Vaccine 37 (2019) 4068–4075

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pneumococcal carriage in children in Ulaanbaatar, Mongolia before and one year after the introduction of the 13-valent pneumococcal conjugate vaccine

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ARTICLE INFO

Article history:
Received 18 February 2019
Received in revised form 24 May 2019
Accepted 26 May 2019
Available online 4 June 2019

Keywords:
13-valent pneumococcal conjugate vaccine impact
Pneumococcal carriage
Children
Mongolia
Herd immunity
Antimicrobial resistance

ABSTRACT

Background: Nasopharyngeal carriage of *Streptococcus pneumoniae* precedes disease, is the source of pneumococcal community spread, and the mechanism for herd protection provided by pneumococcal conjugate vaccines (PCVs). There are few PCV impact studies in low- and middle-income countries, particularly in Asia. In 2016, Mongolia introduced the 13-valent PCV (PCV13) in a phased manner using a 2 + 1 schedule, with catch-up. We aimed to assess the impact of PCV13 introduction on nasopharyngeal pneumococcal carriage and density in children in Mongolia.

Methods: We conducted two cross-sectional carriage surveys (pre- and one year post-PCV) at community health clinics in two districts of the capital city, Ulaanbaatar in both May-July 2015 and 2017. The study analysis included 961 children too young to be vaccinated (5–8 weeks old) and 989 children eligible for vaccination (12–23 months old). Pneumococci were detected by quantitative real-time PCR and molecular serotyping performed using DNA microarray.

Findings: One year post-PCV introduction, PCV13 serotype carriage reduced by 52% in 12–23 month olds (adjusted prevalence ratio [aPR] 0.48 [95% confidence interval [CI] 0.39–0.59]), with evidence of non-PCV13 serotype replacement (aPR 1.55 [95% CI 1.30–1.85]), compared with the pre-PCV period. In 5–8 week olds, PCV13 serotype carriage reduced by 51% (aPR 0.49 [95% CI 0.33–0.73]) with no significant change in non-PCV13 serotype carriage (aPR 1.10 [95% CI 0.83–1.46]). An increase was observed in both PCV13 and non-PCV13 pneumococcal density post-PCV introduction. Antimicrobial resistance (AMR) genes were common, with 82.3% of samples containing at least one of the 10 AMR genes assessed.

Conclusion: This study demonstrates substantive PCV13 impact on pneumococcal carriage one year post-vaccine introduction in Mongolia. The reductions in PCV13 serotype carriage are likely to result in reductions in pneumococcal disease including indirect effects. Increases in non-PCV13 serotypes require further monitoring.

1. Background

Streptococcus pneumoniae (the pneumococcus) remains an important cause of childhood mortality in the post-pneumococcal conjugate vaccine (PCV) period, causing an

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https://doi.org/10.1016/j.vaccine.2019.05.078
0264-410X/

estimated 318,000 deaths globally in children aged 1–59 months in 2015 [1]. Pneumococcal carriage is considered a prerequisite for disease [2] and the source of community pneumococcal spread. Children vaccinated with PCV are less likely to carry vaccine serotypes, and therefore less likely to transmit these serotypes to unvaccinated individuals [3], resulting in indirect effects [4]. There are limited data on herd protection for infants too young to be vaccinated [5,6]. Over time, elimination of vaccine serotypes results in serotype replacement, with increased colonization and disease due to non-vaccine serotypes [7]. Serotype replacement may erode the public health benefit provided by PCV [7,8].

In settings with limited invasive pneumococcal disease (IPD) surveillance to measure vaccine impact, carriage is a valid alternative approach to demonstrate PCV effects [9]. PCV introduction reduces antimicrobial resistance in some settings, as vaccine serotypes are more likely to be resistant compared with non-vaccine serotypes [10]. Although there is increasing evidence documenting the importance of pneumococcal nasopharyngeal density on disease and transmission, the effect of PCVs on pneumococcal density is largely unknown [6,11–13]. PCVs have been introduced into the national immunization programmes of 59 low- and middle-income countries (LMICs) as part of the Gavi pneumococcal Advance Market Commitment funding scheme [14]. There are limited data on vaccine impact using carriage data in LMIC settings, particularly from Asia.

The study aim was to investigate the impact of 13-valent PCV (PCV13) introduction in Mongolia on pneumococcal carriage using two cross-sectional community carriage surveys, conducted pre- and one year post-PCV13 introduction in children aged 12–23 months and 5–8 weeks. These age groups were selected to represent vaccine-eligible children and young, unvaccinated infants, respectively.

2. Methods (see supplement for full details of laboratory methods)

2.1. Study site

Mongolia is a middle-income landlocked country in Central Asia with an estimated population of 3 million. The capital city, Ulaanbaatar, is divided into nine districts. From 2016, the Government of Mongolia introduced PCV13 in a phased manner (by district) into the routine immunization schedule at 2, 4 and 9 months of age. PCV was introduced into the two study districts in Ulaanbaatar (Songinokhairkhan and Sükhbaatar) in June 2016 with a catch-up campaign for children 3 to 23 months of age (given two doses of PCV13, two months apart).

2.2. Study design and participants

Cross-sectional carriage surveys were conducted at family health centres in the two districts from May-July in both 2015 (pre-PCV introduction) and 2017 (one year post-PCV introduction). The study districts included 51 subdistricts; each subdistrict has a family health centre and is defined by a predominant housing type. Family health centres were randomly selected stratified by predominant subdistrict housing type: apartment, ger (traditional portable dwelling) and mixed. Where insufficient patient numbers were available in a given health centre, children were recruited from other health centres in the same district with a similar profile. Similar numbers of children were enrolled from the 5–8 week and 12–23 month age group from a total of 23 clinics in 2015 and 32 clinics in 2017.

Caregivers of children within the two age groups were contacted by telephone to attend the clinic for the study if they were willing. Children in the eligible age groups who were attending the clinics for immunization or other routine well-child clinic visits were also invited to participate. Children were ineligible if they had a fever (>37 °C), had not lived in the area for at least three consecutive months, or if they were 5–8 weeks old and had received PCV13. Clinicians actively excluded children with respiratory symptoms. Study staff completed a questionnaire documenting potential risk factors for pneumococcal carriage. PCV13 vaccination status was verified using the child's hand held vaccination card and confirmed against the electronic immunization register [15].

