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Review

Using pneumococcal carriage studies to monitor vaccine impact in low- and middle-income countries

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ABSTRACT

Pneumococcal disease is a leading cause of childhood mortality, globally. The pneumococcal conjugate vaccine (PCV) has been introduced to many countries worldwide. However there are few studies evaluating PCV impacts in low- and middle-income countries (LMIC) because measuring the impact of PCV on pneumococcal disease in LMICs is challenging. We review the role of pneumococcal carriage studies for the evaluation of PCVs in LMICs and discuss optimal methods for conducting these studies. Fifteen carriage studies from 13 LMICs quantified the effects of PCV on carriage, and identified replacement carriage serotypes in the post-PCV era. Ten studies reported on the indirect effects of PCV on carriage. Results can be used to inform cost-effectiveness evaluations, guide policy decisions on dosing and product, and monitor equity in program implementation. Critically, we highlight gaps in our understanding of serotype replacement disease in LMICs and identify priorities for research to address this gap.

Contents

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1. Introduction

Infections due to *Streptococcus pneumoniae*, including pneumonia and meningitis, are a leading cause of morbidity and mortality, especially in low- and lower middle-income countries (LMICs) worldwide [1]. Two pneumococcal conjugate vaccines (PCVs) are currently licensed for use in children: the 10-valent PCV (Synflorix, GlaxoSmithKline) and the 13-valent PCV (Prevenar 13, Pfizer). They protect against the most common invasive paediatric serotypes of >90 pneumococcal serotypes.

Post-licensure evaluations of PCVs, predominantly in high-income countries (HIC), demonstrated dramatic reductions in pneumococcal disease following vaccine introduction [2]. These reductions include direct effects on vaccinated children, as well as indirect effects across the whole population. Indirect effects are mediated through reductions in carriage and transmission of vaccine-type (VT) pneumococci [2–4] and comprise a substantial proportion of overall effects, driving the cost-effectiveness of the vaccine [5]. However, reductions in VT carriage have led to a corresponding increase in carriage of non-vaccine serotypes (NVT) (i.e. serotype replacement) [6]. Serotype replacement threatens to undermine the public health gains of PCV introduction, especially among older populations who initially benefited from strong indirect effects. While serotype replacement in carriage occurs in all settings, the amount of replacement disease caused is highly variable and the reasons for this are not known [7].

While PCV has been introduced in 144 countries worldwide, there are relatively few studies on the impact of PCV in LMICs [8]. Evaluations of vaccine impact in LMICs are critical because there are key differences in pneumococcal epidemiology, including higher pneumococcal transmission rates [9], which means that studies conducted in HIC are not directly applicable. A recent review highlighted the striking geographical differences in serotype replacement disease in predominantly high-income countries, but in many LMICs the degree of replacement disease and responsible serotypes is not known [7].

Monitoring pneumococcal serotypes and determining PCV impact in LMIC is difficult because the recommended method for assessing vaccine impact, invasive pneumococcal disease (IPD) surveillance, is often not feasible [10]. In 2017, the World Health Organization's Invasive Bacterial Vaccine Preventable Diseases (IB-VPD) global surveillance network, which monitors invasive bacterial diseases, identified just 116 and 55 confirmed IPD cases from the two regions with the highest burden of IPD (Africa and South-East Asia), respectively [11]. These data are insufficient to make policy decisions regarding PCV introduction in LMIC, including choice of the valency of vaccine, vaccine schedule and whether or not to introduce the vaccine [12].

Carriage studies have been used as an adjunct to IPD surveillance and pneumonia studies for assessing PCV impact and monitoring pneumococcal serotypes in LMIC. Reductions in VT carriage following PCV introduction likely correspond to reductions in VT disease, since carriage is a precursor for disease [13]. Carriage studies are easier to conduct, since sample collection is less invasive; and require smaller sample sizes, as incidence and prevalence of pneumococcal carriage are higher than that of IPD. It is challenging to identify the aetiological agent of pneumonia, whereas pneumococcal carriage can be more easily be identified and used to demonstrate the biological effects of PCV on specific serotypes.

There are multiple potential uses of carriage studies for the evaluation of PCVs. Carriage studies can demonstrate the effectiveness of the vaccine against VTs in vaccinated individuals (direct and indirect [i.e. total effects]) and quantify reductions in transmission of VTs to unvaccinated individuals (indirect effects). They can also be used to monitor which serotypes increase in prevalence as VT carriage decreases, which provides an indication of the common circulating serotypes post-PCV introduction which may cause disease. Data from carriage studies can be incorporated into analytical models, alongside information about the serotype-specific invasiveness, to predict impacts on disease [14,15]. These results have important policy implications and can be used to inform cost-effectiveness evaluations, guide policy decisions on dosing and product, and monitor equity in program implementation.

Despite the potential importance of carriage studies for evaluating PCV, and a growing body of literature, there is limited discussion or consensus on the methods for conducting these studies or their interpretation. Current guidelines provided by the World Health Organization focus on carriage methodologies [16], and on measuring the impact of PCV using IPD or pneumonia surveillance [10]. Therefore, we aim to review the utility of carriage studies for evaluating PCV and discuss optimal methods for conducting these studies.

2. Scope of the review

This review has three sections. First, we review the current literature on pneumococcal carriage studies for evaluating PCV in LMIC. Second, we discuss the value of carriage studies for informing PCV policy, and highlight remaining gaps in using carriage studies for PCV program evaluation in LMIC. Lastly, we discuss the implications of different study designs for the interpretation of results and identify optimal designs for future carriage studies.

