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Supplementary appendix

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Supplement to: von Mollendorf C, Mungun T, Ulziibayar M, et al. Effect of pneumococcal conjugate vaccination on pneumococcal carriage in hospitalised children aged 2–59 months in Mongolia: an active pneumonia surveillance programme. *Lancet Microbe* 2024. https://doi.org/10.1016/S2666-5247(24)00171-X

Supplementary material for "Changes in pneumococcal carriage in hospitalised children 2-59 months of age in Mongolia following pneumococcal conjugate vaccine introduction."

1. Supplementary Methods

Sample collection

We used World Health Organization recommended methods for the collection, handling and transport of nasopharyngeal samples.[1] Hospital nurses trained in study procedures collected nasopharyngeal samples using paediatric flocked swabs (Copan Diagnostics). Swabs were immediately placed into 1 ml skim milk tryptone glucose glycerol (STGG) medium. The samples were stored in a dedicated study refrigerator for an average of 2-4 hours before been transported in temperature monitored cooler boxes to the National Center of Communicable Diseases (NCCD) bacteriology laboratory in Ulaanbaatar. Trained laboratory study staff at NCCD vortexed, aliquoted and stored samples at ultra-low temperatures within 7 hours of collection. Samples were shipped on dry ice to the Murdoch Children's Research Institute (Parkville, Australia) for laboratory testing.

Laboratory procedures

Real-time quantitative PCR targeting the *lytA* gene (*lytA* qPCR) was used to detect the presence of pneumococci. Genomic DNA was extracted from one STGG aliquot using the MagNA Pure LC machine (Roche).[2] *LytA* qPCR[3] was performed on all extracted DNA samples. Reactions were run on a Stratagene Mx3005 machine using 2 ml template DNA and Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technologies), according to manufacturer's instructions. Due to machine upgrades, DNA extraction of swabs were performed using the QIAcube HT machine (QIAgen) and *lytA* qPCR using the AriaMX (Agilent Technologies), for all samples collected from May 2018. Samples that were *lytA* qPCR positive (Ct value < 35) or equivocal (Ct value 35–40) were cultured for molecular serotyping by microarray.

Culture, DNA extraction and microarray

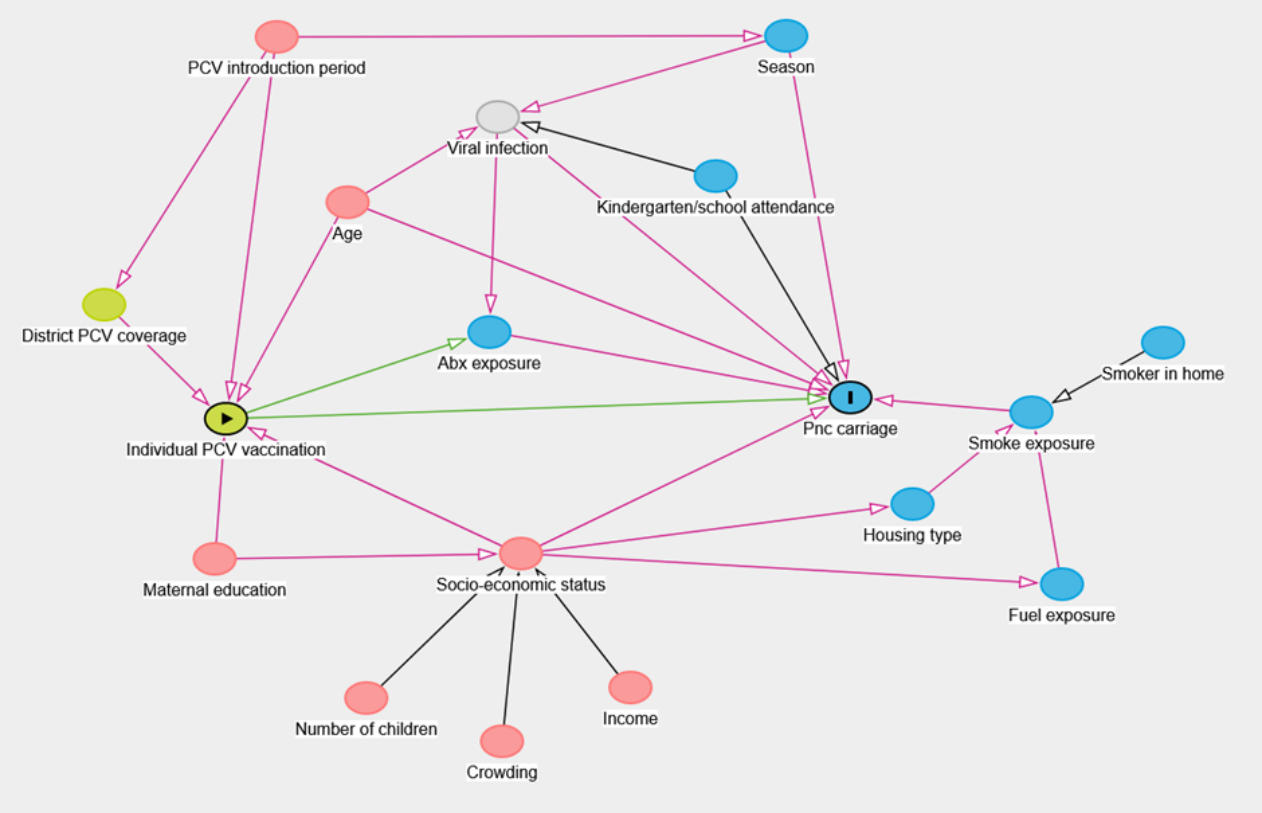
50 µl of STGG were cultured on horse blood agar containing 5 mg/ml of gentamicin (Oxoid). DNA was extracted from the harvested growth with the QIAcube HT instrument (Qiagen) and QIAamp 96 DNA QIAcube HT Kit (Qiagen), using a lysis buffer and RNase A treatment.[4,5] When only a single α-haemolytic colony grew, it was subcultured prior to DNA extraction for microarray. Molecular serotyping by microarray was performed on the extracted DNA using Senti-SPv1.5 microarrays (BUGS Bioscience), as described previously.[4] The microarray data was analysed using a custom web-based software that uses a Bayesian-based model.[6]

