Evaluating impact and effectiveness of the 13-valent pneumococcal conjugate vaccine on *Streptococcus pneumoniae* vaccine-serotype pneumococcal carriage and population incidence of invasive pneumococcal disease in Blantyre, Malawi

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of

Doctor of Philosophy

by

Todd D. Swarthout
BSc, MSc

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Declaration

I, Todd Swarthout, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Chapter 3: While providing scientific input and review, Claudo Fronterre—in collaboration with Peter Diggle—developed and implemented the statistical modelling component (non-linear regression analysis for vaccine-serotype carriage half-life). Additional statistics support was provided by Marc Henrion and Neil French.

Chapter 4: The work on evaluating the concordance of pneumococcal serotyping methods was undertaken with Andrea Gori. The isolates used for whole-genome sequencing were collected during fieldwork that I implemented. The bioinformatic component (including preparing sequence libraries for submission to PneumoCaT software and interpretation of PneumoCaT results) was led by Andrea Gori.

Chapter 5: While providing scientific input and review, Naor Bar-Zeev developed and implemented the statistical modelling component (negative-binomial regression to evaluate secular trend-adjusted incidence rate ratios and predicted counterfactual incidence). Additionally, chapter 5 reports results from a case-control study (assessing vaccine effectiveness) that was entirely led by Naor Bar-Zeev. Archived invasive pneumococcal isolates that were leveraged for serotyping were available as part of ongoing disease surveillance implemented by the Malawi Liverpool Wellcome Clinical Research Programme (MLW) and currently overseen by Brigitte Denis and George Selemani.

Work associated with chapter 3 required adapting existing draft protocols and draft data collection forms (chapter 3). The remaining work (chapters 4-6) required me to (co-)write new grants, develop new study protocols and documents, and oversee governance.

Over the duration of this work I trained and supervised a team of 37 staff (including 5 study coordinators, 12 research nurses, 13 enumerators, 3 research associates, and 4 laboratory technicians). I performed data management, data cleaning, and all analyses other than those specified in the Methods section of each chapter. Analyses were completed with the input and support of Robert Heyderman, Neil French, Naor Bar-Zeev, and Marc Henrion.

The work within this thesis has not been submitted for any other degree or professional qualification.

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Abstract

Title: Evaluating impact and effectiveness of the 13-valent pneumococcal conjugate vaccine on *Streptococcus pneumoniae* vaccine-serotype pneumococcal carriage and population incidence of invasive pneumococcal disease in Blantyre Disttict, Malawi

Background: *Streptococcus pneumoniae* (pneumococcus) is a leading cause of childhood morbidity and mortality worldwide. The 13-valent pneumococcal conjugate vaccine (PCV13) was introduced to the Malawian infant immunization programme on 12 November 2011. In Malawi, PCV continues to be administered using a 3+0 schedule (doses given at 6, 10, and 14 weeks of age, with no booster dose). This thesis aims to evaluate the impact of PCV13 on pneumococcal carriage and invasive pneumococcal disease (IPD) in Blantyre, Malawi, and to provide evidence to inform national stakeholders on the implementation of impactful vaccination strategies in Malawi.

Methods: To evaluate PCV13's impact on pneumococcal carriage, prospective nasopharyngeal carriage surveys were conducted in Blantyre, between 2015 and 2018, among healthy PCV age-eligible and -ineligible children, as well as HIV-infected adults on antiretroviral therapy (ART). The evaluation of PCV13's impact on IPD was achieved by leveraging pneumococcal isolates from ongoing laboratory-based surveillance at Queen Elizabeth Central Hospital in Blantyre, including 6 years before and 7 years after the introduction of PCV13. Multiple pneumococcal serotyping methods were implemented and compared using samples from community carriage surveillance in Blantyre, Malawi, with evaluations of concordance between latex agglutination, whole-genome sequencing (WGS) with Pneumococcal Capsular Typing (PneumoCaT) software, and DNA microarray.

Results: Despite evidence of reduced vaccine-serotype (VT) carriage over the study period, there is persistently high residual carriage among PCV-vaccinated children (16.1% relative reduction from 19.9%-16.7%), PCV-unvaccinated children (40.5%; 26.4%-15.7%), and HIV-infected adults on ART (41.4%; 15.2%-8.9%). A decline in total (VT+non-VT [NVT]) IPD preceded the introduction of PCV13: 19% (incidence rate ratio [IRR], 0.81; 95% confidence interval [CI], 0.74-0.88]; $p<0.001$) among infants, 14% (IRR, 0.86; 95% CI, 0.80-0.93; $p<0.001$) among young children, and 8% (IRR, 0.92; 95% CI, 0.83-1.01; $p=0.08$) among adolescents and adults. Compared with the counterfactually predicted incidence of VT-IPD, the PCV13-associated VT IPD incidence was 38% (95% CI, 37%-40%) lower among infants, 74% (95% CI, 70%-78%) lower among young children, 79% (95% CI, 76%-83%) lower among older children, and 47% (95% CI, 44%-51%) lower among adolescents and adults. Concordance between serotyping methods was high: 90.7% (95% CI, 89.0%-92.2%) between latex and PneumoCaT, 95.2% (95% CI, 93.9%-96.3%)

between latex and microarray, and 96.6% (95% CI, 95.5%-97.5%) between microarray and PneumoCaT. However, by detecting additional VT pneumococcus carried at low relative abundance, microarray increased VT detection by 31.5% across all ages compared with conventional latex serotyping.

Conclusion: Compared with high-income settings, Blantyre had high residual VT carriage 3.6 to 7.1 years after the introduction of PCV13. Though there were significant declines in IPD before PCV implementation, and though the PCV13-attributable impact on IPD was significant among vaccine age-eligible children 7 years after PCV13's introduction, indirect effects benefitting unvaccinated infants and adults were more modest. We report high concordance between three serotyping techniques applicable to routine pneumococcal surveillance in this setting. Latex serotyping, which requires the least expertise and fewest resources for field implementation and analysis, accurately identifies VT pneumococcus. However, WGS (which adds population structure) and microarray (which adds multiple-serotype carriage) should be considered at regional reference laboratories while investigating the impact of VT carriage (in low relative abundance) on transmission and disease. The PAVE study was initiated in March 2021 to evaluate an alternative World Health Organization–approved three-dose schedule, including a booster dose at 9 months of age. Policy decisions should consider multiple alternative public health strategies for reducing carriage and disease burden, including targeted vaccination outside infant immunization programmes to benefit vulnerable populations. Rigorous evaluation of strategies to augment vaccine-induced control of carriage is required, including alternative schedules and catch-up campaigns.

List of abbreviations

ARI	Acute respiratory infection
AMR	Antimicrobial resistance
aPR	Adjusted prevalence ratio
ART	Antiretroviral therapy
BCG	Bacillus Calmette-Guérin
CAP	Community-acquired pneumonia
CDC	Centers for Disease Control and Prevention
CFU	Colony-forming unit
CI	Confidence interval
CLIMB	Cloud Infrastructure for Microbial Bioinformatics
cmPCR	Conventional multiplex PCR serotyping
COM	College of Medicine
COMREC	College of Medicine Research and Ethics Committee
CoPc	Correlate of protection – carriage
cPR	Crude prevalence ratio
CRF	Case report form
CRM	Cross-reactive material
CRT	Cluster-randomized trial
CSF	Cerebrospinal fluid
DE	Design effect
DHO	District health office
DNA	Deoxyribonucleic acid
doi	Digital object identifier
EPI	Expanded Programme on Immunization
FOI	Force of infection
GAVI	Global Alliance for Vaccines and Immunisation
GEE	Generalized estimating equations
GMT	Geometric mean titres
GSK	GlaxoSmithKline
HIC	High-income country
HSA	Health surveillance assistant
HIV	Human immunodeficiency virus
ICC	Intraclass correlation coefficient
IPD	Invasive pneumococcal disease
IgG	Immunoglobulin G

IQR	Interquartile range
IRR	Incidence rate ratio
KUHeS	Kamuzu University of Health Sciences
LRTI	Lower respiratory tract infection
LSTM	Liverpool School of Tropical Medicine
MDHS	Malawi Demographic and Health Survey
MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
MoH	Ministry of Health
MRC	Medical Research Council
NICD	National Institute for Communicable Disease
NITAG	National Immunizations Technical Advisory Group
NP	Nasopharyngeal
NPS	Nasopharyngeal swab
NT	Non-typeable
NVT	Non-vaccine serotype
ODK	Open Data Kit
OP	Oropharyngeal
OR	Odds ratio
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine
PCV7	7-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PHE	Public Health England
PID	Participant identification number
PneumoCaT	Pneumococcal Capsular Typing
PPV23	23-valent polysaccharide vaccine
PRECIS-2	Pagmatic-Explanatory Continuum Indicator Summary 2
QECH	Queen Elizabeth Central Hospital
RCT	Randomized controlled trial
RR	Risk ratio
rRT-PCR	Real-time reverse transcriptase polymerase chain reaction
SBA	Sheep blood + agar
SBG	Sheep blood + gentamicin
SD	Standard deviation
SOP	Standard operating procedure

sSA	sub-Saharan Africa
SII	Serum Institute of India
SSI	Statens Serum Institut
STGG	Skim milk, tryptone, glucose, and glycerol
TB	Tuberculosis
UK	United Kingdom
UoL	University of Liverpool
UR	Uncertainty ratio
USA	United States of America
VCT	Voluntary counselling and testing
VE	Vaccine efficacy or effectiveness
VT	Vaccine serotype
WGS	Whole-genome sequencing
WHO	World Health Organization

List of publications and international presentations arising from my PhD

Published, peer reviewed (* = Joint first author)

1. Betts M, Jarvis S, Jeffries A, Gori A, Chaguza C, Msefula J, Weight CM, Kwambana-Adams B, French N, **Swarthout TD**, Brown JS, Heyderman RS. Complete genome sequence of *Streptococcus pneumoniae* strain BVJ1JL, a serotype 1 carriage isolate from Malawi. Microbiol Resour Announc. 2021;10(39):e0071521. doi: 10.1128/MRA.00715-21. Epub 2021 Sep 30. Erratum in: Microbiol Resour Announc. 2021 Oct 21;10(42):e0098521. PMID: 34591678; PMCID: PMC8483660.

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International presentations

1. Rapid waning of vaccine-induced immunity among PCV13-vaccinted children under 5 years old in Malawi with subsequent acquisition of natural antibody through natural exposure. International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD); 2020; Toronto, Canada. ID 267 [postponed due to COVID-19 pandemic].

2. Pneumococcal serotyping of Malawi carriage samples by latex agglutination, WGS (PneumoCaT) & DNA microarray: Which should I use [oral presentation]? International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD); 2018 Apr 15-19; Melbourne, Australia.

3. Limited vaccine-induced control of pneumococcal carriage amongst children six-years post-introduction of 13-valent pneumococcal conjugate vaccine in Malawi: impact of force of infection and naturally-acquired immunity [poster presentation]. International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD); 2018 Apr 15-19; Melbourne, Australia.

4. Persistent vaccine-type carriage of *Streptococcus pneumoniae* 4 years after introducing PCV13 in a 3+0 schedule in Malawi [oral presentation]. International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD); 2016 26-30 Jun; Glasgow, UK.

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Chapter 1. Introduction

1.1 Overview of the pneumococcus

1.1.1 Characteristics of the pneumococcal bacteria

Streptococcus pneumoniae is a gram-positive, encapsulated diplococcus. The polysaccharide capsule of this bacterium is an essential virulence factor, and the nearly 100 distinct pneumococcal serotypes are defined by differences in the composition of this capsule. In general, serotype-specific immunity follows infection, but cross-protection between related serotypes can occur. While a wide variety of serotypes cause non-invasive pneumococcal diseases, such as otitis media and sinusitis, serotypes 1, 5, 6A, 6B, 14, 19F, and 23F are common causes of invasive pneumococcal diseases (IPD) globally in children <5 years of age. Serotypes 1, 5, and 14 together account for 28% to 43% of IPD across regions and for about 30% of IPD in 20 of the world's poorest countries; serotypes 23F and 19F are responsible for 9% to 18% of cases globally. Serotype 18C is common in regions with large proportions of high-income countries (i.e., Europe, North America, and Oceania).[1] Some serotypes, such as 6B, 9V, 14, 19A, 19F, and 23F, are more likely than others to be associated with drug resistance.[2]

1.1.2 Transmission

Streptococcus pneumoniae frequently colonizes the human nasopharynx and is transmitted mainly through respiratory droplets. Infants and young children are thought to be the main reservoirs of this agent with cross-sectional point prevalences of all-serotype (vaccine-serotype [VT] + non-VT) nasopharyngeal (NP) carriage ranging from 27% in developed to 85% in developing countries.[3]

1.1.3 Clinical presentation

Pneumococcal infection and disease can affect a variety of organ systems and result in a variety of disease syndromes. Although temporary colonization of the NP mucosa—which is the primary focus of infection—rarely results in disease, certain pneumococcal serotypes may occasionally invade the bloodstream causing bacteraemia and possibly infection of secondary sites, such as the meninges. In other instances, contiguous spread from the nasopharynx may cause diseases, such as otitis media or sinusitis. Pneumonia is often caused by aspiration of pneumococci from the nasopharynx. When associated with bacteraemia, pneumonia is classified as IPD.[4]

Case fatality rates can be high for IPD, ranging up to 20% for septicaemia and 50% for meningitis in developing countries. Mortality is greatest among younger infants. Even in industrialized countries, the overall case fatality rates associated with pneumococcal bacteraemia may reach 15% to 20% among adults and 30% to 40% among elderly patients, despite appropriate antibiotic therapy and intensive care. Among meningitis survivors, long-term neurological sequelae, such as hearing loss, cognitive impairment, motor abnormalities, and seizures, have been observed in up to 58% of cases.[5] Pneumococcal middle-ear infection and sinusitis are less severe but considerably more common than other disease manifestations associated with *S. pneumoniae* transmission.

A definitive diagnosis of pneumococcal infection can be made by isolating the bacterium from blood or other normally sterile body sites, such as cerebrospinal fluid, but the aetiological diagnosis is problematic in cases of non-bacteraemic pneumococcal pneumonia.

1.1.4 Pneumococcal epidemiology in sub-Saharan Africa

The distribution of serotypes that cause disease varies by age, disease syndrome, disease severity, geographic region, and over time. Prior to introduction of pneumococcal conjugate vaccines (PCVs), six to eleven serotypes accounted for ≥70% of all IPD occurring in children worldwide.[1] IPD is commonly defined as morbidity associated with the isolation of pneumococci from a normally sterile body site, such as the bloodstream, or morbidity that occurs secondary to bloodstream spread, e.g., meningitis or septic arthritis. IPD does not include, e.g., middle ear infection, which occurs via contiguous spread from the nasopharynx. Most illnesses are sporadic. Outbreaks of pneumococcal disease are uncommon but may occur in closed populations, such as nursing homes, childcare centres, or other institutions. However, in Africa—particularly in the meningitis belt—large outbreaks of meningitis have been caused by serotype 1.[6]

Before widespread immunization with 7-valent PCV (PCV7), the mean annual incidence of IPD among children aged <2 years was 44.4/100 000 per year in Europe and 167/100 000 in the United States.[7,8] In comparison, the annual incidence of IPD among children <2 years in Africa ranged from 60/100 000 in South Africa before the HIV epidemic to 797/100 000 in Mozambique.[9,10,11] Some of the differences could be explained by differences in case ascertainment and surveillance sensitivity, though incidence in Africa did appear to be generally higher than in Europe or North America. In many countries, routine PCV administration has dramatically reduced the incidence of IPD,

and in some places, IPD caused by VTs has virtually disappeared, even among age groups not primarily targeted by the immunization programme (via a herd immunity effect).[12]

1.1.5 Pneumococcus risk groups

A lack of exclusive breastfeeding, nutritional deficiencies, and indoor air pollution are risk factors for pneumonia, including pneumococcal pneumonia, among infants and young children. Apart from the high incidence among children <2 years of age, the risk of pneumococcal disease is higher among adults >65 years of age, and among people who use tobacco or alcohol excessively. This risk is also increased among individuals who suffer from chronic medical conditions, such as heart disease, lung disease, diabetes, or asplenia, or from other conditions that suppress the immune system, such as advanced HIV infection.

1.2 Pneumococcal vaccination

1.2.1 Types of pneumococcal vaccines

Vaccines have been used to prevent pneumococcal disease for more than 30 years. Currently, there are two different types of pneumococcal vaccines on the market: (1) a 23-valent pneumococcal polysaccharide vaccine (PPV23) available since the early 1980s and (2) three PCVs available since 2009—two 10-valent (PCV10) and one 13-valent (PCV13). PCV7 has largely been removed from the market.

1.2.1.1 Polysaccharide vaccines

PPV23 (Pneumovax 23, Merck Sharp & Dohme Corp., Kenilworth, NJ, USA) provides protection against serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B,17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. For primary immunization, PPV23 is administered as a single intramuscular or subcutaneous dose. Revaccination is not normally recommended for immunologically healthy individuals, but one or two revaccinations have been recommended for immunocompromised individuals.[12] In some high- and middle-income countries, PPV23 is recommended for populations proven to be at increased risk of morbidity and mortality from pneumococcal infection, including adults aged >65 years. PPV23 is also used to supplement the immune response following primary vaccination with one of the PCVs (see below).

Developing countries have not prioritized PPV23 administration. Comprehensive meta-analyses of studies assessing PPV23 vaccine efficacy and effectiveness have been conducted, including a 2007 World Health Organization (WHO)–commissioned meta-

analysis and a review of randomized controlled trials (RCTs).[13,14] On balance, as shown in the meta-analyses, the RCT results indicate that PPV23 has a protective effect against IPD and all-cause pneumonia among generally healthy young adults and, to a lesser extent, a protective effect against IPD in the general population of older adults. Such trials have not demonstrated that PPV23 is efficacious against either IPD or all-cause pneumonia in populations at higher risk, such as adults and children with underlying conditions that increase their risk of pneumococcal disease or highly immunosuppressed individuals of any age.[14] PPVs are associated with poor or absent immunogenicity in children <2 years of age and failure, at any age, to induce an anamnestic antibody response upon revaccination.

1.2.1.2 Pneumococcal conjugate vaccines

The term 'pneumococcal conjugate vaccine' (PCV) refers to a vaccine based on chemical coupling of *S. pneumoniae* polysaccharides to an immunogenic protein carrier. This enhances the antibody response and induces immune memory.

PCV7. In high-income countries, mass PCV administration was commonly initiated soon after Prevenar (Wyeth Pharmaceuticals Inc., Philadelphia, PA) became available as the first PCV7 (composed of the capsular polysaccharides purified from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) in 2000. Initially, PCV7 was administered as two (sometimes three) primary (p) doses in infancy and a booster (b) dose at 9 to 18 months of age (i.e., 2p+1b or 3p+1b, commonly referred to as 2+1 or 3+1). Later, the 10-valent Synflorix (PCV10; GlaxoSmithKline [GSK] plc, London, UK) and the 13-valent Prevenar13 (PCV13; Wyeth) became available. PCVs in Africa were predominately introduced from 2011 as supportive funding became available through the Global Alliance for Vaccines and Immunisation (GAVI; now Gavi, the Vaccine Alliance). The vaccination schedules most often implemented with PCV10 or PCV13 were 3+0 in infancy without a booster. South Africa, Rwanda, and The Gambia were the exceptions when they implemented PCV7 in 2009 and then PCV13. South Africa has used a 2+1 schedule, with the third dose given at 9 months of age at the same time as the measles vaccine (see Supplementary Table S3.1 for an overview of the regional differences in vaccine schedules and formulations).

PCV10. Synflorix is a 10-valent conjugate vaccine composed of the capsular polysaccharides purified from 10 serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F. Each is conjugated to a carrier protein, either protein D (an outer membrane protein from non-typable *Haemophilus influenzae*), tetanus toxoid, or diphtheria toxoid. Protein D is

used as carrier protein for 8 of the 10 serotypes (serotypes 1, 4, 5, 6B, 7F, 9V, 14 and 23F); serotype 19F is conjugated to diphtheria toxoid, and serotype 18C is conjugated to tetanus toxoid. PCV10 is adjuvanted with aluminium phosphate and presented in a single-dose syringe or as a one or two-dose vial. The volume per dose is 0.5 mL. Each dose contains 1 μg of polysaccharide for serotypes 1, 5, 6B, 7F, 9V, 14 and 23F, and 3 μg for serotypes 4, 18C, and 19F.

Pneumosil (Serum Institute of India [SII] Pvt. Ltd., Pune, India) is another PCV10 product that was licensed in 2019 and is important in low- and middle-income countries because of its affordability. Its recommended storage temperature is also 2 to 8 °C, and it targets serotypes 1, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, and 23F. Using pre-PCV distribution data from Africa and Asia, it is expected that SII's PCV10 will cover roughly the same percentage of disease (72%-73%) as GSK's PCV10 (72%-74%), assuming cross-protection from 6B to 6A but not from 6A to 6C, which was not estimated in the pre-PCV era.

PCV13. Prevenar13 contains polysaccharide antigens of the pneumococcal capsular serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually conjugated to a non-toxic diphtheria CRM197 carrier protein (CRM, cross-reactive material). A 0.5-mL PCV13 dose contains approximately 2 μg of polysaccharide from each of 12 serotypes and approximately 4 μg of polysaccharide from serotype 6B. The vaccine contains aluminium phosphate as adjuvant. PCV13 is available in single-dose, pre-filled syringes that do not contain latex, as well as in four-dose vials.[15,16]

The recommended storage temperature for both PCV10 and PCV13 is 2 to 8 °C, and the vaccines must not be frozen. A systematic review and meta-analysis of IPD serotype data obtained from children <5 years of age during the period 1980-2007, i.e., before national PCV7 introduction, showed that the serotypes included in PCV7 accounted for ≥49% of IPD in each geographic region, with substantial regional variation (range, 49%-82%) and the highest serotype coverage in North America and Europe. The corresponding estimated coverage for serotypes included in PCV10 and PCV13 reached ≥70% of IPD in every region (range, 70%-84% and 74%-88%, respectively).

1.2.2 Indication and administration

Although the exact labelling details may differ by country, both PCV10 and PCV13 are licensed for active immunization to prevent invasive disease, pneumonia, and acute otitis media caused by the respective *S. pneumoniae* VT in infants and children from 6 weeks to

5 years of age. Additionally, PCV13 is licensed for the prevention of pneumococcal disease among adults >50 years of age.[15,16] PCVs are not indicated for treating pneumococcal disease and are unlikely to prevent infection caused by *S. pneumoniae* serogroups that are not included in the vaccines.

The vaccines are given by injection into the anterolateral aspect of the thigh in infants and into the deltoid muscle in older age groups. For PCV10 and PCV13, the manufacturers recommend three primary doses with an interval of at least 4 weeks between doses, plus a booster at least 6 months after the third dose (3+1 schedule). The first can be given as early as 6 weeks of age; the booster dose should be given between 11 and 15 months of age. An alternative schedule consists of two primary doses given 2 months apart, starting at 2 months of age, followed by a booster at least 6 months after the second dose (2+1 schedule).

1.2.3 Serological criteria for evaluating pneumococcal conjugate vaccines

Results of studies investigating vaccine efficacy or effectiveness against IPD, otitis media, or pneumonia were not available at the time of the initial licensure of PCV10 and PCV13. Since non-inferiority trials comparing these vaccines with PCV7, using clinical end points, would require very large sample sizes and would be prohibitively expensive, licensure of PCV10 and PCV13 was based on non-inferiority trials using serological end points. These trials demonstrated similar immunogenicity profiles between the latter vaccines and PCV7 against most of their common serotypes, as well as favourable immunogenicity profiles for the additional serotypes of PCV10 and PCV13.[17,18]

For the serotypes common to new vaccines and licensed comparators, WHO has defined serological criteria for non-inferiority that should be used in primary analyses.[19] The criteria include (1) the percentage of subjects with serotype-specific IgG ≥0.35 μg/mL using a WHO reference assay (or an alternative and well-justified threshold value based on a specific in-house assay) and (2) the serotype-specific IgG geometric concentration ratios. Additionally, in secondary analyses, it is recommended that comparisons of opsonophagocytic antibody titres of serotypes that are common to the new vaccine and the licensed comparator should focus on serotype-specific geometric mean titre (GMT) ratios rather than the previously used threshold functional titre ≥1:8.

With a dearth in head-to-head studies, there is limited robust evidence to inform the decision for the additional benefit of a booster dose in terms of immunogenicity, reduced NP carriage of VTs, and prevention of IPD.[3,20] One can hypothesize that the two primary

doses of a 2+1 strategy may result in lower antibody levels than a 3+0 strategy during the interval prior to the the booster dose and, in contrast, result in higher antibody levels induced by the third (booster) dose in a 2+1 schedule compared with the third dose in a 3+0 schedule. This boosting effect may be important for optimizing the duration of protection and effectiveness against certain serotypes (e.g., serotype 1). Evidence suggests that differences in clinical outcomes between the 3+0 and 2+1 schedules may be minimal in the presence of herd protection.[21]

1.2.4 Duration of protection

In South Africa, surveillance results have demonstrated that 6.3 years after vaccination with a 9-valent vaccine, vaccine control remained significant against VT-IPD (78%; 95% CI, 34%-92%). This was consistent with immunogenicity data showing that specific antibody concentrations among vaccinated HIV-uninfected children remained above the assumed protective levels observed among unvaccinated HIV-uninfected controls during this period. HIV-infected children who had received a 9-valent PCV at 6, 10, and 14 weeks of age showed some evidence of waning immunity over a mean follow-up period of 2.3 years, with serotype-specific IgG levels below 0.35 μg/ml; the levels were not significantly different between vaccine recipients and controls for three of seven serotypes evaluated.[22] However, about 5 years after vaccination, these vaccinees demonstrated a partial loss of anamnestic responses to PCV.[23]

1.2.5 Impact of pneumococcal conjugate vaccines on serotype replacement

A review of available surveillance data from Australia, Canada, England, Wales, South Africa, and the USA, collected during the period 1998-2009, revealed rapid and substantial reductions in IPD caused by PCV serotypes of the target group for vaccination (children aged <5 years) in all settings, although there was variation in the magnitude of reductions from baseline before mass vaccination implementation. Reductions in IPD rates were also evident among individuals older than the targeted age range for vaccination (reflecting herd protection). In terms of IPD caused by non-PCV serotypes, increased rates were evident among hospitalized patients aged <5 years in some settings and for some age groups in the non-targeted population. In terms of IPD caused by any serotype, the incidence was reduced among those aged <5 years in all settings, whereas among older individuals, some settings experienced decreased IPD rates, some no change, and one setting experienced increases in some age categories.[24] Factors not directly attributable to the vaccines may influence recorded rates of serotype-specific disease and thereby confound the interpretation of the association between mass PCV administration and serotype changes.

Such factors include variations in the proportions of isolates serotyped before and after vaccine introduction, changes in blood culture practice, and outbreaks of pneumococcal disease. Thus, pneumococcal disease surveillance data must be interpreted with caution.[1]

1.2.6 Optimal schedules

Currently, similar proportions of countries that include PCVs in their routine immunization programmes implement the 3+0, 2+1, and 3+1 schedules, respectively. Evidence from systematic reviews strongly supports the 3+0 and 3+1 schedules, which in the included studies typically involved vaccine administration at 6, 10, and 14 weeks or 2, 4, and 6 months of age (plus a booster at 1-2 years of age with the 3+1 schedule).[25,26] Emerging evidence also supports the use of a 2+1 schedule as an alternative, with the third dose (the +1 dose) given between 9 and 15 months of age. Data from observational studies and post-introduction evaluations have reported high levels of protection against IPD following this schedule. In a two-dose schedule with PCV7, the 2-month interval group (vaccination at 2 and 4 months of age) had higher seropositivity levels 1 month after vaccination than the 1-month interval group (with 2-month and 3-month schedules). Similar results were seen at 12 months of age.[27]

1.3 Pneumococcus detection

Carriage studies offer a practical approach for monitoring serotype replacement and can help in assessing the impact of PCVs and other pneumococcal vaccines.[28,29] Pneumococcal carriage is an important end point for efficacy trials of new pneumococcal vaccines.[30] Traditional serotyping methods involve typing a small number of colonies, frequently missing carriage of multiple serotypes, and providing no quantitative data. The gold-standard serotyping method (the quellung reaction) was developed in the early 1900s and is performed by testing colonies with a set of antisera and visualizing the bacteria with a microscope. It is laborious, requires a complete set of type-specific antisera, and is therefore mainly performed by reference laboratories. A pneumococcal serotyping method suitable for use in carriage studies should have high sensitivity (including the ability to detect multiple serotypes), detect most or all serotypes, be amenable to scale-up for large projects, and be practical for resource-poor countries.

Between 1998 and 2001, the WHO convened the Pneumococcal Vaccine Trials Carriage Working Group. This group was charged with formulating a set of core methods for conducting studies investigating NP colonization of *S. pneumoniae,* primarily in the context of PCV efficacy trials.[31] As reported by Satzke et al,[32] the variability in results

from pneumococcal carriage studies across diverse epidemiologic settings can be understood to derive from biologic effects rather than methodological differences, in large part because many of the standard pneumococcal carriage methods have been widely adopted.

