15 isolates were also found in unvaccinated children.

Carriage rate comparison in gender and age subgroups of children
The carriage rate was found to be higher in males (13.0 %) than in females (11.7 %), and there was no age-correlated distribution (Table S2). PCR yielded positive results for
serotype 3 (8.3 %) in children aged less than 1 year; for serotypes 6 and 14 (both 6.3 %) in 1-year-old children; for serotypes 6, 14, 19F and 23F (all 10.0 %) in 2-year-old children; for serotypes 14 and 19A (both 11.1 %) in 3-year-old children; and for serotypes 6 and 19A (both 15.4 %) in 4- to 5-year-old children. For non-vaccine serotypes, serotypes 15A/F (16.7 %) and 23A (8.3 %) were isolated from children aged less than 1 year, and serotypes 15A/F, 15B/C (both 15.4 %) and 23A (7.7 %) were isolated from children aged between 4 and 5 years. Certain serotypes, in particular vaccine serotype 19A, were seen more frequently in older children (aged more than 3 years). Furthermore, a higher proportion of unknown serotypes were observed in younger children (<1 year: 66.7 %, 1 year: 50.0 %), as compared with older children (2 years: 30.0 %, 3 years: 22.2 %, 4–5 years: 23.1 %).

**S. pneumoniae from children with culture-confirmed infections**

From June 2012 to May 2015 a total of 174 clinical pneumococcal isolates were isolated from blood (13), pleural effusion (8), pus (otorrhea) (122) and sputum (31) from children less than 5 years of age. One-hundred-and-eighteen (67.8 %) isolates belonged to PCV13 serotypes, while the remaining 56 (32.1 %) isolates belonged to non-PCV13 serotypes (Fig. 1).

The proportion of PCV13 serotype isolates decreased by 47 % from 83 % in 2012–2013 to 44.1 % in 2014–2015, while the non-PCV13 serotypes increased from 17.0 to 55.9 %. Only six serotypes belonged to PCV13 serotypes, and among them 19A (65 isolates) was the predominant serotype, followed by 6A/B/C/D (24 isolates) and 19F (21 isolates). Among the non-PCV13 serotypes, 15A/F (24 isolates) and 15B/C (20 isolates) were predominant.

Seventy per cent (122 of 174 isolates) of the clinical isolates were isolated from pus (acute otitis media, AOM). Eleven different serotypes were detected in pus, with 19A being the predominant serotype, followed by 19F, 15A/F and 15B/C. The number of serotype 19A isolates detected in AOM patients 2012–2013 (26 isolates) decreased by 77 % when compared with the number of 19A isolates in 2014–2015 (six isolates). A single serotype 19A was found in pleural effusion, and only four isolates each were detected in the first 2 years of the study, while none were detected in the third year. Five different serotypes were isolated in blood in the first year of the study period (2012–2013), but only serotype 19A (two isolates) was isolated in the third year of the study period (2014–2015). Although 12 different serotypes were detected in sputum, serotype 6B (one isolate) was the single serotype isolated in the third year of the study period (2014–2015).

### DISCUSSION

The carriage rate and serotype prevalence of *S. pneumoniae* in children and their households in Taiwan were investigated in the present study. This study is one of the first to survey the epidemiological difference after PCV13 was introduced in Taiwan.

In this region nasopharyngeal pneumococcal carriage rates in the pre PCV7-period were between 19.8 and 26.6 % [15, 16]. During the early PCV7 era pneumococcal carriage rates in northern Taiwan decreased from 16.9 % in 2005 to 9.4 % in 2008 [7]. We found that in the early PCV13 era the