Statistical analysis

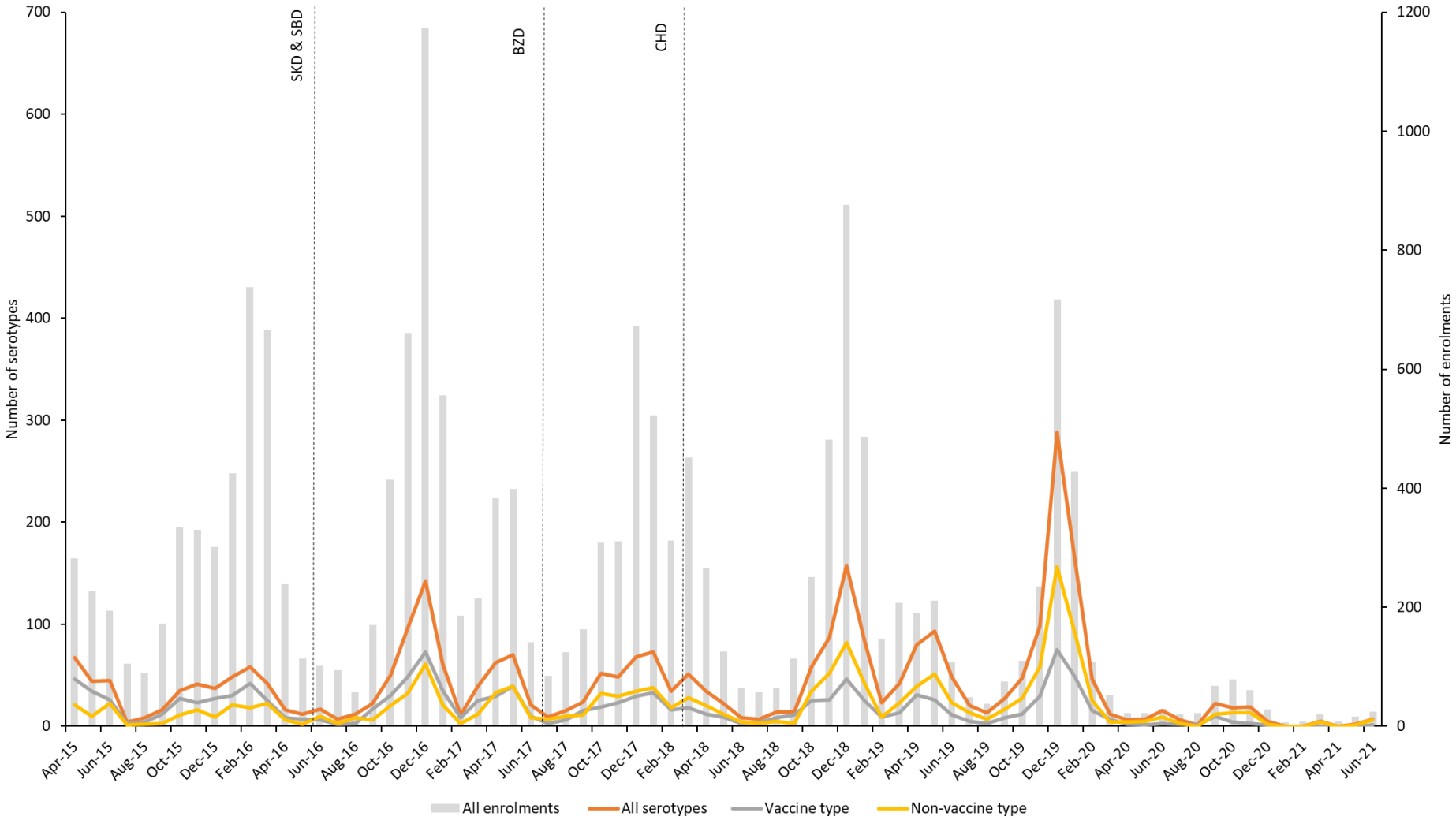
A separate model to determine vaccine impact included year as a continuous exposure variable to show year-on-year changes. Adjusted prevalence ratios were estimated overall, for individual districts and age groups.

Median densities were compared using quantile regression to determine the impact of PCV13 on pneumococcal density. A reduced common set of confounders was used to adjust the regression coefficient. Confounding variables were selected using the directed acyclic graph for carriage and relevance. For all ages combined we adjusted for age, informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding and household income. For stratified age groups we included the same variables except for age.

