nature portfolio

Peer Review File

Effect of pneumococcal conjugate vaccine six years post-introduction on pneumococcal carriage in Ulaanbaatar, Mongolia

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript, effect of PCV six years post-introduction on pneumococcal carriage in Ulaanbaatar, Mongolia provides new information on the impact of PCV13 on carriage in LMICs. The study compared carriage in 5-8 week old infants and 12-23 month old toddlers across a pre vaccine period, an early vaccine period and a 6 year mark. The 6 year time period is 2022 and exactly what influence COVID has on the data is unclear but it does represent a less restrictive time when masking was less frequent. Although use of culture vs molecular detection captures differences (molecular techniques identify current and recent carriage) the use of same technique over time is valid for comparison.
The study has several major results. First a decline in overall SP carriage. Most studies have not shown decline in overall SP carriage following introduction of PCV13. The investigators also note a decline in household smoking, smoky fuel use and crowding – all reported to impact colonization. Clarifying what was PCV13 effect and what was environmental is not possible?
The second major finding was decline in VST carriage in both toddlers wo were immunized, and 5-8 week old infants too young to be immunized, thus herd effect. This is not surprising but valuable and consistent with other LMICs. They also reported increase in NVST, again not surprising and detailed which NVST became dominant. 15A, 10A, 34 emerged; it was unclear if this was linked to antibiotic resistance? They also report the persistence 0f 19F which has been reported globally and lacks complete understanding. The authors reference decreased OPA but OPA is not thought to be a primary mechanism for NP clearance as there is limited complement in NP. Agglutination is thought to be more important. Reference to OPA may be misleading.
The finding of increased density is perplexing. Environmental changes identified above would more likely favor reduced density. PCV13 has been linked to reduced density of VST but this also was not observed. As well, increased density is linked to increase disease (Pneumonia) yet the proportion of children with recent or non-recent pneumonia is markedly lower among toddlers. I think this need more attention in the manuscript.
Lastly, there are several details needed in the methods.
1. What is definition of minor serotype (I assume higher cT cycle but not stated).
2. What is PCV13 regimen – 2-4 -6 or 2-4 9-12??
3. How were AMR genes identified? (again I assume from microarray but unclear)

Reviewer #2 (Remarks to the Author):

This paper details interesting results from three cross-sectional carriage surveys conducted pre-PCV13 introduction (2015) and post-PCV13 introduction (2017 and 2022) among infants and toddlers in Mongolia. It documents substantial residual vaccine type carriage in toddlers in an urban Asian setting – a geography with very limited data on pneumococcal carriage and disease.

I have only very minor comments on the content and flow of the paper:

Abstract

Do you mean that the density of any carriage episode increased in the more recent surveys? Specifying VT and NVT increased is probably unnecessary.

Introduction

The first paragraph introduces a couple of different concepts and serotype replacement would benefit from a little more explanation – the fact that NVTs replace VTs isn't necessarily going to nullify the impact of the vaccine programme if the STs that replace VT are less invasive than the VT were?

The lines 68-70 use the term 'non-susceptibility' is there a reason for this – resistance may be more widely understood by a general reader.

Results

Line 95-98 quotes reductions in carriage but the percentages are difficult to follow – are these the carriage prevalence in the final survey, the 2017 survey or the absolute reductions? If these are the absolute reductions it would be better to put the aPR in brackets with the 95%CI so that it's clear.

I think in general the results may be easier to follow if the results in each age group were described separately.

Lin 119: "PCV13 vaccine serotypes were not more likely to be minor serotypes in 2022" – compared to what? And what does this refer to – are the data for this in supplementary material?

Table 1 – a footnote would be helpful to indicate the 5-8 week olds were all unvaccinated. Was this an eligibility criterion? Eligibility criteria I think need to be added to the methods.