### Table 1. Nasopharyngeal carriage of *S. pneumoniae* in the children’s (n=500) and household (n=631) cohorts

<table>
<thead>
<tr>
<th>S. pneumoniae carriage</th>
<th>48 of the 428 vaccinated children</th>
<th>12 of the 72 unvaccinated children</th>
<th>Positive nasopharyngeal carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children n=60</td>
<td>Household n=23</td>
<td></td>
</tr>
<tr>
<td>Vaccine serotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 (2.0)*</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>6A/B/C/D</td>
<td>4 (8.0)</td>
<td>0 (0.0)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>14</td>
<td>2 (4.0)</td>
<td>1 (14.3)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>18A/B/C/D</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>19A</td>
<td>3 (6.0)</td>
<td>0 (0.0)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>19F</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>23F</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Non-vaccine serotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15A/F</td>
<td>7 (14.0)</td>
<td>1 (14.3)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>15B/C</td>
<td>6 (12.0)</td>
<td>2 (28.6)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>22F</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>23A</td>
<td>3 (6.0)</td>
<td>1 (14.3)</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>35A/C/42</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>Unknown serotypes†</td>
<td>17 (38.0)</td>
<td>7 (58.3)</td>
<td>24 (40.0)</td>
</tr>
</tbody>
</table>

*Number in parentheses, %.
†The samples were only positive for cpsA gene in PCR, but there was no bacterial growth in cultures; therefore the serotypes were unknown.
Fig. 1. Site of isolation and serotype distribution of the 174 pneumococcal isolates from culture-confirmed pulmonary infections in 2012–2015. Blood (B), pleural effusion (PL), pus/otorrhea (PUS) and sputum (SP).
pneumococcal nasopharyngeal carriage rate in children was 12% (2014–2015). Similar carriage rates (12.6%) were reported by Hsieh et al. [17] during the post-PCV7 era, indicating that the pneumococcal carriage rates were similar in the early PCV13 era (2014–2015) and the post-PCV7 era (2010). By contrast, in the Asia-Pacific region pneumococcal carriage rates in children were found to be high in Korea (36.4%) and low in Hong Kong (5.5%) [18, 19].

Carriage rates vary, depending on region, race, age, carriage duration, intra-household member transmissibility and resistance to colonization by competing serotypes [20–22]. Studies that focus on determining the pneumococcal carriage rates in adults and households are rare in Taiwan. In the pre-PCV7 era, Chen et al. [16] found extremely low carriage rates in adolescents (1.35%) and healthcare workers (0%). The carriage rate of 3.6% in adults in our study was similar to that in previous studies from the UK (3.4%) and Israel (4.0%), but lower than that in a study from the southwestern USA (9.6%) [22–24]. It is well documented that the rate of S. pneumoniae carriage is low in adults compared with children: the prevalence rates, risk factors for carriage and factors promoting the spread of the organism are limited among adults [22].

The proportion of PCV13 serotype isolates was higher in clinical isolates (44.1 vs 27.0%) than in nasopharyngeal carriage isolates from vaccinated children. However, the proportion of non-PCV13 serotype isolates was smaller in clinical isolates (55.8 vs 72.9%) than in carriage isolates. While seven of the PCV13 serotypes were found in vaccinated children, only serotype 14 (one isolate) was found in unvaccinated children. We hypothesize that due to herd immunity PCV13 serotypes were less prevalent in unvaccinated children. The presence of serogroup 15 in both vaccinated and unvaccinated children makes it difficult to identify the source of colonization.

Seven of the PCV13 serotype isolates were found in children, but only four PCV13 serotypes were found in households. Overall, serotype 19A was the predominant PCV13 serotype isolate among clinical isolates, while a predominant serotype could not be found in children and household carriage isolates. In this region, in the early PCV7 era serotypes, 19F, 6B, 23F and 14 accounted for 60% of the carriage isolates. We observed that only 18% of the carriage isolates belong to these serotypes, suggesting the replacement of vaccine type serotypes in the community [7]. Although 26.6% of the carriage isolates in children belonged to serogroup 15, none of the household carriers were positive for this serogroup isolate. Based on this finding, we can predict that serogroup 15 pneumococcal carriage and infection in adults in the post-PCV13 era will be due to spread of the organism from children to adults. In this study, among 45 pairs of siblings, three pairs (6.7%) were identified as S. pneumoniae carriers. However, only one pair (2.2%) was colonized with the same non-vaccine serotype 15A. In a similar study, Regev-Yochay et al. [22] examined pairs of siblings for S. pneumoniae carriage in Israel and found that only four of the 32 pairs (12.5%) carried identical strains.