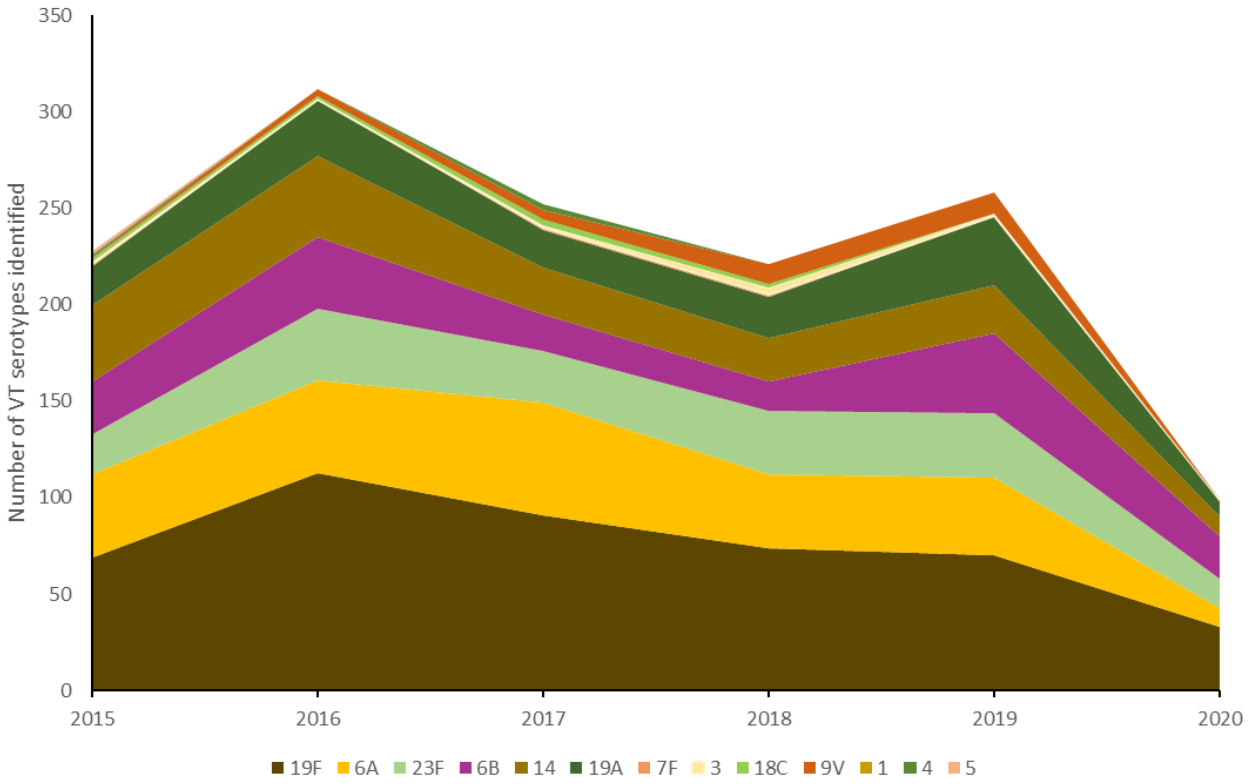
2. Supplementary Figures



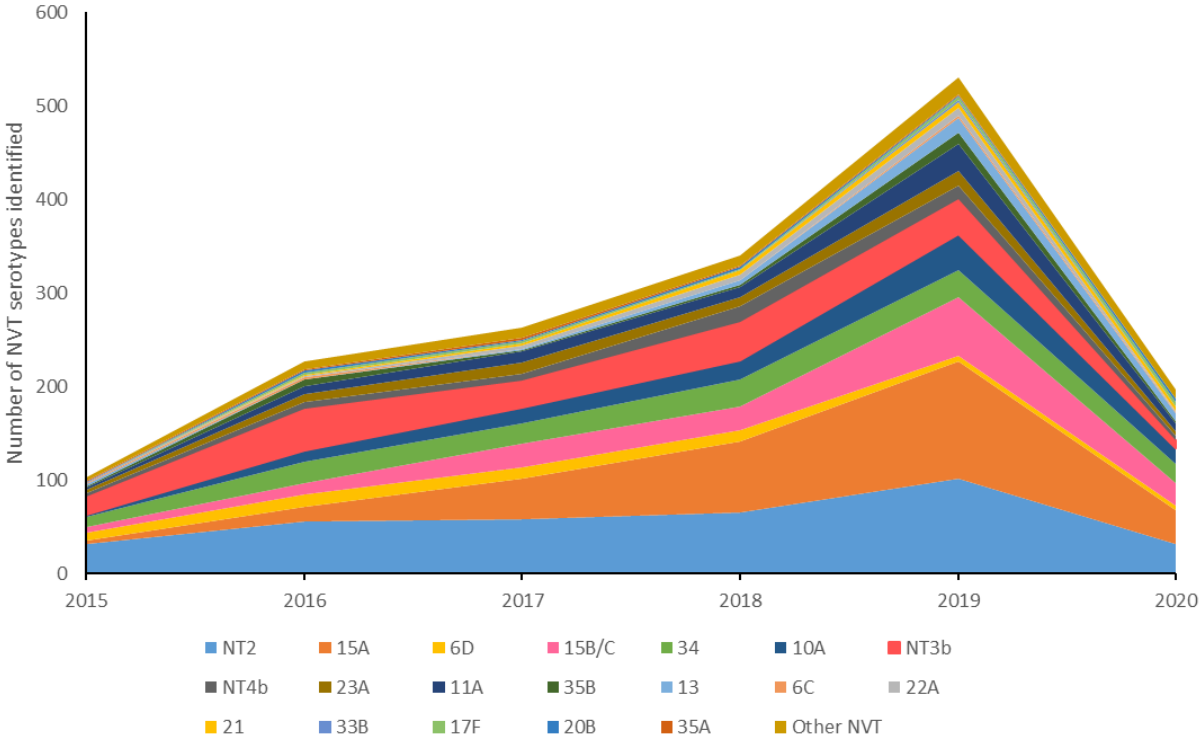
Supplementary Figure S1: Directed acyclic graph (DAG) of the association between PCV13 vaccination (exposure) and pneumococcal carriage (outcome)
The DAG was used to assist with the identification of potential confounders. The green line highlights the causal relationship under investigation and the pink lines highlight potential biasing pathways. The blue variables are ancestors of the outcome, yellow variables ancestors of the exposure and red variables are ancestors of both exposure and outcome. Grey variables represent unobserved variables.
Based on this diagram, we identified that adjusting for PCV introduction period (only for individual PCV vaccination), age group, housing-type, maternal education, household income, household crowding, number of children under five years of age, household fuel type, season and antibiotic exposure may block biasing pathways.



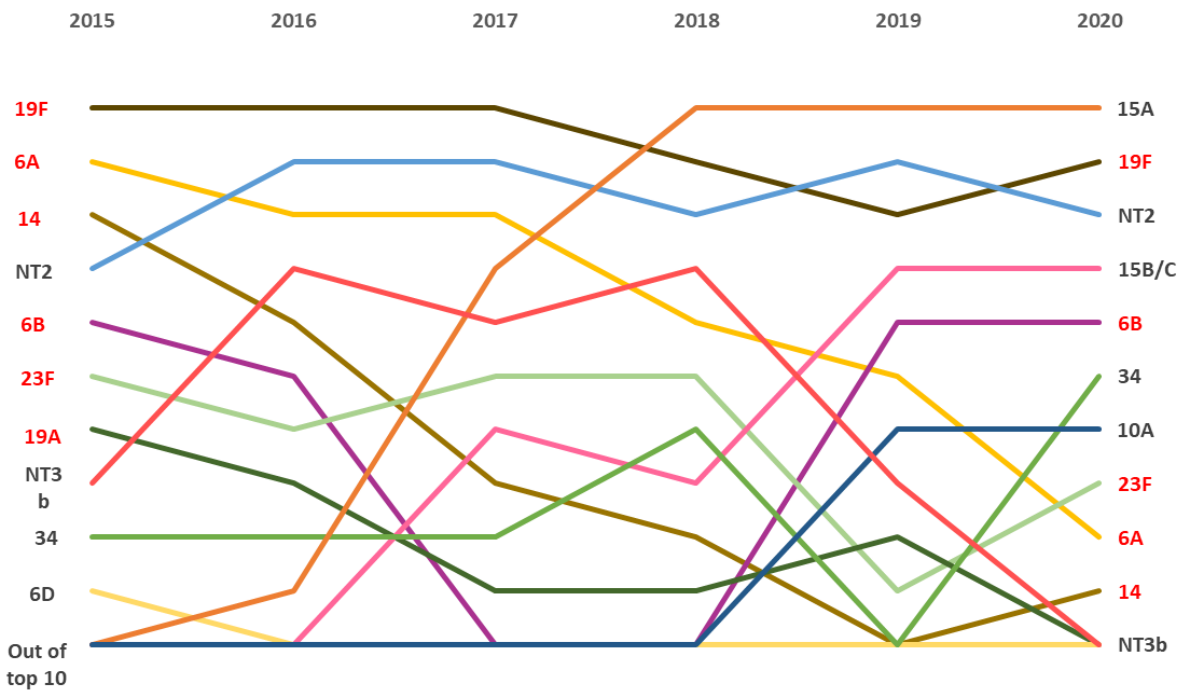
Supplementary Figure S2: Total pneumonia enrolments and pneumococcal serotypes by month for children 2-59 months in Ulaanbaatar, Mongolia.
Dotted lines indicate PCV introduction times in different districts: SKD = Songinokhairkhan District, SBD = Sukhbaatar District, BZD = Bayanzurkh District, CHD = Chingeltei District.



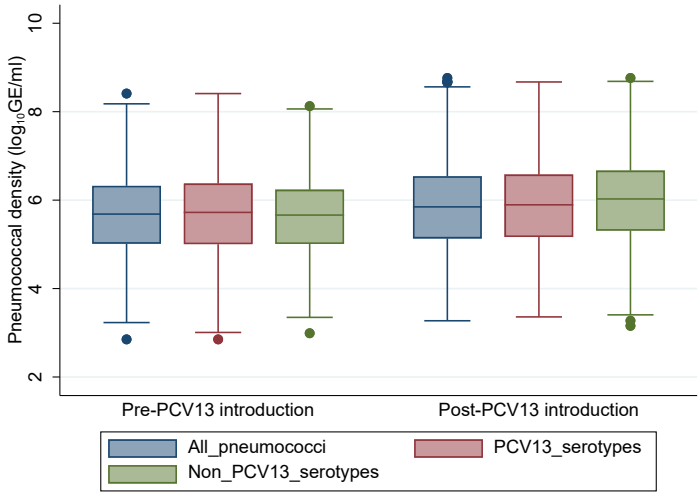
Supplementary Figure S3A: Stacked graph showing the number of individual PCV13 (VT) serotypes identified each year from all participants.



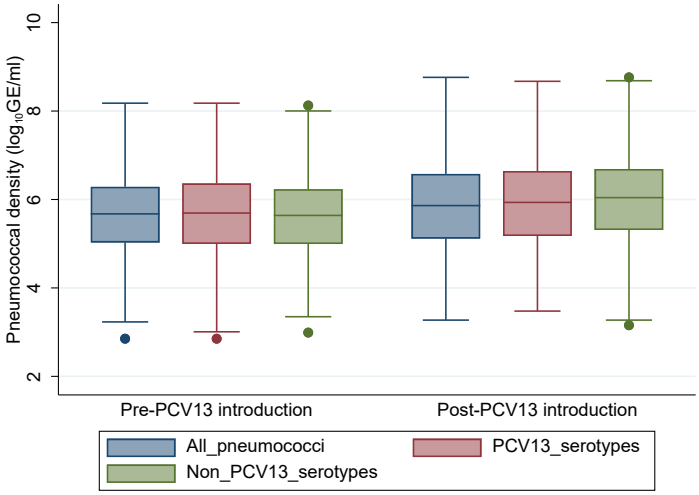
Supplementary Figure S3B: Stacked graph showing the number of individual non-PCV13 (NVT) serotypes identified each year from all participants. The 19 most common non-PCV13 serotypes are shown individually with the remainder grouped as other NVTs.