2.3. Sample size

Sample size calculations assumed that with a baseline PCV13 serotype carriage prevalence of 16%, 281 participants in each age group would detect a 50% reduction, with 90% power at a 5% significance level. As the true prevalence in Mongolia was unknown, the sample size for each age group was increased to 500 per survey.

2.4. Ethics approval

The study was approved by the Mongolian Ministry of Health National Ethics Committee for Health Research and the Royal Children's Hospital Human Research Ethics Committee (HREC 33203A). Written informed consent was obtained from parents/caregivers for all children prior to any study procedures being conducted.

2.5. Sample collection and laboratory procedures

We used World Health Organization recommended methods [16] for nasopharyngeal sample collection, handling and transport. Pneumococcal detection, quantification by real-time PCR, and molecular serotyping by DNA microarray was carried out as previously described [17,18]. Samples that were *lytA* qPCR positive (Ct value <35) but not able to be serotyped (either culture negative or low DNA yield from culture) were considered pneumococcal positive, serotype unknown [17]. PCV13 serotypes were defined as 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. All other serotypes, including non-encapsulated pneumococci as described by Salter et. al. [19], were designated non-PCV13 serotypes. The microarray can detect and discriminate seven genetic variants of non-encapsulated pneumococci [18,19].

The microarray detects 10 antimicrobial resistance (AMR) genes. To examine the presence of AMR genes, we restricted the analysis to samples containing a single pneumococcal type with no other species identified.

2.6. Statistical analysis

Questionnaires were double entered into Microsoft Access databases. The two databases were compared, cleaned and analysed using Stata version 15.1 (College Station, TX: StataCorp LLC). A chi-squared test was used to compare categorical data and the Mann-Whitney test for continuous data.

Univariate log-binomial regression was used to determine associations between overall pneumococcal carriage and potential confounders. Variables with a p-value <0.20 were included in log-binomial regression models for multivariable analysis to estimate adjusted prevalence ratios. All non-significant factors (assessed at p-value >0.05) were dropped from the multivariable models employing stepwise regression starting with predictors with the highest p-values. Reductions in PCV13 carriage were calculated as $(1 - \text{aPR}) * 100\%$, while an increase in non-PCV13 carriage was reported as a fold increase. We used logistic regression to explore risk factors for overall pneumococcal carriage in both age groups as

the log-binomial regression model did not converge for the older age group.

Bacterial density data were $\log_{10}$ transformed and reported as $\log_{10}$ genome equivalents/ml ($\log_{10}$ GE/ml). Median carriage densities were calculated for vaccinated (≥1 PCV13 doses) and unvaccinated 12–23 month old children (0 doses of PCV13), with unvaccinated children from the post-PCV13 period excluded from the analysis. Median densities were compared using quantile regression to determine the impact of PCV13 on pneumococcal density. Median quantile regression estimates the median of the dependent variable (pneumococcal density), conditional on the value of the independent variable (PCV13 vaccination). With a binary predictor, the regression coefficient is the difference in medians between unvaccinated and vaccinated children. No variables were found to be significantly associated with density (assessed at p-value <0.05) and unadjusted results are presented. When the analysis was performed including unvaccinated children from the post-PCV period in the comparison group the results were very similar (data not shown).

3. Results

3.1. Participant characteristics

There were 2000 children enrolled; 50 children did not meet the age inclusion criteria in 2015 and were excluded from the analysis. Characteristics of the 1950 eligible children were similar between the two survey years, with the exception of crowding, income, electricity usage, and housing (Table 1). In the post-PCV13 survey, 84% (418/500) of participants aged 12–23 months had received two or more doses of PCV. No 5–8 week old infants were PCV-vaccinated in either year.

3.2. Risk factors for pneumococcal carriage

On multivariable analysis, risk factors for overall pneumococcal carriage were similar in both age groups in both years (Supplementary Table S1). Living in a ger, and the presence of multiple children aged under 5 years in the household were associated with pneumococcal carriage, whereas higher maternal education was associated with a lower likelihood of carriage in children. Exposure to household cigarette smoke was associated with pneumococcal carriage in the post-PCV period only.

3.3. Carriage prevalence in the pre- and post-PCV period

There was no change in overall pneumococcal carriage prevalence in either age group (Table 2). In children aged 12–23 months, there was a 52% reduction in PCV13 serotype carriage (adjusted prevalence ratio [aPR] 0.48 [95% confidence interval [CI] 0.39–0.59]), with evidence of serotype replacement (1.6 fold increase in non-PCV13 serotypes; aPR 1.55 [95% CI 1.30–1.85]). In 5–8 week old infants there was a 51% reduction in PCV13 serotype carriage (aPR 0.49 [95% CI 0.33–0.73]) compared with the pre-PCV period; there was no change in non-PCV13 serotype carriage prevalence (aPR 1.10 [95% CI 0.83–1.46]).

3.4. Serotypes over time

Serotyping results were obtained from 811 of 824 pneumococcal-positive samples. Thirteen samples were not able to be serotyped because they were either culture negative (n = 12) or had a low DNA yield from culture (n = 1). A total of 997 pneumococci were identified, 861 capsular pneumococci,

Table 1
Characteristics of study participants in cross-sectional pneumococcal carriage surveys, performed pre- and one year post-PCV13 (2015 and 2017 respectively), in healthy children in Mongolia, by age group and time period.