Discussion

I Agree with the first paragraph - the study details substantial indirect effects and I think the discussion of this needs to mention the very high vaccine coverage in the study population to contextualise this (i.e. move the description of high coverage up and move the rest of the discussion of indirect effects up, to avoid repetition).

The 12.5% prevalence of VT (mostly 19F) in 12-23 month olds is high despite very high vaccine coverage in the study population – and potential reasons for this are clearly described.

Line 213 seems to include a hanging sentence: "Published community carriage studies from the WHO Western Pacific region, included post-PCV introduction periods from 1-4 years"

Line 236: "Serotype replacement carriage relevance is dependent on the invasiveness of the increasing serotypes. In hospitalised children with pneumonia in Mongolia, 15A, 15B/C and NT2 were the most common replacement serotypes [19]. Monitoring non-vaccine serotypes in different populations assists in understanding which populations are optimal to predict effects on disease." This is all correct but

could be expanded to infer whether the STs seen to be replacing VTs in Mongolia are in fact at risk of damaging the impact of the vaccine – are they as invasive as VTs? – or if there is still a possibility that the vaccination programme is having a beneficial impact on disease? You could use a review of st-specific invasiveness by Løchen and Croucher here as a reference.

Line 258 – is it also possible that changes in sample handling/ laboratory methods changed over time to create a secular trend in density?

Line 290 – Slightly unclear to the reader which confounders you are missing and whether you have under or overestimated the changes in prevalence that are attributable to PCV?
It is stated that the results are generalisable to other urban areas of Mongolia – but the participants were recruited at health centres. Do you have any data on health seeking behaviour to support the fact that health facility attendees are representative of the general population?

We thank the reviewers for their comments and feedback. We have responded to each query below, with the line numbers referring to the revised clean manuscript file.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The manuscript, effect of PCV six years post-introduction on pneumococcal carriage in Ulaanbaatar, Mongolia provides new information on the impact of PCV13 on carriage in LMICs. The study compared carriage in 5-8 week old infants and 12-23 month old toddlers across a pre vaccine period, an early vaccine period and a 6 year mark. The 6 year time period is 2022 and exactly what influence COVID has on the data is unclear but it does represent a less restrictive time when masking was less frequent. Although use of culture vs molecular detection captures differences (molecular techniques identify current and recent carriage) the use of same technique over time is valid for comparison.

1. The study has several major results. First a decline in overall SP carriage. Most studies have not shown decline in overall SP carriage following introduction of PCV13. The investigators also note a decline in household smoking, smoky fuel use and crowding – all reported to impact colonization. Clarifying what was PCV13 effect and what was environmental is not possible?

There are several factors that affect pneumococcal colonisation and for overall carriage it is difficult to tease out the relative contribution of these factors. To try and account for the influence of these factors on carriage we have adjusted for these factors in our adjusted prevalence ratio calculations and quantile regression models. To recognise this important point, we have now highlighted the difficulty in determining the contribution of other factors to the changes in the limitation section of the text.

Lines 303-307: "Although there was a reduction in the prevalence of some carriage risk factors in children included in the final survey, it is not possible to determine the relative contribution of these factors to changes in carriage rates. However, most of the reduction in carriage is likely driven by vaccine introduction based on observed reductions in vaccine-type serotypes and increases in non-vaccine type serotypes."

2. The second major finding was decline in VST carriage in both toddlers who were immunized, and 5-8 week old infants too young to be immunized, thus herd effect. This is not surprising but valuable and consistent with other LMICs. They also reported increase in NVST, again not surprising and detailed which NVST became dominant. 15A, 10A, 34 emerged; it was unclear if this was linked to antibiotic resistance?

Overall, we demonstrated that AMR genes were more common in PCV13 serotypes compared with non-PCV13 serotypes (Table S3), and that there was a substantial reduction in AMR genes in the post-PCV13 compared with the pre-PCV13 period (Table S4). Although this study was not focused on the identification of the factors that enable the emergence of particular serotypes, we have since conducted some analysis in response to this question about AMR.