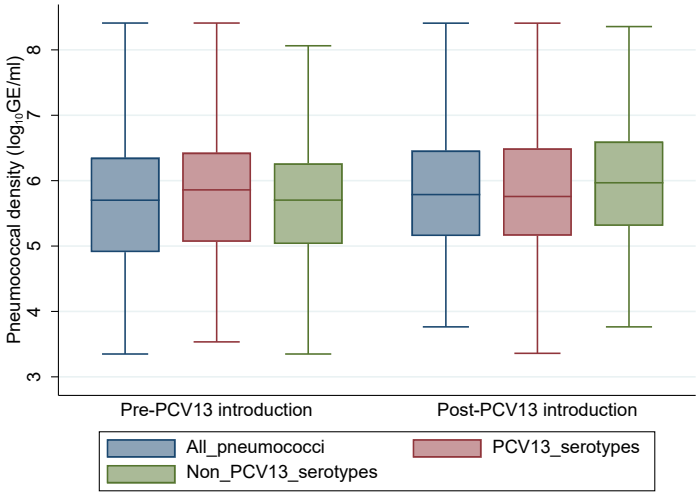
Supplementary Figure S3C: Ranking of the 10 most common individual serotypes by year, with the most common listed at the top. PCV13 serotypes are indicated in red font.



A



B



C

Supplementary Figure S4: Nasopharyngeal pneumococcal carriage density (log_{10} genome equivalents/ml) in (A) children 2-59 months, (B) children 2-23 months and (C) children 24-59 months, who were pneumococcal carriers. 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.

3. Supplementary Tables

Supplementary Table S1: Characteristics of children 2-59 months tested and not tested for pneumococcal carriage, April 2015 – June 2021

Category	Sub-category	Not tested (N=11062) n (%)	Tested (N=6545) n (%)	p-value
Demographics				
Age group	2-23 months	7809 (70·6)	4736 (72·4)	0·01
	24-59 months	3253 (29·4)	1809 (27·6)	
Sex	Male	5966 (53·9)	3567 (54·5)	0·47
	Female	5094 (46·1)	2978 (45·5)	
District	Bayanzurkh	3414 (30·9)	1562 (23·9)	<0·0001
	Chingeltei	2383 (21·5)	1786 (27·3)	
	Songinokhairkhan	3073 (27·8)	2259 (34·5)	
	Sukhbaatar	2192 (19·8)	938 (14·3)	
Primary caregiver	Parent[a]	7862 (89·6)	5842 (90·3)	0·002
	Other relative	722 (8·2)	535 (8·3)	
	Other	190 (2·2)	89 (1·4)	
Risk factors				
Seasons	Summer	854 (7·7)	612 (9·4)	<0·0001
	Autumn	1450 (13·1)	807 (12·3)	
	Winter	6121 (55·3)	3646 (55·7)	
	Spring	2637 (23·8)	1480 (22·6)	
Malnourished[b]	Yes	508 (4·7)	372 (5·8)	0·002
Currently breastfed	Yes	4919 (56·0)	3703 (57·2)	0·15
Caesarean section delivery	Yes	2159 (24·7)	1572 (24·3)	0·66
Asthma	Yes	695 (8·0)	492 (7·7)	0·44
Children aged <5 years in the household	1 child	6045 (70·0)	4377 (68·4)	0·03
	≥2 children	2588 (30·0)	2026 (31·6)	
Child attends daycare /kindergarten[c]	Yes	1940 (22·1)	1251 (19·4)	<0·0001
Chimney in the home	Yes	5562 (63·4)	4170 (64·5)	0·15
Adult smoker living in child's household	Yes	3904 (44·5)	2967 (45·9)	0·09
Adult smoking within the house	Yes	950 (10·8)	702 (10·9)	0·97
Caregiver smokes	Yes	410 (4·7)	302 (4·7)	0·98
Previous hospital admission	Yes	3778 (43·3)	2947 (45·8)	0·003
Antibiotics in 48 hours pre-admission	Yes	5702 (52·3)	3253 (50·2)	0·009
Socioeconomic factors				
Fuel used in the home	Electricity or Gas	3109 (35·5)	2254 (34·9)	0·47
	Coal or Wood	5651 (64·5)	4200 (65·1)	
Housing	Formal	5599 (63·8)	4024 (62·2)	0·05
	Informal	3179 (36·2)	2443 (37·8)	
Mother's education	Primary/Secondary	4293 (49·1)	3431 (53·3)	<0·0001
	Tertiary	4443 (50·9)	3008 (46·7)	
Income level[d]	Above minimum income	4882 (59·3)	3733 (61·3)	0·01
	At or below minimum income	3353 (40·7)	2356 (38·7)	
Crowding (people per room)	≤3	6221 (71·8)	4497 (70·3)	0·04
	>3	2443 (28·2)	1904 (29·7)	
Vaccination status				
PCV13 status[e]	Unvaccinated	5233 (49·2)	1748 (29·2)	<0·0001
	Undervaccinated	3016 (28·4)	1896 (31·7)	
	Vaccinated	2386 (22·4)	2341 (39·1)	
Severity of disease				
Length of hospital stay	≤7 days	8557 (77·4)	5014 (76·6)	0·49
	8-14 days	2291 (20·7)	1402 (21·4)	
	≥15 days	209 (1·9)	129 (2·0)	
Outcome	Died	26 (0.3)	14 (0.2)	0·58
Hypoxic[f]	Yes	1925 (18·3)	1267 (20·0)	0·006
Primary endpoint pneumonia[g]	Yes	74 (1·0)	1739 (29·0)	<0·0001
Severe pneumonia[h]	Yes	8264 (75·9)	5203 (79·9)	<0·0001
Very severe pneumonia[i]	Yes	3722 (34·2)	2712 (41·6)	<0·0001

[a]Mostly mothers (97%); [b]Weight for age -2 standard deviations; [c]Kindergarten for children 2-5 years of age. Daycare for children <2 years; [d]Minimum income was considered 170,000₮ per person/per month; [e]Children recruited in the pre-PCV13 period were considered unvaccinated; children were considered PCV13 vaccinated if they have received at least two doses when administered at less than 12 months of age or at least one dose when administered at greater than or equal to 12 months of age; [f]Hypoxic defined as an oxygen saturation <90%; [g]WHO defined primary end point pneumonia; [h]Severe pneumonia defined according to WHO integrated management of childhood illness 2005 case definition; [i]Very severe pneumonia included severe cases complicated by empyema, intensive care unit admission, persistent severe disease post-discharge, hypoxia or death.

Supplementary Table S2: Pneumococcal carriage positive and negative children 2–59 months of age from four study districts in Ulaanbaatar, Mongolia between April 2015 to June 2021 (N= 6545)