5–8 week old infants	Pre-PCV13 N = 461[a]	Post-PCV13 N = 500[a]	p-value
Median age in weeks (IQR)[b]	6.4 (5.3–7.7)	6.1 (5.4–7.0)	0.05
Female sex, n (%)	222 (48)	244 (49)	0.84
Parent primary caregiver, n (%)	457 (99)	495 (99)	0.83
≥2 children <5 years in household, n (%)	177 (38)	217 (44)	0.11
Exposure to household cigarette smoke, n (%)	28/441 (6)	33 (7)	0.88
Mother completed university, n (%)	260/455 (57)	287/499 (58)	0.91
Crowding[c], n (%)	172 (37)	172 (34)	0.35
Above minimum income[d], n (%)	287/435 (66)	188/489 (39)	<0.001
Born by caesarean section, n (%)	141/457 (31)	147 (29)	0.62
Breastfeeding at time of survey, n (%)	439 (95)	482 (96)	0.36
Living in informal housing (ger), n (%)	153 (33)	154 (31)	0.43
Member of household treated for tuberculosis	15 (3)	12 (2)	0.42
Main source of cooking fuel, n (%)			
Electricity	184 (40)	228 (46)	0.002
Coal	253 (55)	239 (48)	
Wood	16 (4)	33 (7)	
Gas	5 (1)	0 (0)	
Other	3 (1)	0 (0)	
Previous admission for pneumonia, n (%)	7/457 (2)	7 (1)	0.87
12–23 month old children	**Pre-PCV13 N = 489[a]**	**Post-PCV13 N = 500[a]**	**p-value**
Median age in weeks (IQR)[b]	16.4 (14.1–20.7)	15.9 (14.1–19.5)	0.10
Female sex, n (%)	257 (53)	241 (48)	0.17
Parent primary caregiver, n (%)	410 (84)	423 (85)	0.75
≥2 children <5 years in household, n (%)	156 (32)	183 (37)	0.12
Exposure to household cigarette smoke, n (%)	55/488 (11)	54 (11)	0.81
Mother completed university, n (%)	283/487 (58)	296 (59)	0.73
Crowding[c], n (%)	206 (42)	176 (35)	0.03
Above minimum income[d], n (%)	277/465 (60)	188/486 (39)	<0.001
Born by caesarean section, n (%)	142 (29)	161 (32)	0.29
Breastfeeding at time of survey, n (%)	329 (67)	341 (68)	0.76
Living in informal housing (ger), n (%)	189 (39)	156 (31)	0.01
Member of household treated for tuberculosis	19 (4)	6 (1)	0.007
Main source of cooking fuel, n (%)			
Electricity	186 (38)	218 (44)	0.001
Coal	279 (57)	230 (46)	
Wood	16 (3)	37 (7)	
Gas	2 (0.4)	1 (0.2)	
Other	6 (1)	14 (3)	
Previous admission for pneumonia, n (%)	124 (25)	126 (25)	0.95
Vaccinated with any number of PCV13 doses			
Received 1 dose, n (%)	0 (0)	21/439 (5)	
Received 2 doses, n (%)	0 (0)	339/439 (77)	
Received 3 doses, n (%)	0 (0)	79/439 (18)	

[a] Total unless otherwise indicated.
[b] IQR = Interquartile range.
[c] Three or more people per room.
[d] The definition of minimum living standard was changed by the Government of Mongolia between 2015 (170,000₮ per person/per month) and 2017 (241,000₮ per person/per month). This likely accounts for the some of the reduction in people earning above the standard in 2017.

Table 2
Carriage prevalence and prevalence ratios for pneumococcal carriage (overall, PCV13 serotypes, and non-PCV13 serotypes) for 5–8 week old infants and 12–23 month old children before (2015) and one year after (2017) PCV13 introduction.

	Pre-PCV13 n/N	Pre-PCV13 prevalence (%) (95% CI)	Post-PCV13 n/N	Post-PCV13 prevalence (%) (95% CI)	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio[a] (95% CI)
Overall pneumococci[b]						
5–8 week old	131/461	28.4 (24.3–32.8)	120/500	24.0 (20.3–28.0)	0.84 (0.68–1.04)	0.86 (0.70–1.05)
12–23 month old	294/489	60.1 (55.6–64.4)	279/499	55.9 (51.4–60.3)	0.93 (0.84–1.03)	0.96 (0.87–1.05)
PCV13 serotypes						
5–8 week old	59/457	12.9 (10.0–16.3)	31/494	6.3 (4.3–8.9)	0.49 (0.32–0.74)	0.49 (0.33–0.73)
12–23 month old	206/488	42.2 (37.8–46.7)	98/497	19.7 (16.3–23.5)	0.47 (0.38–0.57)	0.48 (0.39–0.59)
Non-PCV13 serotypes						
5–8 week old	74/457	16.2 (12.9–19.9)	86/494	17.4 (14.2–21.0)	1.08 (0.81–1.43)	1.10 (0.83–1.46)
12–23 month old	129/488	26.4 (22.6–30.6)	200/497	40.2 (35.9–44.7)	1.52 (1.27–1.83)	1.55 (1.30–1.85)

[a] The following variables were used to adjust the prevalence ratios in each group: Overall pneumococcal group (two or more children under five years in the household, housing type, mother's education; cooking fuel); PCV13 serotype group (two or more children under five years in the household, housing type, member of household treated for tuberculosis); non-PCV13 serotype group (two or more children under five years in the household, housing type, mother's education) using multivariable log binomial regression models.
[b] Overall carriage prevalence does not necessarily equal the sum of PCV13 serotype and non-PCV13 serotype prevalence. This is due to multiple serotype carriage and/or exclusion of pneumococcal-positive samples for which the serotype was not determined.

and 136 non-encapsulated pneumococci representing four different genetic variants [19]. All PCV13 serotypes were detected, although serotype 1 (n = 2) and 5 (n = 1) were rare.

Fig. 1A shows carriage prevalence of PCV13 serotypes and the most common non-PCV13 serotypes in infants 5–8 weeks, pre- and post-PCV13 introduction. In infants 45% (59/131) of pneumococci were PCV13 serotypes in the pre-PCV13 period, compared with 26% (31/120) in the post-PCV13 period (p = 0.002). The most common serotypes carried in the pre-PCV13 period were 19F (n = 13), 23F (n = 13) and non-encapsulated NT2 (n = 12); while in the post-PCV13 period, serotypes 15A (n = 11) and 23A (n = 11) predominated. PCV13 serotype 23F decreased between the pre-PCV13 (13/457 [2.8%]) and post-PCV13 survey (3/494 [0.6%], p = 0.007), while carriage prevalence of non-PCV13 serotypes 15A and 23A were higher in the post-PCV13 survey (both 11/494 [2.2%]) compared with the pre-PCV13 survey (both 2/457 [0.4%], p = 0.02).