For serotype 15A (n=75) ≥1 AMR gene was detected in all isolates in the final survey (n=40) compared with 75% (n=4) in 2015, while for serotype 10A (n=44) a lower percentage of isolates in the final survey (29%) had AMR genes than in the pre-PCV13 (33%) and first survey (36%). Similarly for serotype 34 (n=57) a lower percentage of isolates in the final survey (32%) had AMR genes than in the pre-PCV13 (43%) and first survey (39%). Therefore, it appears that resistance was not a factor in driving the emergence of all non-vaccine serotypes, although numbers are small especially for 15A in the pre-PCV13 period.

Results Lines 167-171: "With regards to the most common emerging non-PCV13 serotypes, for serotype 15A one (or more) AMR genes were detected in all isolates in the final survey in 2022 (40/40 [100%]) compared with a lower percentage in 2015 (3/4 [75%], p<0.001). For serotypes 10A (7/24 [29%] versus 3/9 [33%], p=0.91) and 34 (8/25 [32%] versus 6/14 [43%], p=0.78) a lower percentage of isolates in the final survey had AMR genes compared with the previous two surveys."

Discussion Lines 267-270: With regards to individual serotypes, it appears that overall antimicrobial resistance was not a factor in driving the emergence of non-vaccine serotypes. However, for serotype 15A the numbers in the pre-PCV13 period were small, and it is unclear what role antimicrobial resistance played in the increase in this serotype.

3. They also report the persistence of 19F which has been reported globally and lacks complete understanding. The authors reference decreased OPA but OPA is not thought to be a primary mechanism for NP clearance as there is limited complement in NP. Agglutination is thought to be more important. Reference to OPA may be misleading.

We have removed the sentence regarding OPA and replaced it with a sentence regarding the uncertainty around the cause of serotype persistence.

Lines 201-202: "The underlying mechanism behind the persistent carriage of certain serotypes such as 19F is still unclear."

4. The finding of increased density is perplexing. Environmental changes identified above would more likely favor reduced density. PCV13 has been linked to reduced density of VST but this also was not observed. As well, increased density is linked to increase disease (Pneumonia) yet the proportion of children with recent or non-recent pneumonia is markedly lower among toddlers. I think this need more attention in the manuscript.

The relationship between pneumococcal vaccination and overall pneumococcal carriage density is complex. Recently, some of the authors undertook a systematic review that found heterogenous findings of the effect of PCV introduction on pneumococcal density across different studies (Jagne2023). Previous studies in healthy children have shown both decreases (Roca2012, Dunne2018, Kandasamy2024) and increases (Satzke2019, von Mollendorf2019) in carriage density with PCV introduction. Variations in density observed across our three cross-sectional surveys may be temporal and/or related to unmeasured factors. For example, asymptomatic viral co-infection may increase pneumococcal carriage density even in the absence of URTI symptoms (DeMuri2018). We have now highlighted this in the discussion with some additional details.

Lines 258-262: "Previous studies in healthy children have shown both decreases (Roca2012, Dunne2018, Kandasamy2024) and increases (Satzke2019, von Mollendorf2019) in carriage density with PCV introduction. Variations in density observed across our three cross-sectional surveys may be temporal and/or related to unmeasured factors. These factors may include viral co-infection, multiple serotype carriage, serotype replacement and prior antibiotic use. It is unclear which factors are driving the changes in pneumococcal density in Mongolia."

Lastly, there are several details needed in the methods.

5. What is definition of minor serotype (I assume higher cT cycle but not stated).

Microarray can detect the presence of carriage of more than one serotype (multiple serotype carriage) and the relative abundance of each serotype. In multiple serotype carriage, the most abundant serotype is considered the major serotype and the other serotypes are considered minor serotypes (Satzke2015). The definition has been added to the text.
Lines 351-354: "Microarray can determine the presence of multiple serotype carriage and the relative abundance of each pneumococcal serotype. The dominant serotype is considered the major serotype and the less abundant serotypes are designated as the minor serotypes (Satzke2015)."