Category	Sub-category	Negative n/N (%)	Positive n/N (%)	p-value
Demographics				
Age group	2-23 months	2502/3489 (71·7)	2234/3056 (73·1)	0.21
	24-59 months	987/3489 (28·3)	822/3056 (26·9)	
Sex	Male	1956/3489 (56·1)	1611/3056 (52·7)	0·007
	Female	1533/3489 (43·9)	1445/3056 (47·3)	
District	Bayanzurkh	936/3489 (26·8)	626/3056 (20·5)	<0·0001
	Chingeltei	880/3489 (25·2)	906/3056 (29·6)	
	Songinokhairkhan	1122/3489 (32·2)	1137/3056 (37·2)	
	Sukhbaatar	551/3489 (15·8)	387/3056 (12·7)	
Primary caregiver	Parent[a]	3118/3477 (90·5)	2724/3019 (90·2)	0.50
	Other relative	287/3477 (8·3)	248/3019 (8·2)	
	Other	42/3477 (1·2)	47/3019 (1·6)	
Risk factors				
Seasons	Summer	335/3489 (9·6)	277/3056 (9·1)	0.64
	Autumn	422/3489 (12·1)	385/3056 (12·6)	
	Winter	1958/3489 (56·1)	1688/3056 (55·2)	
	Spring	774/3489 (22·2)	706/3056 (23·1)	
Malnourished[b]	Yes	213/3428 (6·2)	159/3017 (5·3)	0·11
Currently breastfed	Yes	1982/3453 (57·4)	1721/3021 (57·0)	0·73
Caesarean section delivery	Yes	863/3441 (25·1)	709/3015 (23·5)	0·14
Asthma	Yes	260/3420 (7·6)	232/3001 (7·7)	0·85
Children aged <5 years in the household	1 child	2397/3426 (70·0)	1980/2977 (66·5)	0·003
	≥2 children	1029/3426 (30·0)	997/2977 (33·5)	
Child attends daycare /kindergarten[c]	Yes	639/3441 (18·6)	612/3014 (20·3)	0·08
Chimney in the home	Yes	2099/3444 (60·9)	2071/3017 (68·6)	<0·0001
Adult smoker living in child's household	Yes	1561/3447 (45·3)	1406/3018 (46·6)	0·29
Adult smoking within the house	Yes	371/3440 (10·8)	331/3018 (11·0)	0·81
Caregiver smokes	Yes	148/3445 (4·3)	154/3019 (5·1)	0·13
Previous hospital admission	Yes	1598/3433 (46·5)	1349/3002 (44·9)	0·20
Antibiotics in 48 hours pre-admission	Yes	1827/3460 (52·8)	1426/3019 (47·2)	<0·0001
Socioeconomic factors				
Fuel used in the home	Electricity or Gas	1334/3436 (38·8)	920/3018 (30·5)	<0·0001
	Coal or Wood	2102/3436 (61·2)	2098/3018 (69·5)	
Housing	Formal	2264/3446 (65·7)	1760/3021 (58·3)	<0·0001
	Informal	1182/3446 (34·3)	1261/3021 (41·7)	
Mother's education	Primary/Secondary	1717/3438 (49·9)	1714/3001 (57·1)	<0·0001
	Tertiary	1721/3438 (50·1)	1287/3001 (42·9)	
Income level[d]	Above minimum income	2032/3247 (62·6)	1701/2842 (59·9)	0·03
	At or below minimum income	1215/3247 (37·4)	1141/2842 (40·1)	
Crowding (people per room)	≤3	2513/3413 (73·6)	1984/2988 (66·4)	<0·0001
	>3	900/3413 (26·4)	1004/2988 (33·6)	
Vaccination status				
PCV13 status[e]	Unvaccinated	902/3187 (28·3)	846/2798 (30·2)	0·13
	Undervaccinated	1041/3187 (32·7)	855/2798 (30·6)	
	Vaccinated	1244/3187 (39·0)	1097/2798 (39·2)	
Severity of disease				
Length of hospital stay	≤7 days	2628/3489 (75·3)	2386/3056 (78·1)	<0·0001
	8-14 days	773/3489 (22·2)	629/3056 (20·6)	
	≥15 days	88/3489 (2·5)	41/3056 (1·3)	
Outcome	Died	6/3476 (0.2)	8/3047 (0.3)	0·43
Hypoxic[f]	Yes	698/3388 (20·6)	569/2959 (19·2)	0·17
Primary endpoint pneumonia[g]	Yes	855/3166 (27·0)	884/2830 (31·2)	<0·0001
Severe pneumonia[h]	Yes	2721/3475 (78·3)	2482/3037 (81·7)	<0·0001
Very severe pneumonia[i]	Yes	1476/3475 (42·5)	1236/3037 (40·7)	0·15
Probable pneumococcal pneumonia[j]	Yes	155/3474 (4·5)	384/3052 (12·6)	<0·0001

[a]Mostly mothers (97%); [b]Weight for age -2 standard deviations; [c]Kindergarten for children 2-5 years of age. Daycare for children <2 years; [d]Minimum income was considered 170,000₮ per person/per month; [e]Children recruited in the pre-PCV13 period were considered unvaccinated; children were considered PCV13 vaccinated if they have received at least two doses when administered at less than 12 months of age or at least one dose when administered at greater than or equal to 12 months of age; [f]Hypoxic defined as an oxygen saturation <90%; [g]WHO defined primary end point pneumonia; [h]Severe pneumonia defined according to WHO integrated management of childhood illness 2005 case definition; [i]Very severe pneumonia included severe cases complicated by empyema, intensive care unit admission, persistent severe disease post-discharge, hypoxia or death; [j]Probable pneumococcal pneumonia was defined as elevated C-reactive protein with either PEP or high pneumococcal nasopharyngeal carriage (either high density carriage >1 × 10^6 CFU/mL, or carriage of serotypes 1 or 5).

Supplementary Table S3: Pneumococcal carriage prevalence and prevalence ratios (all pneumococci, PCV13 serotypes and non-PCV13 serotypes) for all four districts in hospitalised vaccinated and undervaccinated children with pneumonia (N=5985).

		n/N (%)	Prevalence rate (%) (95% CI)	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)^
All pneumococci					
All ages	Undervaccinated	1700/3640 (46·7)	46·7 (45·1 - 48·3)	Reference	Reference
	Vaccinated	1098/2345 (46·8)	46·8 (44·8 - 48·9)	1·00 (0·95 - 1·06)	1·03 (0·97 - 1·11)*
2-23 months	Undervaccinated	1190/2510 (47·4)	47·4 (45·4 - 49·4)	Reference	Reference
	Vaccinated	872/1852 (47·1)	47·1 (44·8 - 49·4)	0·99 (0·93 - 1·06)	1·04 (0·96 - 1·13)†
24-59 months	Undervaccinated	510/1130 (45·1)	45·1 (42·2 - 48·1)	Reference	Reference
	Vaccinated	226/493 (45·8)	45·8 (41·4 - 50·3)	1·01 (0·90 - 1·14)	1·01 (0·88 - 1·15)†
PCV13 serotypes					
All ages	Undervaccinated	886/3407 (26·0)	26·0 (24·5 - 27·5)	Reference	Reference
	Vaccinated	332/2149 (15·5)	15·4 (13·9 - 17·0)	0·59 (0·53 - 0·66)	0·74 (0·64 - 0·85)*
2-23 months	Undervaccinated	635/2357 (26·9)	26·9 (25·1 - 28·8)	Reference	Reference
	Vaccinated	273/1697 (16·1)	16·1 (14·4 - 17·9)	0·60 (0·52 - 0·68)	0·79 (0·67 - 0·93)†
24-59 months	Undervaccinated	251/1050 (23·9)	23·9 (21·3 - 26·6)	Reference	Reference
	Vaccinated	59/452 (13·1)	13·0 (10·1 - 16·5)	0·55 (0·42 - 0·71)	0·61 (0·46 - 0·83)†
Non-PCV13 serotypes					
All ages	Undervaccinated	737/3407 (21·6)	21·6 (20·2 - 23·0)	Reference	Reference
	Vaccinated	635/2149 (29·6)	29·5 (27·6 - 31·5)	1·36 (1·25 - 1·50)	1·25 (1·12 - 1·40)*
2-23 months	Undervaccinated	506/2357 (21·5)	21·5 (19·8 - 23·2)	Reference	Reference
	Vaccinated	500/1697 (29·5)	29·5 (27·3 - 31·7)	1·37 (1·23 - 1·53)	1·25 (1·09 - 1·42)†
24-59 months	Undervaccinated	231/1050 (22·0)	22·0 (19·5 - 24·6)	Reference	Reference
	Vaccinated	135/452 (29·9)	29·9 (25·7 - 34·3)	1·36 (1·13 - 1·63)	1·26 (1·02 - 1·56)†

*Adjusted using a common set of confounders: Age, informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding, household income, season, antibiotic exposure prior to admission, and PCV introduction period.