We adhere to previously defined terminology regarding vaccine evaluations [17,18]. In brief, vaccine effectiveness is a measure of direct effects, under field conditions, while vaccine impact encompasses both direct and indirect effects.

We have restricted our discussion to study types suitable for the routine evaluation of PCV programs at a population level (e.g. cross-sectional studies). We exclude data from longitudinal studies and cluster-randomised controlled trials since these study designs are more resource-intensive and better suited to answer specific research questions.

3. Review of existing literature

Our search identified 26 LMICs with ongoing or completed PCV impact studies using nasopharyngeal carriage as an outcome (Supplementary Table 1 [90–95]). There were 15 published or pre-print studies from 13 countries available for this review. All except one were serial or single cross-sectional studies, with one study in Turkey conducting prospective carriage surveillance. All 15 studies were conducted in healthy participants, recruited either from the community or from health facilities. The majority (n = 7) were conducted in the African region. The earliest study was published in 2013 and the greatest number of studies were published in 2018 (n = 5), indicating this is a relatively new and growing area of study. All included studies were found to be at either a moderate or serious risk of bias. The main issue was the degree to which confounding factors were either measured or adjusted for.

3.1. Total effects

Carriage studies in LMIC have consistently reported substantial declines in VT carriage among children within targeted age groups, who benefit from both direct and indirect effects, following PCV introduction (Table 1). For example, in Kenya the prevalence of VT carriage two years following PCV10 introduction among children under five years of age decreased by 64% (PR 0·36, 95%CI 0·26-0·51) [19].

Four studies of the included studies examined trends in VT carriage density (Table 1), which is of interest due the potential role of carriage density in disease (pneumonia) and transmission [20–22]. Only one study, in Fiji, showed a decline in VT carriage density, three years post-PCV introduction, however there were declines in overall pneumococcal carriage density over the same period, indicating the results may not be attributable to vaccine effects [23]. More research is needed to understand the inconsistent results from field-based studies.

3.2. Direct effects

A single study from Brazil reported vaccine effectiveness of three doses of PCV13 of 44.0% (14.2–63.5) against VT carriage among children 7–11 months of age. The study was conducted one year after vaccine introduction, and while the PCV coverage at the time was not reported, 83% of the study population had received at least one dose of PCV [24]. We note that a second study from Brazil also reported vaccine effectiveness, however the unvaccinated group included both children before and after vaccine introduction and therefore reported vaccine effectiveness is in fact a composite measure of both direct and total effects. Accordingly, their estimates of vaccine effectiveness were much higher (92·7% [79·6, 97·4] for three doses of PCV10) [25].

3.3. Indirect effects

Of the 10 studies reporting on indirect effects in older children and adults, all but two studies, both in The Gambia, report some evidence of indirect effects (Table 1). In The Gambia, the VT carriage prevalence in mothers of vaccinated children remained at similar levels following PCV introduction (6.6% pre-PCV, 8.4% one year post-PCV [first study], and 5.6% five years post-PCV [second study] [26]. Among the five studies that report on infants too young to be vaccinated, four studies reported a decline in VT carriage (with only two studies from Fiji and Mongolia achieving statistical significance) [23,27–29]. In Malawi, indirect effects were observed in mothers and older children but not among infants, indicating indirect effects on the infant age group may be harder to achieve than for older age groups [27]. The differences in indirect effects are likely a result of different contact patterns between age groups, alongside differences in naturally acquired immunity [27].

Despite the majority of studies reporting declines in VT carriage among unvaccinated age groups, indicating evidence of indirect effects, many sites, especially in the African region, have reported persistence of VT carriage at high levels in unvaccinated age groups suggesting that full indirect effects are yet to be seen [26–28,30]. This contrasts with studies from HIC such as the US, where carriage of VT serotypes across all age groups were less than 5% four years after vaccine introduction [31]. Ongoing VT transmission despite high levels of coverage is likely due to the higher baseline rates of pneumococcal carriage and transmission [9].

3.4. Serotype replacement in carriage

While VT carriage prevalence declines, overall pneumococcal carriage prevalence remained constant, with replacement by NVTs. All studies demonstrated an increase in NVT carriage rates among vaccinated children. And all studies demonstrated either an increase or non-significant decrease in NVT carriage among unvaccinated children and adults post-PCV introduction, except a study from the early post-PCV period in South Africa, which found a significant decrease in NVTs among individuals >12 years of age. (Supplementary Table 5) We are undertaking a global meta-analysis to compare regional and temporal variations in serotype-specific carriage post-PCV introduction. This will provide critical information to better understand whether differences in replacement carriage serotypes can account for differences in serotype replacement disease observed across different settings.

3.5. Implications for vaccine policy

3.5.1. Informing policy decisions on dosing and product

While carriage studies provide useful information on the impact of PCV on circulating serotypes of pneumococcus, ultimately what is of relevance to policy makers is the impact on disease outcomes. There is substantial heterogeneity in the relative impact of PCV on pneumococcal disease across settings. Understanding the factors that explain this heterogeneity can inform policy recommendations to maximise impacts [2,4].

Carriage studies may help determine the optimal schedule for PCV programs in LMIC. The relative effectiveness of differing vaccine schedules remains contentious. A systematic review compiled for the WHO Strategic Advisory Group of Experts (SAGE) on Immunization in 2017, suggested that there is no evidence of difference between the two most common schedules (three infant doses [3 + 0] or two infant doses plus booster [2 + 1]) in either direct or indirect effects against carriage or IPD [38]. At the time, Australia was the only HIC using a 3 + 0, however, in 2018, policy makers changed to a 2 + 1 schedule based on data on vaccine failures in older children and indirect effects. [39,40] This is supported by results from a meta-regression of carriage studies, showing waning of PCV7 effectiveness with a 3 + 0 schedule compared to a 2 + 1 schedule [41]. An updated of this analysis is warranted, given the availability of more carriage studies from LMIC.