For children aged 12–23 months (Fig. 1B), 206/294 (70.1%) of pneumococci were PCV13 serotypes in the pre-PCV13 survey, compared with 98/279 (35.1%) post-PCV13 introduction (p < 0.001). The most common serotypes identified in 2015 were 6A (n = 61), 19F (n = 47), non-encapsulated NT2 (n = 30) and 23F (n = 29) compared with 19F (n = 41), 15A (n = 36), 34 (n = 34) and 15B/C (n = 26) in 2017. PCV13 serotypes 23F and 6A decreased between the pre-PCV13 (29/488 [5.9%] and 61/488 [12.5%], respectively) and post-PCV13 survey (12/497 [2.4%], p = 0.005 and 17/497 [3.4%], p < 0.001, respectively). Similarly, serotypes 14 (p < 0.001) and 6B (p = 0.02) were significantly reduced post-PCV introduction. Interestingly, PCV13 serotypes 19F (47/488 [9.6%] to 41/497 [8.2%], p = 0.44) and 19A (13/488 [2.7%] to 8/497 [1.6%], p = 0.25) showed no significant reduction. Non-PCV13 serotypes 15A and 34 increased in the post-PCV13 survey (36/497 [7.2%] and 34/497 [6.8%], respectively) compared with the pre-PCV13 survey (3/488 [0.6%] and 7/488 [1.4%], respectively, both p-values < 0.001).

Most pneumococcal-positive samples contained a single serotype (666/811, 82.1%). Less than 10% of colonized infants aged 5–8 weeks (21/241, 8.7%) carried more than one serotype, while 21.8% (124/570) of older colonized children carried two to four serotypes. The proportion of children with multiple serotype carriage did not differ significantly pre- and post-PCV13 introduction: 2.7% and 1.8% (p = 0.37) for 5–8 week old infants; and 13.1% and 12.1% (p = 0.62) for 12–23 month old children, respectively.

3.5. Antibiotic resistance genes

AMR genes were common, with 82.3% of samples containing at least one of the 10 AMR genes assessed. Samples with a PCV13 serotype were more likely to have at least one AMR gene detected (95.0%, 264/278), compared with samples with non-PCV13 serotypes (71.0%, 220/310) (Supplementary Table S2). Four AMR genes were significantly more common in PCV13 serotypes (N = 278) compared with non-PCV13 serotypes (N = 310); *tetM* 258 (92.8%) versus 189 (61.0%), *cat* 87 (31.3%) versus 52 (16.8%), *mefA* 138 (49.6%) versus 62 (20.0%) and *ermB* 199 (71.6%) versus 128 (41.3%).

In children 12–23 months, the proportion of samples containing *cat* decreased post-PCV13 (54/204, 26.5% to 26/189, 13.8%), likely due to decreases in PCV13 serotypes carrying this gene; no significant changes were observed in infants (Supplementary Table S3).

3.6. Pneumococcal densities

The density of pneumococcal carriage for overall pneumococci, PCV13 serotypes, and non-PCV13 serotypes were significantly higher in the post-PCV13 period compared with the pre-PCV13 period for both age groups (all p-values < 0.001, Fig. 2A/B). In 12–23 month old children, the median density of overall pneumococci, PCV13 and non-PCV13 serotypes was higher in PCV13 vaccinated (≥1 dose) compared with unvaccinated (0 doses) children (Table 3).

4. Discussion

There are limited data on PCV impact from LMICs in Asia. We demonstrated a substantial impact of PCV13 introduction in healthy children in Mongolia, one year post-introduction. PCV13 serotype carriage was reduced by more than 50% in children aged 12–23 months and unvaccinated 5–8 week old infants. Indirect protection is particularly important in young infants, as they are at high risk for bacterial infections, and the case fatality rate for pneumococcal infections in neonates is high [20].

Characteristics of children were similar between the two survey years, with the exception of crowding, housing, electricity usage, and income. The reduction in the proportion of households earning above minimum income level between the two years was likely in