†Adjusted using a common set of confounders: Informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding, household income, season, antibiotic exposure prior to admission, and PCV introduction period.

^Used to calculate vaccine effectiveness = (1 - aPR) * 100%.

Supplementary Table S4: Pneumococcal carriage prevalence (all pneumococci, PCV13 serotypes and non-PCV13 serotypes) by year and district for hospitalised pneumonia cases in children aged 2-59 months, April 2015 to December 2020

		Annual prevalence (%) (95% confidence interval)		
District	**Year**	**All pneumococcal carriage**	**PCV13 serotype pneumococcal carriage**	**Non- PCV13 serotype pneumococcal carriage**
All Districts	**2015**	45·1 (41·2 – 48·9)	32·0 (28·3 – 35·7)	15·0 (12·3 – 18·0)
	2016	48·6 (45·6 – 51·7)	28·9 (26·1 – 31·8)	20·8 (18·3 – 23·4)
	2017	51·4 (48·1 – 54·6)	27·2 (24·3 – 30·3)	27·0 (24·1 – 30·1)
	2018	50· 8 (47·8 – 53·8)	20·9 (18·4 – 23·6)	29·7 (26·8 – 32·6)
	2019	45·7 (43·4 – 48·0)	14·6 (13·0 – 16·4)	27·5 (25·4 – 29·7)
	2020	38·4 (35·0 – 41·8)	12·5 (10·3 – 15·0)	23·3 (20·4 – 26·5)
Bayanzurkh District*	**2015**	39·2 (33·1 – 45·5)	26·9 (21·4 – 33·0)	11·8 (8·0 – 16·5)
	2016	40·3 (34·7 – 46·2)	25·5 (20·5 – 31·1)	13·5 (9·7 – 18·1)
	2017	41·5 (34·7 – 48·6)	25·4 (19·4 – 32·1)	15·5 (10·7 – 21·4)
	2018	44·9 (38·6 – 51·4)	17·6 (12·9 – 23·2)	25·5 (20·0 – 31·7)
	2019	38·2 (33·2 – 43·3)	11·1 (7·9 – 14·9)	22·4 (18·1 – 27·3)
	2020	37·1 (30·1 – 44·6)	11·7 (7·3 – 17·5)	23·4 (17·3 – 30·5)
Chingeltei District*	**2015**	56·2 (44·0 – 67·8)	40·0 (28·5 – 52·4)	20·0 (11·4 – 31·3)
	2016	59·0 (51·1 – 66·6)	38·7 (31·2 – 46·8)	21·9 (15·7 – 29·1)
	2017	58·1 (52·0 – 63·9)	32·7 (27·1 – 38·7)	31·6 (26·0 – 37·5)
	2018	55·3 (49·8 – 60·6)	30·3 (25·2 – 35·8)	26·7 (21·8 – 32·0)
	2019	48·3 (44·2 – 52·3)	18·2 (15·0 – 21·7)	28·1 (24·3 – 32·0)
	2020	39·1 (33·6 – 44·9)	12·9 (9·2 – 17·5)	23·7 (18·9 – 29·2)
Songinokhairkhan District*	**2015**	48·5 (41·9 – 55·1)	35·8 (29·6 – 42·5)	17·2 (12·6 – 22·8)
	2016	53·6 (48·4 – 58·7)	30·3 (25·6 – 35·4)	26·4 (21·9 – 31·3)
	2017	54·9 (49·0 – 60·7)	24·7 (19·8 – 30·2)	32·6 (27·1 – 38·4)
	2018	54·6 (49·3 – 59·7)	18·0 (14·1 – 22·4)	37·1 (32·0 – 42·4)
	2019	49·1 (45·3 – 52·8)	13·4 (10·9 – 16·3)	31·6 (28·0 – 35·4)
	2020	40·4 (34·4 – 46·6)	11·2 (7·5 – 15·9)	26·2 (20·8 – 32·3)
Sukhbaatar District*	**2015**	43·7 (33·9 – 53·8)	29·6 (20·8 – 39·7)	14·3 (8·0 – 22·8)
	2016	43·8 (37·3 – 50·4)	23·6 (18·1 – 29·8)	19·9 (14·8 – 25·9)
	2017	45·8 (37·8 – 54·0)	24·5 (17·8 – 32·2)	23·1 (16·6 – 30·8)
	2018	40·3 (32·2 – 48·8)	12·4 (7·2 – 19·3)	24·0 (16·9 – 32·3)
	2019	39·6 (32·7 – 46·8)	14·5 (9·7 – 20·5)	20·7 (15·0 – 27·3)
	2020	32·6 (23·2 – 43·2)	16·3 (9·2 – 25·8)	13·9 (7·4 – 23·1)

*Pneumococcal conjugate vaccine introduction: Bayanzurkh District – July 2017, Chingeltei District – March 2018, Songinokhairkhan and Sukhbaatar Districts – June 2016)

Supplementary Table S5: Pneumococcal carriage prevalence ratios (all pneumococci, PCV13 serotypes and non-PCV13 serotypes) over the study period by age group and district for hospitalised pneumonia cases, April 2015 to December 2020

		Prevalence ratio (95% confidence interval)^		
District	**Age group (months)**	**All pneumococcal carriage**	**PCV13 serotype pneumococcal carriage**	**Non- PCV13 serotype pneumococcal carriage**
All Districts	**2-59**	0.98 (0.96 - 0.99)	0.82 (0.80 - 0.85)	1.07 (1.04 - 1.11)
	2-23	0.97 (0.95 - 0.99)	0.81 (0.78 - 0.84)	1.09 (1.05 - 1.13)
	24-59	0.98 (0.95 - 1.02)	0.87 (0.81 - 0.93)	1.03 (0.97 - 1.09)
Bayanzurkh District	**2-59**	1.01 (0.97 - 1.05)	0.84 (0.78 - 0.90)	1.18 (1.10 - 1.26)
	2-23	1.01 (0.97 - 1.06)	0.85 (0.78 - 0.92)	1.17 (1.08 - 1.27)
	24-59	1.00 (0.92 - 1.08)	0.81 (0.71 - 0.94)	1.24 (1.07 - 1.44)
Chingeltei District	**2-59**	0.93 (0.90 - 0.96)*	0.80 (0.75 - 0.85)	0.99 (0.93 - 1.06)
	2-23	0.92 (0.88 - 0.95)*	0.76 (0.71 - 0.81)*	1.03 (0.95 - 1.11)
	24-59	0.96 (0.89 - 1.04)	0.96 (0.84 - 1.10)	0.88 (0.78 - 1.00)
Songinokhairkhan District	**2-59**	0.97 (0.94 – 1.00)	0.78 (0.74 - 0.83)	1.06 (1.01- 1.11)
	2-23	0.97 (0.94 - 1.00)	0.77 (0.72 - 0.82)	1.09 (1.03 - 1.15)
	24-59	0.96 (0.91 - 1.02)	0.85 (0.75 - 0.96)	0.98 (0.90 - 1.06)
Sukhbaatar District	**2-59**	0.96 (0.91 - 1.01)	0.85 (0.77 - 0.93)	1.00 (0.92 - 1.10)
	2-23	0.95 (0.90 - 1.01)	0.82 (0.74 - 0.91)	1.00 (0.90 - 1.11)
	24-59	0.97 (0.88 - 1.07)*	0.85 (0.72 - 1.01)	0.96 (0.81 - 1.15)

^Prevalence ratio over the study period from April 2015 to December 2020. Year included as a continuous variable. Results were similar when study period was extended to June 2021.

Age group 2-59 months adjusted using a common set of confounders: Age, informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding, household income, season, and antibiotic exposure prior to admission unless otherwise indicated.