6. What is PCV13 regimen – 2-4 -6 or 2-4 9-12??
PCV13 was introduced into the paediatric immunisation program in a 2+1 schedule at 2, 4 and 9 months of age.
Lines 322-323: The Government of Mongolia introduced PCV13 into the routine national immunisation program using a 2+1 schedule (2, 4, 9 months) in a staged manner from 2016 (La Vincente2019).

7. How were AMR genes identified? (again I assume from microarray but unclear)
Indeed, antimicrobial resistance genes were detected by microarray. Details were included in the supplementary material. To make this clearer we have now referred to this in the main text.
Lines 358-359: "AMR genes were also identified using microarray (supplementary methods page 1)."

Reviewer #2 (Remarks to the Author):
This paper details interesting results from three cross-sectional carriage surveys conducted pre-PCV13 introduction (2015) and post-PCV13 introduction (2017 and 2022) among infants and toddlers in Mongolia. It documents substantial residual vaccine type carriage in toddlers in an urban Asian setting – a geography with very limited data on pneumococcal carriage and disease.
I have only very minor comments on the content and flow of the paper:

Abstract
1. Do you mean that the density of any carriage episode increased in the more recent surveys? Specifying VT and NVT increased is probably unnecessary.
We reported the increase in overall pneumococcal density, as well as density of PCV13 and non-PCV13 pneumococci, given that any effect of PCV on density may be different between these groups. However, to simplify the abstract we have amended the sentence accordingly.
Lines 40-41: "An increase in pneumococcal nasopharyngeal density was observed."

Introduction
2. The first paragraph introduces a couple of different concepts and serotype replacement would benefit from a little more explanation – the fact that NVTs replace VTs isn't necessarily going to nullify the impact of the vaccine programme if the STs that replace VT are less invasive than the VT were?
To better convey these concepts, we have added some extra details to the introduction (Also see response to Q11).
Lines 49-53: Reductions in vaccine serotypes are associated with an increase in carriage and disease due to non-vaccine serotypes (serotype replacement) (Weinberger2011). Although replacement can be extensive, there is usually a net benefit from vaccine introduction, as the replacing serotypes are generally less

pathogenic (Lochen2022). However, in some settings this public health benefit of PCV has been eroded overtime (Weinberger2011, Mulholland2012, Betran2024).

3. The lines 68-70 use the term 'non-susceptibility' is there a reason for this – resistance may be more widely understood by a general reader.

We took this approach to be consistent with the cited publication (Andrejko2021), and also to reflect the Clinical & Laboratory Standards Institute (CLSI, 2019) guidelines where intermediate and resistant phenotypes are reported as being non-susceptible. We have added the definition to the main text.

Lines 68-70: Carriage isolates generally had a higher prevalence of penicillin and macrolide non-susceptibility than invasive isolates, where non-susceptibility was defined as an intermediate or resistant phenotype on antimicrobial susceptibility testing (CLSI 2019).

Results

4. Line 95-98 quotes reductions in carriage but the percentages are difficult to follow – are these the carriage prevalence in the final survey, the 2017 survey or the absolute reductions? If these are the absolute reductions it would be better to put the aPR in brackets with the 95%CI so that it's clear.

The percentages reported are the absolute reductions. We have added the adjusted prevalence ratios to clarify this in the text.

Lines 97-102: "Comparing the final survey (2022) with the first post-PCV13 survey (2017) we observed a reduction in all pneumococcal carriage (25% in infants [aPR 0.75, 95%CI 0.58 - 0.96], 15% in toddlers [aPR 0.85, 95%CI 0.76 - 0.96]), a reduction in PCV13 serotype carriage (37% in infants [aPR 0.63, 95%CI 0.35 – 1.12], 35% in toddlers [aPR 0.65, 95%CI 0.48 – 0.90]) and a non-significant change in non-PCV13 serotypes (20% in infants [aPR 0.80, 95%CI 0.59 – 1.08] and 11% in toddlers [aPR 0.89, 95%CI 0.76 – 1.05]) (Supplementary Table S1)."