One the key challenges in using carriage studies to compare schedules is the expected heterogeneity in vaccine impact across different settings, as a function of variability in effectiveness of the vaccine, PCV coverage and timeliness of administration, as well as baseline pneumococcal epidemiology. Meta-regression techniques may be helpful to better understand how such explanatory variables might influence the intervention effect size. Standardising study designs and analysis methods, including period of observation post-PCV introduction, may also help researchers to

Table 1
Summary of published studies evaluating pneumococcal conjugate vaccine impact using carriage in low- and middle-income countries.

WHO Region	Setting	Study type	Study participants	PCV, schedule and catch-up	No. years post-PCV	PCV Coverage[§]	Results – direct effects	Results – indirect effects	Risk of Bias
AFRO	Malawi, 2018 [27]	Serial cross-sectional & longitudinal cohort study	Healthy children up to 15 years of age and mothers, recruited from the community	PCV13, 3 + 0 schedule, catch-up to 1 year of age	3 years post-PCV introduction, compared to pre-PCV introduction	87%	**Infants (18 weeks):** VT carriage decreased from 45·1% to 9·1%; aPR 0·24 (0·08–0·75) **Children (1–4 years), vaccinated:** VT carriage decreased from 28·2 to 16·5%, aPR 0·54 (0·33–0·88)	**Infants (6 weeks):** VT carriage increased from 11·4 to 13·0%; aPR 1·07 (0·38–3·02) **Children (1–4 years), unvaccinated:** VT carriage decreased from 28·2 to 22·9% aPR 0·84 (0·53–1·33) **Children 5–15 years:** VT carriage decreased from 21·2 to 7·9%; aPR 0·37 (0·17–0·78) **HIV-negative mothers:** VT carriage decreased from 6·6 to 2·4%; aPR 0·34 (0·15–0·79)	M
	Mozambique, 2018 [28]	Serial cross-sectional surveys	Healthy children (6 weeks – 59 months); recruited from the community, HIV infected children recruited from OPD	PCV10, 3 + 0 schedule, no catch-up	2 years post-PCV introduction, compared to pre-PCV introduction	80%	**6 weeks–11 months, vaccinated**[*]: VT carriage decreased from 36·6 to 20·6%; PR 0·56 (0·32–0·99) p = 0·04 **12–23 months, vaccinated**[*]: VT carriage decreased from 35·1 to 20·9%; PR 0·59 (0·37–0·97) 0·03 **24–59 months, vaccinated**[*]: VT carriage decreased from 36·0 to 16·7%; PR 0·46 (0·08–2·80) 0·40 **< 5 years, all:** No changes in density for serotypes 19A and 19F	**6 weeks–11 months, unvaccinated**[**]**:** VT carriage decreased from 36·6 to 25·0%; PR 0·68 (0·19–2·36) **24–59 months, unvaccinated**[**]**:** VT carriage decreased from 36·0 to 28·1%; PR 0·78 (0·57–1·07)	S
	Kenya, 2014 [19]	Serial cross-sectional surveys	Healthy population of all ages; recruited from the community	PCV10, 3 + 0 schedule, catch up to 5 years of age	2 years post-PCV10 introduction, compared to pre-PCV introduction	75%	**<5 years**: VT carriage decreased from 34 to 13%; aPR 0·36 (0·26–0·51)	≥**5 years**: VT carriage decreased from 8 to 4%; aPR 0·34 (0·18–0·62)	M
	South Africa, 2013 [32]	Serial cross-sectional surveys	Households with children < 2 years old; recruited from the community	PCV7, 2 + 1, no catch-up	2 years post PCV introduction, compared to survey conducted same year as PCV introduction	72%	**< 2 years old**: VT carriage decreased from 45·1 to 23·5%, p < 0·0001; aRR 0·50 (0·42–0·59)	**6–12 years old**: VT carriage decreased from 19 to 12·6%; aRR 0·66 (0·48–0·92) **13–18 years old**: VT carriage decreased from 5·7 to 2·1%; aRR 0·49 (0·17–1·39) **19–45 year old**: VT carriage decreased from 3% to 1·1%; aRR 0·36 (0·18, 0·74) ≥ **45 years old**: VT carriage decreased from 1·7% to 1·1%; aRR 0·63 (0·16–2·49)	M
	South Africa, 2015 [30]	Serial cross-sectional surveys	HIV-infected and HIV-uninfected mother–child dyads; recruited from HIV clinics or wellness-baby clinics	PCV13, 2 + 1, catch-up to 3 years of age	2 years post PCV13 introduction compared to PCV7 (and pre-PCV13) era	62%	**< 9 months old**: VT carriage decreased from 25·2 to 13·6%; aOR 0·52(0·34, 0·79) **9–24 months old**: VT carriage decreased from 42·2 to 14·8%; aOR 0·26 (0·16, 0·41) ≥**24–48 months old**: VT carriage decreased from 42·1 to 15·5%; aOR 0·25 (0·17, 0·36)	**4–12 years old**: VT carriage decreased from 34·3 to 22·4%; aOR 0·52 (0·37, 0·75); **Mothers**: VT carriage decreased from 7·05 to 3.08%; aOR 0·53 (0·36, 0·78)	M
	The Gambia, 2019 [26]	Serial cross-sectional surveys	Healthy infants (6–11 months) and mothers; recruited from EPI clinics	PCV13, 3 + 0, no catch-up campaign	1 and 5 years post-PCV13 introduction, compared to pre-PCV13/PCV7 era	90%	**6–11 months old:** VT carriage decreased from 33·3 to 11·4%; aRR 0·60 (0·50,0·71), p < 0·001	**Mothers:** VT carriage similar in all three surveys (6.6%, 8.4%, 5.6%); aRR 0·92 (0·67,1·27), p = 0·627 (5 years post-PCV compared to pre-PCV)	M