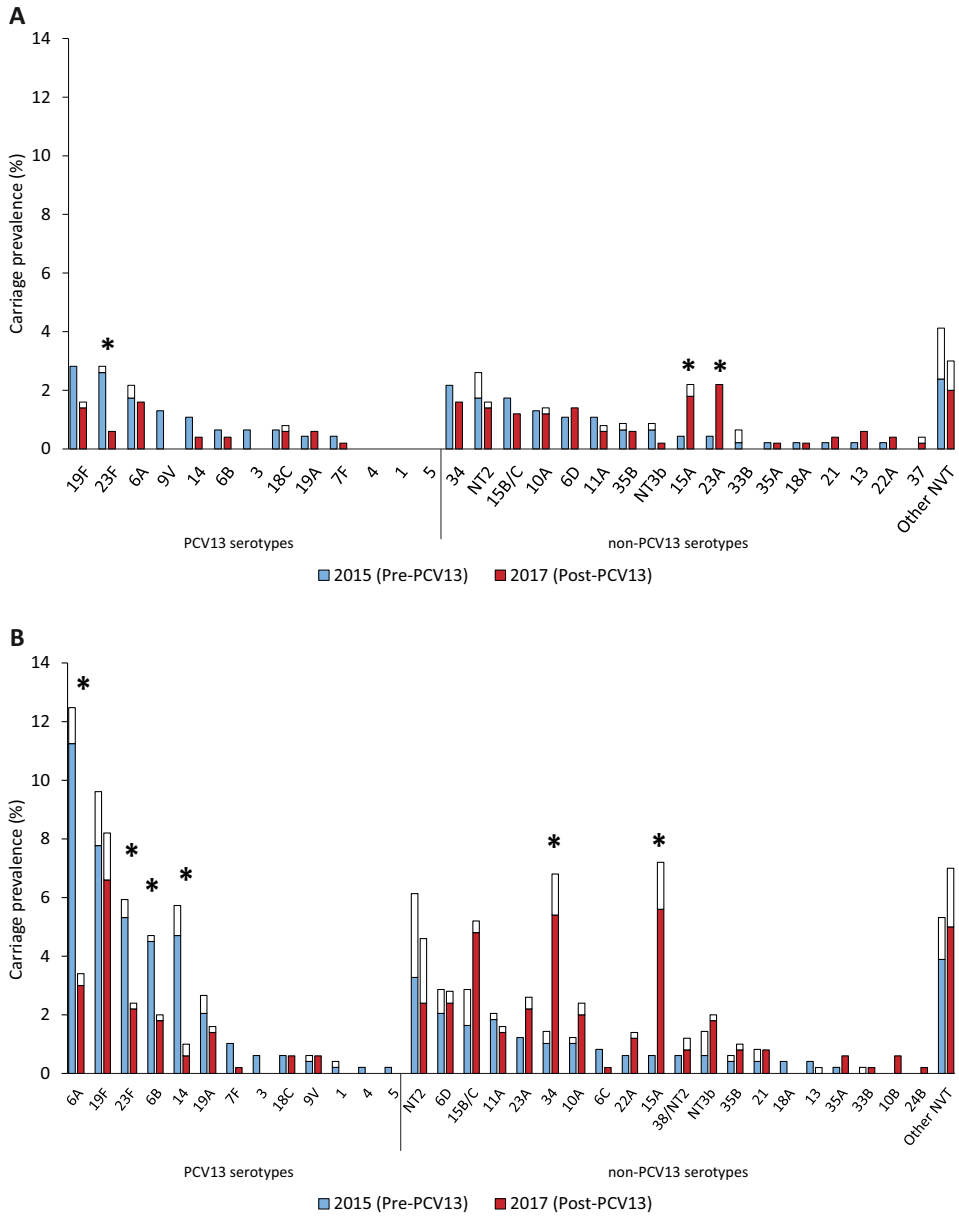

Fig. 1. Carriage prevalence of PCV13 serotypes and the most common non-PCV13 serotypes in (A) 5–8 week old infants and (B) 12–23 month old children, before (pre-PCV13) or after (post-PCV13) vaccine introduction. Solid bars indicate carriage that was detected as a single or major (dominant) serotype, open bars indicate carriage that was detected as a minor (second or third) serotype. Serotypes 15B and 15C were reported as 15B/C [39] and serotype 11F-like were grouped together with 11A serotypes [40]. NT2 refers to non-encapsulated pneumococci containing *nspA*/*pspK* [19]. Significant differences between pre-/post-periods are indicated with an asterisk.

part due to the definition change in minimum living standard in 2017 by the Government of Mongolia.

Most published studies on the effect of PCV introduction are from higher income countries, focus on hospitalized children and in the case of PCV13 introduction had preceding PCV7. Previous work by our group examined community impact of PCV13 on nasopharyngeal carriage in Lao PDR two years following introduction (3 + 0 schedule with limited catch-up) [17], finding a 23% reduction in carriage prevalence of PCV13 serotypes with no significant difference in non-PCV13 serotypes in vaccinated 12–23 month old children, and suggestive but non-significant changes in 5–8 week old infants [17]. Other published work from Greenland (2 + 1 schedule) showed a 56% reduction in PCV13 serotype carriage three years post-PCV13 introduction, compared with one year post introduction [21]. In LMICs four published studies described PCV10 effects on carriage in non-hospitalized children without prior PCV7 use. A cross-sectional carriage study in Kenya (3 + 0 schedule with catch-up) showed a reduction of PCV10 serotype carriage in individuals aged <5 years by 64% two years post-introduction [22]. In Brazil (3 + 1 schedule with catch-up), a 91% reduction in PCV10 serotype carriage in children 12–23 months of age was observed three years following introduction [23]. In Fiji, (3 + 0 schedule with no catch-up) PCV10 carriage in 5–8 week old unvaccinated infants and 12–23 month old children declined by 44% and 66% respectively three years following PCV10 introduction [18]. Lastly, in Mozambique (3 + 0 schedule with no catch-up) PCV10 carriage declined by 42% in HIV-uninfected vaccinated children aged <5 years less than two years post-PCV10 introduction [24].

Even though our study was only one year post-PCV introduction, we showed stronger reductions than Lao PDR, and similar reductions to some of the PCV10 studies that were conducted

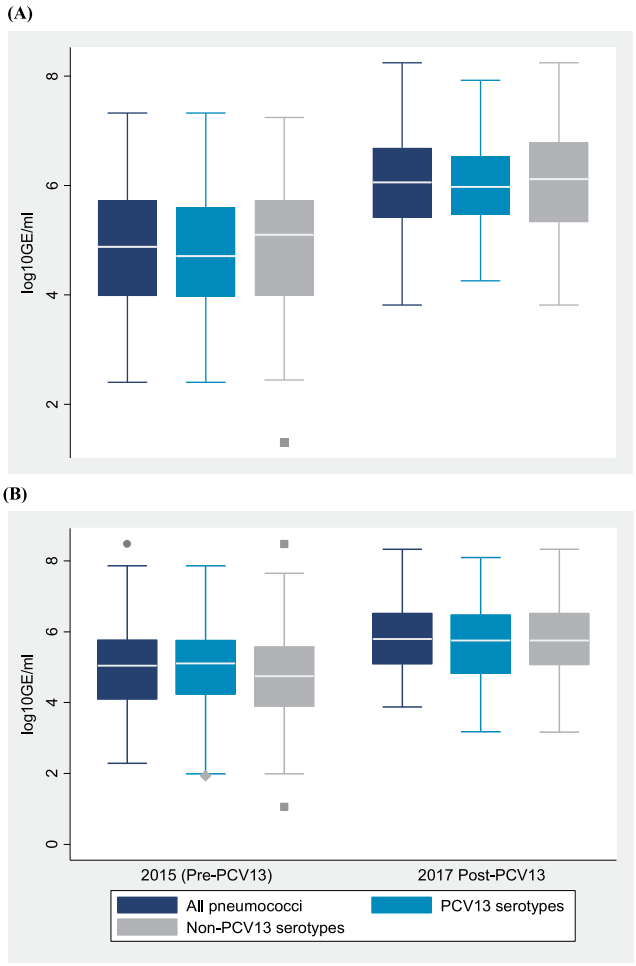

Fig. 2. Nasopharyngeal pneumococcal carriage density (log_{10} genome equivalents/ml) in (A) 5–8 week old infants and (B) 12–23 month old children who were positive for pneumococcal carriage. Boxes depict interquartile range (IQR) with a central line at the median, and whiskers extend 1.5 times IQR past the quartiles. Values outside whiskers plotted as individual points. For both age groups, the median density of overall pneumococci, PCV13 serotypes, and non-PCV serotypes was higher post-PCV13 compared with pre-PCV13 introduction ($p < 0.001$).