5. I think in general the results may be easier to follow if the results in each age group were described separately.

In drafting the results section we considered a number of different structures to organise the findings. We have chosen to report results in themes which we found reduced duplicate information and repetition for the reader.

6. Line 119: "PCV13 vaccine serotypes were not more likely to be minor serotypes in 2022" – compared to what? And what does this refer to – are the data for this in supplementary material?

We explored the likelihood of vaccine serotypes being a major or minor serotype, and how this changed over time. We included any participants who carried vaccine-type serotypes. Although the numbers of participants with multiple serotype carriage were small, there was no evidence of differences by survey years. We have added a table in the supplement (Supplementary Table S2).

Lines 122-124: In participants who carried vaccine serotypes, these were not more likely to be present as a minor serotype in 2022 (compared with 2015 or 2017) for either infants or toddlers (Supplementary Table S2).

Supplementary Table S2: Vaccine serotypes detected as major and minor serotypes by age group and survey year.

		Survey years		
	Vaccine serotypes detected as:	*2015 n (%)*	*2017 n (%)*	*2022 n (%)*
Infants (5-8 weeks)	*Major serotypes*	*55 (93)*	*28 (90)*	*18 (90)*
	Minor serotypes	*3 (5)*	*2 (7)*	*1 (5)*
	Both major and minor serotypes	*1 (2)*	*1 (3)*	*1 (5)*
		206 (100)	*98 (100)*	*62 (100)*
Toddlers (12-23 months)	*Major vaccine serotypes*	*177 (86)*	*80 (82)*	*55 (89)*
	Minor vaccine serotypes	*17 (8)*	*16 (16)*	*7 (11)*
	Both major and minor serotypes	*12 (6)*	*2 (2)*	*0 (0)*
		59 (100)	*31 (100)*	*20 (100)*

7. Table 1 – a footnote would be helpful to indicate the 5-8 week olds were all unvaccinated. Was this an eligibility criterion? Eligibility criteria I think need to be added to the methods.

We have added a summary of the criteria in the methods section and a footnote for Table 1. Detailed eligibility criteria were included in the first publication (von Mollendorf et al., Vaccine 37 (2019) 4068–4075), so we have also added a reference to that paper.

Lines 334-336: "Children were ineligible if they had a fever, had not lived in one of the study districts for at three or more months (toddler group), or if they were infants and had received PCV13 (von Mollendorf2019)."

Table 1: ƒ - Infants were ineligible if they had received PCV13.

Discussion

8. I Agree with the first paragraph - the study details substantial indirect effects and I think the discussion of this needs to mention the very high vaccine coverage in the study population to contextualise this (i.e. move the description of high coverage up and move the rest of the discussion of indirect effects up, to avoid repetition).

We have rearranged the discussion as suggested. The paragraph beginning "Our study population had high PCV13 coverage…" is now the second paragraph and the paragraph on indirect effects "Indirect protection is vital for young infants too young to be vaccinated." is now the third paragraph.

9. The 12.5% prevalence of VT (mostly 19F) in 12-23 month olds is high despite very high vaccine coverage in the study population – and potential reasons for this are clearly described.

Thank you, we have (see reviewer 1, comment 3) highlighted that the reason for persistence of certain serotypes such as 19F is largely still being explored.

10. Line 213 seems to include a hanging sentence: "Published community carriage studies from the WHO Western Pacific region, included post-PCV introduction periods from 1-4 years"

The relevant sentence has been corrected.

Lines 226-227: "Published community carriage studies from the WHO Western Pacific region have included post-PCV introduction periods ranging from 1-4 years."