	The Gambia, 2015 [33]	Serial cross-sectional surveys	Healthy infants (6–11 months) and mothers; recruited from EPI clinics	PCV13, 3 + 0, no catch-up campaign	1 year post-PCV13 introduction, compared to pre-PCV13/PCV7 era	98%	**6–11 months old:** VT carriage decreased from 33.3 to 18·3%; aRR 0·55 (0·42,0·72), p < 0·001	**Mothers:** VT carriage increased from 6·6 to 8·4%; aRR 1·14 (0·65,2·01), p = 0·651	M
EMRO	West Bank, Israel, 2018 [34]	Serial cross-sectional surveys	Healthy children ≤ 5 years ; recruited from paediatric clinics	PCV10, 2 + 1, catch-up to 2 years	3 years post-PCV introduction compared to pre-PCV introduction	N/A	VT carriage decreased from 41·9 to 19%; relative reduction −54·7%, p < 0·0001		S
AMRO	Brazil, 2014 [24]	Single cross-sectional surveys	Healthy children (7–11 months and 15–18 months); recruited from the community	PCV10, 2 + 1, catch-up to 2 years of age	Conducted same year as PCV introduction; vaccinated compared to unvaccinated	82%	VE 44·0% (14·2; 63·5) against VT carriage, p = 0·008		M
	Brazil. 2016 [25]	Serial cross-sectional surveys	Healthy children (12–23 months); recruited during immunisation campaigns	PCV10, 2 + 1, catch-up to 2 years of age	3 years post-PCV introduction, compared to survey conducted same year as PCV introduction	94%	VT carriage decreased from 19·8 to 1·8%; VE against VT carriage: 2 doses – 87·8% (4·9, 98·4); 3 doses – 92·7% (79·6, 97·4); 4 doses – 97·3% (88·7, 99·3)		M
	Colombia, 2013 [35]	Serial cross-sectional surveys	Healthy children (12–18 months); recruited at EPI clinics	PCV7, 2 + 1, no catch-up	3 years post universal PCV10 compared to pre-universal PCV10 introduction	46%	VT carriage decreased from 23·6% to 7·6%, p < 0·001		S
EURO	Turkey, 2016 [36]	Prospective surveillance	Healthy children (0–18 years); recruited from paediatric clinics	PCV13, 3 + 1 schedule	Surveillance starting with PCV introduction, up to 2 years post; vaccinated compared to unvaccinated	97%	OR 0·61 (0·41–0·91), p = 0·01, no association in adjusted analysis (not reported)		S
WPRO	Fiji, 2018 [23]	Serial cross-sectional surveys	Healthy children (5–8 week, 12–23 month, 2–6-years), and caregivers; recruited from villages and health centres	PCV10, 3 + 0 schedule, no catch-up	3 years post PCV introduction, compared to pre-PCV introduction	99%	**12–23 months old**: VT carriage decreased from 22·3 to 7·3%; aPR 0·34 (0·23–0·49); median VT density reduced from 4·98 to 4·29 $\log_{10}$ GE/mL, p = 0·024; density lower in vaccinated compared to vaccinated (adjusted coefficient −0·56, 95%CI −0·98 to −0·15, p = 0·008) **2–6 years old**: VT carriage decreased from 21·7 to 9·1%; aPR 0·47 (0·34–0·66)	**5–8 weeks old**: VT carriage decreased from 9·6 to 5·8%; aPR 0·56 (95% CI 0·34–0·93); **Caregivers**: VT carriage decreased from 2·4 to 0·8%; aPR 0·43 (0·13–1·42)	M
	Laos, 2018 [29]	Serial cross-sectional surveys	Healthy children (5–8 weeks, 12–23 months); recruited from maternal and child health centres	PCV13 3 + 0 schedule, catch-up to 1 year	2 years post PCV introduction compared to pre-PCV introduction	83%	**12–23 months old**: VT carriage decreased from 32·9 to 19·8%; aPR 0·77 (0·61 – 0·96); median VT density increased from 5·50 to 5·99 $\log_{10}$ GE/mL, p = 0·006; density higher in vaccinated compared to unvaccinated (adjusted coefficient 0·40, 95% CI 0·07-0·73, p = 0·017)	**5–8 weeks old**: VT carriage decreased from 6·5 to 5·2% aPR 0·74 (0·43 – 1·27); median VT density increased from 5·19 to 5·95 $\log_{10}$ GE/mL, p = 0·009	M
	Mongolia, 2019 [37]	Serial cross-sectional surveys	Healthy children (5–8 week and 12–23 months); recruited from family health centres	PCV13 2 + 1 schedule, catch-up to 2 years	1 year post PCV introduction compared to pre-PCV introduction	97%[†]	**12–23 months old:** VT carriage decreased from 42·2 to 19·7%; aPR 0·47 (0·38 – 0·58); median VT density increased, p < 0·001; vaccinated compared to unvaccinated (coefficient 0.58, 95% CI 0.19–0.97, p = 0.004)	**5–8 weeks old:** VT carriage decreased from 12.9 to 6.3%; aPR 0·49 (0·33 – 0·74); median VT density increased, p < 0·001	M

Abbreviations: aOR = adjusted odds ratio, OR = odds ratio, aPR = adjusted prevalence ratio, VE = vaccine effectiveness, GE = genome equivalents, M = moderate, S = serious, N/A = not available; P-values included where provided.