In the decade following that pivotal report authored by the working group,[32] there were many key accomplishments, including sequencing of the nearly 100 pneumococcal capsular loci,[33] the advent of molecular detection and quantification of pneumococci in NP specimens and serotype-specific detection, including improved detection of multiple-serotype colonization. There were also significant advances in molecular typing, as well as in modelling and statistical methods for longitudinal studies of carriage dynamics. Considering these advances, and the importance of carriage studies, WHO invited an ad hoc group of experts, some of whom were members of the previous working group, to evaluate the state of knowledge, revise the core methods where appropriate, and outline the important scientific questions for the future. The resulting document formed the basis of a review meeting in Geneva, which took place on 29 and 30 March 2012, and a second important published set of methods outlining minimum requirements for pneumococcal carriage studies.[32] That document was especially useful in that it constituted updated recommendations from the WHO, and it provided clear needs for future research.

Despite these advances in alternative serotyping methods, few data formally comparing the performance of these methods to the gold-standard quellung reaction, or to each other, were available.[34] The PneuCarriage project—a large multicentre, international study—was established with the aim of identifying the best pneumococcal serotyping methods for carriage studies.[35] The project identified microarray with a culture amplification step as the top-performing method. Microarray has multiple advantages, including its capacities to detect all known serotypes, differentiate multiple serotypes in a biological sample, accurately measure per cent relative abundance (when multiple serotypes are present), and facilitate high-throughput testing. While robust and systematic, the decision algorithm did not consider variables, such as requisite costs, skill level, and resources for assay implementation and maintenance, as well as output processing and interpretation—an observation addressed in chapter 4 of this thesis.

1.3.1 Detection of multiple serotypes

As mentioned earlier in this chapter, microarray has multiple advantages, including its capability to detect all known serotypes and differentiate multiple serotypes in a biological

sample. While latex agglutination is accurate, rapid, and requires relatively minimal expertise and resources for field implementation and analysis, standard latex approaches are not optimal for high-resolution surveillance of vaccine impact, including the detection of multiple-serotype and VT carriage when *S. pneumoniae* is present in low relative abundance.[36] There have been attempts to implement latex agglutination for detecting multiple-serotype carriage. Gratten et al[37] serotyped up to six colonies from nasal secretion culture plates and found multiple-serotype carriage in 29.5% of children in a Papua New Guinean sample. The authors went on to serotype at least 50 colonies from 10 selected nasal secretion cultures and concluded that, among individuals with multiple-serotype carriage, the respective minor serotypes accounted for 4% to 27% of the total pneumococcal population. A review of published data on multiple-serotype carriage concluded that, to detect a minor serotype, it would be necessary to serotype at least five colonies to have a 95% chance of detecting the serotype if it accounted for 50% of the total pneumococcal population, and one would need to examine 299 colonies if the serotype was present at a relative abundance of 1%. In the PneuCarriage project, for thorough sample characterization, up to 120 colonies from each sample were selected to achieve >99% power to detect a minor serotype of 5% abundance.[35] This approach would not be cost- or time-effective. Though dependent on technical capacity to develop in-house reagents, researchers in The Gambia developed a technique in which colonies from the primary culture plate are suspended in saline and serotyped by latex agglutination.[38] In their longitudinal infant cohort study, while they did not differentiate NVT serotypes, they showed that up to 10.4% of pneumococcal acquisitions were of multiple serotypes. Though less cost- and time-effective, the use of commercial products (including from Statens Serum Institut, Copenhagen, Denmark) in conjunction with the production of in-house latex serotyping reagents has been well documented to significantly expand the number of NVT serotypes that can be differentiated by latex, including as part of quality control procedures.[39]

1.4 Malawi

1.4.1 Demographic and health factors

Malawi is in southern Africa (Figure 1) and had a population of approximately 17.6 million as of 2018; this is estimated to exceed 26 million by 2030.[40,41] The average life expectancy at birth is 54.9 years for males and 55.2 years for females. Malawi has a young population, with a median age of 17 years; 46% of the population is under 15 years of age.[41] One of the poorest countries in the world, Malawi has a gross domestic product of US$355 per capita and is ranked 174th of 187 countries in terms of Human Development

Index.[42] The economy is predominantly agricultural, with approximately 80% of the population living in rural areas. A substantial proportion of Malawians rely on subsistence farming and are, therefore, vulnerable to natural disasters, such as drought or heavy rain. Blantyre, the commercial and finance centre, is situated in southern Malawi. It has a population of 1.3 million, around two-thirds of which resides in urban dwellings. Public healthcare is free at the point of delivery. Under the jurisdiction of the Blantyre District Health Office, the government healthcare system in Blantyre consists of Queen Elizabeth Central Hospital (QECH), the largest referral hospital in the country, and 28 health centres. QECH provides secondary and tertiary care to the population of greater Blantyre and receives referrals from all over Malawi. Blantyre also has several private hospitals and clinics, including many operated by non-governmental organizations, but these facilities generally have relatively few inpatient beds.

Figure 1.1. Map of Malawi, highlighting study location (Blantyre)

Malawi is characterized by a heavy burden of disease, as evidenced by high mortality rates among both children (infant and under-five mortality, 66/1000 and 112/1000 live births, respectively) and adults (maternal mortality 675/100 000 live births),[40] as well as high rates of diseases, such as tuberculosis, malaria, HIV, and other tropical diseases. Despite this, Malawi has only 1.9 physicians per 100 000 population, constituting one of the lowest physician densities in the world.[43]

1.5 Aim and hypothesis

The overall research question of this thesis is: "What is the impact of the 13-valent pneumococcal conjugate vaccine (PCV), introduced as part a national Expanded Programme on Immunization, in a setting with a high HIV prevalence and a high pneumococcal carriage prevalence?" The primary hypothesis is that PCV13 has not provided optimal direct or indirect protection to reduce VT carriage and IPD in Malawi, compared to high-income settings. Thus, policymakers should consider available evidence when developing vaccine strategies in Malawi and other similar settings.

The aims of the investigative work contributing to this thesis, in answering the overall research question, were to implement robust research activities, including:

1. Implementation of rolling cross-sectional pneumococcal carriage surveys in Blantyre, evaluating populations at risk of pneumococcal carriage
2. Supporting and leveraging existing IPD surveillance to evaluate the impact of PCV13 on VT IPD
3. Evaluating optimal pneumococcal serotyping methods that are both robust and appropriate for Malawi and other similar settings
4. Propose a pragmatic study design for evaluating an alternative vaccine strategy in Malawi

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Chapter 2. General methods

This chapter discusses methodological aspects that are common to the studies described in chapters 3 through 6, including study sites, laboratory procedures, data management, and ethical considerations. Methods pertaining to each study—namely study design, population, clinical definitions, procedures, sample size, statistical analyses, and timescale—will be discussed within each individual chapter.

2.1 Study sites

2.1.1 Queen Elizabeth Central Hospital

Queen Elizabeth Central Hospital (QECH) is the largest government inpatient health facility (1250 beds) in Malawi and is the referral centre for patients in the Southern Region. It provides secondary and tertiary care to the population of greater Blantyre (around 1.3 million), including internal medicine, general surgery, paediatrics, obstetrics and gynaecology, anaesthesia, and radiology, in addition to surgical subspecialties, such as orthopaedics, otorhinolaryngology, neurosurgery, and ophthalmology.

2.1.2 Antiretroviral therapy and voluntary counselling and testing clinics

The antiretroviral therapy (ART) clinic at QECH was founded initially as a fee-paying clinic in 2000 but has been providing ART free of charge to children and adults since 2004 as part of the Ministry of Health's scale-up programme.[1,2] The voluntary counselling and testing (VCT) service is located adjacent to the ART clinic. Trained counsellors provide HIV counselling and testing to approximately 60 walk-in patients daily.

2.1.3 Malawi-Liverpool-Wellcome Trust Clinical Research Programme

The Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW) conducts laboratory, clinical, epidemiological, and social science research on health problems affecting Malawi and sub-Saharan Africa. Established in 1995, MLW is now part of the Kamuzu University of Health Sciences (KUHeS; formerly the University of Malawi College of Medicine [COM]) and has close links with the University of Liverpool (UoL) and the Liverpool School of Tropical Medicine (LSTM). MLW has also established collaborative links with numerous research institutions worldwide. Since 2003, it has been recognized as one of the Wellcome Trust's African and Asian Programmes.

MLW is located within the grounds of QECH, with field research sites around urban Blantyre, Chikwawa, and Thyolo Districts, all within Malawi's Southern Region. The programme hosts more than 200 local and international PhD and master's students, as well as postdoctoral researchers. It also provides operational support, including administration, finance, information technology, human resources, data management, laboratory capacity, and ordering and storage of research consumables. Most MLW fellows with a clinical background contribute to clinical duties, as well as undergraduate and postgraduate teaching programmes at QECH and KUHeS. MLW's research activities are focused under six themes: (1) Population Health; (2) Social Sciences; (3) Maternal, Neonatal, and Child Health; (4) Vaccines; (5) Clinical and Experimental Medicine; and (6) Infection Biology.

2.2 Laboratory procedures

2.2.1 Nasopharyngeal swabs

Nasopharyngeal swab collection is described in greater detail elsewhere.[3] In brief, nasopharyngeal swab samples were collected using nylon flocked swabs (FLOQSwabs, Copan Diagnostics, Murrieta, CA, USA) and then immediately placed into 1.5 mL of STGG (skim milk, tryptone, glucose, and glycerol) medium and processed at the MLW laboratory in Blantyre, according to World Health Organization recommendations.[4] Samples were frozen on the same day at −80 °C (Figure 4.2).

2.3 Data management

Data for this study were collected in accordance with the ethical principles of good clinical practice, as defined by the International Council for Harmonisation guidelines. MLW standard operating procedures were adhered to in relation to data handling and consent. Each participant was assigned a unique study number at enrolment. Data collection forms for a particular participant were linked by a scannable barcode containing this number. Data were collected by electronic data capture using Open Data Kit (ODK Collect v1.24.0; Get ODK Inc., CA, USA) open-source software, uploaded to a secure server at MLW. No patient identifiable information was kept in the electronic database, which was password protected and accessible by the principal investigator. Data files were exported to Stata 13 (StataCorp LLC, College Station, TX, USA) for analysis. Any paper case report forms (CRFs) were stored in locked filing cabinets in the MLW research office that were accessible only to members of the respective study team.

The patient/guardian consent forms and assent forms contained the only sources of person-identifiable information. These forms contained the participants' names, contact details,

and unique study identification numbers to provide a link with the anonymized clinical data. Consent forms were kept in a secured and locked cabinet held separately from the CRF hard copies. Paper CRFs are stored in the MLW archives for a minimum 5 years following study completion, after which time they will be destroyed.

2.4 Statistical analysis

Statistical analysis details are described in the Methods sections of the subsequent chapters of this thesis.

2.5 Ethical considerations

Ethical approval for all work described in chapters 3 through 6 was obtained from the College of Medicine Research Ethics Committee (COMREC), University of Malawi (P.07/10/958) and relevant research ethics committees in the United Kingdom. The Methods sections in chapters 3 through 6 include descriptions of ethical considerations. For the work reported in chapter 3, the only chapter reporting results from study-specific recruitment, written informed consent was obtained from all participants or their guardians. Written informed assent was obtained from all participants aged 8 to 17 years in addition to written informed consent from their guardians. The approved version of the CRF is included in Appendix C.

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Chapter 3. High residual carriage of vaccine-serotype *Streptococcus pneumoniae* after introduction of pneumococcal conjugate vaccine in Malawi

N.B., Associated published peer-reviewed article: **Swarthout TD**, Fronterre C, Lourenço J, Obolski U, Gori A, Bar-Zeev N, Everett D, Kamng'ona AW, Mwalukomo TS, Mataya AA, Mwansambo C, Banda M, Gupta S, Diggle P, French N, Heyderman RS. High residual carriage of vaccine-serotype *Streptococcus pneumoniae* after introduction of pneumococcal conjugate vaccine in Malawi. Nat Commun. 2020 May 6;11(1):2222. doi: 10.1038/s41467-020-15786-9. PMID: 32376860; PMCID: PMC7203201.

Chapter introduction: This chapter, and much of the work reported in this document, was largely based on the PCVPA (**P**neumococcal **C**arriage among **V**ulnerable **P**opulations in **A**frica) study. This study was designed to evaluate the impact of Malawi's 2011 introduction of the 13-valent pneumococcal vaccine (PCV13), examining its impact on nasopharyngeal carriage among children PCV13-vaccinated through the routine Malawi Expanded Programme on Immunization (in whom vaccine-induced immunity begins to wane after the first year of life), children too old to have received PCV13, and HIV-infected adults on antiretroviral therapy who do not routinely receive pneumococcal vaccination.

Abstract

Introduction: There are concerns that pneumococcal conjugate vaccines (PCVs) in sub-Saharan Africa sub-optimally interrupt *Streptococcus pneumoniae* vaccine-serotype (VT) carriage and transmission.

Methods: Here we assess PCV carriage using rolling, prospective nasopharyngeal carriage surveys between 2015 and 2018, 3.6 to 7.1 years after Malawi's 2011 introduction of PCV13. Carriage decay rate was analysed using non-linear regression.

Results: Despite evidence of reduction in VT carriage over the study period, there is high persistent residual carriage. This includes among PCV-vaccinated children 3 to 5 years old (16.1% relative reduction from 19.9%-16.7%); PCV-unvaccinated children 6 to 8 years old (40.5% reduction from 26.4%-15.7%); HIV-infected adults 18 to 40 years old on antiretroviral therapy (41.4% reduction from 15.2%-8.9%). VT carriage prevalence half-life was similar among PCV-vaccinated and PCV-unvaccinated children (3.34 and 3.26 years, respectively).

Conclusion: Compared with high-income settings, there is high residual VT carriage 3.6 to 7.1 years after PCV introduction. Rigorous evaluation of strategies to optimise vaccine-

induced control of carriage, including alternative schedules and catch-up campaigns is required.

3.1 Introduction

Streptococcus pneumoniae is estimated to be responsible for over 318 000 (uncertainty ratio [UR]: 207 000-395 000) deaths every year in children aged 1 to 59 months worldwide, with the highest mortality burden among African children.[1] *S. pneumoniae* has over 90 immunological serotypes and is a common coloniser of the human nasopharynx, particularly in young children, as well as in resource-poor and HIV-affected populations.[2] Although most carriers are asymptomatic, pneumococcal colonization is a prerequisite for transmission and the development of disease, including pneumonia, meningitis, and septicaemia.[3] In high-income country (HIC) settings (including Europe and North America), routine infant administration of pneumococcal conjugate vaccine (PCV) has rapidly reduced vaccine-serotype (VT) invasive pneumococcal disease (IPD)[4-6] and carriage.[7-9] Importantly, this has occurred in vaccinated and unvaccinated age groups. Thus, indirect protection resulting from a reduction in carriage and transmission amplifies PCV impact and cost-effectiveness.[10] In HICs, PCV was commonly initiated soon after becoming available in 2000, initially with 7-valent Prevenar (PCV7; Wyeth Pharmaceuticals Inc., Philadelphia, PA) usually with a booster dose in the second year of life in addition to doses in infancy (e.g., 2+1 or 3+1), subsequently switching to 10-valent Synflorix (PCV10; GlaxoSmithKline plc, London, UK) or 13-valent Prevenar13 (PCV13; Wyeth). PCVs in Africa were predominately introduced from 2011 as supportive funding became available through the Global Alliance for Vaccines and Immunisation (GAVI; now Gavi, the Vaccine Alliance). The schedules most often implemented, with PCV10 or PCV13, were given in infancy without a booster (3+0). South Africa, Rwanda, and The Gambia were the exceptions, implementing PCV7 in 2009 and then PCV13. South Africa has used a 2+1 schedule, with the third dose given at 9 months of age at the same time as the measles vaccine. See Supplementary Table S3.1 for an overview of the regional differences in vaccine schedules and formulations.

Pneumococcal epidemiology in sub-Saharan Africa (sSA) is characterized by higher rates of carriage and transmission than HIC settings.[11-15] Carriage studies predating PCV introduction in Kenya,[11] Mozambique,[12] Malawi,[13] The Gambia,[14] and South Africa,[15] for example, reported pneumococcal carriage prevalence values ranging from 59% to 90% among children <5 years old, with colonization occurring rapidly early in life.[16] This differed markedly from high-income settings including, for example, the

UK,[17] the USA,[18] and the Netherlands,[19] which reported PCV pneumococcal carriage prevalence estimates between 48% and 68%.

Vaccine trials and post-routine-introduction studies in sSA have demonstrated substantial direct effects of PCV against IPD, pneumonia, and all-cause mortality among young children.[20-23] Though PCV impact is not directly comparable between countries and regions given differences in PCV implementation strategies (formulations and schedules), these countries (e.g., Malawi [PCV13; 3+0],[13] The Gambia [PCV7 & PCV13; 2+1],[20,21] Kenya [PCV10; 3+0],[22] South Africa [PCV7 & PCV13; 2+1],[23,24] and Mozambique [PCV13; 3+0][25]) have consistently reported higher residual VT carriage than HICs (UK [PCV7 and PCV13; 2+1],[17] Netherlands [PCV7 and PCV10; 3+1 and 2+1],[26] and USA [PCV7 and PCV13; 3+1][27]). See Supplementary Table S3.2 for an overview of regional differences in residual VT carriage prevalence after the introduction of PCV into routine Expanded Programmes on Immunization [EPIs]).

As reported for HIC settings,[17,27,28] there is also increasing evidence of non-VT (NVT) carriage replacement in sSA.[29,30] While NVTs typically cause less invasive disease,[31] capsule switching[32] and genotypic shifts[33] may lead to increases in replacement. This is of particular concern in many countries in sSA where the 3+0 schedule has been implemented into infant EPI programmes.[34]

In November 2011, Malawi (previously PCV naïve) introduced PCV13 as part of the national EPI, using a 3+0 schedule (6, 10, and 14 weeks of age). A three-dose catch-up vaccination campaign included infants <1 year of age at the date of first dose, receiving three doses at 1-month intervals. Previous field studies in Malawi have reported high PCV13 uptake rates of 90% to 95%,[35,36] similar to the 92% full PCV vaccination coverage reported by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF).[37] A 2015-2016 Malawian Ministry of Health assessment reported a 12.8% HIV prevalence among adult women (aged 15-64) and an HIV prevalence of 8.2% among adult men.[38] In 2011, Malawi adopted Option B+, whereby all HIV-infected pregnant or breastfeeding women commence lifelong full antiretroviral therapy (ART) regardless of clinical or immunological stage, dramatically reducing mother-to-child-transmission.[39]

An earlier, small community surveillance study in Karonga District, northern Malawi, compared pneumococcal carriage before and 2 years after PCV introduction.[13] VT carriage among young PCV-vaccinated children (1-4 years of age) was 28.2% before vs

16.5% after PCV introduction; Supplementary Table S3.5). These data led us to hypothesize that despite evidence of PCV13 impact on IPD and pneumonia in Malawi,[40,41] in the longer term after vaccine introduction there would be persistently high residual VT carriage and that this would maintain transmission in both childhood and adult reservoirs. In this large population-based study we investigated this among children who received PCV13 through the routine EPI (in whom vaccine-induced immunity begins to wane after the first year of life[42]), children too old to have received PCV13, and HIV-infected adults on ART who do not routinely receive pneumococcal vaccination but were previously demonstrated to have a high carriage prevalence.[43,44] We found that, despite evidence of reduction in VT carriage since PCV13 introduction, there is high persistent residual carriage among PCV-vaccinated and PCV-unvaccinated study populations. We show a VT carriage prevalence half-life that is similar among older PCV-vaccinated and PCV-unvaccinated children. These results, not dissimilar to those observed in several other sub-Saharan African countries, underline the need for rigorous evaluation of strategies to augment vaccine-induced control of carriage.

3.2 Methods

3.2.1 Study design

This was a prospective observational study using stratified random sampling to measure pneumococcal nasopharyngeal (NP) carriage in Blantyre, Malawi. Sampling consisted of twice-annual rolling cross-sectional surveys over 3.5 years.

3.2.2 Study population

Blantyre is located in southern Malawi and has an urban population of approximately 1.3 million. Recruitment included four groups: (1) healthy infants 4 to 8 weeks old prior to the first dose of PCV, recruited from vaccination centres using systematic sampling; (2) randomly sampled healthy children 18 weeks to 7 years old who received PCV as part of routine EPI or via the catch-up campaign, recruited from households and public schools; (3) randomly sampled healthy children 3 to 10 years old who were age-ineligible (born on or before 11 November 2010 and, therefore, too old) to receive PCV as part of routine EPI or via the catch-up campaign), recruited from households and public schools; and (4) HIV-infected adults 18 to 40 years old and on ART, recruited from Blantyre's Queen Elizabeth Central Hospital ART clinic using systematic sampling. After evidence of persistent carriage among children 3 to10 years old during the early surveys, recruitment of infants 4 to 8 weeks old and children 18 weeks to 2 years old was implemented starting from survey 5 and survey 4, respectively. Exclusion criteria for all participants included current

tuberculosis treatment, pneumonia hospitalization ≤14 days prior to screening, or terminal illness. Exclusion criteria for children included parent/guardian not providing written informed consent, child 8 to 10 years old not providing written informed assent, reported immunocompromising illness (including HIV), having received antibiotics ≤14 days prior to screening, having received PCV if age-ineligible or not having received PCV if age-eligible. Individuals were not purposely resampled but were eligible if randomly reselected in subsequent surveys.

3.2.3 Site selection and recruitment

3.2.3.1 Declaration

The field activity methods (including household selection, recruitment, sample collection and storage) were developed by Todd Swarthout. Daily oversight of the field team, including quality control measures, was provided by the study coordinator, Andrew Mataya. Todd Swarthout provided initial training in relevant techniques and provided overall study supervision.

3.2.3.2 Study sites

Households, schools, and vaccination centres were selected from within three non-administrative zones representative of urban Blantyre's socioeconomic spectrum in medium- to high-density townships. These zones were further divided into clusters, allowing for approximately 25 000 adults per zone and 1200 adults per cluster. At the start of each rolling cross-sectional survey, eight clusters were randomly selected. Clusters were not purposely resampled but were eligible if randomly selected in subsequent surveys. Within each cluster, after randomly choosing a first house, teams moved systematically, recruiting one eligible child per household until the required number of children were recruited from each cluster. If no parent/guardian was available or no age-eligible child was at home for household recruitment (including when no one was home), the study team moved systematically to the next house without attempting to revisit the skipped household. At the start of each survey, updated school registers were collected from schools. Individual schoolgoers were randomly selected from school registers, and letters were sent home inviting parents/guardians to travel to the school within the following 3 school days to discuss the study and consider consenting to their child's participation. If the parent/guardian did not respond to the letter or did not visit the school within the specified 3 days, another child was randomly selected from the school's register.

3.2.3.3 Determining pneumococcal vaccination status

A child was considered PCV vaccinated if she or he had received at least one dose of PCV before screening. Vaccination status and inclusion/exclusion criteria were further assessed from subject-held medical records (known as health passports). If a child was reported by the parent/guardian to be PCV vaccinated but no health passport was available, a questionnaire was applied. The questionnaire was developed by identifying, among a subset of 60 participants, four questions most commonly answered correctly by parents/guardians of children with proof of PCV vaccination. The questions included the child's age when vaccinated, vaccine administration route (oral or injectable), anatomical site of vaccination, and which other (if any) vaccines were received at the time of PCV vaccination. If the child was PCV age-eligible and the parent/guardian answered all four questions correctly, the child was recruited as PCV vaccinated.

3.2.3.4 Sample size

The sample size strategy was pragmatic to allow for adequate precision of the carriage prevalence estimates. VT carriage was considered the primary end point, and the sample size was calculated based on the precision of the prevalence estimation, assuming an infinite sampling population. Among children 3 to 7 years old (vaccinated), an absolute VT prevalence up to 10% was expected, with a sample of 300 per survey providing a 95% confidence interval (CI) of 6.6% to 13.4%. Among children 3 to 10 years old (unvaccinated) and HIV-infected adults, an absolute VT prevalence of 20% was expected, with a sample of 200 per survey providing a 95% CI of 14.5% to 25.5%.

3.2.4 Laboratory

3.2.4.1 Declaration

The laboratory methods (including sample collection, processing, serotyping and archiving) were developed by Todd Swarthout in collaboration with Brigitte Denis (lead, laboratory services at Malawi-Liverpool-Wellcome Trust Clinical Research Programme [MLW]) and Maaike Alaerts (lead, MLW molecular testing services). Latex serotyping, including result interpretation, was completed by study lab technicians: Nelson Simwela, Arnold Botomani, Comfort Brown, Jacquline Msefula, Chikondi Jassi, and Mphatso Mayuni. Todd Swarthout provided training in relevant laboratory techniques, provided routine supervision and was ultimately responsible for data quality control and interpretation.

3.2.4.2 Nasopharyngeal swab collection

An NP swab (NPS) sample was collected from each participant using a nylon flocked swab (FLOQSwabs, Copan Diagnostics, Murrieta, CA, USA) and then immediately placed into 1.5 mL of STGG (skim milk, tryptone, glucose, and glycerol) medium and processed at the MLW laboratory in Blantyre, according to WHO recommendations.[68] Samples were frozen on the same day at −80 °C.

3.2.4.3 Pneumococcal identification and latex serotyping

After being thawed and vortexed, 30 µL NPS-STGG was plated on sheep blood agar + gentamicin (SBG; 7% sheep blood agar, 5 µL gentamicin/mL) and incubated overnight at 37 °C in 5% CO_2. Plates showing no *S. pneumoniae* growth were incubated overnight a second time before being reported as negative. *S. pneumoniae* was identified by colony morphology and optochin disc (Oxoid, Basingstoke, UK) susceptibility. The bile solubility test was used on isolates with no or intermediate optochin susceptibility (zone diameter <14 mm). A single colony of confirmed pneumococcus was selected and grown on a new SBG plate as before. Growth from this second plate was used for serotyping by latex agglutination (ImmuLex 7-10-13-valent Pneumotest; Statens Serum Institut, Copenhagen, Denmark). This kit allows for differential identification of each PCV13 VTs but not for differential identification of NVTs; NVT and non-typeable isolates were, therefore, reported as NVTs. Samples were batch tested on a weekly basis, blinded to the sample source. Latex serotyping results showed good concordance with whole-genome sequence and DNA microarray serotyping.[69]

3.2.5 Statistical analysis

3.2.5.1 Declaration

While I provided input on relevant parameters, mechanisms, and biological plausibility, the non-linear regression analysis for VT carriage half-life was completed by Claudio Fronterre, under the supervision of Peter Diggle. I led the interpretation of the results from the non-linear regression analysis, in collaboration with Claudio Fronterre and Peter Diggle.

3.2.5.2 Variables and analysis overview

Participant demographic characteristics were summarized using means, standard deviations, medians, and ranges for continuous variables and frequency distributions for categorical variables. Non-ordinal categorical variables were assessed as indicators. Comparison of covariate distribution between study groups was done by independent group t-test for continuous covariates and by chi-square (χ^2) analysis for categorical covariates. Potential confounders were identified by testing associations between variables and VT carriage and included in the multivariable models when $p<0.1$. Carriage crude prevalence ratios (cPRs) and adjusted prevalence ratios (aPRs) were calculated over the study duration by log-binomial regression using years (365.25 days) between Malawi's introduction of PCV13 (12 November 2011) and participant recruitment, coded as a single time variable. This allowed for an estimated prevalence ratio per annum. Prevalence ratio analyses were restricted to children 3 to 5 years old (PCV vaccinated), children 6 to 8 years old (PCV unvaccinated), and adults. This (1) limited bias due to age, (2) maximized the chance that any change in carriage prevalence over time to be represented primarily by change in calendar time (not ageing in time), and (3) maximized the inclusion of age category representatives with sequential years of the same PCV vaccination status. The formula for relative change in carriage prevalence was: ([carriage prevalence of final survey − carriage prevalence of initial survey]/carriage prevalence of initial survey) $\times$ 100%. CIs are binomial exact. Statistical significance was inferred from two-sided $p<0.05$. Participant data collection was completed using Open Data Kit (ODK Collect v1.24.0; Get ODK Inc., CA, USA) open-source software. Statistical analyses were completed using Stata 13.1 (StataCorp LLC, College Station, TX, USA).

3.2.5.3 Non-linear regression analysis for vaccine-type carriage half-life

To better understand the rate at which VT and NVT carriage prevalence was decreasing, we developed a non-linear model to describe the variation in individual probability of VT or NVT carriage with age, adjusted for age at recruitment. The model is fitted using carriage data from children 3.6 to 10 years of age, maximizing overlap with empiric data and allowing direct comparisons of parameters between vaccinated and unvaccinated children. Model outputs for individual VT and NVT carriage probability were then transformed into a population-level (decay) half-life of each VT and NVT carriage (i.e., time in years for VT and NVT carriage prevalence in the sampled cohort to reduce to one-half of its peak), using $\log(2)/\delta$, where δ = rate of decay of VT or NVT carriage prevalence with age. Model parameters were estimated by maximum likelihood, and 95% confidence bands for the

predicted exponential decay curves were obtained through parametric bootstrap. This analysis used R open-source software, version 3.5.0 (The R Foundation for Statistical Computing, Vienna, Austria). Details of the analysis framework are included as Supplementary Note S3.1.