After analysing the pairs of S. pneumoniae isolates recovered from children and their parents, we found that none (0%) of them were infected with identical S. pneumoniae serotypes. A high percentage of paired children and parents carried identical serotypes in Finland (31.5%) and Japan (62.1%), but the percentage was very low in Israel (0.6%) [20–22]. Among the healthy carrier children, serogroup 15 and serotype 23 were most frequently isolated in our study and they were found in all the age groups (<5 years) and were independent of the vaccine doses received. The presence of serogroup 6 isolates among carriers who were fully immunized might be due to the high prevalence of serogroup 6 isolates in Taiwan, low antibody response to serogroup 6 by PCV13, or an increase in specific clones after the introduction of PCV13. Serogroup 6 isolates were also found in children who were fully immunized and in catch-ups during the PCV7 era [11, 25]. Two mechanisms might explain the increase of non-PCV13-serotype replacement: demographic expansion of non-vaccine serotype lineages and capsular switching [26]. Both epidemiological and genome sequencing studies are required to understand the mechanism behind the serotype replacements in Taiwan.

One of the limitations in this study resulted from the narrow research area, with most of the children (85.0%) being enrolled from the Linkou district of northern Taiwan. The carriage rate may not reflect the overall S. pneumoniae prevalence in Taiwan. The socioeconomic status of the study subjects (residence in the metropolitan area) was a possible factor affecting the carriage rate in this study. There were other limitations regarding the sampling method and examination approach in this study. Although nasopharyngeal swabbing is considered to be the gold standard for ascertaining S. pneumoniae carriage, just swabbing the nasopharynx may underestimate the level as it is not the only site where S. pneumoniae resides. The respiratory tract, sinuses and nasal cavity are parts of the host body that are usually infected as well. Throat swabs, nasal swabs, or sputum may also be used for detecting S. pneumoniae colonization [27].

In Taiwan, serotypes 14, 23F, 6B, 19F and 3 were the most common invasive pneumococcal isolates in the pre-PCV7 era (before 2005) [28]. We observed that in the clinical isolates only six serotypes belonged to PCV13 serotypes, and in the third year of the current study (2014–2015) pneumococcal isolates related to serotype 14, 23F and 3 isolates were not detected. The proportion of PCV13 serotype isolates decreased by 47% from 83%, while the non-PCV13 serotypes increased from 17 to 56%. This remarkable turnaround in the prevalence of invasive pneumococcal isolates in children could be directly linked to the introduction of PCV7 in 2005 and PCV13 in 2011 [9, 28, 29]. After the introduction of PCV7, serogroup 19 (19A and 19F) clinical isolates increased with time, but the percentage of serotype 19A increased significantly from 5.5% in 2008 to 25.3% in 2012, with it becoming
the most prevalent serotype in 2010 [29, 30]. In the current study we observed that in the early PCV13 era serotype 19A isolates decreased from 54.9% in 2012–2013 to 23.5% in 2014–2015. Recently, Kaur et al. [31] from the USA reported that the most common serotypes identified during the PCV7 era were 19A and 23B, compared with 35B, 23B and 21 in the PCV13 era. Our data show that in the early PCV13 era in Taiwan a single ‘19A-like’ serotype did not emerge, but isolates belonging to serotypes 15A, 15B and 23A might emerge as the most prevalent serotypes with time [9, 18]. Finally, during the study period 2014–2015 the serotypes found in carriage isolates in children and clinical isolates were similar. Among the carriage isolates, a higher percentage belonged to serogroup 15 when compared to serogroup 19 (26.6 vs 6.66%, 2014–2015; P=0.003). Therefore, clinical isolates belonging to serogroup 15 were more prevalent in this region than those belonging to serogroup 19 (44.1 vs 32.3%, 2014–2015). Similarly, in Hong Kong, carriage of serogroup 15 was more common among children vaccinated with PCV13 [32]. In conclusion, the carriage rate in the study population was 12.4% in children and very low (3.6%) in their households. However, the isolation of non-vaccine serotypes (serogroup 15) and unknown serotypes after the introduction of PCV13 in children highlights the serotype replacement phenomenon and the importance of continued surveillance for emerging serotypes.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

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