[*] Vaccinated defined as having received three doses.

[**] Unvaccinated defined as having received no doses.

[§] PCV coverage from WHO/UNICEF Estimates of National Immunization Coverage (WUENIC) estimates for final year of study unless otherwise specified [8].

[†] Administrative estimates.

determine whether observed variability is due to true differences in the biological effect of different schedules. This is particularly important when making comparisons between vaccine schedules, since differences in effect sizes are likely to be smaller.

Another key question in the field is the potential use of reduced dose schedules. Following the control of VT disease, transitioning to a schedule comprising of a single priming followed by a booster dose (1 + 1) may be sufficient to sustain VT disease control at reduced costs [42]. The success of this approach will largely be determined by the schedule's ability to maintain indirect protection for infants receiving insufficient doses for direct protection. In LMICs, different strategies may be required to achieve and maintain control of VTs [9,43,44]. Modelling from Malawi and Kenya indicate that VT elimination is unlikely, despite high vaccine coverage using a 3 + 0 schedule for period of six to ten years [45,46]. Vaccine programs may need to cover broader age ranges and use different schedules to control VT transmission [9,43,44]. Carriage studies can also highlight target age groups for control of VT strains and identify settings which have successfully eliminated VT transmission and are suitable reduced dose schedules.

Monitoring of serotype replacement disease, in particular, remains a critical gap within LMICs. In the absence of IPD data in many LMIC, it is not known the degree to which reductions in VT disease have been supplanted by increases in NVT disease. Data on replacement serotypes is also important to inform the development of higher-valency vaccine formulations. Discussion of whether carriage studies can be used to model replacement disease are discussed further below.

3.5.2. Modelling vaccine impact on disease

There are a number of studies that have modelled the impact of PCV on both VT and NVT IPD using carriage data. A method proposed by Weinberger et al. employs static regression models populated by a combination of pre-PCV IPD data and change in VT carriage before and after PCV introduction to estimate impact of PCV on overall IPD [14]. This method has been validated using data from the five sites across three HIC, where the predicted changes in incidence of VT and NVT IPD was within 95% predictive intervals of the observed impact for all sites except England and Wales [47]. This method was also validated in South Africa, where it accurately predicted impacts in older children and adults but not in the age group of children targeted for vaccination – this may be due to the model not accounting for the direct effectiveness of PCV in preventing VT-IPD and/or not accounting for reduced population susceptibility to IPD due to increased anti-retroviral therapy uptake in the HIV population [48]. A similar method used by Flasche et al. showed that variations in proportion of VT carriage and IPD pre-PCV was responsible for the observed heterogeneity in PCV impact across different nine sites in five HIC [15]. This method was also applied to assess the cost-effectiveness of PCV globally, finding that PCV is likely to be a cost-effective measure to reduce childhood mortality and morbidity across all UN regions and most countries.

Dynamic models have also been used to predict PCV impacts on disease using carriage data, and can be of particular use to policymakers since they are able to make projections into the future and simulate different intervention scenarios. Using a compartmental dynamic model fitted to pre-vaccination carriage data in Kenya and validated using post-vaccination carriage and IPD data, Ojal et al predicted a sustained reduction in VT carriage and a 56% reduction of overall IPD incidence in the 10 years post-PCV introduction [46]. A similar model explored the potential impacts of catch-up campaigns in Vietnam, finding that most of the additional benefit of catch-up campaigns in children occur within the first three years after PCV introduction [49].

The question of whether we can confidently model pneumococcal vaccine impact on disease in the absence of IPD surveillance remains unclear, given our limited understanding of serotype replacement disease. Drawing conclusions about reductions in VT disease following reduction or elimination of VT carriage are relatively straightforward. However, in order to accurately predict replacement disease, we need to understand the factors contributing to variation in replacement disease observed in HIC. Recent results from a whole-genome sequencing study suggests local antibiotic selective pressure influences trends in pneumococcal lineages and therefore serotype prevalence observed in IPD post-PCV. For example, a lineage (GPSC55) with penicillin resistance increased alongside common use of beta-lactams antibiotics for treating pneumonia in Israel. Whereas, in The Gambia and South Africa – where cotrimoxazole is widely used to prevent bacterial infection in people with HIV, a lineage (GPSC26) with cotrimoxazole resistance predominated [50].

The key piece of information added by carriage studies, used by the models above to accurately model PCV impact on disease in most settings, is the composition of replacement carriage serotypes in the post-PCV era. In order to predict disease, the models rely on the assumption that the invasiveness of a particular serotype is an intrinsic property of the capsule and does not change following PCV introduction, and there is some evidence for this [51–53]. However, a recent review by Lewnard and Hanage notes that serotype distributions in carriage are similar in the US and UK and contribute little to explain the greater IPD burden from serotypes 8, 9N, 12F, 15A in the UK [7]. This may be due to the inability for cross-sectional carriage studies among healthy children to detect highly invasive serotypes. For example, serotypes 1, 12F and 5 are highly invasive serotypes that commonly cause outbreaks of disease but are rarely carried and may only be transiently detectable in carriage during outbreaks [54–57]. In the UK, replacement serotypes 8 and 12F were not detected in carriage studies despite being increasingly found in invasive disease [58]. Carriage studies performed in children with ARI, may better identify such invasive serotypes.