two or more years post-introduction. Differences in the extent of PCV impact on carriage reduction are likely impacted by a number of factors including number of years' post-PCV introduction, differences in baseline demographics, baseline serotype distribution, vaccination schedule, catch-up campaigns and PCV coverage. Of note, there is high PCV13 coverage in Mongolia (estimated at 97% in 2017 [25]) and extensive catch-up campaigns in children up to 24 months of age were conducted in the two study districts.

Although PCV13 has been shown to generate good functional antibody responses against 19F and 19A [26], we observed very little reduction in these two serotypes; however variable reductions have been seen for 19A [27,28] and 19F [18] carriage in other studies.

Our results are consistent with other studies demonstrating serotype replacement in carriage in vaccinated children [22,23]. Non-vaccine serotypes 15A, 15B/C and 34 increased post-introduction in our older age group, while in the 5–8 week infants 15A and 23A increased. Increases in nasopharyngeal carriage of non-PCV13 serotypes were shown in healthy US children aged 6–30 months in the 5 years following PCV13 introduction [29]. Serotypes 15A and 23B have been shown to cause increases in IPD post-PCV13 introduction [30]. Even though there was no change in overall carriage prevalence of non-PCV13 serotypes in the 5–8 week olds in our study, we expect that this will increase over time.

The proportion of children with multiple serotype carriage was low in our study, and we did not find any changes in multiple serotype carriage following the introduction of PCV, which is similar to some previous studies [17,31].

We identified a number of risk factors for pneumococcal carriage which were similar to previous studies. This included the presence of multiple children under the age of 5 years in the household [32] and exposure to cigarette smoke in the household [33].

In many Asian countries, community usage of antibiotics results in high levels of AMR [34] and data from Mongolia are consistent. Inappropriate prescription of antibiotics for pneumonia [35] and high antibiotic resistance in ICU patients [36] has been shown. In our study, 82% of pneumococcal positive samples had at least one AMR gene, with a higher proportion of four AMR genes in PCV13 serotypes than non-vaccine serotypes. PCV-mediated effects on AMR in Mongolia may be less than those observed in other settings [10] given the high background level of circulating AMR genes. Analysis of AMR genes in our study was limited to the 10 mobile-genetic element associated genes included on the microarray, and some antibiotics of clinical importance, such as penicillin, were not assessed, nor was phenotypic testing conducted.

This study showed an increase in both PCV13 and non-PCV13 density post-PCV introduction. Some studies [6,11–13,17,18] have evaluated PCV impact on pneumococcal density, with varying results, but methodology and study timing differed. These include a cluster-randomized trial in The Gambia which showed a decline

Table 3
Median density and quantile regression analysis of overall pneumococci, PCV13 serotypes, and non-PCV13 serotypes in PCV13 vaccinated (≥1 dose) and unvaccinated (0 doses) 12–23 month old children who were pneumococcal carriers in 2015 and 2017.

	Median density (IQR)[a]	Unadjusted coefficient (95% CI)[b]	p-value[c]
Overall pneumococci			
Unvaccinated (294)[d]	5.04 (4.10, 5.76)	Reference	
PCV13 vaccinated (239)[d]	5.87 (5.10, 6.56)	0.83 (0.55–1.10)	<0.001
PCV13 serotypes			
Unvaccinated (206)[d]	5.11 (4.23, 5.76)	Reference	
PCV13 vaccinated (78)[d]	5.80 (4.78, 6.51)	0.65 (0.23–1.06)	0.002
Non-PCV13 serotypes			
Unvaccinated (129)[d]	4.79 (4.02, 5.67)	Reference	
PCV13 vaccinated (178)[d]	5.83 (5.08, 6.51)	1.08 (0.72–1.44)	<0.001

[a] Density reported in log_{10} genome equivalents/ml and interquartile range (IQR).
[b] Unadjusted coefficient (difference in medians) as determined by quantile regression, reported with 95% confidence intervals (CI).
[c] P-value refers to difference in median densities between vaccinated and unvaccinated groups.
[d] Number of pneumococcal carriers shown in parentheses. Unvaccinated children only included those from the pre-PCV13 period. Vaccinated children included children who had received one or more doses of vaccine.

in vaccine and non-vaccine serotype density in vaccinated and control villages [13], and a study from Lao PDR [17] which found that both VT and NVT pneumococcal density increased post-PCV introduction, which was thought to be due to temporal variation and/or secular trends. All the available literature does not show a consistent long-term effect of PCV on pneumococcal density.

This study has some limitations. Firstly, older age groups were not included, however we would expect to see indirect effects of PCV introduction as observed in other settings [37]. Secondly, our study had a relatively short follow-up post-PCV introduction, and so an effect on pneumococcal density may not yet be apparent [24]. Lastly, it is possible that information on all relevant confounders influencing the vaccine type carriage endpoint were not collected in this study. This study also has a number of strengths. Firstly, we sampled clinics randomly according to subdistrict to be representative of the different housing types in Ulaanbaatar, including to account for difference in health seeking behaviour. Therefore, it is likely that our findings are generalizable to other urban populations in Mongolia. Secondly, we used sensitive molecular methods [16,38] to measure the prevalence and density of pneumococcal carriage, and were able to detect all pneumococcal serotypes present in a sample. Although carriage does not measure the impact of PCV on disease, it is a practical and biologically relevant end-point, with surveys able to be implemented quickly ahead of vaccine introduction. Thirdly, due to the high vaccine coverage achieved by the routine and catch-up campaigns for PCV, we were able to demonstrate evidence of PCV impact and serotype replacement one year following PCV13 introduction in Mongolia.