3.3 Results

3.3.1 Recruitment

Between 19 June 2015 and 6 December 2018, seven rolling carriage surveys were completed. The dates for each survey were, respectively, (1) June through August 2015; (2) October 2015 through April 2016; (3) May through October 2016; (4) November 2016 through April 2017; (5) May through October 2017; (6) November 2017 through June, 2018; (7) June through December 2018. This spanned a period of 3.6 to 7.1 years after Malawi's 12 November 2011 introduction of PCV13. Given that seasonality was found to be associated with pneumococcal carriage in a previous study in Malawi,[16] enrolment was evenly distributed across seasons of high and low carriage incidence.

7554 individuals were screened (Figure 3. 1), including 371 infants 4 to 8 weeks old (prior to receiving the first dose PCV), 602 PCV-vaccinated children 18 weeks to 1 year old, 538 PCV-vaccinated children 2 years old, 2696 PCV-vaccinated children 3 to 7 years old, 1505 PCV-unvaccinated children 3 to 10 years old, and 1842 HIV-infected adults 18 to 40 years old and on ART (PCV unvaccinated). Among those screened, 24 infants (6.5%), 196 children age-eligible for PCV (5.1%), 96 children age-ineligible for PCV (6.4%), and 67 HIV-infected adults (3.6%) were excluded (Figure 3.1) from recruitment after screening. Twenty-three participants (18 children, 5 HIV-infected adults) did not allow a swab to be

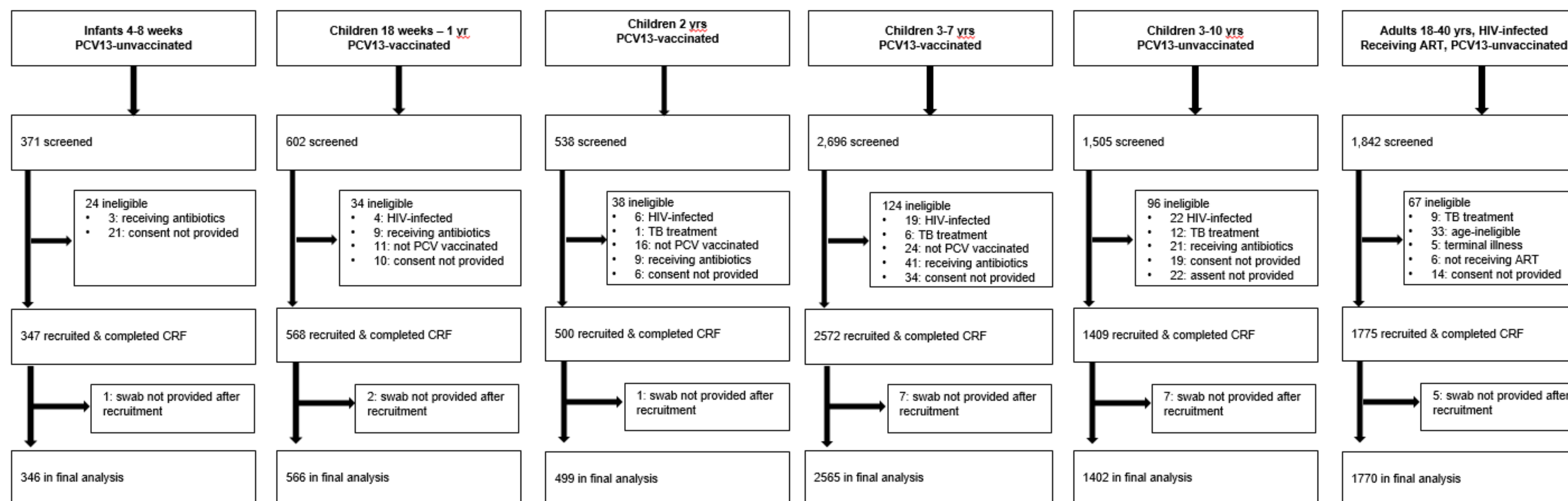

Figure 3.1. Recruitment flow diagram. Among schoolgoers (6-10 years old), the number reported as screened does not include parents/guardians who did not come to the school after written invitation. Overall, 3781 letters of invitation were sent to parents/guardians during recruitment of schoolgoers. Of these, 1493 (39.5%) came to the school to be further informed of the study and consider their child's participation. Reasons for not accepting the invitation were not routinely collected. A total 1427 schoolgoers were recruited. For children recruited from households (18 weeks to 5 years old), study teams maintained a diary of the number of homes visited (i.e., knocked on gate/door of main house). An average of 7.2 households were approached for every child screened. Reasons for failing to screen at households included (1) no one home, (2) someone home but no age-eligible child home, and (3) age-eligible child home but no parent/guardian available. ART, antiretroviral therapy; CRF, case report form; PCV, pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; TB, tuberculosis

collected after recruitment. The final analysis included 7148 participants: 346 infants recruited before receiving their first dose PCV, 566 children 18 weeks to 1 year old and PCV vaccinated, 499 children 2 years old and PCV vaccinated, 2565 children 3 to 7 years old and PCV vaccinated, 1402 children 3 to 10 years old and PCV unvaccinated, and 1770 HIV-infected adults on ART and PCV unvaccinated. Among schoolgoers, of the 3781 invitation letters sent home, 1493 (39.5%) resulted in a parent/guardian visiting the school for further study information and screening. The number of letters received by the parents/guardians and the reasons for parents/guardians not accepting the invitations were not routinely documented. During household recruitment, study teams maintained a diary to record the number of homes visited. An average of 7.2 households were approached for every child screened. Reasons for failing to screen at households included, (1) no one home, (2) someone home but no age-eligible child available, and (3) age-eligible child home but no parent/guardian available. Among the children in the final analysis, 3605 were recruited from households and 1427 from schools.

3.3.2 Demographics and vaccination history

When assessing the distribution of demographics in Table 3.1, to optimize the overlap in ages we restricted the analysis to children 3 to 7 years of age among the PCV-unvaccinated and PCV-vaccinated children. Though the surveyed groups had similar demographics, a higher proportion of older PCV-unvaccinated children (recruited from schools) lived in houses with some more costly infrastructure (walls of burnt brick, a water toilet, a water tap to house); these children also had a higher index of household possessions and a higher crowding index. Among those screened and age-eligible for PCV vaccination, 98.7% (3785/3836) reported receiving at least one dose of PCV. Among the 3630 PCV-vaccinated children recruited and providing an NPS, 1209 (33.3%) had a documented vaccination status with vaccination dates (in the health passport), ranging from 86.5% among the youngest vaccinated age group (18 weeks to 1 year old) to 25.7% among the oldest vaccinated age group (3-7 years old). Among those with health passports confirming dates of vaccination, the median (interquartile range) ages at the first, second, and third doses of PCV were 6.3 (3.2), 11.2 (5.0), and 16.4 (8.1) weeks, respectively; 1143 (94.5%) had received three doses of PCV, 24 (2.0%) had received only two doses, and 42 (3.5%) had received only one dose.

3.3.3 Pneumococcal carriage

Figure 3.2 reports the carriage dynamics of *S. pneumoniae* per rolling survey, stratified by study group. Refer to Supplementary Tables S3.3 and S3.4 for the VT and NVT prevalence data used for Figure 3.2.

Table 3.1. Demographic and household characteristics of study participants

Variable	age 4-8 wk PCV-unvacc'd (n=346)	age 18 wk-1 y PCV-vacc'd (n=566)	age 2 y PCV-vacc'd (n=499)	age 3-7 y PCV-vacc'd (n=2565)	age 3-10 y PCV-unvacc'd (n=1402)	age 18-40 y HIV-infected on ART PCV-unvacc'd (n=1770)
Demographics						
Age, years, median, (SD) [range]	0.13 (0.018) [0.08-0.17]	1.19 (0.46) [0.35-1.99]	2.50 (0.28) [2.0-2.99]	4.14 (0.95) [30-7.9]	8.49 (1.63) [3.6-10.99]	3355 (5.83) [18.0-40.9]
Gender, male n (%)	179 (51.7)	296 (52.3)	244 (48.9)	1271 (49.6)	718 (51.2)	559 (31.96) [c]
PCV received n (%)[a,,b]						
1 dose only	--	1 (02)	0	26 (1.2)	--	--
2 doses only		7 (1.2)	1 (0.2)	19 (0.9)		
3 doses		558 (98.6)	498 (99.8)	2051 (97.9)		
Household crowding[d]						
Crowding index, mean (median)[e]	2.5 (2.3)	2.6 (2.5)	2.5 (2.3)	2.6 (2.5)	2.9 (2.5)	2.1 (2.0)
Children <5 y in HH[e]						
Median [range]	1 [1-3]	1 [1-3]	1 [1-2]	1 [1-3]	1 [1-3]	0 [0-4]
Smoker in household[f,g]						
Yes, n (%)	28 (8.1)	41 (7.2)	43 (8.6)	124 (7.7)[g]	61/674 (9.1)	33/1092 (3.0)
House structure[f], n (%)						
Walls[e]						
Burnt brick/concrete	174 (50.4)	168 (29.6)	169 (33.9)	941 (36.7)	901 (64.3)	1212 (68.5)
Unburnt brick	166 (48.1)	397 (70.2)	330 (66.1)	1621 (63.2)	488 (34.8)	292 (16.5)
Mud, thick/thin	6 (1.5)	1 (0.2)	0	3 (0.10)	13 (0.9)	266 (15.0)
Floor						
Tiles	1 (0.3)	1 (0.2)	0	3 (0.1)	4 (0.3)	20 (1.1)
Concrete	325 (93.9)	447 (89.9)	406 (89.2)	2125 (87.3)	1257 (91.9)	1619 (91.5)
Mud	20 (5.8)	48 (9.9)	49 (10.8)	307 (12.6)	107 (7.8)	130 (7.4)
Latrine[e]						
Water toilet	16 (4.3)	13 (2.6)	11 (2.4)	57 (2.4)	238 (17.4)	279 (15.8)
Simple pit latrine	330 (95.7)	480 (97.0)	441 (97.6)	2368 (97.5)	1126 (82.4)	2 (0.1)
Other	0	2 (0.4)	0	2 (0.1)	3 (0.2)	1484 (84.1)
Water[e]						
Tap to house	48 (13.9)	42 (8.5)	39 (8.6)	242 (9.9)	425 (31.1)	591 (33.4)
Communal tap	293 (84.9)	448 (90.1)	414 (91.0)	2156 (88.6)	884 (64.6)	947 (53.5)
Bore hole	3 (0.9)	7 (1.4)	2 (0.4)	30 (1.2)	45 (3.3)	181 (10.2)
Well (covered/open)	2 (0.3)	0	0	7 (0.3)	14 (1.0)	50 (2.8)
Electricity at household						
Yes	274 (79.4)	379 (76.3)	342 (75.2)	1742 (71.5)	1021 (74.6)	1275 (72.1)
Possessions index[h]						
mean (SD)[e]	7.1 (2.7)	6.4 (3.5)	6.4 (3.4)	6.8 (3.3)	8.2 (3.2)	(3.3)

Recruitment location: Infants 4-8 weeks were recruited from household or health centre (present for EPI); PCV-vaccinated children 18 weeks to 5 years old were recruited from household; PCV-unvaccinated children 5-10 years old were recruited from schools; HIV-infected adults on ART were recruited from Queen Elizabeth Central Hospital ART clinic. PCV, pneumococcal conjugate vaccine; ART, antiretroviral therapy; SD, standard deviation; vacc'd, vaccinated, HH, household

[a]Among a subset of PCV-vaccinated children with written evidence of vaccination. [b]n=2096 among children 3-7 years old. [c]The gender distribution among HIV-infected adults recruited from the ART clinic is representative of the gender distribution among those attending the clinic. [d]Crowding index: Calculated as number of household residents divided by number of bedrooms in main house; data only collected starting survey 4. [e]Distribution of these covariates was statistically significant when comparing unvaccinated children 3-7 years of age and vaccinated children 3-7 years of age. Crowding index (mean 2.9 vs 2.6, respectively) $p<0.000$; number of children < 5 years old in household (mean, 0.7-1.1) $p<0.000$; Walls of burnt bricks or concrete (61.5 vs 36.7) $p<0.000$; Latrine, water toilet (17.4 vs 2.4) $p<0.000$; Water tap to house (31.1 vs 9.5) $p<0.000$; Possession index (7.8 vs 6.8) $p<0.000$. [f]Smoker in household: the percentage of households with at

least one household member who smokes tobacco; data only collected starting survey 4. Ranking of household structure variables: each variable is presented with the costliest category at top and least costly at bottom of list. [g]n=1615 among children 3-7 years old. [h]Possession index: calculated as a sum of positive responses for household ownership of each of one of fifteen different functioning items: watch, radio, bank account, iron (charcoal), sewing machine (electric), mobile phone, CD player, fan (electric), bed net, mattress, bed, bicycle, motorcycle, car, and television.

Using aggregated survey data, VT and NVT carriage prevalence were respectively, 8.4% (95% CI, 5.7%-11.8%) and 33.8% (95% CI, 28.8%-39.1%) among children 4 to 8 weeks (prior to first dose PCV; surveys 5-7); 17.1% (95% CI, 14.2%-20.5%) and 62.7% (58.6%-66.6%) among PCV-vaccinated children 18 weeks to 1 year old (surveys 4-7); 18.4% (95% CI, 15.3%-22.1%) and 58.5% (95% CI, 54.1%-62.8%) among PCV-vaccinated children 2 years old (surveys 4-7); 18.0% (95% CI, 16.5%-19.5%) and 56.2% (95% CI, 54.2%-58.1%) among PCV-vaccinated children 3 to 7 years old (surveys 1-7); 18.2% (95% CI, 16.2%-20.3%) and 38.5% (95% CI, 35.9%-41.0%) among PCV-unvaccinated children 3 to 10 years old (surveys 1-7); 12.3% (95% CI, 10.8-13.9) and 28.1% (26.0-30.2) among HIV-infected adults on ART (surveys 1-7).

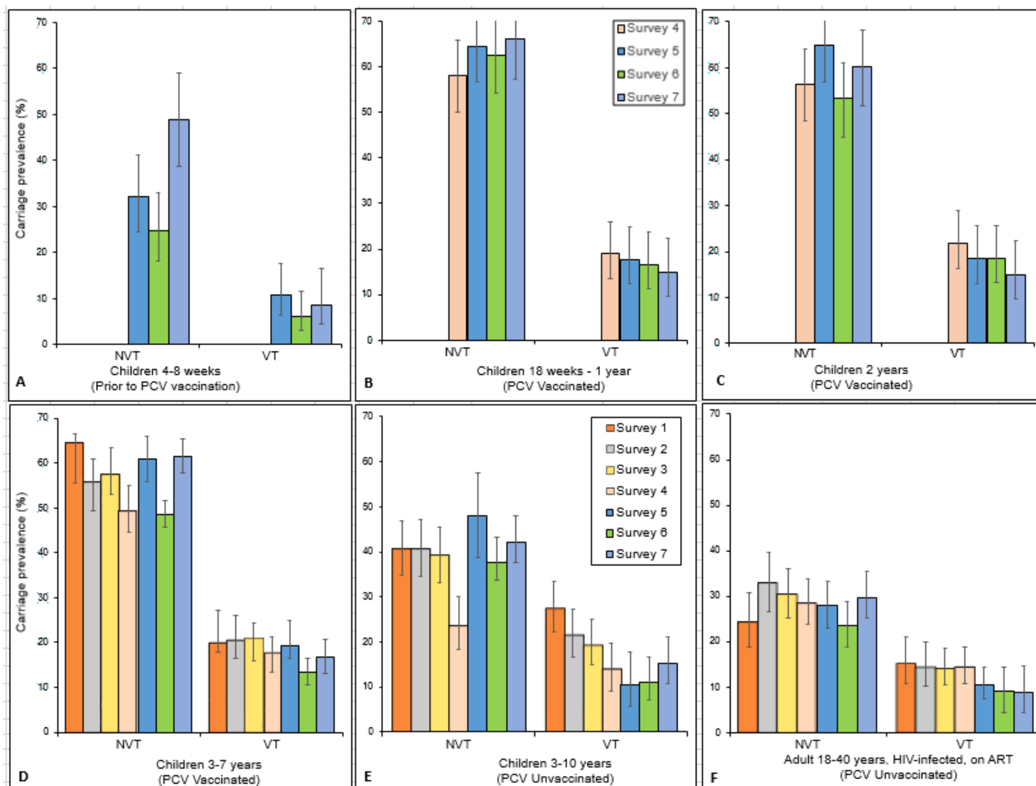

Figure 3.2. *Streptococcus pneumoniae* carriage prevalence per survey, stratified by study group. Surveys 1-7 spanned a time of 3.6-7.1 years after the 12 November 2011 introduction of PCV into Malawi's EPI. Younger children (4-8 weeks up to 2 years of age; cells A-C) were recruited starting survey 4 or 5. Prevalence of non-carriers is calculated by 1 − (NVT+VT). Aggregated sample size for each study group: [A]n=346 children 4-8 weeks old (PCV unvaccinated), [B]n=566 children 18 weeks to 1 year old (PCV vaccinated), [C]n=499 children

2 years old (PCV vaccinated), [D]n=2565 children 3-7 years old (PCV vaccinated), [E]n=1402 children 3-10 years old (PCV unvaccinated), [F]n=1770 HIV-infected adults on ART (PCV unvaccinated). Refer to Supplementary Tables S3.3 and S3.4 for the sample sizes used in calculating per-survey VT and NVT prevalence data and error bars in this figure. For each coloured bar, 95% confidence interval error bars are shown. The confidence interval bounds are calculated by exponentiating the bounds in the logit scale. EPI, Expanded Programme on Immunization; NVT, non–vaccine serotype; PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

A sensitivity analysis among PCV-vaccinated age groups showed that neither the overall VT prevalence nor the VT distribution changed significantly when limiting these analyses to children (1) who received only one, only two, or all three PCV doses; (2) with document-confirmed PCV vaccination; or (3) who adhered to the vaccination schedule to within 2 weeks of each scheduled dose. As shown in Supplementary Figure S3.1, when stratified by age (in years) and aggregating survey data, reduction in VT carriage was not exponential among vaccinated children. VT carriage increased slightly during the first 4 years of life, from 16.6% (95% CI, 11.9%-22.6%) among children 18 weeks to 11 months old to 17.4% (95% CI, 13.6%-21.7%), 18.6% (95% CI, 15.1%-22.1%), and 19.5% (95% CI, 17.3%-22.1%) among 1-, 2-, and 3-year-old children, respectively. VT carriage then decreased to 18.5% (95% CI, 16.0%-20.9%), 14.4% (95% CI, 10.8%-19.0%), 12.0% (95% CI, 6.7%-19.3%), and 7.0% (95% CI, 1.4%-17.1%) among 4-, 5-, 6-, and 7-year-old children, respectively.

Although all 13 VTs were identified in each of the three older (3-40 years old) study groups, with serotype 3 the predominant VT in each, serotype carriage dynamics were more heterogeneous among those <3 years old (Figure 3.3). Serotype 1, a common cause of IPD in Africa,[45,46] contributed 3.0% to the all-ages VT carriage prevalence. Supplementary Tables S3.7 and S3.8 show the proportion of total VT carriage attributed to individual VTs, stratified by study group and survey among PCV-vaccinated and PCV-unvaccinated study groups, respectively.

Table 3.2. Carriage prevalence among children and adults

	Children 3-5 years PCV vaccinated % (n); 95% CI	Children 6-8 years PCV unvaccinated % (n); 95% CI	Adult, HIV-infected on ART PCV unvaccinated % (n); 95% CI
Survey 1	n=286	n=91	n=198
Total carriage	84.2 (241) 79.5-88.3	67.1 (61) 56.4-76.5	39.4 (78)
VT	19.9 (57); 15,7-25.0	26.4 (24) 18.3-36.4	15.2 (30) 10.8-20.9
NVT	64.3 (184); 58.6-69.7	40.7 (37) 31.0-51.1	24.2 (48) 18.8-30.7
Survey 2	n=303	n=111	n=201
Total carriage	76.0 (230) 70.7-80.6	65.7 (73) 56.2-74.5	47.2 (95)
VT	20.5 (62) 16.3-25.4	21.6 (24) 14.9-30.3	14.4 (29) 10.2-20.0
NVT	55.5 (168) 49.8-61.0	44.1 (49) 35.2-53.5	32.8 (66) 26.7-39.6
Survey 3	n=361	n=139	n=279
Total carriage	78.1 (282) 73.5-82.3	63.3 (88) 54.7-71.3	44.5 (124)
VT	20.8 (75) 16.9-25.3	21.6 (30) 15.5-29.2	14.0 (39) 10.4-18.6
NVT	57.3 (207) 52.2-62.4	41.7 (58) 33.8-50.1	30.5 (85) 25.3-36.1
Survey 4	n=378	n=128	n=308
Total carriage	67.0 (253) 61.9-71.7	42.9 (55) 34.3-52.0	42.9 (132)
VT	17.5 (66) 14.0-21.6	14.8 (19) 9.6-22.2	14.3 (44) 10.8-18.9
NVT	49.5 (187) 44.4-54.5	28.1 (36) 21.0-36.6	28.6 (88) 23.8-33.9
Survey 5	n=371	n=56	n=305
Total carriage	80.6 (299) 76.2-84.5	55.3 (31) 41.5-68.7	38.4 (117)
VT	19.4 (72) 15.7-23.8	8.9 (5) 3.7-19.9	10.5 (32) 7.5-14.5
NVT	61.2 (227) 56.1-66.0	46.4 (26) 33.8-59.6	27.9 (85) 23.1-33.2
Survey 6	n=382	n=100	n=277
Total carriage	67.3 (257) 62.3-72.0	52.0 (52) 41.8-62.1	32.5 (90)
VT	15.2 (58) 11.9-19.2	15.0 (15) 9.2-23.5	9.0 (25) 6.2-13.0
NVT	52.1 (199) 47.1-57.1	37.0 (37) 28.1-46.9	23.5 (65) 18.8-28.8
Survey 7	n=324	n=70	n=202
Total carriage	79.7 (258) 74.8-83.9	61.4 (43) 49.0-72.8	38.6 (78)
VT	16.7 (54) 13.0-21.1	15.7 (11) 8.9-26.3	8.9 (18) 5.7-13.7
NVT	63.0 (204) 57.6-68.1	45.7 (32) 34.4-57.5	29.7 (60) 23.8-36.4
Total (Survey 1-7)	n=2405	n=695	n=1770
Total carriage	75.7 (1820) 73.9-77.4	58.0 (403) 54.2-61.7	40.4 (714)
VT	18.5 (444) 16.9-20.1	18.4 (128) 15.6-21.5	12.3 (217) 10.8-13.9
NVT	57.2 (1376) 55.2-59.2	39.6 (275) 35.9-43.3	28.1 (497) 26.0-30.2
	cPR[a] (95% CI) p-value	cPR[a] (95% CI) p-value	cPR[a] (95% CI) p-value
No carriage	--	--	--
VT	0.912 (0.840-0.990) 0.028	0.806 (0.690-0.942)	0.847 (0.750-0.957) 0.008
NVT	0.972 (0.939-1.007) 0.112	0.964 (0.881-1.056)	0.967 (0.899-1.040) 0.362
	aPR[a] (95% CI) p-value	aPR[a] (95% CI) p-value	aPR[a] (95% CI) p-value
Total carriage	--	--	--
VT	0.912 (0.840-0.990) 0.028	0.839 (0.712-0.990)	0.831 (0.735-0.938) 0.003
NVT	0.972 (0.939-1.007) 0.112	0.974 (0.886-1.070)	0.963 (0.895-1.036) 0.307
	Relative change[b]	Relative change[b]	Relative change[b]
Total carriage	--	--	--
VT	−16.1%	−40.5%	−41.4%
NVT	−2.0%	+12.3%	+22.7%

Surveys 1-7 spanned a time of 3.6-7.1 years after Malawi's November 2011 introduction of PCV. aPR, adjusted prevalence ratio (adjusted for age [years old] at recruitment); ART, antiretroviral therapy; CI, confidence interval; cPR, crude prevalence ratio; NVT, non–vaccine serotype; VT, vaccine serotype. [a]Carriage prevalence ratios (crude and adjusted) were calculated over the study duration of seven surveys by log-binomial regression using years (365.25 days) between date of Malawi's PCV introduction and participant recruitment, coded as a single time variable, allowing an estimate of (adjusted) prevalence ratio per annum. [b]Relative change = [(VT prevalence of final survey − VT prevalence of initial survey)/VT prevalence of initial survey] × 100%.

3.3.4 Change in carriage prevalence over time

To minimize confounding, we calculated the relative change in carriage prevalence and prevalence ratios on a narrower age range within each study group (Table 3.2). Among children 3 to 5 years old (PCV vaccinated), aggregated (surveys 1-7) VT and NVT carriage prevalences were, respectively, 18.5% (95% CI, 16.9%-20.1%) and 57.2% (95% CI, 55.2%-59.2%). There was a 16.1% relative reduction in VT carriage, from 19.9% (95% CI, 15.7%-25.0%) in survey 1 to 16.7% (95% CI, 13.0%-21.1%) in survey 7. There was a 2.0% relative decrease in NVT carriage, from 64.3% (95% CI, 58.6%-69.7%) to 63.0% (95% CI, 57.6%-68.1%). When adjusted for age (in years) at recruitment, the adjusted prevalence ratio (aPR) over the 3.5-year study was 0.919 (95% CI, 0.845-0.999; p=0.047) for VT carriage and 0.978 (95% CI, 0.944-1.013, p=0.208) for NVT carriage. Among children 6 to 8 years old (PCV unvaccinated), aggregated (surveys 1-7) VT and NVT carriage prevalences were, respectively, 18.4% (95% CI, 15.6%-21.5%) and 39.6% (95% CI, 35.9%-43.3%). There was a 40.5% relative reduction in VT carriage, from 26.4% (95% CI, 18.3%-36.4%) in survey 1 to 15.7% (95% CI, 8.9%-26.3%) in survey 7. There was a 12.3% relative increase in NVT carriage, from 40.7% (95% CI, 31.0%-51.1%) to 45.7% (34.4%-57.5%). Among HIV-infected adults on ART (PCV unvaccinated), aggregated (surveys 1-7) VT and NVT carriage prevalence were respectively, 12.3% (95% CI, 10.8%-13.9%) and 28.1% (95% CI, 26.0%-30.2%). There was a 41.4% relative reduction in VT carriage, from 15.2% (95% CI, 10.8%-20.9%) in survey 1 to 8.9% (95% CI, 5.7%-13.7%) in survey 7. There was a 22.7% relative increase in NVT carriage, from 24.2% (95% CI, 18.8%-30.7%) to 29.7% (95% CI, 23.8%-36.4%). When adjusted for age at recruitment, the aPR over the 3.5-year study was 0.831 (95% CI, 0.735-0.938; p=0.003) for VT carriage and 0.963 (95% CI, 0.895-1.036; p=0.307) for NVT carriage. To further assess for potential confounders, we investigated whether the demographic characteristics, as reported in Table 3.1, changed over the study period. Although there was change in some covariates (crowding index, smoker in household, latrine type, electricity at household, and possession index), the magnitude was limited and, when adjusted for in the model, they had no meaningful influence on the relationship between VT carriage and time.