Age groups 2-23 and 24-59 months adjusted using a common set of confounders: Informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding, household income, season, and antibiotic exposure prior to admission unless otherwise indicated.
*Adjusted as above, excluding season, and antibiotic exposure prior to admission as model did not converge.

Supplementary Table S6: Pneumococcal carriage prevalence and prevalence ratios for individual serotypes for all four districts in hospitalised children with pneumonia and serotyping results in the pre-PCV13 and post-PCV13 period (N=6046).

	Pre-PCV13 N=1742 n	Pre-PCV13 prevalence (%) (95% CI)	Post-PCV13 N=4304 n	Post-PCV13 prevalence (%) (95% CI)	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)[a]
PCV13 serotypes						
1	0	0 (0 – 0·21)	0	0 (0 – 0·08)	Not calculated	Not calculated
3	3	0·17 (0·03 – 0·50)	5	0·12 (0·04 – 0·27)	0·65 (0·15 – 2·72)	0·71 (0·17 – 2·96)
4	3	0·17 (0·03 – 0·50)	1	0·02 (0·001 – 0·13)	0·13 (0·01 – 1·25)	0·17 (0·02 – 1·69)
5	2	0·11 (0·01 – 0·41)	0	0 (0 – 0·08)	Not calculated	Not calculated
6A	111	6·37 (5·27 – 7·62)	126	2·93 (2·44 – 3·47)	0·44 (0·34 – 0·57)	0·46 (0·35 – 0·60)
6B	60	3·44 (2·64 – 4·41)	96	2·23 (1·81 – 2·72)	0·62 (0·45 – 0·86)	0·58 (0·42 – 0·81)
7F	1	0·06 (0·001 – 0·32)	2	0·05 (0·006 – 0·17)	0·78 (0·07 – 8·60)	0·95 (0·08 – 10·77)
9V	4	0·23 (0·06 – 0·59)	27	0·63 (0·41 – 0·91)	2·63 (0·92 – 7·52)	2·65 (0·92 – 7·62)
14	87	4·99 (4·02 – 6·12)	77	1·79 (1·41 – 2·23)	0·34 (0·25 – 0·47)	0·36 (0·27 – 0·50)
18C	4	0·23 (0·06 – 0·59)	4	0·09 (0·02 – 0·24)	0·39 (0·10 – 1·56)	0·58 (0·12 – 2·89)
19A	49	2·81 (2·09 – 3·70)	86	2·00 (1·60 – 2·46)	0·68 (0·48 – 0·97)	0·69 (0·48 – 0·99)
19F	200	11·48 (10·02 – 13·07)	251	5·83 (5·15 – 6·57)	0·49 (0·41 – 0·58)	0·51 (0·42 – 0·61)
23F	70	4·02 (3·14 – 5·05)	97	2·25 (1·83 – 2·74)	0·54 (0·40 – 0·73)	0·56 (0·41 – 0·76)
Non-PCV13 serotypes[b]						
6C	3	0·17 (0·03 – 0·50)	5	0·12 (0·04 – 0·27)	0·65 (0·15 – 2·72)	0·64 (0·15 – 2·71)
6D	27	1·55 (1·02 – 2·25)	30	0·70 (0·47 – 0·99)	0·43 (0·26 – 0·73)	0·47 (0·27 – 0·79)
10A	19	1·09 (0·66 – 1·70)	92	2·14 (1·73 – 2·61)	1·89 (1·16 – 3·09)	2·57 (1·46 – 4·50)
11A	11	0·63 (0·31 – 1·13)	60	1·39 (1·06 – 1·79)	2·13 (1·12 – 4·04)	1·98 (1·04 – 3·79)
13	3	0·17 (0·03 – 0·50)	29	0·67 (0·45 – 0·97)	3·77 (1·15 – 12·37)	3·47 (1·05 – 11·43)
15A	28	1·61 (1·07 – 2·31)	277	6·43 (5·72 – 7·21)	3·86 (2·63 – 5·67)	3·90 (2·61 – 5·82)
15B/C	25	1·43 (0·93 – 2·11)	130	3·02 (2·53 – 3·58)	2·03 (1·33 – 3·10)	2·19 (1·40 – 3·45)
17F	3	0·17 (0·03 – 0·50)	12	0·28 (0·14 – 0·49)	1·56 (0·44 – 5·52)	1·49 (0·42 – 5·34)
20B	1	0·06 (0·001 – 0·32)	10	0·23 (0·11 – 0·43)	3·90 (0·50 – 30·46)	3·95 (0·50 – 31·19)
21	1	0·06 (0·001 – 0·32)	17	0·39 (0·23 – 0·63)	6·63 (0·88 – 49·80)	Not calculated
22A	3	0·17 (0·03 – 0·50)	18	0·42 (0·25 – 0·66)	2·34 (0·69 – 7·94)	1·83 (0·53 – 6·31)
23A	17	0·97 (0·57 – 1·56)	37	0·86 (0·60 – 1·18)	0·85 (0·48 – 1·50)	0·85 (0·46 – 1·56)
33B	1	0·06 (0·001 – 0·32)	3	0·07 (0·01 – 0·20)	1·17 (0·12 – 11·24)	0·79 (0·07 – 8·91)
34	33	1·89 (1·31 – 2·65)	102	2·37 (1·94 – 2·87)	1·21 (0·82 – 1·78)	1·45 (0·95 – 2·21)
35A	1	0·06 (0·001 – 0·32)	5	0·12 (0·04 – 0·27)	1·95 (0·23 – 16·69)	2·15 (0·25 – 18·60)
35B	6	0·34 (0·13 – 0·75)	22	0·51 (0·32 – 0·77)	1·43 (0·58 – 3·52)	1·30 (0·53 – 3·23)
NT2	94	5·40 (4·38 – 6·56)	248	5·76 (5·08 – 6·50)	1·03 (0·82 – 1·30)	1·05 (0·82 – 1·33)
NT3b	54	3·10 (2·34 – 4·02)	124	2·88 (2·40 – 3·42)	0·89 (0·65 – 1·23)	0·95 (0·68 – 1·33)
NT4b	12	0·69 (0·36 – 1·20)	44	1·02 (0·74 – 1·37)	1·43 (0·76 – 2·70)	1·30 (0·68 – 2·48)

[a] Adjusted using a common set of confounders: Age, informal housing, other children <5 years in the home, maternal education, season and antibiotics prior to admission.
[b] Only the 19 most common non-PCV13 serotypes that were detected were included in the table.

Supplementary Table S7: Median density and quantile regression analysis of all pneumococci, PCV13 serotypes and non-PCV13 serotypes in pre-PCV13 and post-PCV13 period in children who were pneumococcal carriers.