5. Conclusion

This study provides evidence of substantial PCV impact following introduction in a middle income country in Asia. The reduction in PCV13 serotype carriage will likely result in decreases in pneumococcal disease in both vaccine-eligible and unvaccinated age groups. Serotype replacement warrants ongoing monitoring.

Declaration of Competing Interest

CS and EMD received the Robert Austrian Research Award in Pneumococcal Vaccinology, which was funded by Pfizer, but awarded by ISPPD. CVM, EMD, EKM, CS and TM are investigators and MU, BS and DL are team members on a clinical research collaboration with Pfizer investigating the impact of PCV on adult pneumonia in Mongolia. JH is an investigator on studies conducted on behalf of St George's, University of London or BUGS Bioscience that are sponsored and/or funded by vaccine manufacturers, including Pfizer, GlaxoSmithkline and Sanofi Pasteur. JH is co-founder and board member of BUGS Bioscience, a not-for-profit spin-out company of St George's, University of London but JH receives no personal financial benefit from these activities. All the other authors have no declarations of interest to report with regards to this work.

Acknowledgements

We would like to acknowledge the Ministry of Health in Mongolia for their support in this project. We would also like to thank study staff, laboratory staff and participating families. Thanks to Katherine Gould from the Bacterial Microarray Group, St. George's University of London for technical advice regarding microarray.

Author's contributions

EKM, SLV and CS conceived the idea and designed the study. CVM, SLV, MU, BS, DL, DN, SD, CN and EKM supported the protocol development and coordinated study implementation for fieldwork. EKM oversaw the study with support from CvM and SLV. TM led the study in Mongolia. CS oversaw the microbiology. CS and EMD devised the microbiological approach and laboratory protocols, and supported field-based protocols for microbiological aspects. BS, BDO, CLP, MLN and AA managed and/or tested laboratory samples and cleaned laboratory data. JH interpreted microarray data. CVM, SLV, CN and EKM devised the analysis plan. CVM conducted data analysis. CVM drafted the manuscript with EMD, CS and EKM. All authors provided feedback to the draft manuscript and have read and approved the final version.

Funding statement

The project was funded by the Gavi Alliance (contract number PP61690717A2), with support from the Victorian Government's Operational Infrastructure Support Program. CS was supported by a NHMRC Career Development Fellowship (1087957) and a veski Inspiring Women Fellowship.

Data availability statement

All relevant data are within the paper and the supplementary information files.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.078.

References

[1] Wahl B, O'Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. Lancet Glob Health 2018;6:e744–57.
[2] Simell B, Auranen K, Kayhty H, Goldblatt D, Dagan R, O'Brien KL, et al. The fundamental link between pneumococcal carriage and disease. Expert Rev Vaccines 2012;11:841–55.
[3] Davis SM, Deloria-Knoll M, Kassa HT, O'Brien KL. Impact of pneumococcal conjugate vaccines on nasopharyngeal carriage and invasive disease among unvaccinated people: review of evidence on indirect effects. Vaccine 2013;32:133–45.
[4] Klugman KP. Herd protection induced by pneumococcal conjugate vaccine. Lancet Glob Health 2014;2:e365–6.
[5] Egere U, Townend J, Roca A, Akinsanya A, Bojang A, Nsekpong D, et al. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal carriage in newborns in rural Gambia: a randomised controlled trial. PLoS One 2012;7:e49143.
[6] O'Brien KL, Millar EV, Zell ER, Bronsdon M, Weatherholtz R, Reid R, et al. Effect of pneumococcal conjugate vaccine on nasopharyngeal colonization among immunized and unimmunized children in a community-randomized trial. J Infect Dis 2007;196:1211–20.
[7] Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet 2011;378:1962–73.
[8] Mulholland K, Satzke C. Serotype replacement after pneumococcal vaccination. Lancet. 2012;379:1387; author reply 8–9.
[9] Weinberger DM, Bruden DT, Grant LR, Lipsitch M, O'Brien KL, Pelton SI, et al. Using pneumococcal carriage data to monitor postvaccination changes in invasive disease. Am J Epidemiol 2013;178:1488–95.
[10] Ginsburg AS, Klugman KP. Vaccination to reduce antimicrobial resistance. Lancet Glob Health 2017;5:e1176–7.
[11] Dagan R, Juergens C, Trammel J, Patterson S, Greenberg D, Givon-Lavi N, et al. PCV13-vaccinated children still carrying PCV13 additional serotypes show similar carriage density to a control group of PCV7-vaccinated children. Vaccine 2017;35:945–50.
[12] Collins AM, Wright AD, Mitsi E, Gritzfeld JF, Hancock CA, Pennington SH, et al. First human challenge testing of a pneumococcal vaccine. Double-blind randomized controlled trial. Am J Respir Crit Care Med 2015;192:853–8.
[13] Roca A, Bottomley C, Hill PC, Bojang A, Egere U, Antonio M, et al. Effect of age and vaccination with a pneumococcal conjugate vaccine on the density of pneumococcal nasopharyngeal carriage. Clin Infect Dis 2012;55:816–24.
[14] International Vaccine Access Center (IVAC), Johns Hopkins Bloomberg School of Public Health. VIEW-hub Report: Global Vaccine Introduction and Implementation; June 2018.