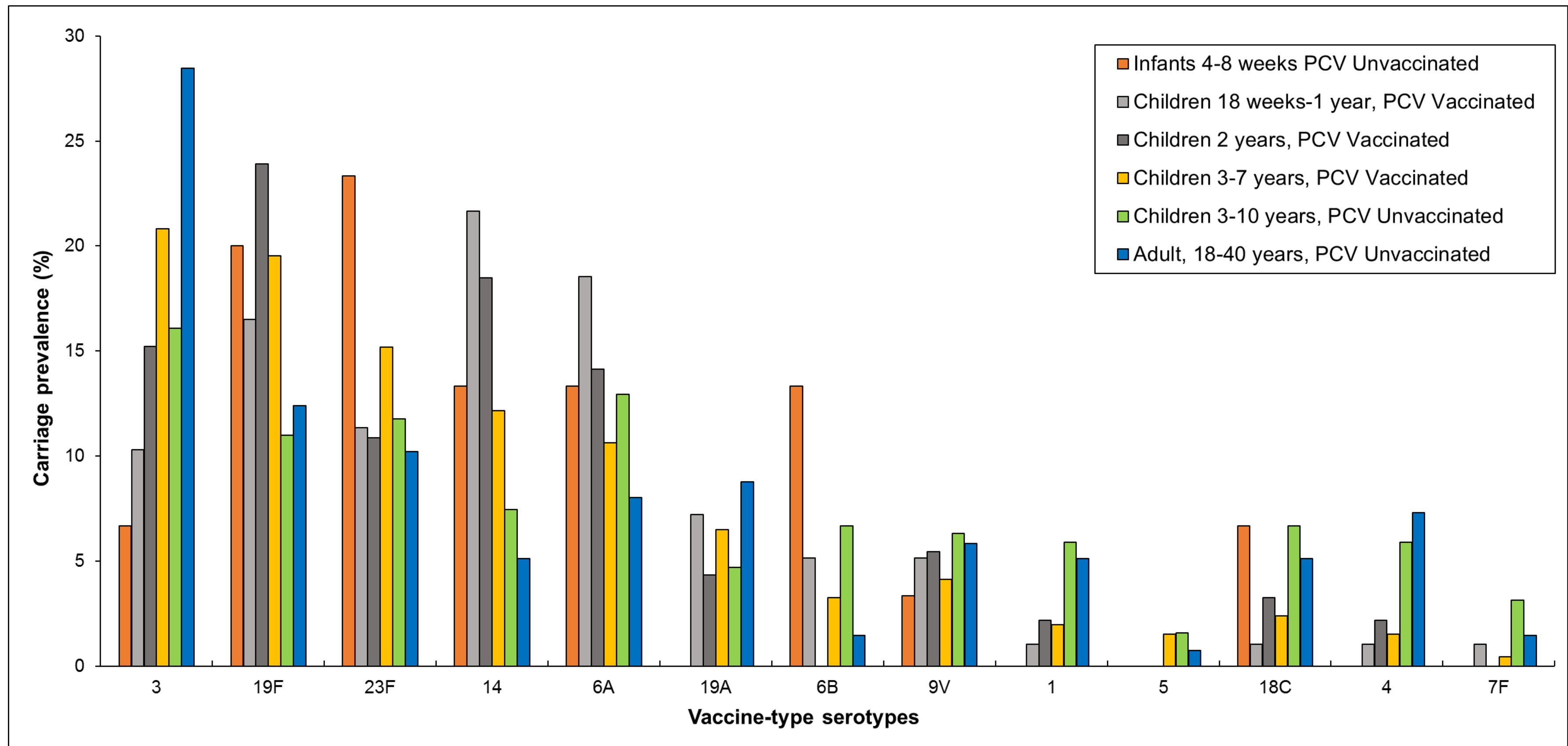

Figure 3.3. Distribution of vaccine-serotype carriage, aggregated across study period and stratified by study group. Proportion of VT carriage attributed to individual VTs across all surveys, stratified by study group. The denominator for each serotype is the total VT isolates in each study group. PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

3.3.5 Contribution of serotype 3 to change in carriage prevalence

Several post–PCV13-introduction studies have reported that PCV13 is less immunogenic for serotype 3 compared with other vaccine serotypes.[47,48] We, therefore, addressed this potential bias by classifying serotype 3 as an NVT in a separate analysis (Supplementary Table S3.9). The aPRs for VT carriage prevalence among PCV-vaccinated children 3 to 5 years of age followed the same trend but was no longer statistically significant (serotype 3 as VT aPR, 0.919; 95% CI, 0.845-0.999; $p=0.047$ vs serotype 3 as NVT aPR, 0.942; 95% CI, 0.856-1.036; $p=0.218$).

3.3.6 Probability of vaccine-serotype carriage with age and carriage half-life

Using non-linear regression analysis, with carriage data censored below 3.6 years of age, to investigate the individual probability of VT carriage as a function of a child's age (years), the probability of VT carriage declined with age for both vaccinated and unvaccinated children (Figure 3.4). However, the population-averaged effect of not receiving PCV more than doubled an individual's probability of VT carriage at 3.6 years of age, $\beta=2.15$ (95% CI, 1.47-2.83) (Figure 3.4 and Table 3.3). While there were different estimated probabilities of VT carriage at the individual level (e.g., 0.22 for PCV-vaccinated and 0.47 [$\alpha\times\beta$] for PCV-unvaccinated children at 3.6 years of age), the estimated population-level half-lives (derived from individual carriage probability data) of VT carriage prevalence were similar to one another ($T_{½}$: 3.34 years [95% CI, 1.78-6.26] vs $T_{½}$: 3.26 years [95% CI, 2.42-4.38], respectively). In terms of the probability of NVT carriage, β was 0.91 (95% CI, 0.73-1.09), with similar estimated probabilities of NVT carriage for individual vaccinated and unvaccinated children at 3.6 years of age (0.59 and 0.54, respectively [$\alpha\times\beta$]). The estimated half-life of NVT carriage prevalence was also similar among PCV-vaccinated children ($T_{½}$: 9.46 years [95% CI, 4.69-19.04 years]) and PCV-unvaccinated children ($T_{½}$: 9.83 years [95% CI, 5.69-16.99 years]). The goodness-of-fit assessment indicated a good fit, with no discernible relationship between the residual and predicted values and the range of residuals compatible with the theoretical mean and standard deviations of 0 and 1, respectively (Supplementary Figure S3.3).

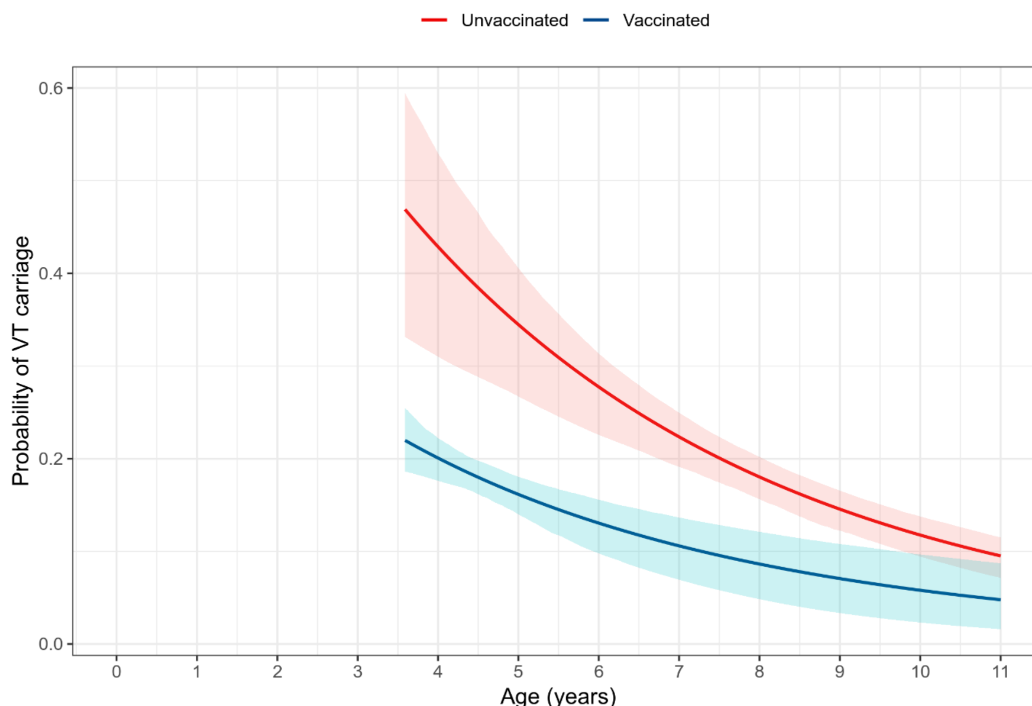

Figure 3.4. Modelling the relationship between a child's probability of VT carriage and age. Estimated probabilities and pointwise 95% confidence intervals (shaded regions) of the probability of an individual child's vaccine-serotype (VT) carriage as a function of a child's age (in years), for an unvaccinated child (red line) and a vaccinated child (blue line). The fitted line for unvaccinated children includes the range of the empiric data. The fitted line for vaccinated children is left censored at 3.6 years old and extrapolated beyond the oldest vaccinated child (7.9 years old). The model shows significantly different estimated probabilities of VT carriage for an individual (distance between lines), while the estimated population-level half-life of VT carriage (derived from individual carriage probability data; refer to Table 3.3) translates to very similar estimates among PCV-vaccinated (3.34 years) and PCV-unvaccinated (3.26 years) children.

Table 3.3. Estimates for probability of carriage with age and carriage half-life

	VT		NVT	
Parameter	Estimate	95% CI	Estimate	95% CI
Carriage prevalence at censoring age (3.6 years) for vaccinated children (α)	0.22	0.19, 0.25	0.59	0.55, 0.63
Carriage prevalence at censoring age (3.6 years) for unvaccinated children ($\alpha\times\beta$)	0.47	0.33, 0.60	0.54	0.43, 0.64
Decay rate of carriage prevalence with age for vaccinated children (δ_v)	0.21	0.11, 0.39	0.07	0.04, 0.15
Carriage half-life for vaccinated children *(log (2)/δ_v)*	3.34	1.78, 6.26	9.46	4.69, 19.04
Decay rate of carriage prevalence with age for unvaccinated children (δ_u)	0.21	0.16, 0.29	0.07	0.04, 0.12
Carriage half-life for unvaccinated children *(log (2)/δ_u)*	3.26	2.42, 4.38	9.83	5.69, 16.99
Effect of not receiving PCV (β)	2.15	1.47, 2.83	0.91	0.73, 1.09

CI, confidence interval; PCV, pneumococcal conjugate vaccine; NVT, non–vaccine serotype; VT, vaccine serotype

3.4 Discussion

In this population-based assessment of pneumococcal carriage, we surveyed potential reservoir populations from 3.6 to 7.1 years after the introduction of routine PCV13 administration in Malawi. At the start of the study, we found high VT carriage in all age groups, including younger PCV-vaccinated children, older PCV-unvaccinated children, and HIV-infected adults on ART. Despite a statistically significant relative reduction in VT prevalence over the next 3.5 years, residual carriage was higher than reported in high-income settings (Supplementary Table S3.2). All 13 VTs were isolated despite high vaccine uptake and good schedule adherence. There was no significant change in NVT carriage prevalence. The 18.7% residual VT carriage prevalence we report among PCV-vaccinated children 1 to 4 years old was consistent with the 16.5% reported in northern Malawi by Heinsbroek et al[13] for 2014 (Supplementary Table S3.5). Though the residual VT carriage prevalence we report for Blantyre was lower than that observed by Heinsbroek et al[13] before vaccine introduction (28%) and may have missed the greatest fall in VT prevalence soon after PCV introduction, we did not observe the substantially lower levels rapidly achieved in high-income, low-carriage-prevalence settings (<5%) associated with control of carriage and transmission.[17,26,27] When aggregating surveys, the reduction in VT carriage among PCV-vaccinated children did not conform to a simple exponential distribution, with a statistically non-significant increase among children <4 years of age, before evidence of a decrease among older vaccinated children (Supplementary Figure S3.1). In light of the 2017 WHO Technical Expert Consultation Report on Optimization of PCV Impact,[49] these data started to address the paucity of information on the long-term impact of the widely implemented 3+0 vaccine schedules on serotype-specific disease and carriage in this region.

Despite the reporting of high residual VT carriage, our non-linear statistical analysis shows a lower probability of VT carriage among vaccinated children, starting at 3.6 years of age, when the model is censored. From this, we speculate that vaccine-induced protection, despite evidence of early waning of immunity (perhaps within the first 6-12 months of life),[42,50] does provide a longer-term benefit to this population in providing a lower VT carriage set point at an age when naturally acquired immunity and herd immunity begin to play roles in carriage control. A lower carriage set point among vaccinated children is of benefit in both direct protection and in reducing transmission. We propose that a comparable VT half-life at 3.6 years of age is due to a limited role of vaccine-induced immunity that started to wane within the first 12 months of life. The mechanism underlying the vaccine effect could be prevention of carriage (reduced incidence) or shortening of carriage duration (reduced point prevalence). There is also evidence that, in older

vaccinated and unvaccinated children, the reductions in carriage prevalence are due to the indirect benefits of vaccination augmented by naturally acquired immunity to subcapsular protein antigens.[51,52]

These indirect benefits augmented by naturally acquired immunity explains, in part, the more pronounced decline in VT carriage among PCV-unvaccinated children 6 to 8 years old (40.5%) and HIV-infected adults on ART (41.4%), compared with younger vaccinated children 3 to 5 years old (16.1%; Table 3.2). Using a dynamic transmission model fitted to data from this population in Blantyre, Malawi, we have shown that the force of infection (FOI; the rate by which a certain age group of susceptible individuals is infected) is characterized by different transmission potentials within and between age groups.[53] This analysis suggests that the time period of fastest FOI reduction for the 0- to 5-year-olds was between vaccine introduction and 2015 (when no carriage data were collected), which contrasted with the older age groups, for which the period of fastest FOI reduction was predicted to be just before or during the first three surveys.

To achieve herd protection in settings with high carriage prevalence, such as Malawi, we need to effectively interrupt person-to-person transmission. In Finland, a microsimulation model suggested a moderate transmission potential of pneumococcal carriage, predicting the elimination of VT carriage among those vaccinated within 5 to 10 years of PCV introduction, assuming high (90%) vaccine coverage and moderate (50%) vaccine efficacy against acquisition.[54] Thus, vaccine impact predicted by transmission models from low carriage prevalence settings probably does not translate to high carriage prevalence settings. Although it has previously been assumed that PCVs would eliminate VT carriage in mature PCV programmes,[55] our data bring into question the potential for either a sustained direct or indirect effect on carriage using a 3+0 strategy. Alternative vaccine schedules, including those with a booster, should be evaluated to determine whether a higher rate of vaccine-induced VT carriage decay can be achieved.

In Malawi, the vaccine impact on carriage prevalence has been less than that observed in Kenya, The Gambia, and South Africa, which have used different vaccination strategies. Kenya reported a reduction from 34% to 9% VT carriage among PCV-vaccinated children under 5 years of age, 6 years after introduction of PCV10.[22] The Gambia reported a reduction from 50% to 13% VT carriage among children 2 to 5 years old, 20 months after introducing PCV7.[56] Likewise, a study from South Africa showed reduced PCV13-serotype colonization from 37% to 13% within 1 year of transitioning from PCV7 to PCV13.[57] However, these countries have also not achieved the low carriage prevalences

seen in Europe and North America 2 to 3 years after the introduction of PCVs.[4,18] As presented by Lourenço et al,[53] we propose that a high FOI in settings, such as Malawi, limits a 3+0 schedule to achieving only a short duration of VT carriage control in infants. While a 2+1 schedule, as deployed in South Africa, may improve colonization control, this remains unproven in other African settings. Given the likely importance of an early reduction in transmission intensity to maintain a reduced carriage prevalence, a catch-up-campaign with booster doses over a broader age range (i.e., <5 years of age) may also be required. Although Gavi has considerably reduced PCV costs for low-income countries,[58,59] vaccine impact must be optimized (particularly indirect effects) to achieve financial sustainability. The FOI and determinants of transmission between and within age groups need to be considered, as new approaches to improving vaccine-induced carriage reduction are proposed and tested.

Unlike low-transmission settings,[60] as well as The Gambia[29] and South Africa,[57] we observed a very modest decrease in NVT carriage among young PCV-vaccinated children in Malawi. Given evidence elsewhere of rapid serotype replacement after PCV introduction, it is possible that serotype replacement and redistribution had already occurred before the start of this study, and that as part of a stochastic secular trend, we are now observing an overall decrease in pneumococcal carriage prevalence. There may have also been individual NVTs that increased, while other NVTs decreased in prevalence. Though distribution of individual NVTs warrants further analysis, our latex serotyping methods did not allow for identifying individual NVT serotypes. It is also plausible that overall improvement in living conditions (improved nutrition, sanitation, and disease control) and healthcare (ART roll-out and rotavirus vaccination) have resulted in an overall sustained drop in pneumococcal carriage, evidenced by falling under-5 mortality in recent years.[61] Either way, the importance of this in NVT carriage will become clearer as the trends in NVT-associated invasive disease become available from these different settings.

We have previously shown incomplete pneumococcal protein antigen-specific reconstitution of natural immunity and high levels of pneumococcal colonization in HIV-infected Malawian adults on ART.[43,44] While the significant decline (41.4% relative reduction, Table 3.2) in VT carriage among HIV-infected adults suggests some indirect benefit in this population following the introduction of routine infant PVC13 administration, the residual 8.9% VT carriage may represent a persistent reservoir of VT carriage and transmission. Previous studies in Malawi and South Africa have suggested that despite a higher risk of VT pneumococcal colonization among HIV-infected women, they are still unlikely to be a significant source of transmission to their children.[24]

However, in the context of routine infant PCV13 and persistent pneumococcal carriage, the balance of transmission may now be different. Given the higher risk of IPD, ongoing burden of pneumococcal pneumonia,[62] and the evidence that PCV protects HIV-infected adults from recurrent VT pneumococcal infections,[63] targeted vaccination benefitting this at-risk population may help reduce overall carriage and disease prevalence.

Though this work provides a robust community-based estimate of VT and NVT pneumococcal carriage in Blantyre, there are some limitations worth noting. The study was conducted over a relatively short time frame for understanding long-term temporal trends. For this reason, the statistical analysis has some limitations in its ability to disentangle the effects of calendar time and age-since-vaccination time, given the small overlap in ages of vaccinated and unvaccinated children in our data (Supplementary Figure S3.2). This limited overlap is also a limitation in terms of results interpretation. For example, with estimates of individual VT carriage probabilities as a function of age (Figure 3.4 and Table 3.3), the age variations by vaccine group tend to exaggerate the differences between vaccinated and unvaccinated groups (to different degrees across the age ranges). However, given the relatively narrow age band and time span, the impact of this exaggeration is likely minimal. It is also possible that readjustment of carriage dynamics (VT and NVT prevalence, as well as serotype-specific trends) had already occurred between PCV introduction and our first carriage survey. As reported elsewhere,[64,65] a major challenge with field-based surveillance studies is ascertainment of vaccine coverage. Measurement of vaccination status depends predominantly on health passports with limited capacity for verification of either recorded or reported vaccination statuses. However, our reported high coverage is concordant with coverage reported by other studies in Malawi, and any misclassification is, therefore, likely to be small and would not significantly change the findings. Jahn et al[65] showed that Bacillus Calmette-Guérin (BCG) scar data allows inference of population vaccination coverage independently from vaccination records, reporting a similar prevalence of BCG scars among children <5 years old with no health passports relative to those with a health passport and BCG reported (70% vs 78%, respectively). Subsequently, a 2015 Malawi cluster vaccination coverage survey reported that 94% of children 12 to 23 months of age had a BCG scar.[36] Although there are pre–vaccine-introduction data from elsewhere in Malawi, there are no equivalent historical carriage data for urban settings in Malawi using the same sampling frame. However, this does not detract from the finding of high levels of residual VT carriage in these reservoir populations. Finally, given evidence that more sensitive serotyping methods that detect multiple serotype carriage (e.g., by DNA microarray)[66,67] will increase VT carriage

estimates, our carriage prevalence data likely underestimate the true residual VT (and NVT) prevalence levels.

3.5 Conclusions

Despite success in achieving direct protection of infants against disease, a 3+0 PCV13 schedule in Malawi has not achieved the low universal VT carriage prevalence reported in high-income settings and that is required to control carriage and transmission. We propose that, although vaccine-induced immunity reduces the risk of VT carriage in children, in the context of a high residual FOI, this impact is limited by rapid waning (perhaps within the first 6-12 months of life) of vaccine-induced mucosal immunity and pneumococcal recolonization. Therefore, alternative schedules and vaccine introduction approaches in countries with high levels of pneumococcal carriage and disease should be revisited through robust evaluation rather than through programmatic change without supporting evidence. Furthermore, we need to better understand the relative impact of waning vaccine-induced immunity, indirect vaccine protection, and naturally acquired immunity on VT carriage in the 2 to 3 years after vaccination.

3.6 Data availability

The data supporting the findings of this study has been deposited in the Figshare repository (doi: 10.6084/m9.figshare.11985255).[70] The source data underlying Figures 3.2 and 3.3 are available in the Supplementary Information, Supplementary Tables S3.3 and S3.4, respectively. Figure 4 data are available with the coding data at: https://github.com/claudiofronterre/pneumococco. The computer coding used to generate results in this manuscript are available at: https://github.com/claudiofronterre/pneumococco.

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3.8 Supplementary Information

Supplementary Table S3.1. Regional differences in vaccine formulations and schedules

Country	WHO region	World Bank income group	Year PCV introduced	Current formulation	Current dosing schedule	Current timing	Initial formulation (if different than current)
Central African Republic	AFRO	Low income	2011	PCV13	3+0	6, 10, 14 weeks	--
Ethiopia	AFRO	Low income	2011	PCV10	3+0	6, 10, 14 weeks	--
Gambia	AFRO	Low income	2009	PCV13	3+0	2, 3, 4 months	PCV7
Kenya	AFRO	Lower middle income	2011	PCV10	3+0	6, 10, 14 weeks	--
Lesotho	AFRO	Lower middle income	2015	PCV13	3+0	6, 10, 14 weeks	--
Madagascar	AFRO	Low income	2012	PCV10	3+0	6, 10, 14 weeks	--
Mozambique	AFRO	Low income	2013	PCV13	3+0	6, 10, 14 weeks	PCV10
Namibia	AFRO	Upper middle income	2014	PCV13	3+0	6, 10, 14 weeks	--
Rwanda	AFRO	Low income	2009	PCV13	3+0	6, 10, 14 weeks	PCV7
South Africa	AFRO	Upper middle income	2009	PCV13	2+1	6, 14 weeks & 9 months	PCV7
Tanzania	AFRO	Low income	2012	PCV13	3+0	6, 10, 14 weeks	--
Uganda	AFRO	Low income	2013	PCV10	3+0	6, 10, 14 weeks	--
Zambia	AFRO	Lower middle income	2013	PCV10	3+0	6, 10, 14 weeks	--
Zimbabwe	AFRO	Lower middle income	2012	PCV13	3+0	6, 10, 14 weeks	--
Canada*	AMRO	High income	2002	PCV13	2+1	2, 4, 6 months	PCV7
					3+1	2, 4, 6, & 12-18 months	--
Haiti	AMRO	Low income	2018	PCV13	3+0	6, 10, 14 weeks	--
Mexico	AMRO	Upper middle income	2008	PCV13	2+1	2, 4, & 12 months	PCV7
Nicaragua	AMRO	Lower middle income	2010	PCV13	3+0	2, 4, 6 months	--
United States	AMRO	High income	2000	PCV13	3+1	2, 4, 6 & >12 months	PCV7
Kuwait	EMRO	High income	2007	PCV13	3+1	2, 4, 6 & 18 months	PCV7
Morocco	EMRO	Lower middle income	2010	PCV10	2+1	2, 4 & 12 months	--
Saudi Arabia	EMRO	High income	2009	PCV13	3+1	2, 4, 6 & 12 months	PCV7
Denmark	EURO	High income	2007	PCV13	2+1	3, 5 & 12 months	--
France	EURO	High income	2006	PCV13	2+1	2, 4 & 11 months	PCV7
Germany	EURO	High income	2006	PCV13	2+1	2, 4 & 11-14 months	PCV7
Ireland	EURO	High income	2008	PCV13	2+1	2, 6 & 12 months	PCV7
Israel	EURO	High income	2009	PCV13	2+1	2, 4 & 12 months	PCV7
Italy	EURO	High income	2005		2+1	3, 5-6 & 11-13 months	PCV7
Netherlands	EURO	High income	2006	PCV10	2+1 (3+1)	2, 4 & 11 months	PCV7
Portugal	EURO	High income	2015	PCV13	2+1	2, 4 & 12 months	
Russian Federation	EURO	Upper middle income	2014	PCV13	2+1	2, 4.5 & 15 months	PCV10
Spain	EURO	High income	2001	PCV13	2+1 (3+1)	2, 4 & 12 months	PCV7
Switzerland	EURO	High income	2006	PCV13	2+1	2, 4 & 12 months	PCV7
United Kingdim	EURO	High income	2006	PCV13	2+1	2, 4 & 13 months	PCV7
Bangladesh	SEARO	Lower middle income	2015	PCV10	3+0	6, 10, 14 weeks	--
India	SEARO	Lower middle income	2017	PCV13	2+1	6, 14 weeks & 9 months	--
Indonesia	SEARO	Lower middle income	2018	PCV13	2+1	2, 3 & 12 months	--
Myanmar	SEARO	Lower middle income	2016	PCV13	3+0	2, 4, 6 months	PCV10
Nepal	SEARO	Low income	2015	PCV10	2+1	6, 10 weeks & 9 months	--
Australia	WPRO	High income	2005	PCV13	2+1	2, 4, & 12 months	PCV7
Cambodia	WPRO	Lower middle income	2015	PCV13	3+0	6, 10, 14 weeks	--
Fiji	WPRO	Upper middle income	2012	PCV10	3+0	6, 10, 14 weeks	--
Japan	WPRO	High income	2011	PCV13	3+1	2, 3, 4 months & 1 year	PCV7
New Zealand	WPRO	High income	2008	PCV13	3+1	6 weeks, 3, 5, & 15 months	PCV7

Data collected from: http://www.view-hub.org/

*Canada: According to Jan 2016 schedule, Northwest Territories and Nunavut province use a 3+1 schedule (2, 4, 6, 18 months); all the other provinces use a 2+1 schedule (either 2, 4, 12 months or 2, 4, 18 months).

Supplementary Table S3.2. Regional VT carriage prevalence, pre- and post-PCV introduction

Country	Region	Carriage Prevalence Pre-Vaccine	Carriage Prevalence Post-Vaccine	Percent Reduction	Time (years) since PCV introduction	Age	PCV	Ref
Fiji	WPRO	22.3	5.8	74.0	3	12-23 m	PCV10	[1]
Australia	WPRO	23.7	12.0	49.4	1.5	< 5y	PCV13	[2]
Mozambique	AFRO	35.1	20.9	40.5	2	24-59 m	PCV10	[3]
Kenya	AFRO	33.8	13	61.5	2	< 5y	PCV10	[4]
Kenya	AFRO	"	8.8	73.9	5	"	"	"
Malawi	AFRO	28.2	17.9	36.6	4.5	1-4 y	PCV13	[5]
USA	AMRO	55.4	10.9	80.3	2	<5 y	PCV7	[6]
USA	AMRO	"	4.8	91.4	3	"	"	"
Netherlands	EURO	38	8	80.1	3	11 m	PCV7	[7]
Netherlands	EURO	36	4	88.1	3	2 y	PCV7	"
UK	EURO	31.9	4.2	86.4	7	<5 y	PCV7	[8]
UK	EURO	"	0.4	98.9	11	"	"	"
UK	EURO	39.9	1.0	97.5	7	<5 y	PCV13	[9]

"Indicates that the information for this cell is the same as the information in the cell directly above.

Supplementary Table S3.3. PCV-vaccinated study groups: Pneumococcal carriage prevalence, stratified by survey

18 weeks-1 year old (PCV-vaccinated)	Survey 1[§]	Survey 2[§]	Survey 3[§]	Survey 4 (n=153)	Survey 5 (n=147)	Survey 6 (n=139)	Survey 7 (n=127)	Total (n=566)
Total carriage								
% (n)	--	--	--	77.1 (118)	82.3 (121)	79.1 (110)	81.1 (103)	79.9 (452)
95% CI				69.6, 83.5	75.2, 88.1	71.4, 85.6	73.2, 87.5	76.3, 83.1
VT								
	--	--	--	19.0 (29)	17.7 (26)	16.5 (23)	15.0 (19)	17.1 (97)
				13.5, 26.0	12.3, 24.8	11.2, 23.7	9.7, 22.3	14.2, 20.5
NVT								
	--	--	--	58.1 (89)	64.6 (95)	62.6 (87)	66.1 (84)	62.7 (355)
				50.2, 65.8	56.6, 72.9	54.2, 70.3	57.4, 73.9	58.6, 66.6
2 years old (PCV vaccinated)	**Survey 1[§]**	**Survey 2[§]**	**Survey 3[§]**	**Survey 4 (n=124)**	**Survey 5 (n=114)**	**Survey 6 (n=135)**	**Survey 7 (n=126)**	**Total (n=499)**
Total carriage								
% (n)	--	--	--	78.3 (97)	83.3 (95)	71.8 (97)	75.4 (95)	76.9 (384)
95% CI				69.9, 85.1	75.2, 89.6	63.5, 79.2	66.9, 82.6	73.0, 80.6
VT								
	--	--	--	21.8 (27)	18.4 (21)	18.5 (25)	15.1 (19)	18.4 (92)
				15.3, 30.0	12.3, 26.7	12.8, 26.0	9.8, 22.5	15.3, 22.1
NVT								
	--	--	--	56.5 (70)	64.9 (74)	53.3 (72)	60.3 (76)	58.5 (292)
				47.6, 64.9	55.7, 73.2	44.9, 61.6	51.5, 68.5	54.1, 62.8
3-7 years old (PCV vaccinated)	**Survey 1 (n=286)**	**Survey 2 (n=303)**	**Survey 3 (n=361)**	**Survey 4 (n=380)**	**Survey 5 (n=382)**	**Survey 6 (n=475)**	**Survey 7 (n=378)**	**Total (n=2565)**
Total carriage								
% (n)	84.2 (241)	76.0 (230)	78.1 (282)	66.8 (254)	80.4 (307)	61.7 (293)	78.1 (295)	74.2 (1902)
95% CI	79.5, 88.3	70.7, 80.6	73.5, 82.3	61.9, 71.6	76.0, 84.2	57.4, 66.1	73.5, 82.1	72.4, 75.8
VT								
	19.9 (57)	20.5 (62)	20.8 (75)	17.6 (67)	19.4 (74)	13.3 (63)	16.7 (63)	18.0 (461)
	15.7, 25.0	16.3, 25.4	16.9, 25.3	14.1, 21.8	15.7, 23.7	10.5, 16.6	13.2, 20.8	16.5, 19.5
NVT								
	64.3 (184)	55.5 (168)	57.3 (207)	49.2 (187)	61.0 (233)	48.4 (230)	61.4 (232)	56.2 (1441)
	58.6, 69.7	49.8, 61.0	52.2, 62.4	44.2, 54.2	56.0, 65.8	43.9, 52.9	56.4, 66.2	54.2, 58.1

[§] There was no recruitment for these age groups during these surveys. CI, confidence interval; VT, vaccine serotype; NVT, non–vaccine serotype.