	N§	Median density (IQR)*	Unadjusted coefficient (95% CI)†	P value	Adjusted coefficient (95% CI)‡	P value
Children 2-59 months						
All pneumococci						
Pre-PCV13 period	882	5·68 (5·01 - 6·32)	Reference		Reference	
Post-PCV13 period	2174	5·85 (5·13 - 6·54)	0·16 (0·06 - 0·27)	0·002	0·15 (0·04 - 0·26)	0·007
PCV13 serotypes						
Pre-PCV13 period	548	5·72 (5·00 - 6·37)	Reference		Reference	
Post-PCV13 period	742	5·89 (5·17 - 6·58)	0·17 (0·03 - 0·31)	0·02	0·16 (0·01 - 0·32)	0·04
Non-PCV13 serotypes						
Pre-PCV13 period	325	5·66 (5·01 - 6·23)	Reference		Reference	
Post-PCV13 period	1159	6·03 (5·31 - 6·67)	0·36 (0·21 – 0·52)	<0·0001	0·38 (0·21 – 0·54)	<0·0001
Children 2-23 months						
All pneumococci						
Pre-PCV13 period	644	5·67 (5·03 - 6·29)	Reference		Reference	
Post-PCV13 period	1590	5·86 (5·11 - 6·58)	0·18 (0·06 - 0·30)	0·004	0·19 (0·06 - 0·32)	0·005
PCV13 serotypes						
Pre-PCV13 period	418	5·69 (4·99 - 6·36)	Reference		Reference	
Post-PCV13 period	538	5·93 (5·17 - 6·64)	0·24 (0·08 - 0·40)	0·004	0·23 (0·04 - 0·42)	0·02
Non-PCV13 serotypes						
Pre-PCV13 period	224	5·64 (4·99 - 6·23)	Reference		Reference	
Post-PCV13 period	855	6·04 (5·31 - 6·69)	0·42 (0·21 – 0·62)	<0·0001	0·46 (0·27 – 0·66)	<0·0001
Children 24-59 months						
All pneumococci						
Pre-PCV13 period	238	5·70 (4·91 - 6·35)	Reference		Reference	
Post-PCV13 period	584	5·79 (5·15 - 6·46)	0·09 (-0·10 - 0·29)	0·35	0·05 (-0·16 - 0·26)	0·65
PCV13 serotypes						
Pre-PCV13 period	130	5·86 (5·06 - 6·43)	Reference		Reference	
Post-PCV13 period	204	5·76 (5·16 - 6·49)	-0·06 (-0·34 - 0·22)	0·66	-0·09 (-0·37 - 0·19)	0·54
Non-PCV13 serotypes						
Pre-PCV13 period	101	5·70 (5·03 - 6·26)	Reference		Reference	
Post-PCV13 period	304	5·97 (5·31 - 6·60)	0·26 (0·01 – 0·51)	0·04	0·22 (-0·07 – 0·52)	0·13

*Density reported in $\log_{10}$ genome equivalents/ml and interquartile range (IQR).
†Coefficient is the difference in medians as determined by quantile regression, reported with 95% confidence intervals (CI).
‡Children 2-59 months adjusted for age, informal housing, other children <5 years in the home, coal used for fuel, maternal education crowding and household income; Children 2-23 and 24-59 months adjusted for informal housing, other children <5 years in the home, coal used for fuel, maternal education crowding and household income.
§Number of pneumococcal carriers.

Supplementary Table S8: Median density and quantile regression analysis of all pneumococci, PCV13 serotypes and non-PCV13 serotypes in PCV13 vaccinated (2 or 3 doses) and undervaccinated (0 or 1 dose) children who were pneumococcal carriers.

	N§	Median density (IQR)*	Unadjusted coefficient (95% CI)†	P value	Adjusted coefficient (95% CI)‡	P value
Children 2-59 months						
All pneumococci						
Undervaccinated	1700	5·76 (5·08 - 6·43)	Reference		Reference	
Vaccinated	1098	5·85 (5·13 - 6·54)	0·09 (-0·01 - 0·18)	0·08	0·04 (-0·06 - 0·15)	0·41
PCV13 serotypes						
Undervaccinated	886	5·77 (5·05 - 6·45)	Reference		Reference	
Vaccinated	332	5·91 (5·20 - 6·58)	0·13 (-0·03 - 0·30)	0·11	0·12 (-0·05 - 0·29)	0·18
Non-PCV13 serotypes						
Undervaccinated	728	5·89 (5·22 - 6·47)	Reference		Reference	
Vaccinated	629	6·00 (5·27 - 6·64)	0·12 (-0·02 – 0·26)	0·09	0·11 (-0·02 – 0·25)	0·09
Children 2-23 months						
All pneumococci						
Undervaccinated	1190	5·78 (5·08 - 6·45)	Reference		Reference	
Vaccinated	872	5·85 (5·11 - 6·55)	0·07 (-0·04 - 0·18)	0·23	0·04 (-0·08 - 0·16)	0·47
PCV13 serotypes						
Undervaccinated	635	5·76 (5·03 - 6·46)	Reference		Reference	
Vaccinated	273	5·94 (5·20 - 6·59)	0·18 (-0·01 - 0·37)	0·06	0·16 (-0·04 - 0·35)	0·68
Non-PCV13 serotypes						
Undervaccinated	500	5·88 (5·28 - 6·48)	Reference		Reference	
Vaccinated	495	5·99 (5·22 - 6·67)	0·11 (-0·06 – 0·28)	0·19	0·06 (-0·11 – 0·22)	0·49
Children 24-59 months						
All pneumococci						
Undervaccinated	510	5·72 (5·08 - 6·39)	Reference		Reference	
Vaccinated	226	5·83 (5·19 - 6·50)	0·10 (-0·09 - 0·30)	0·30	-0·01 (-0·21 - 0·20)	0·96
PCV13 serotypes						
Undervaccinated	251	5·79 (5·06 - 6·43)	Reference		Reference	
Vaccinated	59	5·60 (5·12 - 6·50)	-0·19 (-0·55 - 0·18)	0·31	0·03 (-0·38 - 0·43)	0·89
Non-PCV13 serotypes						
Undervaccinated	228	5·90 (5·17 - 6·42)	Reference		Reference	
Vaccinated	134	6·04 (5·37 - 6·63)	0·14 (-0·09 – 0·37)	0·24	0·15 (-0·11 – 0·42)	0·25

*Density reported in $\log_{10}$ genome equivalents/ml and interquartile range (IQR).
†Coefficient is the difference in medians as determined by quantile regression, reported with 95% confidence intervals (CI).
‡Children 2-59 months adjusted for age, informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding and household income; Children 2-23 and 24-59 months adjusted for informal housing, other children <5 years in the home, coal used for fuel, maternal education, crowding and household income.
§Number of pneumococcal carriers.

Supplementary Table S9: Antimicrobial resistance genes detected by microarray in nasopharyngeal samples from hospitalised children with pneumonia aged 2-59 months.* Detection rate of antimicrobial resistance genes shown for all pneumococci, PCV13 and non-PCV13 serotypes.

Antimicrobial resistance gene	Encodes resistance to	Detected in all pneumococci (N=1647) n (%)	Detected in PCV13 serotypes (N=785) n (%)	Detected in non-PCV13 serotypes (N=853) n (%)	P value†
tetM	Tetracycline	1357 (82·4)	749 (95·4)	608 (71·3)	<0·0001
tetK	Tetracycline	85 (5·2)	33 (4·2)	50 (5·9)	0·13
tetO	Tetracycline	2 (0·1)	0 (0·0)	2 (0·2)	0·17
tetL	Tetracycline	6 (0.4)	2 (0.2)	4 (0·5)	0·47
cat	Chloramphenicol	263 (16·0)	148 (18·8)	114 (13·4)	0·002
mefA	Macrolides	588 (35·7)	441 (56·2)	147 (17·2)	<0·0001
aphA3	Kanamycin	24 (1·5)	10 (1·3)	14 (1·6)	0·54
sat4	Streptothricin	24 (1·5)	10 (1·3)	14 (1·6)	0·54
ermB	Erythromycin	1084 (65·8)	598 (76·2)	486 (57·0)	<0·0001
ermC	Erythromycin	133 (8·1)	57 (7·3)	74 (8·7)	0·29
Any antimicrobial resistance gene		1441 (87·5)	756 (96·3)	682 (79·9)	<0·0001
≥3 antimicrobial resistance genes		554 (33·6)	427 (54·4)	126 (14·8)	<0·0001

*Only samples that contained a single pneumococcal serotype with no other species identified were included in the analysis.
†p-values compared VT versus NVT serotypes using chi-squared test.

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