In order to accurately model replacement disease, models may also need to include other factors, such as prevalence of comorbidities [59,60], variations in patterns of vaccine coverage (including subnational heterogeneity in coverage), and different contact patterns between children and older age groups – all of which are likely to vary considerably both within LMICs and between LMICs and HICs [61,62]. While including all these elements within a model are technically feasible, increasing the complexity of models often comes at the cost of having to make more assumptions around parameters for which we have limited data. For example, all of the models above treat VT and/or NVT carriage as a group. Modelling specific serotypes would require assumptions about how different serotypes coexist and compete, and there may be less certainty around serotype-specific parameters. At this stage, the minimum components needed to robustly model serotype replacement disease and identify key replacement disease serotypes is not clear.

To our knowledge, carriage studies have not been used to predict the impact of PCV on pneumococcal pneumonia, which has a much higher disease burden than IPD. This would be useful since current studies of vaccine impact on pneumonia in LMIC are limited by challenges in accurately diagnosing pneumonia and assessing aetiology. Greenberg et al. have compared carriage in healthy children against children with radiological pneumonia, in order to estimate serotype-specific disease potential for childhood pneumonia [63]. There are a growing number of carriage studies among children with pneumonia or ARI. As we begin to amass data about the impacts of PCV on carriage, pneumonia and IPD in a range of settings, there is an opportunity to use mathematical models to better understand and predict vaccine impacts on different disease outcomes in LMIC.

3.5.3. Monitoring reductions in inequity

It is well established that there are disparities in the burden of pneumococcal carriage and disease within populations [64]. Examining vaccine impact by risk groups enables policy makers to determine whether the vaccine is working as intended for those most in need. In Fiji, where rates of pneumococcal carriage and disease are higher in the Indigenous population compared with the non-Indigenous population, carriage studies were able to demonstrate the role of PCV in reducing differences in VT carriage between the two groups. VT carriage prevalence decreased from 14.8 to 5.6% among non-Indigenous children and from 26.9 to 8.4% among Indigenous children 12–23 months of age [23,65]. Similar trends have been observed in both carriage and disease in Australia and the US [52,66,67].

3.5.4. Antimicrobial resistance

There is growing appreciation of the potential for vaccines to address the problem of antimicrobial resistance [68]. PCVs can reduce antibiotic resistant infections through (1) direct reductions in VT carriage and disease (often more resistant than NVTs) and (2) reduction in pneumonia and otitis media, therefore less antibiotic use [68]. Since resistance is associated with antibiotic pressure on strains carried in the nasopharynx, initial reductions in AMR due to reductions in VTs are often counteracted by rising resistance in NVTs over time [69]. Therefore careful monitoring, through carriage studies, is required ascertain long-term impacts of PCVs on AMR.

3.6. Methodological considerations

3.6.1. Study design

The design of pneumococcal carriage studies depends on the study objectives, feasibility and setting. There are two main study designs commonly used for carriage studies: serial cross-sectional surveys, in which participants from a particular population are recruited for a once-off survey periodically, and prospective carriage surveillance, in which participants fulfilling a certain case definition are recruited over time in an ongoing manner (Table 2). While the majority of early impact evaluations using carriage were conducted as cross-sectional surveys, an increasing number of evaluations are using a combination of study designs or carriage surveillance alone to evaluate impact. The former is more suited to community-based studies and the latter is typically health-centre based. Cross-sectional studies need to be conducted at similar time points of the year, since pneumococcal carriage and density is known to vary according to season [70,71]. Importantly, cross-sectional studies may not be able to detect transient changes in carriage e.g. during outbreaks [72].

3.6.2. Study population and recruitment

The populations targeted by carriage studies can be broadly classified into three main groups: (a) healthy populations recruited from the community; (b) healthy populations recruited from health facilities; and (c) populations with acute respiratory infection (ARI) recruited from health facilities (Table 3).

Studies of healthy children are ideal for describing circulating pneumococcal serotype populations. They have the advantage of being easily translated to disease outcomes, using case-carrier ratios. Community-based studies can avoid the selection bias that can be associated with recruiting from health facilities, although they are commonly more resource-intensive to conduct [73].

The rationale for sampling children with respiratory illness is that the patterns of carriage may better reflect the serotypes causing disease [74]. A study of 31 paired lung aspirate and nasopharyngeal aspirates in The Gambia, reported that for all but five cases, serotypes found in lung aspirates were also found in

Table 2
Key features, advantages and disadvantages in the type of pneumococcal carriage study type to determine vaccine impact and monitor pneumococcal serotypes.

	Key features	Select examples	Advantages	Disadvantages
Serial cross-sectional surveys	Cross-sectional surveys completed regularly at a similar season, usually annually or periodically	Kenya [19] Malawi [79] South Africa [30] Fiji[23] Laos[29]	• Suitable study design for community-based sampling • Completed in short timeframe	• Logistical challenges related to hiring staff for short periods • Need to ensure consistency in methods across different survey years • Need to adjust analyses for changing characteristics of study participants between surveys • Single snapshots in time, therefore results are more sensitive to temporal effects, such as outbreaks, which could confound vaccine effects • If PCV coverage is high in the post-PCV survey, there may be insufficient unvaccinated children in target age groups to determine vaccine effectiveness
Prospective carriage surveillance	Persons fulfilling pre-specified case definitions are routinely sampled in an ongoing manner	Turkey [36]Mozambique [74]Laos, PNG & Mongolia [80]	• Able to monitor seasonality of carriage and changes over shorter time periods e.g. outbreaks • Recruitment over time, as PCV programs are rolled out, is more likely to include both vaccinated and unvaccinated individuals needed to determine vaccine effectiveness	• Not suited to community-wide surveys • Need to adjust analyses for changing characteristics of study participants over time

Table 3
Key features, advantages and disadvantages of different study populations for determining vaccine impact and monitoring pneumococcal serotypes.