11. Line 236: "Serotype replacement carriage relevance is dependent on the invasiveness of the increasing

serotypes. In hospitalised children with pneumonia in Mongolia, 15A, 15B/C and NT2 were the most common replacement serotypes [19]. Monitoring non-vaccine serotypes in different populations assists in understanding which populations are optimal to predict effects on disease." This is all correct but could be expanded to infer whether the STs seen to be replacing VTs in Mongolia are in fact at risk of damaging the impact of the vaccine – are they as invasive as VTs? – or if there is still a possibility that the vaccination programme is having a beneficial impact on disease? You could use a review of st-specific invasiveness by Løchen and Croucher here as a reference.

We have added some extra details to the introduction (Also see response to Q2).

Lines 49-53: Reductions in vaccine serotypes are associated with an increase in carriage and disease due to non-vaccine serotypes (serotype replacement) (Weinberger2011). Although replacement can be extensive, there is usually a net benefit from vaccine introduction, as the replacing serotypes are generally less pathogenic (Lochen2022). However, in some settings this public health benefit of PCV has been eroded overtime (Weinberger2011, Mulholland2012, Betran2024).

12. Line 258 – is it also possible that changes in sample handling/ laboratory methods changed over time to create a secular trend in density?

The same main study staff worked across all three surveys. Sample collection and handling was standardised across all three surveys with standard operating procedures. Real-time quantitative PCR was performed using the Stratagene Mx3005 machine for all surveys.

The only difference we could identify was that DNA was extracted using the MagNA Pure LC machine for 2015 and 2017 samples, and the QIAcube HT machine for 2022 survey samples. However, we consider it unlikely that the change in DNA extraction equipment contributed to this finding, given that a change in density was already noted in 2017 (prior to the change in laboratory equipment). In addition, a sample of specimens was included in internal bridging studies at the time of machine changeover and the effect on density was minimal (data not shown).

13. Line 290 – Slightly unclear to the reader which confounders you are missing and whether you have under or overestimated the changes in prevalence that are attributable to PCV?

Data on viral infections circulating in the community during our surveys were not collected, although we excluded children with respiratory symptoms in the past two weeks. We did not collect data on some other factors which may affect carriage, including air pollution levels and recent antibiotic use. By not controlling for all potential confounders, we may have underestimated the impact of PCV on pneumococcal carriage.

We have added some text to clarify these issues.

Lines 300-303: Secondly, we did not collect information on all confounders that may potentially effect vaccine type carriage, for example air pollution levels and recent antibiotic use, and some of these factors may have resulted in us underestimating the impact of PCV on pneumococcal carriage.

14. It is stated that the results are generalisable to other urban areas of Mongolia – but the participants were recruited at health centres. Do you have any data on health seeking behaviour to support the fact that health facility attendees are representative of the general population?

We believe that the children recruited at family health clinics are representative of children living in Ulaanbaatar, on the basis that:

- *In Mongolia all citizens, including children under five years of age, have free access to primary health care which is funded by the government.*
- *Primary health care is provided by subdistrict level family health centres in Ulaanbaatar (Dorjdagva2017). The majority of children (99.6%) are registered at birth and receive care at the relevant subdistrict family health clinic (WHO situation report2024). All children are therefore able to readily access care at family health clinics in the relevant districts.*
- *In addition, immunisation coverage is high with coverage >95% in all the districts where PCV has been introduced (WHO immunization data 2024). The high coverage observed in the 12-23 month old children in our survey is therefore reflective of all children living in Ulaanbaatar.*

Lines 291-293: "Our findings are therefore likely generalisable to other urban populations in Mongolia, as primary health care is free for children and immunisation coverage is high across the population."

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have responded to my concerns in a clear manner, adding limitations of the study where necessary. I would recommend acceptance of the manuscript. It provides additional information about impact of vaccine as 2+1 regimen in a LMIC country.

Reviewer #2 (Remarks to the Author):

Thank you for clearly addressing all comments