Supplementary Table S3.4. PCV-unvaccinated study groups: Pneumococcal carriage prevalence, stratified by survey

4-8 weeks old (PCV unvaccinated)	Survey 1[§]	Survey 2[§]	Survey 3[§]	Survey 4[§]	Survey 5 (n=121)	Survey 6 (n=133)	Survey 7 (n=92)	Total (n=346)
Total carriage								
% (n)	-	-	-	-	42.9 (52)	30.8 (41)	57.6 (53)	42.2 (146)
95% CI					34.0, 52.3	23.1, 39.4	46.9, 67.9	36.9, 47.6
VT								
	-	-	-	-	10.7 (13)	6.0 (8)	8.7 (8)	8.4 (29)
					6.3, 17.7	3.0, 11.6	4.4, 16.5	5.7, 11.8
NVT								
	-	-	-	-	32.2 (39)	24.8 (33)	48.9 (45)	33.8 (117)
					24.5, 41.1	18.2, 32.9	38.8, 59.1	28.8, 39.1

3-10 years old (PCV unvaccinated)	Survey 1 (n=255)	Survey 2 (n=231)	Survey 3 (n=242)	Survey 4 (n=198)	Survey 5 (n=106)	Survey 6 (n=173)	Survey 7 (n=197)	Total (n=1402)
Total carriage								
% (n)	68.3 (174)	62.4 (144)	58.7 (142)	37.8 (75)	58.5 (62)	48.6 (84)	57.4 (113)	56.7 (794)
95% CI	62.1, 73.9	55.7, 68.6	52.2, 64.9	31.1, 45.0	48.5, 68.0	40.9, 56.3	50.1, 64.4	53.9, 59.2
VT								
	27.5 (70)	21.7 (50)	19.4 (47)	14.1 (28)	10.4 (11)	11.0 (19)	15.2 (30)	18.2 (255)
	22.3, 33.3	16.7, 27.4	14.9, 24.9	9.9, 19.7	5.8, 17.8	7.1, 16.6	10.8, 21.0	16.2, 20.3
NVT								
	40.8 (104)	40.7 (94)	39.3 (95)	23.7 (47)	48.1 (51)	37.6 (65)	42.1 (83)	38.5 (539)
	34.9, 46.9	34.5, 47.2	33.2, 45.6	18.3, 30.2	38.7, 57.6	30.6, 45.0	35.4, 49.2	35.9, 41.0

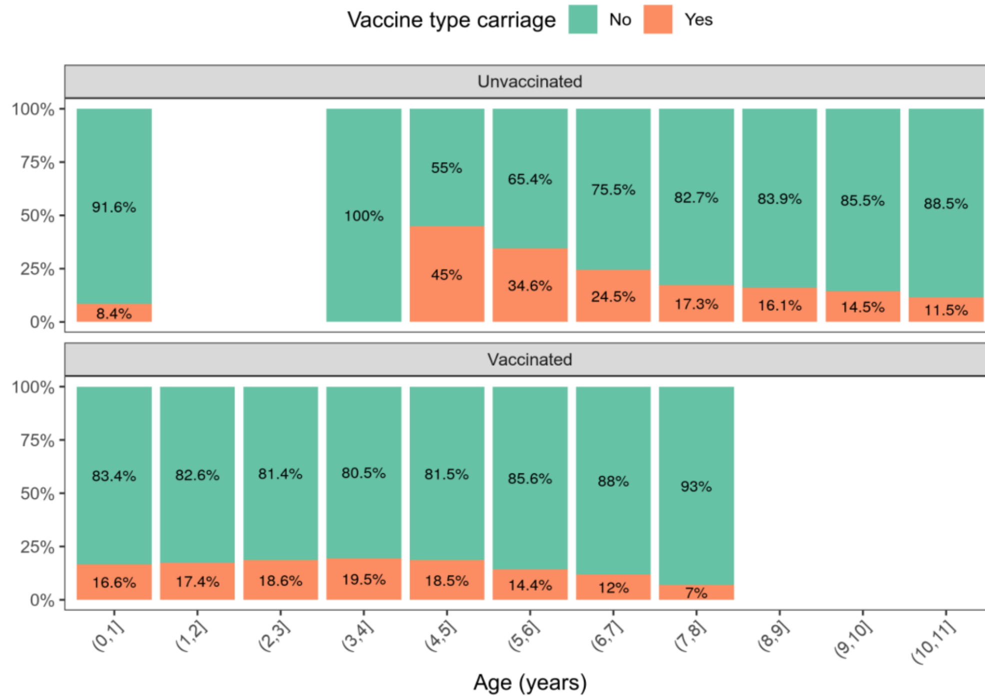
18-40 years old (PCV unvaccinated)	Survey 1 (n=198)	Survey 2 (n=201)	Survey 3 (n=279)	Survey 4 (n=308)	Survey 5 (n=305)	Survey 6 (n=277)	Survey 7 (n=202)	Total (n=1770)
Total carriage								
% (n)					38.4 ()	32.5 (90)	38.6 (78)	40.4 (714)
95% CI								
VT								
	15.2 (30)	14.4 (29)	14.0 (39)	14.3 (44)	10.5 (32)	9.0 (25)	8.9 (18)	12.3 (217)
	10.8, 20.9	10.2, 20.0	10.4, 18.6	10.8, 18.9	7.5, 14.5	6.2, 13.0	5.7, 13.7	10.8, 13.9
NVT								
	24.2 (48)	32.8 (66)	30.5 (85)	28.6 (88)	27.9 (85)	23.5 (65)	29.7 (60)	28.1 (497)
	18.8, 30.7	26.7, 39.6	25.3, 36.1	23.8, 33.9	23.1, 33.2	18.8, 28.8	23.8, 36.4	26.0, 30.2

[§]There was no recruitment for these age groups during these surveys. CI, confidence interval; VT, vaccine serotype; NVT, non–vaccine serotype.

Supplementary Table S3.5. Population-based pneumococcal carriage prevalence studies, Malawi

Age	Carriage	Karonga (rural), Malawi[1] 2009-2011 Pre PCV intro.	Karonga (rural), Malawi[1] 2014 Post PCV intro.		Blantyre (urban), Malawi 2015-2019 Post PCV intro.
6 weeks old PCV-unvaccinated[2]	Spn	38.6	43.8		57.3
	VT	11.4	13.0		8.4
	NVT	27.1	30.8		48.9
	Total N	70	146		346
18 weeks old PCV-vaccinated[3]	Spn	74.7	50.0		81.8
	VT	45.1	9.1		16.8
	NVT	29.6	40.9		65.0
	Total N	71	44		203
			Unvacc'd	**Vacc'd**	**Vacc'd**
1-4 years old PCV-unvaccinated and vaccinated	Spn	59.4	63.9	70.0	77.2
	VT	28.2	22.9	16.5	18.7
	NVT	31.2	41.0	53.4	58.5
	Total N	330	83	103	2958
			Unvacc'd[4]	**Vacc'd**	
18 weeks-4years (<5y) PCV-unvaccinated and vaccinated	Spn	62.1	63.9	63.9	77.5
	VT	31.2	22.9	14.3	18.6
	NVT	30.9	41.0	49.7	58.9
	Total N	401	83	147	3161
5-15 years PCV-unvaccinated[5]	Spn	49.5	37.1		53.8
	VT	21.2	7.9		16.3
	NVT	28.3	29.2		37.5
	Total N	325	89		1748

[1]Heinsbroek et al[5] [2]Blantyre study data includes infants 4-8 weeks of age. [3]Blantyre study data includes ages 18 weeks-12 months of age. [4]No children <1 year old were unvaccinated; therefore, same results as among children 1-4 years old. [5]Blantyre study data among PCV-unvaccinated is limited to ages 3-10 years. PCV, pneumococcal conjugate vaccine; intro., introduction; Spn, *Streptococcus pneumoniae*; VT, vaccine serotype; NVT, non–vaccine serotype; Vacc'd, PCV vaccinated; Unvacc'd, PCV unvaccinated.



Supplementary Figure S3.1. VT and NVT carriage among PCV-vaccinated and PCV-unvaccinated children. Proportions reported in the lower (orange) section of each bar represent the prevalence of VT carriage in each age group. Proportions reported in the upper (green) section of each bar represent the prevalence of no VT carriage (NVT + no carriage). The (0, 1] bar among unvaccinated children includes only children 4-8 weeks of age. The (A, B] notation used for each age group includes all children whose age was greater than A and less than or equal to B ($A < age \leq B$). When modelling the entire range of data (i.e., not censoring at 3.6 years) with an exponential decay curve the estimated decay parameter for vaccinated was not significantly different from zero (i.e., an almost flat curve was the resulting fit). Among PCV-vaccinated children there is an observed increase in pneumococcal carriage until the (3,4] year age range and only then does VT carriage prevalence start to decrease. Carriage profiles are susceptible to multiple confounders, including time (e.g., change in vaccine coverage and herd protection over time) and age, the effects of which are difficult to disentangle. Our conjecture is that the overall trend in pneumococcal carriage for vaccinated children starting from birth is more complex than an exponential decay curve. NVT, non–vaccine serotype; PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

Supplementary Table S3.6. Denominator (N) for each age bar in Supplementary Figure S3.1

	(0, 1]	(1, 2]	(2, 3]	(3, 4]	(4, 5]	(5, 6]	(6, 7]	(7, 8]	(8, 9]	(9, 10]	(10, 11]
Unvaccinated, N	346	--	--	2	16	136	150	243	302	272	281
Vaccinated, N	203	363	499	1105	991	309	117	43	--	--	--

For cells showing '--', there was no recruitment for these age groups.

Supplementary Table S3.7. Proportion of VT carriage attributed to individual VT (PCV vaccinated)

Vaccine serotype	Survey-1 % (n)	Survey-2 % (n)	Survey-3 % (n)	Survey-4 % (n)	Survey-5 % (n)	Survey-6 % (n)	Survey-7 % (n)	Total % (n)
Children 18 weeks to 1 year, PCV-vaccinated §								
1	--	--	--	0	0	(4.4) 1	0	1.0 (1)
3	--	--	--	6.9 (2)	7.7 (2)	8.7 (2)	21.0 (4)	10.3 (10)
4	--	--	--	0	0	0	5.3 (1)	1.0 (1)
5	--	--	--	0	0	0	0	0
6A	--	--	--	20.7 (6)	15.4 (4)	21.7 (5)	15.8 (3)	18.6 (18)
6B	--	--	--	13.8 (4)	3.9 (1)	0	0	5.2 (5)
7F	--	--	--	0	3.9 (1)	0	0	1.0 (1)
9V	--	--	--	3.5 (1)	7.7 (2)	4.4 (1)	5.3 (`)	5.2 (5)
14	--	--	--	13.8 (4)	15.4 (4)	34.8 (8)	26.3 (5)	21.7 (21)
18C	--	--	--	0	0	0	5.3 (1)	1.0 (1)
19A	--	--	--	13.8 (4)	3.9 (1)	8.7 (2)	0	7,2 (7)
19F	--	--	--	13.8 (4)	23.1 (6)	17.4 (4)	10.5 (2)	16.5 (16)
23F	--	--	--	13.8 (4)	19.2 (5)	0	10.5 (2)	11.3 (11)
Total	--	--	--	100 (29)	100 (26)	100 (23)	100 (19)	100 (97)
Children 2 years, PCV-vaccinated §								
1	--	--	--	0	(2)	0	0	2.2 (2)
3	--	--	--	18.5 (5)	14.3 (3)	16.0 (4)	10.5 (2)	15.2 (14)
4	--	--	--	0	0	0	10.5 (2)	2.2 (2)
5	--	--	--	()	()	()	()	()
6A	--	--	--	22.2 (6)	9.5 (2)	8.0 (2)	15.8 (3)	14.1 (13)
6B	--	--	--	()	()	()	()	()
7F	--	--	--	()	()	()	()	()
9V	--	--	--	7.4 (2)	9.5 (2)	0	5.3 (1)	5.4 (5)
14	--	--	--	11.1 (3)	14.3 (3)	28.0 (7)	21.1 (4)	18.5 (17)
18C	--	--	--	3.7 (1)	4.8 (1)	4.0 (1)	0	3.3 (3)
19A	--	--	--	7.4 (2)	0	4.0 (1)	5.3 (1)	4.4 (4)
19F	--	--	--	25.9 (7)	14.3 (3)	28.0 (7)	26.3 (5)	23.9 (22)
23F	--	--	--	3.7 (1)	23.8 (5)	12.0 (3)	5.3 (1)	10.9 (10)
Total	--	--	--	(100) 27	(100) 21	(100) 25	(100) 19	(100) 92
Children 3-7 years, PCV-vaccinated								
1	5.3 (3)	1.6 (1)	1.3 (1)	0	4.1 (3)	1.6 (1)	0	2.0 (9)
3	17.5 (10)	22.6 (14)	25.3 (19)	17.9 (12)	24.3 (18)	11.1 (7)	25.4 (16)	20.8 (96)
4	0	3.2 (2)	1.3 (1)	0	0	6.4 (4)	(0)	1.5 (7)
5	3.5 (2)	4.8 (3)	0	1.5 (1)	0	1.6 (1)	0	1.5 (7)
6A	14.0 (8)	6.5 (4)	12.0 (9)	9.0 (6)	9.5 (7)	17.5 (11)	6.4 (4)	10.6 (49)
6B	1.8 (1)	4.8 (3)	6.7 (5)	1.5 (1)	2.7 (2)	1.6 (1)	3.2 (2)	3.3 (15)
7F	0	0	0	1.5 (1)	0	1.6 (1)	0	0.4 (2)
9V	3.5 (2)	6.5 (4)	1.3 (1)	3.0 (2)	4.1 (3)	4.8 (3)	6.4 (4)	4.1 (19)
14	8.8 (5)	11.3 (7)	6.7 (5)	14.9 (10)	10.8 (8)	14.3 (9)	19.1 (12)	12.2 (56)
18C	1.8 (1)	0	4.0 (3)	3.0 (2)	1.4 (1)	3.2 (2)	3.2 (2)	2.4 (11)
19A	7.0 (4)	12.9 (8)	9.3 (7)	6.0 (4)	6.8 (5)	1.6 (1)	1.6 (1)	6.5 (30)
19F	24.6 (14)	8.1 (5)	21.3 (16)	28.4 (19)	13.5 (10)	17.5 (11)	23.8 (15)	19.5 (90)
23F	12.3 (7)	17.7 (11)	10.7 (8)	13.4 (9)	23.0 (17)	17.5 (11)	11.1 (7)	15.2 (70)
Total	100 (57)	100 (62)	100 (75)	100 (67)	100 (74)	100 (63)	100 (63)	100 (461)

§ There was no recruitment of these age groups during surveys 1-3.
PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

Supplementary Tables S3.7 and 8 show the proportion of total VT carriage attributed to individual vaccine serotypes, among PCV-vaccinated and PCV-unvaccinated children, respectively. Low sample sizes do not allow for a robust analysis of relative change over time. Children 18 weeks to 2 years of age were recruited starting survey 4. The denominator

is the total VTs identified in each survey. Surveys 1-7 spanned a period of 3.6 to 7.1 years after Malawi's 12 November 2011 introduction of PCV13. For total sample size per survey, refer to Supplementary tables S3.3 and S3.4.

Supplementary Table S3.8. Proportion of VT carriage attributed to individual VT (PCV unvaccinated)

Vaccine serotype	Survey-1 % (n)	Survey-2 % (n)	Survey-3 % (n)	Survey-4 % (n)	Survey-5 % (n)	Survey-6 % (n)	Survey-7 % (n)	Total % (n)
Infants 4-8 weeks, PCV-unvaccinated §								
1	--	--	--	--	0	0	0	0
3	--	--	--	--	7.7 (1)	0	12.5 (1)	6.7 (2)
4	--	--	--	--	0	0	0	0
5	--	--	--	--	0	0	0	0
6A	--	--	--	--	15.4 (2)	25.0 (2)	0	4 (13.3)
6B	--	--	--	--	7.7 (1)	0	37.5 (3)	4 (13.3)
7F	--	--	--	--	0	0	0	0
9V	--	--	--	--	7.7 (1)	0	0	1 (3.3)
14	--	--	--	--	7.7 (1)	25.0 (2)	12.5 (1)	13.3 (4)
18C	--	--	--	--	7.7 (1)	12.5 (1)	0	6.7 (2)
19A	--	--	--	--	0	0	0	0
19F	--	--	--	--	15.4 (2)	25.0 (2)	25.0 (2)	20.0 (6)
23F	--	--	--	--	30.8 (4)	12.5 (1)	12.5 (1)	23.3 (6)
Total	--	--	--	--	100 (13)	100 (8)	100 (8)	100 (29)
Children 3-10 years, PCV-unvaccinated								
1	14.3 (10)	6.0 (3)	2.1 (1)	0	0	5.3 (1)	0	5.6 (15)
3	12.9 (9)	20.0 (10)	23.4 (11)	14.3 (4)	0	26.3 (5)	6.7 (2)	16.1 (41)
4	5.7 (4)	2.0 (1)	10.6 (5)	7.1 (2)	0	15.8 (3)	0	5.9 (15)
5	1.4 (1)	0	0	0	0	10.5 (2)	3.3 (1)	1.6 (4)
6A	10,0 (7)	20.0 (10)	6.4 (3)	21.4 (6)	18.2 (2)	5.3 (1)	13.3 (4)	12.9 (33)
6B	2.9 (2)	10.0 (5)	8.5 (4)	7.1 (2)	9.1 (1)	0	10.0 (3)	6.7 (17)
7F	5.7 (4)	2.0 (1)	2.1 (1)	0	9.1 (1)	5.3 (1)	0	6.7 (8)
9V	8.6 (6)	6.0 (3)	4.3 (2)	7.1 (2)	9.1 (1)	0	6.7 (2)	6.3 (16)
14	0	8.0 (4)	17.0 (8)	7.1 (2)	9.1 (1)	5.3 (1)	10.0 (3)	7.5 (19)
18C	7.1 (5)	4.0 (2)	8.5 (4)	0	27.3 (3)	5.3 (1)	6.7 (2)	6.7 (17)
19A	4.3 (3)	4.0 (2)	4.3 (2)	7.1 (2)	0	0	10.0 (3)	4.7 (12)
19F	12.9 (9)	10.0 (5)	8.5 (4)	10.7 (3)	0	10.5 (2)	16.7 (5)	11.0 (28)
23F	14.3 (10)	8.0 (4)	4.3 (2)	17.9 (5)	18.2 (2)	10.5 (2)	16.7 (5)	11.8 (30)
Total	100 (70)	100 (50)	100 (47)	100 (28)	100 (11)	100 (19)	100 (30)	100 (255)
Adults 18-40 years, HIV-infected on ART, PCV-unvaccinated								
1	3.3 (1)	13.8 (4)	7.7 (3)	0	0	0	0	3.7 (8)
3	23.3 (7)	34.5 (10)	28.2 (11)	27.3 (12)	37.5 (12)	36.0 (9)	55.6 (10)	32.7 (71)
4	13.3 (4)	6.9 (2)	7.7 (3)	2.3 (1)	9.4 (3)	8.0 (2)	0	6.9 (15)
5	0	0	2.6 (1)	0	0	0	0	0.5 (1)
6A	13.3 (4)	3.5 (1)	10.3 (4)	4.6 (2)	6.3 (2)	4.0 (1)	5.6 (1)	6.9 (15)
6B	3.3 (1)	3.5 (1)	0	0	3.1 (1)	4.0 (1)	0	1.8 (4)
7F	0	0	0	4.6 (2)	0	0	0	0.9 (2)
9V	3.3 (1)	3.5 (1)	5.1 (2)	9.1 (4)	3.1 (1)	4.0 (1)	0	4.6 (10)
14	0	0	7.7 (3)	9.1 (4)	3.1 (1)	8.0 (2)	0	4.6 (10)
18C	6.7 (2)	3.5 (1)	0	9.1 (4)	0	8.0 (2)	16.7 (3)	5.5 (12)
19A	10.0 (3)	6.9 (2)	7.7 (3)	9.1 (4)	9.4 (3)	12.0 (3)	5.6 (1)	8.8 (19)
19F	20.0 (6)	17.2 (5)	5.1 (2)	13.6 (6)	15.6 (5)	8.0 (2)	16.7 (3)	13.4 (29)
23F	3.3 (1)	6.9 (2)	18.0 (7)	11.4 (5)	12.5 (4)	8.0 (2)	0	9.7 (21)
Total	100 (30)	100 (29)	100 (39)	100 (44)	100 (32)	100 (25)	100 (18)	100 (217)

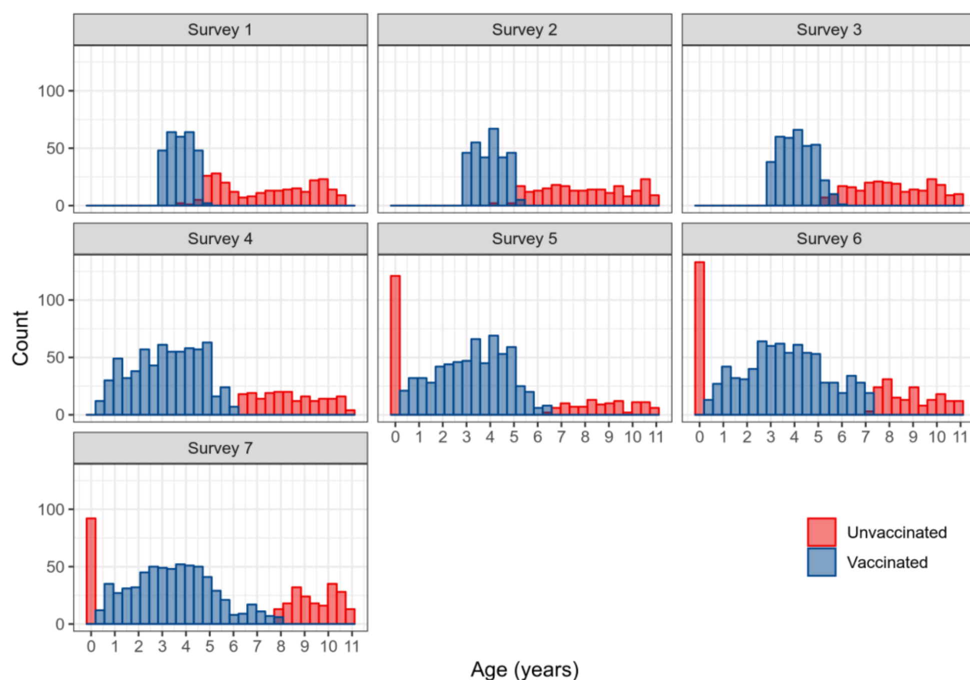
§ There was no recruitment of these age groups during surveys 1-4
ART, antiretroviral therapy; PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

Supplementary Table S3.9. Serotype 3: impact of classifying serotype-3 as NVT on carriage prevalence and prevalence ratio

Children 3-5 years (PCV vaccinated)	Survey 1 (n=286)	Survey 2 (n=303)	Survey 3 (n=361)	Survey 4 (n=378)	Survey 5 (n=371)	Survey 6 (n=382)	Survey 7 (n=324)	Total (n=2405)	cPR¥ (95% CI) p-value	aPR¥ (95% CI) p-value	Relative change
Total carriage % (n) 95% CI	84.2 (241) 79.5, 88.3	75.9 (230) 70.7, 80.6	78.1 (282) 73.5, 82.3	67.0 (253) 61.9, 71.7	80.6 (299) 76.2, 84.5	67.3 (257) 62.3, 72.0	79.7 (258) 74.8, 83.9	75.7 (1820) 73.9, 77.4	--	--	--
VT, Excluding serotype 3	16.4 (47) 12.6, 21.2	15.8 (48) 12.1, 20.4	15.5 (56) 12.1, 19.6	14.6 (55) 11.3, 18.5	14.6 (54) 11.3, 18.5	13.4 (51) 10.3, 17.2	13.0 (42) 9.7, 17.1	14.7 (353) 13.3, 16.2	0.926 (0.842, 1.018) 0.111	0.942 (0.856, 1.036) 0.218	−20.7%
NVT, Including serotype 3	67.8 (194) 62.2, 73.0	60.1 (182) 54.4, 65.4	62.6 (226) 57.5, 67.5	52.4 (198) 47.3, 57.4	66.0 (245) 61.1, 70.7	53.9 (206) 48.9, 58.9	66.7 (216) 61.3, 71.6	61.0 (1467) 59.0, 63.0	0.964 (0.934, 0.996) 0.026	0.968 (0.937.1.000) 0.048	−1.6%
Children 6-8 years (PCV-unvaccinated)	Survey 1 (n=91)	Survey 2 (n=111)	Survey 3 (n=139)	Survey 4 (n=128)	Survey 5 (n=56)	Survey 6 (n=100)	Survey 7 (n=70)	Total (n=695)	cPR¥ (95% CI) p-value	aPR¥ (95% CI) p-value	
Total carriage % (n) 95% CI	67.1 (61) 56.4, 76.5	65.8 (73) 56.2, 74.5	63.3 (88) 54.7, 71.3	43.0 (55) 34.3, 52.0	55.3 (31) 41.5, 68.7	52.0 (52) 41.8, 62.1	61.4 (43) 49.0, 72.8	58.0 (403) 54.2, 61.7	--	--	--
VT, Excluding serotype 3	22.0 (20) 14.6, 31.7	17.1 (19) 11.2, 25.3	15.8 (22) 10.6, 22.9	12.5 (16) 7.8, 19.5	8.9 (5) 3.7, 19.9	10.0 (10) 5.4, 17.7	15.7 (11) 8.9, 26.3	14.8 (103) 12.3, 17.7	0.812 (0.680, 0.970) 0.021	0.844 (0.699, 1.019) 0.078	-28.6%
NVT, Including serotype 3	45.1 (41) 35.1, 55.4	48.7 (54) 39.4, 57.9	47.5 (66) 39.3, 55.8	30.5 (39) 23.1, 39.0	46.4 (26) 33.8, 59.6	42.0 (42) 32.7, 51.9	45.7 (32) 34.4, 57.5	43.2 (300) 39.4, 46.9	0.948 (0.872, 1.031) 0.214	0.959 (0.878, 1.047) 0.347	+1.3%
Adult, HIV-infected on ART (PCV-unvaccinated)	Survey 1 (n=198)	Survey 2 (n=201)	Survey 3 (n=279)	Survey 4 (n=308)	Survey 5 (n=305)	Survey 6 (n=277)	Survey 7 (n=202)	Total (n=1770)	cPR¥ (95% CI) p-value	aPR¥ (95% CI) p-value	
Total carriage % (n) 95% CI	39.4 (78) 32.5, 46.6	47.3 (95) 40.2, 54.4	43.4 (124) 38.5, 50.5	42.9 (132) 37.3, 48.6	38.4 (117) 32.9, 44.1	32.5 (90) 27.0, 38.4	38.7 (78) 31.9, 45.7	40.4 (714) 38.0, 42.7	--	--	--
VT, Excluding serotype 3	11.6 (23) 7.8-16.9	9.5 (19) 6.1-14.4	10.0 (28) 7.0-14.2	10.4 (32) 7.4-14.3	6.6 (20) 4.3-10.0	5.8 (16) 3.6-9.2	4.0 (8) 2.0-7.7	8.3 (146) 7.1-9.6	0.783 (0.673, 0.912) 0.002	0.763 (0.655, 0.888) 0.000	-65.5%
NVT, Including serotype 3	27.8 (55) 22.0, 34.4	37.8 (76) 31.4, 44.7	33.4 (96) 29.1, 40.2	32.5 (100) 27.5, 37.9	31.8 (97) 26.8, 37.3	26.7 (74) 21.8, 32.2	34.7 (70) 28.4, 41.5	32.1 (568) 29.9, 34.3	0.970 (0.908, 1.037) 0.373	0.966 (0.904, 1.032) 0.309	+24.8%

cPR, crude prevalence ratio; aPR, adjusted ratio; CI, confidence interval; VT, vaccine serotype; NVT, non–vaccine serotype; PCV, pneumococcal conjugate vaccine

3.8.1 Supplementary Note S3.1. Details of the non-linear regression analysis framework



Supplementary Figure S3.2. Age distribution of PCV-unvaccinated and PCV-vaccinated children throughout the duration of the PCVPA study. Red and blue bars represent PCV-unvaccinated and PCV-vaccinated children, respectively. The x-axis is age in years old. The y-axis shows the count (frequency) of children. Each bar represents a 4-months progression in age. PCV, pneumococcal conjugate vaccine

Summary description of the non-linear regression analysis: To better understand the rate at which VT and NVT carriage prevalence was decreasing, we developed a model to describe the variation in an individual's probability of VT or NVT carriage with age, adjusted for age at recruitment. The model is fitted using carriage data from children 3.6 to 10 years of age, maximizing overlap of empiric data. Model outputs for individual carriage probability were then transformed into a population-level (decay) half-life of each VT and NVT carriage. By calculating the effect of not receiving the vaccination (β), we can then begin to define the benefit of vaccine-induced immunity in lowering an individual's risk of VT carriage at different ages after receiving PCV vaccination. This was then be extrapolated to define the rate of reduced VT carriage within the studied population. Further analyses, not currently available, should include a measure of other contributing factors, including waning maternal and developing naturally acquired immunity.