Study population	Key features and examples	Select examples	Advantages	Disadvantages
Healthy populations – community based	Community-wide surveys	South Africa [32]Brazil [24]	• Ideal for describing circulating serotypes in the community • Can be completed in a short timeframe • Can be used infer PCV impacts on IPD using case-carrier ratios	• Resource-intensive to conduct • Less likely to detect invasive serotypes in healthy children compared to children with acute respiratory infection[63] • Random population sampling may be difficult or not feasible (e.g. if lacking population register) • Ready access to appropriate infrastructure (including minus 80° freezers), may be difficult in remote areas and therefore findings may not be generalisable to the wider community
Healthy populations in health or educational facilities	Recruitment of healthy populations in day-care facilities, schools, aged care facilities or attending immunisation clinics	The Gambia [26] Colombia [35]	• Easily accessible population • Easier access to appropriate infrastructure and equipment (including minus 80° freezers) • Can be used infer PCV impacts on disease using case-carrier ratios	• Sampling is biased towards populations with access to health or educational infrastructure, therefore findings may not be generalisable to the wider community; • Less likely to detect invasive serotypes in healthy children compared to children with acute respiratory infection[63]
Populations with acute respiratory infection	Recruitment based at a health-care facilities – may include a range of severities	Laos, PNG & Mongolia [80] Mozambique [74]	• Easily accessible population • Can be more reflective of carriage serotypes causing pneumonia [63] • Easier access to appropriate infrastructure (including minus 80° freezers)	• Carriage may be affected by prior antibiotic use • Findings may not be generalizable to describe pneumococcal serotypes circulating in the wider community • Unable to translate outcomes to IPD using case-carrier ratios

nasopharyngeal aspirates [75]. A study from Mozambique comparing serotype distribution of carriage between healthy children and children with pneumonia after PCV introduction reported that while distributions were similar, serotype 1 was only detected in pneumonia cases and pneumonia was associated with VT carriage [74]. Therefore sampling in this population may better identify the highly invasive serotypes. However, interpretation of these studies in terms of their implications for IPD are less clear since case-carrier ratios, which are based on carriage in healthy children, are not directly applicable. The degree to which carriage patterns reflect disease-causing pneumococcal serotypes may also depend on the specificity of the case-definition for pneumococcal pneumonia. Analyses would also have to account for antibiotic use in the population, which decreases pneumococcal yield in nasopharyngeal samples by 30% when using culture and 5–7% using molecular methods [76].

Another important consideration are the age and vaccination status of study populations. In order to evaluate indirect effects, studies need to include either unvaccinated children or age groups. A meta-analysis demonstrated a strong correlation between VT carriage in children and adults, indicating that it is possible to make informed predictions about adult carriage based on data from children, which are more widely available [77]. In particular, studying toddlers or pre-school age children may be useful proxy for adults, since these age groups drive indirect protection [78]. However, the relevant age group might differ in LMICs, as transmission patterns differ.

3.6.3. Study outcomes

To date, VT and NVT carriage prevalence as a proportion of all participants, which can be interpreted as a proxy for transmission, has been the most widely used outcome for carriage studies. Some studies have also reported VT and NVT carriage as a proportion of pneumococci identified, which may better account for fluctuations in pneumococcal carriage and highlight early vaccine effects on circulating pneumococcal populations [23,29].

Pneumococcal density has been suggested as an important outcome to consider in PCV evaluations due to emerging evidence for the role of carriage density in disease and transmission [20–22]. However, there is uncertainty about how best to use and interpret this outcome given that carriage density is a dynamic process with no clear cut-offs for either disease or transmission [20]. The Pneumonia Etiology Research for Child Health (PERCH) group determined an optimum density cut-off for pneumococcal pneumonia, however the cut-off had only limited sensitivity (64%) [20]. Studies using carriage density as an outcome will also need to consider other factors known to affect density including age, antibiotic exposure, acute respiratory infection and viral co-infection [81,82].

Individuals can be colonised by multiple serotypes of pneumococci simultaneously, and multiple serotype carriage is common in LMIC, however the importance of detecting multiple serotype carriage in vaccine impact studies remains an open question [83]. Lastly, an emerging area of interest is the impact of PCV on antimicrobial resistance (AMR) – studies may consider monitoring resistance patterns and measuring the vaccine effectiveness against AMR.

3.6.4. Comparison groups and potential confounders

To determine direct effects (also known as vaccine effectiveness), studies can compare carriage outcomes among vaccinated children with unvaccinated children in the post-PCV period– since both groups benefit from indirect effects, comparison of the two groups allows researchers to determine the additional direct benefits experienced by vaccinated children [84]. If indicated, we recommend a study design, where outcome status is assessed for a cohort of individuals by nasopharyngeal swab and immunisation records are used to retrospectively ascertain PCV status. While case-control studies are commonly conducted to assess vaccine effectiveness against disease outcomes, this study design is challenging to apply to carriage outcomes, since carriage is asymptomatic and expedited testing (often not feasible in LMICs) is required to determine case status. We note that vaccine effectiveness may be difficult to determine in settings with high coverage

since strong indirect effects will result in low VT carriage in both vaccinated and unvaccinated groups (insufficient power), and the population that remains unvaccinated is likely to be quite different from the general population and there is therefore a strong potential for confounding. Surveillance studies are often more suited to determining vaccine effectiveness, since recruitment over time is more likely to include unvaccinated individuals as programs are rolled out, whereas carriage surveys conducted one year following PCV introduction may comprise mostly vaccinated children, especially if vaccine coverage is high.