[15] Chan J, Mungun T, Dorj N, Volody B, Chuluundorj U, Munkhbat E, et al. High agreement between the new Mongolian electronic immunization register and written immunization records: a health centre based audit. Western Pac Surveill Response J. 2017;8:5–10.
[16] Satzke C, Turner P, Virolainen-Julkunen A, Adrian PV, Antonio M, Hare KM, et al. Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. Vaccine 2013;32:165–79.
[17] Satzke C, Dunne EM, Choummanivong M, Ortika BD, Neal EFG, Pell CL, et al. Pneumococcal carriage in vaccine-eligible children and unvaccinated infants in Lao PDR two years following the introduction of the 13-valent pneumococcal conjugate vaccine. Vaccine 2019;37:296–305.
[18] Dunne EM, Satzke C, Ratu FT, Neal EFG, Boelsen LK, Matanitobua S, et al. Effect of ten-valent pneumococcal conjugate vaccine introduction on pneumococcal carriage in Fiji: results from four annual cross-sectional carriage surveys. Lancet Glob Health 2018;6:e1375–85.
[19] Salter SJ, Hinds J, Gould KA, Lambertsen L, Hanage WP, Antonio M, et al. Variation at the capsule locus, cps, of mistyped and non-typable *Streptococcus pneumoniae* isolates. Microbiology 2012;158:1560–9.
[20] Okike IO, Johnson AP, Henderson KL, Blackburn RM, Muller-Pebody B, Ladhani SN, et al. Incidence, etiology, and outcome of bacterial meningitis in infants aged <90 days in the United Kingdom and Republic of Ireland: prospective, enhanced, national population-based surveillance. Clin Infect Dis 2014;59:e150–7.
[21] Navne JE, Koch A, Slotved HC, Andersson M, Melbye M, Ladefoged K, et al. Effect of the 13-valent pneumococcal conjugate vaccine on nasopharyngeal carriage by respiratory pathogens among Greenlandic children. Int J Circumpolar Health 2017;76:1309504.
[22] Hammitt LL, Akech DO, Morpeth SC, Karani A, Kihuha N, Nyongesa S, et al. Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* in Kilifi, Kenya: findings from cross-sectional carriage studies. Lancet Glob Health 2014;2:e397–405.
[23] Brandileone MC, Zanella RC, Almeida SCG, Brandao AP, Ribeiro AF, Carvalhanas TMP, et al. Effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* among children in Sao Paulo. Brazil. Vaccine 2016;34:5604–11.
[24] Sigauque B, Moiane B, Massora S, Pimenta F, Verani JR, Mucavele H, et al. Early declines in vaccine type pneumococcal carriage in children less than 5 years old after introduction of 10-valent pneumococcal conjugate vaccine in Mozambique. Pediatr Infect Dis J 2018;37:1054–60.
[25] World Health Organization. WHO and UNICEF estimates of national immunization coverage; 2018.
[26] Grant LR, O'Brien SE, Burbidge P, Haston M, Zancolli M, Cowell L, et al. Comparative immunogenicity of 7 and 13-valent pneumococcal conjugate vaccines and the development of functional antibodies to cross-reactive serotypes. PLoS One 2013;8:e74906.
[27] Desai AP, Sharma D, Crispell EK, Baughman W, Thomas S, Tunali A, et al. Decline in pneumococcal nasopharyngeal carriage of vaccine serotypes after the introduction of the 13-valent pneumococcal conjugate vaccine in children in Atlanta, Georgia. Pediatr Infect Dis J 2015;34:1168–74.
[28] Lee GM, Kleinman K, Pelton SI, Hanage W, Huang SS, Lakoma M, et al. Impact of 13-valent pneumococcal conjugate vaccination on *Streptococcus pneumoniae* carriage in young children in Massachusetts. J Pediatric Infect Dis Soc 2014;3:23–32.
[29] Kaur R, Casey JR, Pichichero ME. Emerging *Streptococcus pneumoniae* strains colonizing the nasopharynx in children after 13-valent pneumococcal conjugate vaccination in comparison to the 7-valent era, 2006–2015. Pediatr Infect Dis J 2016;35:901–6.
[30] van der Linden M, Perniciaro S, Imohl M. Increase of serotypes 15A and 23B in IPD in Germany in the PCV13 vaccination era. BMC Infect Dis 2015;15:207.
[31] Brugger SD, Frey P, Aebi S, Hinds J, Muhlemann K. Multiple colonization with *S. pneumoniae* before and after introduction of the seven-valent conjugated pneumococcal polysaccharide vaccine. PLoS One 2010;5:e11638.
[32] Koliou MG, Andreou K, Lamnisos D, Lavranos G, Iakovides P, Economou C, et al. Risk factors for carriage of *Streptococcus pneumoniae* in children. BMC Pediatr 2018;18:144.
[33] Turner P, Melchiorre S, Moschioni M, Barocchi MA, Turner C, Watthanaworawit W, et al. Assessment of Streptococcus pneumoniae pilus islet-1 prevalence in carried and transmitted isolates from mother-infant pairs on the Thailand-Burma border. Clin Microbiol Infect 2012;18:970–5.
[34] Kim SH, Song JH, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in antimicrobial resistance and serotypes of *Streptococcus pneumoniae* isolates in Asian countries: an Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. Antimicrob Agents Chemother 2012;56:1418–26.
[35] Dorj G, Hendrie D, Parsons R, Sunderland B. An evaluation of prescribing practices for community-acquired pneumonia (CAP) in Mongolia. BMC Health Serv Res 2013;13:379.
[36] Bataar O, Khuderchuluun C, Lundeg G, Chimeddorj S, Brunauer A, Gradwohl-Matis I, et al. Rate and pattern of antibiotic resistance in microbiological cultures of sepsis patients in a low-middle-income country's ICU. Middle East J Anaesthesiol 2013;22:293–300.
[37] Loughlin AM, Hsu K, Silverio AL, Marchant CD, Pelton SI. Direct and indirect effects of PCV13 on nasopharyngeal carriage of PCV13 unique pneumococcal serotypes in Massachusetts' children. Pediatr Infect Dis J 2014;33:504–10.
[38] Satzke C, Dunne EM, Porter BD, Klugman KP, Mulholland EK. PneuCarriage project group. The pneucarriage project: a multi-centre comparative study to identify the best serotyping methods for examining pneumococcal carriage in vaccine evaluation studies. PLoS Med. 2015;12:e1001903.
[39] van Selm S, van Cann LM, Kolkman MA, van der Zeijst BA, van Putten JP. Genetic basis for the structural difference between *Streptococcus pneumoniae* serotype 15B and 15C capsular polysaccharides. Infect Immun. 2003;71:6192–8.
[40] Manna S, Ortika BD, Dunne EM, Holt KE, Kama M, Russell FM, et al. A novel genetic variant of *Streptococcus pneumoniae* serotype 11A discovered in Fiji. Clin Microbiol Infect 2018;24(428):e1–7.