Model specification, VT

In this cross-sectional study, the response (i.e. VT carrier or not) for each child is a single binary variable, $Y_i = 1/0$ representing presence/absence of VT carriage at the time of measurement (i.e. sample collection), with respective probabilities p_i and $1 - p_i$.

The modelled probability, p_i, of VT carriage for an individual child i is $\alpha\beta\exp\{-\delta_u(age_i - t_c)\}$ if the child is unvaccinated and $\alpha\exp\{-\delta_v(age_i - t_c)\}$ if vaccinated, where age_i is the age in years of child i at time of measurement, t_c is the time at which we censor the data (3.6 years), β is the effect of not receiving the vaccination, δ_u and δ_v are the rate of decay of VT carriage prevalence with age for unvaccinated and vaccinated children, respectively, and α is the VT carriage prevalence for vaccinated children at time t_c.

All parameters estimated in the model are calculated using the maximum likelihood, as also specified in the main manuscript. Y_i is distributed as a Bernoulli with probability p_i specified according to the equations above. From this we can calculate the likelihood and then find the parameters that maximize that likelihood.

Supplementary Table S3.10. Summary of parameters

	VT carriage, Yes	VT carriage, No (NVT or no carriage)
Variable for carriage status	1	0
Probability of VT carriage	p_i	$1 - p_i$
Modelling parameter (p_i) (=probability of VT carriage for an individual child, i)		
Unvaccinated	$\alpha\beta\exp\{-\delta_u(age_i - t_c)\}$	
Vaccinated	$\alpha\exp\{-\delta_v(age_i - t_c)\}$	
age_i	age in years of child i at time of measurement	
t_c	time at which we censor the data (3.6 years)	
β	effect of not receiving the vaccination	
δ_u	rate of decay of VT carriage prevalence with age for unvaccinated children	
δ_v	rate of decay of VT carriage prevalence with age for vaccinated children	
α	VT carriage prevalence for vaccinated children at time t_c.	

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S3.11. Alpha and beta values for PCV-vaccinated children and PCV-unvaccinated children[1]

Parameter	3.6 years old	4 years old	4.5 years old	5 years old	5.5 years old	6 years old	6.5 years old	7 years old
VT carriage prevalence (with 95% CI) at censoring age (3.6 years) for vaccinated children (α)	0.22 0.19, 0.25	0.22 0.19, 0.25	0.20 0.17, 0.23	0.20 0.16, 0.24	0.17 0.12, 0.23	0.16 0.10, 0.26	0.19 0.08, 0.38	0.06 0.03, 0.13
VT Carriage prevalence (with 95% CI) at censoring age (3.6 years) for unvaccinated children (α×β)	0.47 0.43, 0.51	0.47 0.43, 0.51	0.45 0.42, 0.48	0.40 0.36, 0.45	0.36 0.30, 0.41	0.30 0.23, 0.40	0.25 0.12, 0.45	0.20 0.16, 0.27
Effect of not receiving the vaccination (β)	2.21 1.51, 2.92	2.21 1.51, 2.92	2.26 1.57, 2.95	2.0 1.38, 2.62	2.13 1.29, 2.98	1.85 0.83, 2.88	1.33 0.23, 2.43	3.39 0.59, 6.18

[1]Low sample sizes does not allow for a robust analysis beyond seven years of age.

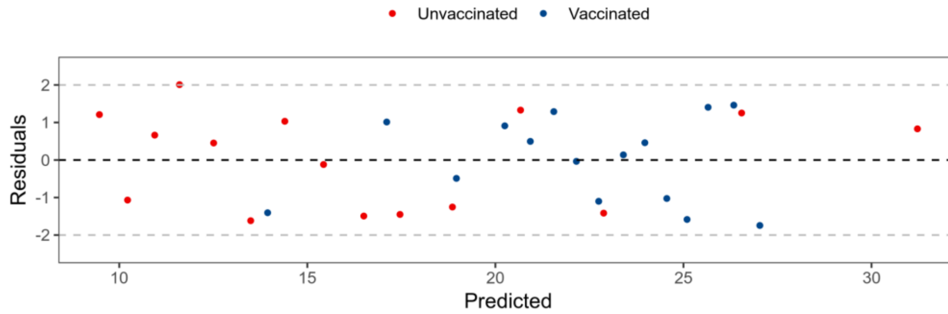
CI, confidence interval; PCV, pneumococcal conjugate vaccine; VT, vaccine serotype

Goodness-of-fit and limitations of the non-linear regression analysis

The conventional way to assess goodness-of-fit of a regression model is to plot standardized residuals against predicted (fitted) values; in a well-fitting model, the plot should show no discernible structure. However, individual residuals from a model with binary response are unreliable. Instead, we use grouped residuals as follows. Within each of the unvaccinated and vaccinated sets of children, order the estimated probabilities p_i from smallest to largest. Choose a group size, k. The first grouped predicted value is the sum $s_1 = p_1 + \cdots + p_k$, and the first standardized grouped residual is $r_1 = \{(Y_1 + \cdots + Y_k) - s_1\}/\sqrt{(v_1)}$ where $Y_i = 0/1$ denotes absence/presence of VT carriage in the ith child and $v_1 = p_1(1 - p_1) + \cdots + p_k(1 - p_k)$. The second grouped predicted value and standardized residual are calculated in the same way using the next k ordered p_i, and so on.

Supplementary figure S3.3 shows the resulting plot, using $k = 125$ and $k = 93$ for unvaccinated and vaccinated children, respectively, to give 15 groups in each of the two sets. The plot indicates a good fit in that (a) there is no discernible relationship between the residual and predicted values, and (b) the range of the residuals, from approximately -2 to +2, is compatible with their theoretical mean and standard deviation of 0 and 1, respectively, if the model is correct.

The major limitation of the analysis reported here is the inability of our model to extrapolate before the censoring time t_c with a reasonable amount of uncertainty. This is due to the small overlap in the age ranges of vaccinated and unvaccinated children and to the fact that the VT carriage dynamic for vaccinated children is too complex to capture, with the available data, in the early years of life.



Supplementary Figure S3.3. Plot of standardized grouped residuals against predicted values. The major limitation of the analysis reported here is the inability of our model to extrapolate to before the censoring time t_c with a reasonable amount of uncertainty. This is because of the small overlap in the age ranges of vaccinated and unvaccinated children and because the vaccine-serotype carriage dynamic for vaccinated children is too complex to capture, with the available data, in the early years of life.

3.8.2 References – supplementary material

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Chapter 4. Evaluation of pneumococcal serotyping in nasopharyngeal-carriage isolates by latex agglutination, whole-genome sequencing (PneumoCaT), and DNA microarray in a high-pneumococcal-carriage-prevalence population in Malawi

N.B., Associated published peer-reviewed article: *Swarthout TD, *Gori A, Bar-Zeev N, Kamng'ona AW, Mwalukomo TS, Bonomali F, Nyirenda R, Brown C, Msefula J, Everett D, Mwansambo C, Gould K, Hinds J, Heyderman RS, French N. Evaluation of pneumococcal serotyping of nasopharyngeal-carriage isolates by latex agglutination, whole-genome sequencing (PneumoCaT), and DNA microarray in a high-pneumococcal-carriage-prevalence population in Malawi. J Clin Microbiol. 2020 Dec 17;59(1):e02103-20. doi: 10.1128/JCM.02103-20. PMID: 33087431; PMCID: PMC7771446.

Chapter introduction: Chapter 3 reports the primary findings of the PCVPA carriage surveillance study, showing that, compared with high-income settings, Blantyre has high residual vaccine-serotype carriage 3.6 to 7.1 years after the introduction of the 13-valent pneumococcal conjugate vaccine in Malawi. Given the importance of such findings in informing national and international vaccine policy, one must ensure both robust and site-appropriate techniques for assessing serotype distribution associated with pneumococcal colonization and disease. Chapter 4 describes, in the context of the PCVPA study, the level of concordance between three methods commonly used during ongoing routine pneumococcal surveillance activities in our work: latex agglutination, microarray, and serotyping by sequencing. The chapter also addresses parameters that researchers, reference laboratories, and policymakers can consider when deciding which assay to implement in their local setting.

Abstract

Background: Accurate assessment of the serotype distribution associated with pneumococcal colonization and disease is essential for the evaluation and formulation of pneumococcal vaccines and informing vaccine policy.

Methods: Using samples from community carriage surveillance in Blantyre, Malawi, we evaluated pneumococcal serotyping concordance between latex agglutination with the ImmuLex 7-10-13-valent Pneumotest kit (Statens Serum Institut, Cophenhagen, Denmark), whole-genome sequencing with the HiSeq4000 platform (Illumina Inc., San Diego, CA, USA) and PneumoCaT (Pneumococcal Capsular Typing; Public Health England, London,

UK) software, and DNA microarray using the SP-CPS v1.4.0 microarray (Bacterial Microarray Group at St. George's [*BμG@S*], London, UK). Nasopharyngeal swabs were collected, following World Health Organization recommendations, between 2015 and 2017, using stratified random sampling among study populations. Participants included healthy children 3 to 6 years old (vaccinated with the 13-valent pneumococcal conjugate vaccine [PCV13] as part of the Expanded Programme on Immunization), healthy children 5 to 10 years (age-ineligible for PCV13), and HIV-infected adults (18-40 years old) on antiretroviral therapy. For phenotypic serotyping, we used a 13-valent latex kit (Statens Serum Institut). For genomic serotyping, we applied the PneumoCaT pipeline to whole-genome sequence libraries. For molecular serotyping by microarray, we used the Senti-SP microarray (BUGS Bioscience Ltd, London, UK).
Results: 1347 samples were analysed. Concordance was 90.7% (95% confidence interval [CI], 89.0%-92.2%) between latex and PneumoCaT; 95.2% (95% CI, 93.9%-96.3%) between latex and microarray; and 96.6% (95% CI, 95.5%-97.5%) between microarray and PneumoCaT. By detecting additional vaccine-serotype (VT) pneumococcus carried at low relative abundance (median, 8%), microarray increased VT detection by 31.5% compared with latex serotyping.

Conclusion: All three serotyping methods were highly concordant in identifying dominant serotypes. Latex serotyping is accurate for identifying vaccine serotypes and requires the least expertise and resources for field implementation and analysis. However, whole-genome sequencing, which adds population structure, and microarray, which adds multiple-serotype carriage, should be considered at regional reference laboratories while investigating the importance of VT (in low relative abundance) to transmission and disease.

4.1 Introduction

Streptococcus pneumoniae colonizes the nasopharynx of healthy individuals. Although carriage is usually asymptomatic, nasopharyngeal (NP) colonization is a prerequisite for disease, including otitis media, sinusitis, (bacteraemic) pneumonia, bacteraemia, and meningitis.[1] The pneumococcus is estimated to be responsible for over 318 000 (uncertainty ratio [UR]: 207 000-395 000) deaths every year among children aged 1 to 59 months, with the highest mortality burden among African children.[2] Evidence also shows that HIV-infected children and adults are at significantly higher risk of invasive pneumococcal disease (IPD) than their HIV-uninfected counterparts.[3,4]

Current multivalent pneumococcal conjugate vaccines (PCVs) target subsets of the 100 capsular serotypes known to be expressed by the pneumococcus. PCV reduces NP carriage

of the subset of pneumococcal serotypes they contain, known as vaccine serotypes (VTs). With reduced carriage among vaccinated individuals, there is then reduced risk of VT IPD among vaccinated individuals (direct protection), reduced transmission, and therefore reduced risk of VT IPD among PCV-unvaccinated individuals (indirect protection). However, non-VTs (NVTs) have the potential to fill the ecological niche, becoming more common in carriage and disease.[5-7] This phenomenon, known as serotype replacement, may be more pronounced in low-income settings because of the associated higher prevalence, density, and diversity of pneumococcal carriage, and it represents a considerable risk to the global pneumococcal immunization strategy.[8] Serotype distribution differs between continents and individual countries.[9] Given these differences, accurate assessment of the serotype distribution associated with both pneumococcal colonization and pneumococcal disease is needed in the evaluation, formulation, and delivery of pneumococcal vaccines.

A pneumococcal serotyping method suitable for use in robust carriage and surveillance studies should, therefore, at least be accurate in its serotype assignment, particularly in relation to VTs. Additional desirable parameters include ability to detect most or all serotypes, ability to detect multiple serotypes in carriage (common in high-burden settings),[10,11] more in-depth information on genotype, amenability to scale-up for large projects, and practicality for resource-poor settings. Unfortunately, work in resource-poor settings can too often limit the number of these parameters that can be achieved.

The gold-standard serotyping method, the quellung reaction, was developed in the early 1900s and is performed by testing colonies with a set of type-specific antisera.[12] Bacteria are observed by microscopy, with serotypes defined by observing apparent capsular swelling in reaction to the type-specific antisera. It is laborious, requires frequent use to maintain skills, requires a complete set of type-specific antisera, and is therefore mainly performed by reference laboratories. The PneuCarriage project, a large multicentre study, was established with the aim of identifying the best pneumococcal serotyping methods for carriage studies.[13] The project identified microarray with a culture amplification step as the top-performing method. While robust and systematic, their decision algorithm did not account for variables such as cost, required skill level, and resources needed for assay implementation and maintenance, as well as output processing and interpretation.

Here we describe, in the context of a field-based pneumococcal carriage study,[14] the level of concordance between three methods commonly used during ongoing routine pneumococcal surveillance activities in our work: latex agglutination, microarray, and

serotyping by sequencing. We also address considerations for researchers and policymakers when deciding which assay to implement in their local settings.

4.2 Methods

4.2.1 Study setting

Blantyre is in southern Malawi and has an urban population of approximately 1.3 million.

4.2.2 Study population and recruitment

Samples were collected as part of a larger 3.5-year pneumococcal carriage surveillance project, as described elsewhere.[14] In brief, this was a prospective rolling cross-sectional observational study using stratified random sampling to measure pneumococcal NP carriage in Blantyre. Samples used in the analysis described in this chapter were collected during the first 2 years of twice-annual cross-sectional surveys, from June 2015 through April 2017. Recruitment included three groups: (1) healthy children 3 to 6 years old who received PCV13 as part of routine immunization, (2) healthy children 5 to 10 years old who were age-ineligible to receive PCV13 as part of routine immunization, and (3) HIV-infected adults (18-40 years old) on antiretroviral therapy (ART).

4.2.2.1 Sample selection

For concordance analyses between the three methods, all samples were included that had serotyping results available from each of the three methods (latex, microarray, serotyping by sequencing). From among all NP swab (NPS) samples collected during the larger surveillance project (including 1044 from children 3-6 years old [PCV vaccinated], 531 from children 5-10 years old [PCV unvaccinated, age-ineligible], and 428 from HIV-infected adults on ART), 1347 samples were culture confirmed for *S. pneumoniae* and had results available from microarray and serotyping-by-sequencing analyses. The final concordance analysis included samples from 846 children 3 to 6 years old (PCV13 vaccinated), 422 children 5 to 10 years old (age-ineligible for PCV13 vaccination), and 79 adults (HIV infected and PCV13 unvaccinated) (Figure 4.1). Sample selection for microarray and serotyping by sequencing was done independently and by individuals blinded to latex serotype data.

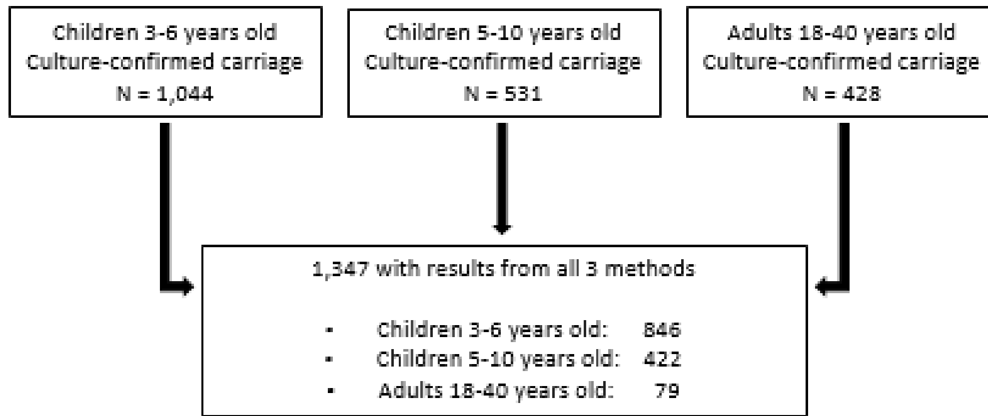


Figure 4.1. Sample selection for analysis. Samples were collected during four rolling cross-sectional surveys from June 2015 through April 2017. Out of all nasopharyngeal swab samples collected, 1347 had results available from the three assays under review. Selection for the microarray and PneumoCaT was done independently of available serotyping data.

4.2.3 Laboratory

4.2.3.1 Nasopharyngeal swab collection

Collection of NPS samples is described elsewhere.[14] In brief, an NPS sample was collected from each participant using a nylon flocked swab (FLOQSwabs, Copan Diagnostics, Murrieta, CA, USA) and then immediately placed into 1.5 mL of STGG (skim milk, tryptone, glucose, and glycerol) medium and processed at the Malawi-Liverpool-Wellcome Trust (MLW) laboratory in Blantyre, according to World Health Organization recommendations.[15] Samples were frozen on the same day at −80 °C (Figure 4.2).

4.2.3.2 Nasopharyngeal swab culture for pneumococcal screening and serotyping

NPS-STGG (30 µL) was plated on a sterile sheep blood + gentamicin (SBG; 7% SBA, 5µl gentamicin/mL) agar plate (primary plate) and incubated overnight at 37 °C in ~5% CO_2. Plates showing no *S. pneumoniae* growth were incubated overnight a second time before being reported as negative. *S. pneumoniae* was identified by colony morphology and optochin disc (Oxoid, Basingstoke, UK) susceptibility. The bile solubility test was used on isolates with no or intermediate optochin susceptibility (zone diameter <14 mm). A single colony of confirmed pneumococcus was selected and grown on a sterile SBG plate (secondary plate), following the same process as the primary plate (Figure 4.2).

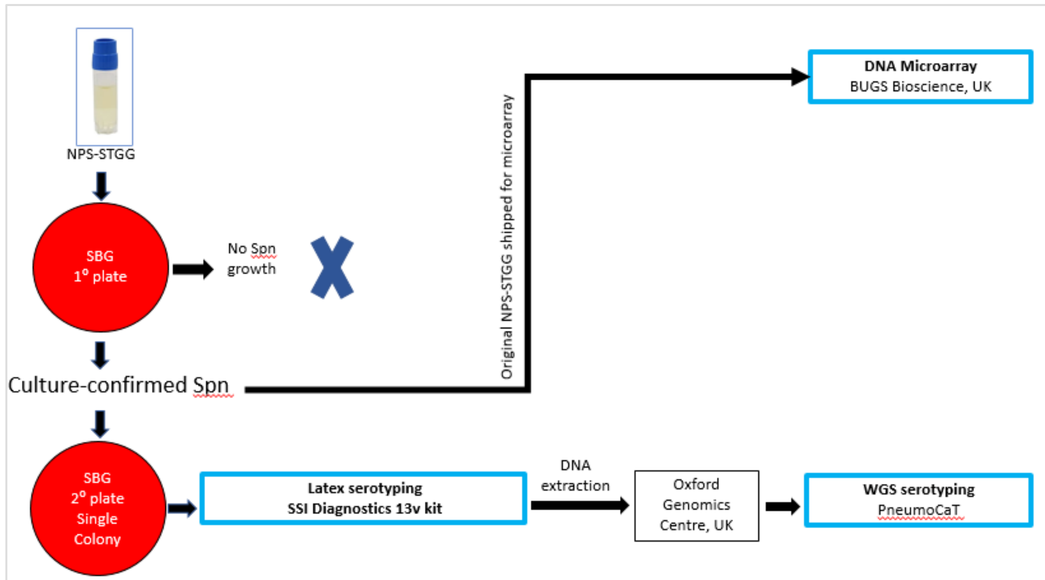


Figure 4.2. Laboratory procedures. NPS samples were inoculated into STGG medium and subsequently plated on an SBG agar growth medium. Bacterial growth (from single-colony picks) from samples culture confirmed for *Streptococcus pneumoniae* was used for latex serotyping. The remaining pure-growth isolates, retained at −80 °C in sterile STGG, were later grown for DNA extraction and WGS. Aliquots of original samples (NPS-STGG) that were culture confirmed for *Streptococcus pneumoniae* were assessed by microarray. NPS, nasopharyngeal swab; STGG, skim milk, tryptone, glucose, and glycerol; WGS, whole-genome sequencing; Spn, *Streptococcus pneumoniae*; SBG, sheep blood + gentamicin; SSI, Statens Serum Institut; 13v, 13-valent; NPS-STGG, NPS inoculated into STGG

4.2.3.3 Latex serotyping

Declaration: Latex serotyping, including results interpretation, was completed by study lab technicians: Nelson Simwela, Arnold Botomani, Comfort Brown, Jacquline Msefula, Chikondi Jassi, and Mphatso Mayuni. They were trained in relevant laboratory techniques and routinely supervised by Todd Swarthout.

Pneumococcal growth from secondary plates was used for serotyping by latex agglutination (ImmuLex 7-10-13-valent Pneumotest; Statens Serum Institut, Copenhagen, Denmark), following manufacturer guidelines. Using a reaction card and a sterile inoculation loop, a small sweep of an overnight bacterial culture was mixed with saline and a series of individual Pneumotest-Latex reagents in suspension. The card was rocked manually and observed for agglutination. A Pneumotest-Latex chessboard was used to determine the serotype associated with the observed set of agglutination reactions. The kit allows for differential identification of each PCV13 VT (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F). Other than for a limited number of serogroups (6, 7, 9, 18, 19, 23), for which the kit provides serogroup differentiation, there is no further differential

identification of NVT serogroups or serotypes. NVT and non-typeable isolates were reported as NVT. Samples were batch tested on a weekly basis, with technicians blinded to the sample source. After serotyping was complete, the remaining growth from each secondary plate was archived at −80 °C in sterile STGG. Refer to subsection 4.7.1.1 in the supplemental material for a more detailed description of latex serotyping.

4.2.3.4 Molecular serotyping by microarray

Declaration: Microarray work, using original NP swab samples in STGG, was completed by Jason Hinds and Katherine Gould (BUGS Bioscience, London Bioscience Innovation Centre, London, UK). Jason Hinds and Katherine Gould contributed to the interpretation of microarray results.

For samples with culture-confirmed pneumococcal carriage, the original inoculated STGG was thawed and vortexed. Aliquots of 100 µL were shipped in 1.8 mL cryovials to BUGS Bioscience (BUGS Bioscience Ltd, London, UK) on dry ice (Figure 4.2). The remaining steps for microarray serotyping (including sample processing, culturing, DNA extraction, microarray, and analysis) were completed entirely by BUGS Bioscience.[16,17] Final microarray results were retrieved by the study team from BUGS Bioscience's web-based SentiNET platform and imported into Stata 13.1 (StataCorp LLC, College Station, TX, USA) for analysis. Refer to subsection 4.7.1.2 in the supplemental material for a more detailed description of microarray serotyping.

4.2.3.5 DNA extraction and whole-genome sequencing

Declaration: The DNA extraction methods were developed by Todd Swarthout, with input from Maaike Alaerts (lead, MLW molecular testing services). DNA extraction (including sample preparation and quality control) was completed by study lab technicians: Nelson Simwela, Arnold Botomani, Comfort Brown, Jacquline Msefula, Chikondi Jassi, and Mphatso Mayuni. They were trained in relevant laboratory techniques and routinely supervised by Todd Swarthout.

Archived secondary growth isolates were used to develop sequence libraries for serotyping by sequencing. To optimize the total retrieved DNA, 30 µL of thawed isolate-STGG was incubated overnight in 6 mL Todd Hewitt broth + yeast enrichment culture. DNA was extracted from the overnight culture using the QIAampTM DNA Mini Kit (Qiagen, Hilden, Germany), following manufacturer guidelines for bacterial DNA. Quality control measures,

as required by the guidelines of the sequencing institution, included DNA quantification (QubitTM; Thermo Fisher Scientific, Waltham, MA, USA) of all DNA samples and gel electrophoresis imaging on 0.7% agarose to assess DNA integrity. After attaining quantity and quality requirements, 100 µL of extracted DNA were aliquoted into skirted 96-well microwell plates and stored at −80 °C until shipped on dry ice to the Oxford Genomics Centre (University of Oxford, UK) for sequencing. Whole-genome sequencing (WGS) was performed at the Oxford Genomics Centre on a HiSeq4000 platform (Illumina Inc., San Diego, CA, USA), with paired-end libraries and a read length of 150 bp.

4.2.3.6 Serotyping by sequencing

Declaration: All work associated with securing whole-genome sequence libraries (including liaising with the Oxford Genomics Centre) was completed by Andrea Gori. Subsequent work with the PneumoCaT (Pneumococcal Capsular Typing; https://github.com/phe-bioinformatics/PneumoCaT; Public Health England, London, UK) software, including cleaning raw sequencing reads and interpretation of serotype results, was led by Andrea Gori.

WGS data were retrieved by the study team from a web-based FTP link. Serotype was inferred from the isolates' genome sequences using the PneumoCaT software pipeline, an open-source bioinformatic tool.[18] PneumoCaT requires raw sequencing reads for each isolate, which were trimmed and cleaned. Reads were trimmed of the Illumina adapters and cleaned of low-quality ends using Trimmomatic version 0.38 (available at http://www.usadellab.org/cms/?page=trimmomatic). Minimum read length after trimming was 80 bp, with the minimum average quality for a sliding window of 4 nucleotides being 15. A subset of 700 000 reads per end (1.4 million total) was used for any subsequent analysis. XML result files were parsed with ad hoc bash scripts to extract and tabulate the serotyping result for each isolate. PneumoCaT was installed and used on a Linux machine at the Medical Research Council's Cloud Infrastructure for Microbial Bioinformatics (CLIMB; https://www.climb.ac.uk/). Each serotype identification required an average of 5 to 8 minutes. Refer to subsection 4.7.1.3 in the supplemental material for a more detailed description of serotyping by sequencing.

4.2.4 Definitions

Concordance was calculated with all samples aggregated and according to the level of discrimination provided by the method. Concordance is reported using two criteria: (1) a criterion based on whether both assays reported NVT or both reported VT (VT/NVT

criterion) and (2) a criterion based on whether the final serotype reported by each assay was equivalent (serotype-specific criterion).

4.2.4.1 Concordance between latex and serotyping by sequencing (PneumoCaT)

Other than a limited number of serogroups (6, 7, 9, 18, 19, 23) for which the latex kit provides serogroup differentiation, there was no opportunity for further differential identification of NVT serogroups to serotype. NVT and non-typeable isolates were reported as NVT. Concordance at the serotype level (serotype-specific criterion) was reported only if latex reported VT carriage. If latex reported NVT, any NVT reported by PneumoCaT was considered concordant. For example: 23F reported by both latex and PneumoCaT was considered concordant, as was NVT and 15B. However, 19F and 19A was considered discordant, as was NVT and 6B.

4.2.4.2 Concordance between latex and microarray

Concordance at the serotype level (serotype-specific criterion) was reported only if latex reported VT carriage. If latex reported NVT, any NVT reported by microarray was considered concordant. Because microarray reports multiple-serotype carriage, 23F reported by latex and 23F+34 reported by microarray was considered concordant, as was NVT and 18C+33D. However, 19F and 33D+19A was considered discordant, as was NVT and 3+7F.

Note that for microarray, some closely related serotypes are reported as a group, with the final individual serotype call in brackets (e.g., 6A/B [6B]). In this case, results were analysed using the individual serotype call. For example, if microarray reported 6A/B [6B], this was considered discordant with a 6A latex result and concordant with a 6B latex result. For simplicity of analysis, if a method did not claim to detect a serotype, but the sample contained that serotype, this result was deemed discordant. For example, if microarray detected both serotypes 23F and 19A but latex detected only serotype 3, this result was considered discordant.

4.2.4.3 Concordance between microarray and serotyping by sequencing (PneumoCaT)

Microarray and PneumoCaT both differentiate VT and NVT to the serotype level, allowing concordance to be calculated on serotype concordance (serotype-specific criterion) for both VT and NVT *S. pneumoniae*.

4.2.5 Statistical analysis

The formula for percent increase in VT prevalence was: ([VT prevalence using latex − VT prevalence using microarray]/VT prevalence using latex) × 100%. Confidence intervals are binomial exact. Statistical significance was inferred from two-sided p<0.05. Participant data collection was completed using Open Data Kit (ODK Collect v1.24.0; Get ODK Inc., CA, USA) open-source software. Statistical analyses were completed using Stata 13.1.