To determine indirect effects, researchers can compare unvaccinated people within populations where PCV has been introduced against a separate populations where PCV has not been introduced. The comparison population can be separated temporally (e.g. pre-post comparisons) or geographically (e.g. staged introduction of PCV [either intentionally or unintentionally since programs often rollout gradually across a country for administrative reasons]) [84]. Comparisons pre- and post PCV introduction need to account for other factors influencing in carriage prevalence that may have changed over time. Important risk factors for carriage include: age, season, viral infection, crowding, smoke exposure and use of antibiotics [85,86]. Most studies have only a limited period of baseline carriage data, making it difficult to determine whether changes post-PCV introduction may in fact be attributable to secular trends or random variation. In this context, trends in overall pneumococcal carriage can provide a useful indicator of likely secular trends in the absence of PCV introduction.

3.6.5. Laboratory methods

The World Health Organization guidelines for pneumococcal carriage studies for recommend collection of a nasopharyngeal swab with prompt storage in skim milk-tryptone-glucose-glycerol medium. Culture on selective blood agar media followed by optochin susceptibility and bile solubility testing remain the gold standard for pneumococcal identification, however molecular methods are becoming widely used, with *lytA* real-time PCR the recommended option [16]. An advantage of PCR-based methods is that they can applied quantitatively.

The capsular reaction/swelling test (Quellung reaction) is considered the gold standard for pneumococcal serotyping, with latex agglutination being less expensive and cumbersome [16]. Molecular methods, including PCR, can also be used to conduct pneumococcal serotyping [87]. The PneuCarriage Project evaluated pneumococcal serotyping methods for use in carriage studies and identified DNA microarray with a culture-amplification step as the top-performing method, with a latex sweep method also performing well [88]. Regardless of methodology, serotyping methods require trained microbiologists and quality control/quality assurance procedures, and their establishment requires time and resources. In some settings, collaboration with a regional reference laboratory may be a more practical option rather than conducting serotyping in country.

4. Conclusion

This review highlights the growing body of pneumococcal carriage studies in LMICs. While carriage studies have consistently demonstrated strong direct and indirect vaccine effects of PCV on carriage, the magnitude of the impact of PCV has varied widely, likely due to variations in pneumococcal serotype distribution, transmission patterns, PCV coverage and timeliness of administration, vaccine effectiveness by setting, population risk factors and more [7].

Furthermore there remains critical gap in our ability to monitor serotype replacement disease in LMIC. Our ability to infer disease patterns using carriage studies is limited by our lack of understanding about replacement disease, demonstrated by our inability to account for the marked variation in replacement disease across HIC [7]. A more detailed understanding of the factors and processes involved in the transition from carriage to disease could guide modelling studies to translate carriage results to disease outcomes.

Carriage studies in LMICs are often structured and funded as research studies, and the sustainability of this model is uncertain. In particular, current laboratory methods require substantial expertise time and resources. Given the important functions that carriage studies are providing in monitoring PCV impact in LMIC, the public health community should consider how to improve feasibility and costs, and how these programs can be better integrated within routine vaccine preventable disease surveillance.

Robust assessments of vaccine impact are required as policy makers consider adopting or funding PCV programs, review potential changes to PCV schedules, and plan for the roll-out of newer generations of pneumococcal vaccines. Some middle-income countries are yet to introduce the vaccine and many former low-income countries are transitioning from receiving Gavi support and need to consider self-financing existing programs. From a global perspective, not every country requires an impact evaluation, however there needs to be sufficient evidence generated across different settings in order to inform vaccine policy. In this context, carriages studies can provide useful information to inform cost-effectiveness assessments and evaluate newer vaccines and novel vaccination strategies. However, comparisons across settings, in order to assess vaccination strategies for example, may be challenging given differences in baseline pneumococcal epidemiology and health systems.

5. Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published from 2000 to July 2018, by use of the terms "pneumococcal vaccine", "pneumococcal conjugate vaccine", "carriage", and "colonization" (supplemented post-hoc with studies from late 2018 and early 2019). We adapted filters prepared by the Cochrane Collaboration to restrict references to those reporting on LMICs. Relevant studies were also identified by reviewing abstracts from the 11th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD), reports from the International Vaccine Access Center, Johns Hopkins Bloomberg School of Public Health and expert consultation. Data extraction (Table 1) was performed on published and preprint studies only. Risk of bias was assessed using the Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool.[89] Detailed assessments are available in Supplementary Tables 2–4.

6. Contributors

FMR conceived the idea for the review. JC conducted the literature review and completed the initial draft. FMR and CDN supervised the drafting of the review. EMD and CS drafted the laboratory section. CDN, EMD, EKM, TM, WP, ER, CS, DMW, AX and FMR provided critical feedback on subsequent drafts.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: DMW reports personal fees from Pfizer, personal fees from Affinivax, outside the submitted work; EKM, CDN, CS, EMD, TM reports grants from Pfizer, outside the submitted work; JC, FMR, ER, and WP have nothing to disclose.

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Appendix A. Supplementary material

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