4.3 Results

4.3.1 Concordance in terms of correctly identifying pneumococcal carriage

Pneumococcal carriage prevalence results from the larger surveillance project are reported elsewhere.[14] Comparing latex with PneumoCaT, the adjusted concordance in terms of the techniques correctly identifying pneumococcal carriage as VT or NVT was 90.7% (1216/1341; 95% CI, 89.0%-92.2%) (Figure 4.3). Based on the serotype-specific criterion, concordance between latex and PneumoCaT was 87.5% (1174/1341; 95% CI, 85.7%-89.3%). Comparing latex with microarray, the concordance in terms of the techniques correctly identifying pneumococcal carriage as VT or NVT was 97.3% (1311/1347; 95% CI, 96.3%-98.1%). Based on the serotype-specific criterion, the concordance was 95.2% (1282/1347; 95% CI, 93.9%-96.3%). Comparing microarray with PneumoCaT, the concordance in terms of the techniques correctly identifying pneumococcal carriage as VT or NVT was 96.6% (1295/1341; 95% CI, 95.5%-97.5%). Based on the serotype-specific criterion, the concordance was 82.8% (1110/1341; 95% CI, 80.6%-84.8%).

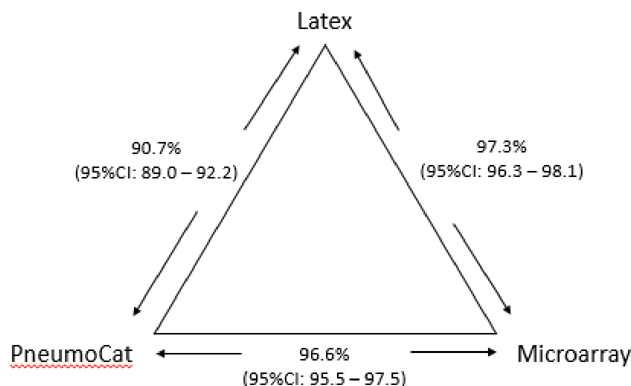


Figure 4.3. Concordance between assays. Concordance between two assays was defined as both assays identifying pneumococcal carriage as VT or as NVT. Latex agglutination and PneumoCaT reported one result per sample, both using the same pure-growth culture. The microarray, using an aliquot of the original NPS-STGG, differentiated individual serotypes in multiple-serotype carriage, when present. In comparing the three assays, concordance was based on serotype if latex serotyping reported VT carriage. If latex serotyping reported NVT carriage, this was considered concordant with any NVT reported by PneumoCaT or the microarray, as long as PneumoCaT and microarray reported the same NVT. NPS, naspoharyngeal swab; NVT, non–vaccine serotype; STGG, skim milk, tryptone, glucose, and glycerol; VT, vacine serotype

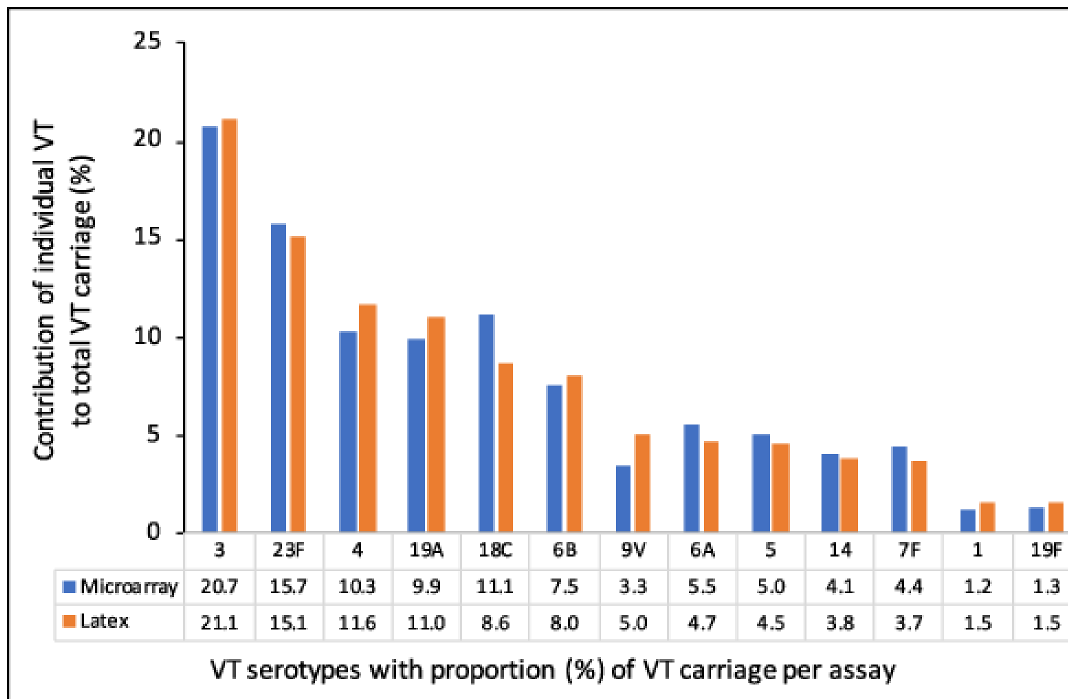
4.3.2 Increased vaccine serotype detection using microarray

Using a larger study database of 1949 samples from the same study, we evaluated latex and microarray data. When we aggregated all ages (i.e., child and adult), there was an increase of 31.5% in VT prevalence by microarray compared with latex serotyping: a 43.0% increase in VT carriage among children 3 to 6 years old, a 21.7% increase among children 5 to 10 years old, and a 10.8% increase among HIV-infected adults on ART (Table 4.1). This was due to samples being reported as NVT by latex but also carrying VT, as detected by microarray. These VTs, undetected by latex, were carried in lower relative abundance (median, 8%; range, 2%-48%). The prevalence of multiple-serotype carriage (range, 2-6 serotypes) was 35.2% (686/1949). The prevalences among the respective age groups were 44.4% (457/1029) among children 3 to 6 years old, 32.8% (169/515) among children 5 to 10 years old, and 14.8% (60/405) among HIV-infected adults. Among samples with multiple-serotype carriage, latex identified the dominant serotype in 85.3% of samples (585/686; 95% CI, 82.4%-87.8%). Despite the overall increase in detection of VT carriage, there was no significant difference between the proportions of individual VTs detected by microarray vs latex (Figure 4.4). Refer to Table S4.1 in the supplemental material for the reported frequency of each VT detected by microarray and latex.

Table 4.1. Increased detection of VT carriage, latex vs microarray

	Latex VT prevalence (n) 95% CI	Microarray VT prevalence (n) 95% CI	% Increase in VT prevalence
Children 3-6 years, PCV vaccinated (n=1360)	20.0% (272) 17.9, 22.2	28.6% (389) 26.2, 31.1	43.0%
Children 5-10 years, PCV unvaccinated (n=904)	21.1% (191) 18.5, 23.9	26.5% (240) 23.7, 29.6	21.7%
Adults, 18-40 years, HIV-infected, PCV unvaccinated (n=963)	14.2% (137) 12.1, 16.6	16.6% (160) 14.3, 19.1	10.8%
Total (n=3227)	18.6 (600) 17.3, 20.0	24.4 (789) 23.0, 26.0	31.5%

VT, vaccine serotype; CI, confidence interval; PCV, pneumococcal conjugate vaccine



	3	23F	4	19A	18C	6B	9V	6A	5	14	7F	1	19F
Microarray	20.7	15.7	10.3	9.9	11.1	7.5	3.3	5.5	5.0	4.1	4.4	1.2	1.3
Latex	21.1	15.1	11.6	11.0	8.6	8.0	5.0	4.7	4.5	3.8	3.7	1.5	1.5

Figure 4.4. Proportions of individual vaccine serotypes contributing to total vaccine-serotype carriage. The proportions of individual VTs detected by microarray and by latex agglutination were not significantly different from one another. Refer to Table S4.1 in the supplemental material for the reported frequency of each VT detected by microarray and latex agglutination. VT, vaccine serotype

4.3.3 Key variables of selected serotyping methods

Table 4.2 presents key variables to further consider when deciding which assay is appropriate for a particular setting. Estimated costs and feasibility of implementation and maintenance are specific to the setting in Malawi at the MLW Clinical Research Programme in Blantyre. Extrapolation would need further validation outside the scope of this evaluation. Though more limited in that it reports only single serotypes, latex agglutination is highly accurate while being less costly and requiring less expertise and fewer resources for field implementation and analysis. While microarray is the costliest option, it provides greater accuracy of total pneumococcal carriage, including information about multiple-serotype carriage and the relative abundance of the individual serotypes detected. WGS is a strong alternative to latex and would be nearly cost free if the sequence libraries were already available. Additionally, WGS provides opportunities for further analyses, including to determine population structure and antibiotic resistance profiles.

Table 4.2. Key comparative variables of serotyping methods

Variable	Latex agglutination (phenotypic)	Microarray (genomic)	PneumoCaT (genomic)
Assay implementation			
Sample used in assay	• Pure growth from single isolate	• Original sample in STGG	• Pure growth from single isolate
Cost estimate[1]	• Lowest of three assays	• Highest of three assays	• Middle of three assays
Implementation of assay[1]	• Least difficult (relatively simple)	• Most difficult	• Moderate difficult
Training required for implementation	• Minimal	• Advanced	• DNA extraction: moderate • WGS library manipulation: advanced • PneumoCaT tool: moderate
Training required for processing and interpretation of results	• Minimal	• Moderate	• Moderate
Assay output and interpretation			
Serotypes reported	• Single	• Multiple, if present	• Single
NVT differentiation	• No[2]	• Yes	• Yes
Relative abundance of individual serotypes reported	• No	• Yes	• No
Additional outputs	• Isolates archived and available for further analyses	• AMR profile[3] • NT differentiation	• WGS library accessible for further analyses, including population structure and AMR
Conclusions	• Adequate for surveillance • Limited resolution for optimal VE estimation	• Cost and technical complexity limit ability to decentralize implementation • Detection of VT in low relative abundance is of critical importance • Sentinel sites should be considered for regional NVT and VT resolution for optimal VE estimation	• Limited resolution for optimal VE estimation • No benefit over latex unless WGS library already available

[1]The estimated costs and feasibility of implementation and maintenance are specific to the study requirements and laboratory capacity (including no capacity for WGS or microarray) at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme in Blantyre, Malawi. [2]NVT and NT isolates are reported as NVT. The use of both commercial products and latex serotyping reagents produced in house can significantly increase the number of NVT serotypes that can be differentiated by latex agglutination. [3]An AMR profile cannot be assigned to a single strain in a sample with multiple-serotype or multiple-pathogen carriage. AMR, antimicrobial resistance; NT, non-typeable; NVT, non–vaccine serotype; STGG, skim milk, tryptone, glucose, and glycerol; VE, vaccine effectiveness; VT, vaccine serotype; WGS, whole-genome sequencing

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4.4 Discussion

We report high concordance between three serotyping techniques applicable to routine pneumococcal surveillance. Importantly, we have extended the analysis to include relevant variables beyond accuracy including cost, time to result, and measures of input required for assay implementation and maintenance. These are variables that researchers and policymakers should consider when deciding which assay to implement. All three assays appear accurate and concordant in identifying the dominant serotype.

While latex agglutination is rapid, accurate, and requires the least expertise and fewest resources for field implementation and analysis, standard latex approaches are not optimal for optimal surveillance of vaccine impact, including the detection of multiple-serotype carriage and VTs in low relative abundance.[19] There have been attempts to implement latex for detecting multiple-serotype carriage. Gratten et al[20] serotyped up to six colonies from nasal secretion culture plates and found multiple-serotype carriage in 29.5% in a sample of Papua New Guinean children. The authors went on to serotype at least 50 colonies from 10 selected nasal secretion cultures and concluded that the minor serotype accounted for 4% to 27% of the total pneumococcal population. A review of published data on multiple carriage concluded that, to detect a minor serotype it would be necessary to serotype at least five colonies to have a 95% chance of detecting the serotype if it accounted for 50% of the total pneumococcal population, and one would need to examine 299 colonies if the serotype was present at a relative abundance of 1%. As part of the PneuCarriage project, for thorough sample characterization, up to 120 colonies from each sample were selected to achieve >99% power to detect a minor serotype of 5% abundance.[13] This approach would not be cost- or time-effective. Though dependent on technical capacity to develop in-house reagents, researchers in The Gambia developed a technique in which colonies from the primary culture plate are suspended in saline and serotyped by latex agglutination.[21] In their longitudinal infant cohort study, while they did not differentiate NVT serotypes, they showed that up to 10.4% of pneumococcal acquisitions were of multiple serotypes. While latex is limited in its output, the process can be leveraged for additional end points, including measuring carriage density through counting of colony-forming units on agar culture plates. Moreover, though less cost- and time-effective, the use of both commercial products and production of in-house latex serotyping reagents has been well documented to significantly expand the number of NVT serotypes that can be differentiated by latex, including quality control procedures.[11,22]

With open-source bioinformatic tools, such as PneumoCaT, serotyping by sequencing can be less costly than microarray, even accounting for the costs of DNA extraction and WGS,

while still facilitating differentiation between non-typeable pneumococci and nearly every known VT and NVT. Though we would not recommend initiating DNA extraction and WGS for the use of PneumoCaT alone, sequence libraries can be further leveraged for extensive informative bioinformatic analyses, useful in population biology, antimicrobial resistance investigations, and vaccine monitoring. Moreover, using PneumoCaT for serotyping would be essentially cost free if the sequence libraries were already available, apart from the limited bioinformatic skills needed. While microarray is more costly, it differentiates NVT and multiple-serotype carriage with relative abundance, as well as non-*S. pneumoniae* contaminants (i.e., *Streptococcus mitis*, *Streptococcus salivarius*, *Staphylococcus aureus*) with a degree of precision. This technique stands out for its sensitivity, being able to detect serotypes in low relative abundance, which is of critical importance for understanding the transmission patterns of *S. pneumoniae*. Having the extra counts for each serotype from the same number of samples, as provided by microarray, also has the advantage of adding power to a study's statistics.

There are several limitations to mention, including several serotyping methods that were not evaluated, including polymerase chain reaction (PCR) and the SeroBA (Wellcome Sanger Institute, Hinxton UK) pipeline. SeroBA is a relatively new serotyping-by-sequencing software application. With similar accuracy to PneumoCaT, SeroBA does have operational advantages.[23] SeroBA can correctly call a serotype with a read coverage as low as 10× (20× is required for PneumoCaT). Using a k-mer–based approach, rather than the raw sequence alignment, SeroBA requires much lower computational power and time. On the other hand, the PneumoCaT source code can be easily adapted to the operator's needs, and both software progams are likely to run on a standard server configuration. Alternative culture-independent methods, such as isolation-independent conventional multiplex PCR serotyping (cmPCR) could be important for confirming carriage when re-culturing of original NP swab samples is not feasible. Though cmPCR has been successfully applied on DNA extracted directly from NPS-STGG, evidence suggests that cmPCR serotyping after culture enrichment returns a higher sensitivity and an ability to identify multiple-serotype carriage.[9] Nonetheless, cmPCR can be confounded by non-pneumococcal streptococci (including *Streptococcus mitis*, *Streptococcus oralis* and *Streptococcus parasanguinis*).[24] Due to a high sequence similarity with target serotype-specific amplicons, cmPCR can overestimate pneumococcal carriage. Carvalho et al,[24] for example, reported that 82.5% of samples (combined nasopharyngeal and oropharyngeal swabs) that were positive for pneumococcus by cmPCR were culture negative by latex. Similarly, 35.0% of those positive for pneumococcus by cmPCR were negative by lytA-pnemococcal–specific PCR. This is particularly problematic when bacterial DNA is

extracted from culture-enriched NP and oropharyngeal samples, requiring PCR results to be confirmed by latex agglutination or other serotyping procedures. Additional PCR limitations include the need for region-specific reaction protocols, implementing a high number of primer pairs to identify the same range of serotypes identified by microarray or WGS, and the increased risk of detecting non-viable pneumococci. As there is no evidence of a viable but non-culturable state in *S. pneumoniae*,[25] identifying non-viable pneumococci could be disadvantageous for field-based research. While a formal economic analysis of the methods would be justified, we were unable to extrapolate the individual costing components between sites. Such components would include local salaries and additional labour costs, procurement and shipping of equipment and consumables, equipment maintenance, local health and safety requirements, and institutional costs. For this reason, comparative costing is grossly categorized. Though we did not include invasive isolates (from blood or cerebrospinal fluid, for example), it is important to identify serotypes associated with IPD, including in post-PCV impact studies. For invasive isolates, with a single-serotype sample expected, microarray would have limited advantage. Application of serotyping by sequencing would then be the most informative option, including insight into population structure, antimicrobial resistance patterns, and disease associated with serotype replacement.

4.5 Conclusions

Selection of the appropriate assay should be based on the intended analysis and end points. While accuracy and concordance are high between the three assays, implementation and cost considerations vary significantly. In a setting of limited resources, as is true throughout much of sub-Saharan Africa, latex is the best overall option for decentralized surveillance of vaccine impact. However, WGS (which adds population structure) and microarray (which adds multiple-serotype carriage) should be considered at regional reference laboratories while investigating the impact of VT (in low relative abundance) on transmission and disease.

4.6 Data availability

The data supporting the findings of this study has been deposited in the Figshare repository.[26]

4.7 References

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4.8 Supplementary material

4.8.1 Description of each assay

4.8.1.1 Serotyping by latex agglutination

This is performed on pure growth cultures of pneumococci. It is an agglutination method using anti-rabbit IgG-coated latex particles sensitized to pooled and select individual pneumococcal serotype-specific antisera (PCV13 serotypes 1, 3, 4, 5 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) for serogrouping and serotyping *S. pneumoniae*. Visible agglutination occurs when the *S. pneumoniae* capsular antigen reacts with the antibody-coated latex beads. Commercially available kits, including the Pneumotest-Latex kit (Statens Serum Institut) are intended for partial typing of pure cultures of pneumococci, differentiating VTs, with both non-typeable pneumococci and NVTs reported as NVT.

4.8.1.2 Molecular serotyping by microarray

This assay is performed on DNA extracts of original NPS samples using the SP-CPS v1.4.0 microarray (Bacterial Microarray Group at St. George's [*BμG@S*], London, UK), designed for *S. pneumoniae*. Serotyping by microarray utilizes the DNA extracted from a primary culture plate, where an aliquot of the original NPS storage medium is grown. The main strength of this technique is to differentiate and report nearly all known serotypes, including the relative abundance of each in the case of multiple-serotype carriage. Other outputs, though less robust, include presence of other bacterial species and genetic characteristics, including antimicrobial resistance (AMR) and genome group based on the presence or absence of core genes in the original sample. Characteristics such as AMR are less robust, for example, because the AMR profile cannot be assigned to a single strain in a sample with multiple-serotype or multiple-pathogen carriage. Final microarray results are accessible from BUGS Bioscience's password-protected SentiNet website as a Microsoft Excel spreadsheet or a tab-separated text file. As with PneumoCaT, described below, microarray outputs are generally accessed with limited guidelines for use or interpretation. Though less formidable than PneumoCaT, microarray interpretation required some further reading and discussion with the BUGS Bioscience's team. Examples of terminology include '-like' (e.g., 23F-like) and including an '*' (asterisk) with some reported serotypes. Some closely related serotypes are reported as a group, with the individual serotype call in brackets (e.g., 6A/B [6B]). BUGS Bioscience produces a very useful web-based dashboard of pre-programmed analyses.

Note: For this analysis, identified serotypes marked with an '*' (asterisk) were removed.

4.8.1.3 Serotyping by sequencing using PneumoCaT

The rapid reduction in the cost of WGS has led to its extensive use in the monitoring of pneumococcal serotypes. Developed in 2015 by Public Health England (PHE), PneumoCaT (Pneumococcal Capsule Typing) offers a fully functional automated pipeline for serotyping *S. pneumoniae* WGS data.[1] As an opensource bioinformatic tool, PneumoCaT was developed to meet the needs of the initial owner, in this case PHE. The main drive for PHE's developer was the serotyping of invasive pneumococcal isolates, wherein multiple-serotype carriage is rare and a limited variability of serotypes is recorded. As such, in studies investigating IPD, PneumoCaT was initially reported to identify over 90% of pneumococcal serotypes, but the database is regularly corrected and updated.

Although the PneumoCaT tool includes all the steps to determine serotype from raw (unassembled) DNA sequences, the user is required to perform a number of steps to obtain these sequences, including DNA extraction from bacterial isolates and DNA sequencing (in house or externally). Bioinformatic and information technology competencies are needed if working with raw WGS and the user is not familiar with the command line environment. The user will need to review the raw WGS data files for contamination and cleaning of low-quality DNA segments in the sequence libraries. If the user has WGS libraries available (e.g., for other study objectives), then there are no additional material costs. If DNA extraction and sequencing is required, costings increase accordingly. As a tool designed by scientists for scientists, PneumoCaT's interface can be challenging to those not familiar with the format for inputting sequence libraries and interpreting output. As with microarray, PneumoCaT is generally accessed by researchers with relevant training with limited guidelines for use or interpretation of outputs. This requires time for reviewing the relevant websites and publications or a willingness to contact the tool owner directly.

Table S4.1. Frequency of vaccine serotype detected by microarray and latex, as reported in Figure 4.4

Serotype	Microarray (%)	n	Latex (%)	n	% increase
3	20.7	174	21.1	127	37.0%
23F	15.7	86	15.1	70	22.9%
4	10.3	34	11.6	23	47.8%
19A	9.9	63	11.0	48	31.3%
18C	11.1	37	8.6	22	68.2%
6B	7.5	42	8.0	27	55.6%
9V	3.3	46	5.0	28	64.3%
6A	5.5	83	4.7	66	25.8%
5	5.0	10	4.5	9	11.1%
14	4.1	93	3.8	52	78.8%
7F	4.4	11	3.7	9	22.2%
1	1.2	28	1.5	30	−6.7%
19F	1.3	132	1.5	91	45.1%
Total		**839**		**602**	

[1]Percent increase: ([frequency of serotype by microarray − frequency of serotype by latex]/frequency of serotype by latex) × 100%

Having the extra counts for each serotype from the same number of samples, as provided by microarray, has the advantage of adding power to a study's statistics.

4.8.2 References – supplementary material

1. Kapatai G, Sheppard CL, Al-Shahib A, Litt DJ, Underwood AP, Harrison TG, Fry NK. Whole genome sequencing of *Streptococcus pneumoniae*: development, evaluation and verification of targets for serogroup and serotype prediction using an automated pipeline. PeerJ. 2016 Sep 14;4:e2477. doi: 10.7717/peerj.2477. PMID: 27672516; PMCID: PMC5028725.

Chapter 5. Impact and effectiveness of 13-valent pneumococcal conjugate vaccine on population incidence of vaccine and non-vaccine serotype invasive pneumococcal disease in Blantyre, Malawi, 2006-2018: prospective observational time-series and case-control studies

N.B., Associated published peer-reviewed article: *Bar-Zeev N, ***Swarthout TD**, Everett DB, Alaerts M, Msefula J, Brown C, Bilima S, Mallewa J, King C, von Gottberg A, Verani JR, Whitney CG, Mwansambo C, Gordon SB, Cunliffe NA, French N, Heyderman RS; VacSurv Consortium. Impact and effectiveness of 13-valent pneumococcal conjugate vaccine on population incidence of vaccine and non-vaccine serotype invasive pneumococcal disease in Blantyre, Malawi, 2006-18: prospective observational time-series and case-control studies. Lancet Glob Health. 2021 Jul;9(7):e989-e998. doi: 10.1016/S2214-109X(21)00165-0. PMID: 34143997; PMCID: PMC8220129.

*Joint first author

Chapter introduction: Chapters 2 and 3 report high residual pneumococcal carriage post–PCV introduction based on highly accurate laboratory techniques. Recognizing that carriage is a prerequisite for disease, chapter 5 further evaluates the impact of Malawi's 2011 introduction of PCV13 on disease, this time examining its impact on vaccine-serotype and non–vaccine-serotype invasive pneumococcal disease among vaccine age-eligible and age-ineligible children and adults. We conducted prospective surveillance for invasive pneumococcal disease from which we derived incidence rates as described below to infer vaccine impact (population reduction in disease incidence). We also conducted a prospective case-control study to estimate vaccine effectiveness among age-eligible infants (those aged ≥6 weeks on the date of PCV introduction, 12 November 2011) as described in detail below.

Abstract

Background: The population-level impact of pneumococcal conjugate vaccines (PCVs) depends on direct and indirect protection. Following Malawi's 2011 introduction of the 13-valent PCV (PCV13), we examined impact on vaccine-serotype (VT) and non-VT (NVT) invasive pneumococcal disease (IPD) among vaccine age-eligible and age-ineligible children and adults.

Methods: Laboratory-based surveillance at a government hospital in Malawi included the 6 years before and 7 years after PCV13 introduction. Using negative binomial regression,

we evaluated secular trend-adjusted incidence rate ratios (IRRs) in VT and NVT IPD before and after the introduction of PCV13. We compared the predicted counterfactual incidence (in the hypothetical absence of the vaccine) with the empirically observed incidence following vaccine introduction. A case-control study assessed vaccine effectiveness, comparing PCV uptake among vaccine age-eligible IPD cases vs matched community controls.

Results: Surveillance covered 10 281 476 person-years of observation, with 140 498 blood cultures and 63 291 cerebrospinal fluid cultures. A decline in total (VT+NVT) IPD incidence preceded PCV introduction: 19% (IRR, 0.81; 95% CI, 0.74-0.88; $p<0.0001$) among infants, 14% (IRR, 0.86; 95% CI, 0.80-0.93; $p<0.0001$) among young children, and 8% (IRR, 0.92; 95% CI, 0.83-1.01; $p=0.08$) among adolescents and adults. Among older children, there was a 2% increase in total IPD (IRR, 1.02; 95% CI, 0.93-1.11; $p=0.72$). Compared with the counterfactually predicted incidence, the vaccine-era VT IPD incidence was 38% (95% CI, 37%-40%) lower among infants, 74% (95% CI, 70%-78%) lower among young children, 79% (95% CI, 76%-83%) lower among older children, and 47% (95% CI, 44%-51%) lower among adolescents and adults. Though NVT IPD incidence had increased since 2015, the observed incidence remained low. The case-control study, (19 cases and 76 controls), showed a vaccine effectiveness against VT IPD of 80.7% (95% CI, −73.7% to 97.9%).

Conclusion: In a high-mortality, high-HIV-prevalence setting in Africa, there were significant pre–vaccine introduction (2006-2011) declines in IPD incidence. Seven years after PCV introduction, while the vaccine-attributable impact among vaccine age-eligible children was significant, indirect effects benefitting unvaccinated infants and adults were more modest. Policy decisions should consider multiple alternative strategies for reducing disease burden, including targeted vaccination outside the infant Expanded Programme on Immunization to benefit vulnerable populations.

5.1 Introduction

Streptococcus pneumoniae is estimated to be responsible for over 318 000 (uncertainty ratio [UR]: 207 000-395 000) deaths every year among children aged 1 to 59 months worldwide, with the highest mortality burden in Africa.[1] *S. pneumoniae* has almost 100 serotypes and is a common colonizer of the human nasopharynx, particularly in young children, as well as in resource-poor and HIV-affected populations.[2] The contribution to adult mortality is poorly quantified but is thought to be large, particularly in the elderly and high-risk groups, such as people living with HIV. Although most carriers are asymptomatic, pneumococcal colonization is a prerequisite for transmission and may also lead to the development of disease, including pneumonia, meningitis, and septicaemia.

Despite evidence of residual vaccine-serotype (VT) carriage across sub-Saharan Africa, including Malawi,[3,4] vaccine trials and post–routine-introduction studies have demonstrated substantial direct protection by pneumococcal conjugate vaccines (PCVs) against invasive pneumococcal disease (IPD) among age-eligible children.[5,6] Importantly, in many settings, PCVs have also been associated with herd protection, with resulting declines in IPD among vaccine-ineligible groups through indirect means, presumably through their impact on reduced nasopharyngeal carriage and subsequent reduced transmission to unvaccinated individuals.[6-8] In high-income countries, groups benefiting from the indirect effects of infant vaccination include the very young,[9] older adults,[10] and HIV-infected adults.[11] However, there is good evidence of replacement disease, with an absolute increase in non-VT (NVT) IPD reported in several countries that has eroded the impact seen initially after PCV introduction.[5,12]

In Malawi, a southern African country consistently ranked by the World Bank in the lowest income category, IPD prevalence by age closely mirrors HIV prevalence. HIV-infected adults remain at high risk of IPD and, therefore, would likely benefit from PCV vaccination.[13,14] In 2015, the mean HIV prevalence in Malawi was 8.8%, reaching 19.8% among urban women.[15] Free antiretroviral therapy (ART) has been provided in Malawi since 2004, initially to patients with clinical AIDS and then to an increasing number of HIV-infected people, now reaching >90% of people living with HIV through the support of the Global Fund. Life expectancy among HIV-infected persons has increased markedly since ART was first introduced in 2004 and became widely available since 2006.[14,16] In 2011, Malawi adopted Option B+, whereby all HIV-infected pregnant or breastfeeding women commence lifelong ART regardless of clinical or immunological stage, dramatically reducing mother-to-child-transmission. On 12 November 2011, Malawi (previously PCV-naïve) introduced 13-valent PCV (PCV13) as part of the national Expanded Programme on Immunization, using a 3+0 schedule (three primary doses at 6, 10, and 14 weeks of age with no booster dose). A catch-up vaccination campaign included infants <1 year of age at the date of first dose, receiving three doses at 1-month intervals. Field studies in Malawi have reported high PCV13 uptake of 90%-95%,[17] similar to the 92% PCV13 coverage recently reported by WHO/UNICEF.[18] Furthermore, we have reported good adherence to the dosing schedule in Malawi, with the median (IQR) ages at first, second, and third doses of PCV being 6.3 (3.2; 4.9-8.1), 11.2 (5.0; 9.1-14.1), and 16.4 (8.1; 11.4-19.5) weeks, respectively.[4]

In Malawi, we have demonstrated the early onset of pneumococcal colonization,[19] a high prevalence of residual VT carriage among children in the PCV era,[4] and a persistently high prevalence of pneumococcal carriage among HIV-infected adults receiving ART.[20,21] We also project that the 10-year VT carriage reduction among children 0 to 9 years old will be lower than what has been observed in other settings, mainly driven by a high local force of infection (FOI; the rate by which a certain age group of susceptible individuals is infected).[22] Here, we assess the direct impact of PCV13 on VT IPD and the magnitude of indirect effects of PCV13 on VT IPD in populations ineligible for routine vaccination. We have leveraged our long-standing and comprehensive sentinel invasive bacterial disease surveillance, conducted in the era of ART, to assess the direct and indirect population-level impacts of PCV13 on VT and NVT IPD incidence across age groups. We also investigated individual vaccine effectiveness (VE).

5.2 Methods

5.2.1 Case ascertainment and laboratory confirmation

Declaration: The work in this chapter was made possible by leveraging an archive of invasive IPD isolates. Archiving of IPD isolates, as part of sentinel surveillance for laboratory-confirmed IPD, was established in 1998 at Queen Elizabeth Central Hospital (QECH), in collaboration with the Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW). Establishment and maintenance of the MLW archive can be attributed to a significant number of persons since 1998, including institutional leads, clinicians (collecting biological samples as part of routine care), and lab teams (sample processing, identification, and archiving of isolates identified as part of routine diagnostic services). For the purposes of this chapter, molecular serotyping of archived isolates was completed (prior to this PhD being initiated) by study lab technicians (Nelson Simwela and Arnold Botomani) under the supervision of Naor Bar-Zeev and Maaike Alaerts. Latex serotyping of archived isolates was completed by study lab technicians (Nelson Simwela, Arnold Botomani, Comfort Brown, Jacquline Msefula, Chikondi Jassi, and Mphatso Mayuni) under the supervision of Todd Swarthout.

MLW has conducted ongoing sentinel surveillance for laboratory-confirmed IPD, including bloodstream infection and meningitis among all age groups at the QECH since 1998, as described previously.[23,24] QECH is the government referral hospital providing free medical care to the 1.3 million urban, peri-urban, and rural residents of Blantyre District, Malawi. In accordance with long-standing clinical guidelines, all adults and children presenting to QECH with fever (axillary temperature >37.5 °C) or clinical

evidence of sepsis or meningitis undergo blood draws for culture and, where appropriate, lumbar puncture. Though generally stable over time, the ratio of blood cultures to population size fell during 2006 before increasing again in 2010 and thereafter reducing again to a ratio similar to 2006.[23] We report data generated after the national introduction of ART, from 1 January 2006 through 31 December 2018.

Specimens were processed at the MLW (co-located with QECH) laboratory, using BacT/Alert 3D (Biomerieux, Marcy l'Etoile France). Those positive by BacT/Alert 3D were Gram stained, and cocci were further assessed using the catalase test. For subsequent serotyping, archived pneumococcal isolates were plated on sheep blood agar + gentamicin (SBG; 7% sheep blood, 5 µL gentamicin/mL) and incubated overnight at 37 °C in 5% CO_2. *S. pneumoniae* growth was confirmed by colony morphology and optochin disc (Oxoid, Basingstoke, UK) susceptibility. The bile solubility test was used on isolates with no or intermediate optochin susceptibility (zone diameter <14 mm). A single colony of confirmed pneumococcus with the predominant morphological phenotype was selected and grown on a new SBG plate as before. Growth from this second plate was used for serotyping by latex agglutination (ImmuLex 7-10-13-valent Pneumotest; Statens Serum Institut, Copenhagen, Denmark). The ImmuLex kit allows for differential identification of each PCV13 VT but not for differential identification of NVTs; NVTs and non-typeable isolates were, therefore, reported as NVT. Latex agglutination was used on all samples collected after 2013. Nucleic acid amplification-based serotyping was performed on samples collected between 2006 and 2013, using the Centers for Disease Control and Prevention's 'triplex sequential real-time PCR-serotyping Africa' protocol.[25] Both assays have been shown to be highly accurate and concordant in pneumococcal serotyping.[26,27] For identified serogroups that contained VTs but for which the RT-PCR assay did not provide serotype differentiation (6A/B/C/D, 9A/V, 18A/B/C/F, 7A/F), latex agglutination was used to determine VT presence. A random selection of serotyped isolates was sent for confirmatory serotyping by quellung reaction at the regional pneumococcal reference laboratory at the National Institute for Communicable Disease (NICD) in Johannesburg, South Africa. Since 2011, serotyping occurs in real time with specimen processing. Isolates collected before 2011 were retrospectively serotyped. Demographic information was collected at the time of sampling from the patient.

5.2.2 Statistical analysis

Demographic characteristics were summarized using means, standard deviations, medians, and interquartile ranges (IQRs) for continuous variables and frequency distributions for categorical variables. Comparison of covariate distributions between study groups was

done by χ^2 analysis (Mantel-Haenszel χ^2 where stratified) unless there were fewer than 5 observations, wherein Fisher's exact test was used. For comparisons among continuous covariates, the t-test of means and the rank-sum test of medians were applied.

5.2.3 Invasive pneumococcal disease incidence

Declaration: While providing scientific input and review, Naor Bar-Zeev completed the statistical modelling component (including negative binomial regression to evaluate secular trend-adjusted incidence rate ratios [IRRs]).

IPD surveillance at QECH (providing care to the 1.3 million residents of Blantyre District, with very few inpatient beds outside QECH) included, therefore, systematic recruitment from the whole population of Blantyre District. IPD events by age category were multiplied by 100 000 and divided by annual age-specific population estimates for Blantyre District published by the Malawi National Statistics Office.[15] Since not all historical isolates were recoverable or serotypeable, we applied each year's proportion of PCV13 VT to impute VT and NVT rates to the unserotyped pneumococcal isolates of each year by age category. The relative rarity of laboratory-confirmed IPD makes incidence rates subject to annual fluctuations even in the presence of moderately large surveillance population denominators. To account for longer-term trends but dampen year-on-year fluctuation, we used a 3-year locally weighted moving average smoothed for incidence $\hat{I}$, calculated as follows: $\hat{I} = \frac{[I_{(t-1)}+2I_{(t)}+I_{(t+1)}]}{4}$; where $I_{(t)}$ indicates observed incidence at year t. We were unable to consistently identify persons who had simultaneous meningitis and bacteraemia (i.e., with both blood and cerebrospinal fluid [CSF] samples collected), thus these episodes are potentially double counted in overall incidence calculations. We, therefore, also performed the same steps separately for pneumococci derived from blood culture and those derived from CSF (Supplementary Figure S5.2).

5.2.4 Vaccine coverage

We evaluated population vaccine coverage in a convenience sample of vaccine age-eligible children admitted with diarrhoeal disease to the same institution as IPD cases. Through this, we showed that community controls in Blantyre and disease-unaligned hospitalized children have comparable vaccine coverage.[28]

5.2.5 Vaccine impact

Declaration: While providing scientific input and review, Naor Bar-Zeev completed the statistical work for predicting counterfactual incidences and IRRs.

To estimate empirically observed IPD incidence before and during the PCV13 era, we fitted negative binomial models, adjusted for time (year of sample collection), to smoothed long-term VT and NVT IPD incidence against year and vaccine introduction. To predict the counterfactual that would be expected in the period 1 January 2014 through 31 December 2018 in the hypothetical absence of PCV13, we developed a model that fitted IPD incidences against year for the pre-vaccine period 1 January 2006 through 31 December 2011. We compared the predicted counterfactual against the same model fitted to the smoothed empirically observed incidence for the ongoing PCV era, 1 January 2014 through 31 December 2018, excluding the 2 years following PCV13 introduction when vaccination coverage among infants had not yet plateaued at high population coverage. This comparison of empirically observed incidence against the counterfactual allowed us to capture not only a step change in incidence rate following vaccine introduction, but also a change in the rate of reduction in the incidence and allowed us to observe whether vaccine introduction further enhanced the rate of reduction of an already (prior to PCV introduction) declining incidence. Confidence bounds about the model-derived counterfactual were constructed from the negative binomial model. However, since we report all observed cases in the population over a period of 13 years rather than a sample thereof, we did not calculate an a priori sample size for predefined power. IRRs were calculated as (IPD incidence estimated by negative binomial model/IPD incidence predicted by the counterfactual model). The 95% confidence intervals for IRRs were constructed manually by predicting the standard error (se) and using the equation: exp(estimate +/- 1.96*se). The percent reduction comparing the counterfactually predicted IPD incidence and the empirically observed VT IPD incidence was calculated as ([1−IRR]×100%).

5.2.6 Case-control study

Declaration: Naor Bar-Zeev entirely led the case-control study (assessing VE) investigating the impact and effectiveness of PCV13 on population incidence of VT and NVT IPD, which is an integral component of this chapter.

To estimate VE from identified IPD patients admitted to QECH between 12 November 2011 and 31 December 2016, we selected as study case patients all children who had VT

IPD, were vaccine eligible (i.e., born 6 weeks before PCV13 introduction or later), and whose parents consented to participate. We then conducted a random walk method in each case patient's community to systematically identify four healthy age-matched controls, as described previously.[29] Among children <1 year old and among those ≥1 year old, the acceptable age differences for matching were date of birth ±30 days and ±90 days, respectively. We recorded the date of vaccination documented on the patient-held record (known as a health passport) among these controls and classified their vaccine statuses as they were on the admission date of the matched case. Using Stata 13.1 (StataCorp LLC, College Station, TX, USA), we fitted an unadjusted conditional logistic regression model comparing vaccine receipt between cases and matched controls. VE was defined as 1 minus the ratio of odds of receiving three doses of PCV13 among cases compared with matched controls. We calculated that we would need 16 cases of VT IPD to achieve 80% power at 5% significance to detect a VE of 80% against VT IPD with four matched controls per case, a vaccine uptake among controls of 60%, and a vaccine coverage correlation of 0.5 among cases and matched controls.

5.3 Results

5.3.1 Impact evaluation

Between 1 January 2006 and 31 December 2018, surveillance at QECH covered 10 281 476 person-years of observation. We performed 140 498 blood cultures and 63 291 cultures of CSF, yielding 2638 positive *S. pneumoniae* cultures. Among these isolates, 2005 were successfully serotyped: 1133 (57%) were PCV13 VTs, and 872 (43%) were NVTs. Supplementary Tables S5.1-S5.20 report serotyping results, stratified by individual serotypes (13 VT and NVT) and by age group. Analysis of isolates that were and were not recoverable showed no statistically significant differences in terms of age, gender, or sample type (data not shown). All samples sent to the South Africa regional pneumococcal reference laboratory for testing by quellung reaction were concordant for confirmatory testing.

A decline in total (VT+NVT) IPD incidence was observed after the national introduction of antiretroviral therapy (1 January 2006) and prior to PCV13 introduction (both bacteraemia and meningitis; Supplementary Figure S5.1). The reduction in total IPD was 19% (IRR, 0.81; 95% CI, 0.74-0.88; p<0.0001) among infants, 14% (IRR, 0.86; 95% CI, 0.80-0.93; p<0.0001) among children aged 1 to 4 years, and 8% (IRR, 0.92; 95% CI, 0.83-1.01; p=0.08) among persons aged ≥15 years. Among children aged 5 to 14 years there was a 2% increase in total IPD (IRR, 1.02; 95% CI, 0.93-1.11; p=0.72). Pre-PCV trends in both

VT and NVT IPD reductions followed similar patterns (Table 5.1). Following PCV13 introduction in November 2011, vaccine coverage in the age-eligible birth cohort in the years 2012, 2013, and 2014 for dose 1 was 84.6%, 95.1%, and 97.5%; for dose 2, it was 70.3%, 90.2%, and 96.6%; and for dose 3, it was 47.7%, 79.9%, and 90.1%, respectively. In the years that followed, coverage of all doses exceeded 95%.

Table 5.1. Pre–vaccine introduction reductions in IPD (2006 to 2011)

Serotype	Age group	IRR	95% CI	p-value
Total IPD	Infants	0.81	0.74, 0.88	<0.0001
	Children 1-4 years	0.86	0.80, 0.93	<0.0001
	Children 5-14 years	1.02	0.93, 1.11	0.72
	Persons ≥15 years	0.92	0.83, 1.01	0.08
VT IPD	Infants	0.81	0.72, 0.90	<0.0001
	Children 1-4 years	0.92	0.83, 1.01	0.07
	Children 5-14 years	1.01	0.90, 1.13	0.89
	Persons ≥15 years	0.89	0.81, 0.99	0.03
NVT IPD	Infants	0.80	0.75, 0.86	<0.0001
	Children 1-4 years	0.78	0.68, 0.90	0.001
	Children 5-14 years	1.04	0.89, 1.21	0.62
	Persons ≥15years	0.96	0.85, 1.08	0.49

CI, confidence interval; IPD, invasive pneumococcal disease; IRR, incidence rate ratio; NVT, non–vaccine serotype; VT, vaccine serotype

Compared with the vaccine-era counterfactually predicted VT IPD incidence (i.e., in the hypothetical absence of the vaccine), the vaccine-era empirically observed VT IPD incidence was lower by 38% (95% CI, 37%-40%) among infants aged 0 to 11months, 74% (95% CI, 70%-78%) among children aged 1 to 4 years, 79% (95% CI, 76%-83%) among children aged 5 to 14 years, and 47% (95% CI, 44%-51%) among persons aged ≥15 years (Table 5.2). In terms of absolute IPD case numbers from 2006 to 2018, there has been a reduction in all age groups in both VT and NVT IPD. When aggregating all ages, for example, there was a reduction in VT IPD from 120 confirmed IPD cases in 2006 to 13 in 2018. Similarly, the NVT IPD frequency decreased from 102 to 14. Supplementary Tables S5.1-S5.20 report serotyping results for these cases, stratified by individual serotypes (13 VTs individually and NVT as a group) and by age group. Supplementary Table S5.21 presents an abridged version of the case number data stratified by year, with serotype data categorized as VT or NVT.

After 2015 there was an increase in the incidence of NVT disease, which became the commonest cause of IPD and led to a transient increase in total IPD incidence in 2015 and 2016. Nonetheless, total IPD incidence remained low by 2018 (Supplementary Figure S5.1). Among children aged 1 to 4 years, as well as among those aged 5 to 14 years, the vaccine-era empirically observed VT IPD incidence was lower than was anticipated from the background (counterfactual) secular trend (Figure 5.1). The model also showed a lower-

than-anticipated incidence of NVT disease among children 5 to 14 years old, which cannot be attributed to vaccine introduction (Figure 5.1). Serotype 1 and 5 dominated in a 5-yearly cycle prior to vaccine introduction, with a peak still observed in serotype 1 after PCV13 introduction (Supplementary Figure S5.3).

As shown in Supplementary Table S5.22, statistically significant reductions in empirically observed (non-counterfactual) VT IPD incidence were seen in the 2014-2018 period, starting approximately 2 years after vaccine introduction. However, similar to the counterfactual analysis, reductions in empirically observed incidence were both more modest and statistically non-significant among infants aged <1 year and those aged ≥15 years.

5.3.2 Case-control study of vaccine effectiveness

Between 7 October 2011 and 27 June 2016, we identified 34 IPD cases among vaccine age-eligible children, including 19 VT IPD cases (Table 5.3). We recruited 136 controls, matched by age and neighbourhood, between 20 February 2012 and 19 May 2017. Demographic features and vaccine receipt among all-serotype IPD cases and controls are presented in Table 5.3. Among 19 VT IPD cases and their 76 matched controls, 13 groups (65%) had concordant vaccination status so could not contribute statistically to conditional logistic regression. Among 37 all-serotype IPD case-control groupings, 17 (46%) had concordant vaccination statuses. Conditional logistic regression of PCV13 receipt among VT IPD cases and their matched controls found an odds ratio of 0.19 (95% CI, 0.02-1.74; p=0.14), equivalent to a VE of 80.7% (95%CI, −73.7 to 97.9). The final analysis was an unadjusted conditional logistic regression; adjusting for anthropometry, household size, and mother’s vital status did not materially affect the outcome (data not shown).

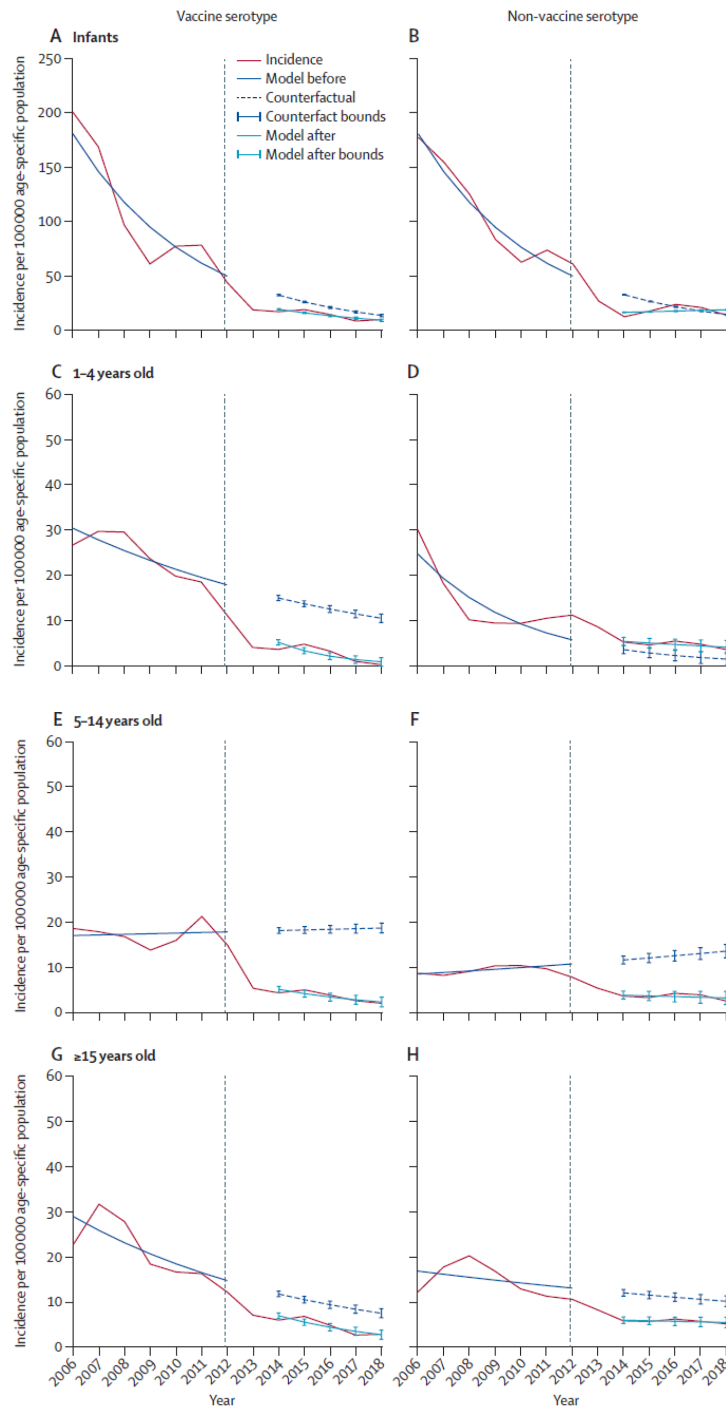

Figure 5.1. Incidence as a 3-year moving average of VT and NVT IPD incidence per 100 000 age-specific population from 1 January 2006 through 31 December 2018. Shown is VT IPD in (A) infants, (C) children 1-4 years old, children 5 to 14 year old , and (G) individuals aged ≥15 years, as well as NVT IPD in (B) infants, (D) children 1-4 years old, (F) children 5-14 years old, and (H) individuals aged ≥15 years. Negative binomial model fit for 1 January 2006 through 31 December 2011 (before PCV) and predicted counterfactual therefrom for 1 January 2014 through 31 December 2018 (after PCV). Negative binomial model fit for 1 January 2014 through 31 December 2018. PCV, pneumococcal conjugate vaccine. Vertical dashed line indicates introduction of PCV13. Negative binomial model fit for 1 January 2006 through 31 December 2011 (pre-PCV) and predicted counterfactual therefrom for 1 January 2014 through 31 December 2018 (PCV era). Negative binomial model

fit for 1 January 2014 through 31 December 2018. IPD, invasive pneumococcal disease; VT, vaccine serotype; NVT, non–vaccine serotype

Table 5.2. Post–vaccine introduction empirical and counterfactually predicted incidence per 100 000 age-specific population (2014 to 2018)

Serotype	Age group	Vaccine era, empirical	95% CI	Vaccine era, counterfactual	95% CI	IRR	95% CI[¥]
Total IPD	Infants	30.62	30.02-31.23	49.94	49.34-50.54	0.61	0.61-0.62
	Children 1-4 years	7.96	7.38-8.54	13.74	13.15-14.32	0.58	0.56-0.60
	Children 5-14 years	7.22	6.57-7.88	29.69	29.04-30.35	0.24	0.23-0.26
	Persons ≥15 years	10.94	10.21-11.67	20.29	19.56-21.02	0.54	0.52-0.56
VT IPD	Infants	15.51	14.68-16.35	25.18	24.35-26.02	0.62	0.60-0.63
	Children 1-4 years	3.50	2.80-4.21	13.29	12.58-14.00	0.26	0.22-0.30
	Children 5-14 years	3.76	2.94-4.57	18.09	17.28-18.91	0.21	0.17-0.24
	Persons ≥15 years	5.37	4.61-6.13	10.18	9.41-10.94	0.53	0.49-0.56
NVT IPD	Infants	16.17	15.67-16.68	24.26	23.75-24.76	0.67	0.66-0.67
	Children 1-4 years	4.67	3.56-5.77	2.50	1.39-3.61	1.87	1.60-2.56
	Children 5-14 years	3.53	2.44-4.63	12.27	11.18-13.36	0.29	0.22-0.35
	Persons ≥15 years	5.65	4.77-6.54	11.19	10.30-12.08	0.51	0.46-0.54

Negative binomial regression of locally weighted incidence, adjusted for year. The 95% confidence intervals for the IRR were constructed manually by predicting the standard error (se) and using the equation exp(estimate +/- 1.96*se). [¥]Note: Strictly speaking, all results in Table 5.2 are statistically significant in the sense that none contain the null value. Even the rate at which NVT IPD incidence increased among children 1-4 years old is a statistically significant increase and may indirectly be due to serotype replacement or to non–vaccine-related factors. However, the issue here is more nuanced and relates to magnitude of effect by age and by VT. There are direct vaccine effects, indirect vaccine effects, and non-vaccine effects at play here. CI, confidence interval; IPD, invasive pneumococcal disease; IRR, incidence rate ratio; NVT, non–vaccine serotype; VT, vaccine serotype

Table 5.3. Demographic features of vaccine age-eligible all-serotype IPD cases vs age- and community-matched controls

Covariate	Cases (n=34)	Controls (n=136)	p-value*
Male	23 (68%)	69 (51%)	0.220
Median (IQR) age, months	8.7 (5.6-18.6)	8.4 (5.3-18.9)	0.960
Vaccine record observed	33 (97%)	132 (97%)	0.065
Mean (SD) household occupants	4.3 (1.5)	4.3 (1.4)	0.99
Mean (SD) maternal age, years	25.6 (5.8)	26.5 (6.4)	0.53
Mother deceased	1 (3%)	0	0.21
Father deceased	0	1 (1%)	0.79
Weight for age Z-score	-1.5	0.1	<0.0001
Height for age Z-score	-0.3	-0.5	0.66
Weight for height Z-score	-1.8	0.6	<0.0001
Number of PCV doses received			
0	6 (18%)	25 (18%)	
1	6 (18%)	11 (8%)	
2	5 (15%)	15 (11%)	
3	17 (50%)	85 (63%)	

*Categorical covariates: χ^2 test (Mantel-Haenszel χ^2 where stratified), unless fewer than five observations where Fisher's exact test used. Continuous covariates: t-test of means, rank-sum test of medians. IPD, invasive pneumococcal disease; IQR, interquartile range; SD, standard deviation; PCV, pneumococcal conjugate vaccine

5.4 Discussion

In this high-disease-burden, low-income sub-Saharan African population where IPD incidence was already declining, we used our robust long-term hospital-based surveillance to show a substantial additional decline in VT IPD incidence following PCV13 introduction among children aged 1 to 4 years, as well as among those aged 5 to 14 years. Importantly, we also report a more modest, non-significant decline in VT IPD incidence among infants <1 year old and persons aged ≥15 years. Although, as has been observed elsewhere,[30] the power of our case-control analysis was undermined by high levels of vaccine uptake (the confidence intervals are wide and include 1), our VE point estimate derived from the case-control analysis suggests a level of protection among vaccine age-eligible children comparable with other post-introduction studies in similar populations.[5,6] Serotype replacement by NVT has occurred since PCV introduction but remains at low levels in absolute terms. The more modest indirect effect among very young and older persons is similar to those seen after PCV introduction in other sub-Saharan African countries, including The Gambia[5] and Kenya.[6] The decline in NVT-associated disease incidence in these age groups suggests a relatively small contribution of routine vaccination to the fall in VT IPD. This is in marked contrast to the United States and some European countries

where the impact on adult VT IPD exceeded, in absolute terms, that seen among vaccine-eligible age groups.[31]

There are several possible causes for the lack of indirect protection observed, despite high vaccine coverage in the 7 years following PCV introduction in Malawi. Malawi's accelerated 3+0 vaccine schedule with no booster and a limited catch-up (among children <1 year of age) on initial introduction has not led to the same reduction in VT carriage seen in high-income settings where schedules frequently include doses in the second year of life.[4] Older siblings are often the source of pneumococcal transmission to younger infants in rural Malawi. Owing to a large carriage pool in this age group, it might take many years of vaccine roll-out before the FOI is substantially reduced and the full indirect protection of PCV becomes measurable. Additionally, median age at first pneumococcal colonization is 6 to 8 weeks of age, before receipt of the full course of PCV13.[19,32] Once colonization occurs, it is plausible that serotype-specific vaccine effectiveness could be blunted owing to immunological changes predating vaccination. Among Malawian adults, IPD is partially driven by HIV coinfection. Ubiquitous availability of ART has been temporally associated with reductions in the incidence of IPD in Malawi,[14] as has occurred in South Africa,[33] but a similar impact of ART on pneumococcal carriage has not been observed.[4,20]

It is possible that the improved survival of HIV-infected persons on ART paradoxically contributes to increased residual VT pneumococcal carriage and transmission in the community, reducing potential indirect vaccine effects. Adults at particularly high risk of pneumococcal colonization and disease (including those living with HIV) or those whose pneumococcal acquisition is derived from other adults may benefit less from an infant schedule.[34] Although our laboratory surveillance did not collect individual HIV status data, we have reported HIV seroprevalence among adult bacterial meningitis and pneumonia patients as 85% and 78%, respectively.[35] This is similar to South Africa, where 89% of adult IPD cases were in HIV-coinfected persons, with IPD incidence rates far higher than among HIV-uninfected persons.[34] Given the persistence of VT carriage and IPD in this vulnerable population, reconsideration should be given to the possible benefit of adult vaccination in this context.[13,36]

Following 7-valent PCV (PCV7) introduction in Alaska, USA, replacement disease was seen.[37] Over a decade later, similar observations had been made in The Gambia,[38] United Kingdom,[12] and South Africa[39] following iterative PCV7 then PCV13 introductions. In Malawi, although NVT IPD events now outnumber VT IPD events, the absolute number of such events is low, and the overall burden of all-cause IPD has declined.

This is likely due to concurrent non-vaccine interventions, such as availability of ART and other health-improving measures, such as rotavirus vaccination, improved control of malaria, and improved nutrition security. The cumulative impact of these public health measures in reducing the overall burden of all-cause IPD underlines that no one public health intervention (including vaccines) is adequate to significantly reduce disease burden. Rather, a strategy of implementing multiple public health interventions including a pneumococcal vaccine that provides both direct and indirect protection is optimal. Ongoing serotype-specific surveillance is required to monitor for the emergence of NVT disease, particularly for isolates that are antimicrobial resistant.

It is challenging to use observational data to attribute routine vaccination to PCV-era declines in IPD incidence, especially in the face of pre-existing declines.[30] Nonetheless, the strengths of our surveillance include its longevity and methodological stability in a stable setting with a limited risk of changes in treatment-seeking behaviour. The duration and scale of the downward trend in IPD incidence prior to vaccine introduction is an important finding and missing from many studies in Africa. While declines in all-serotype IPD were observed in Malawi well before PCV13 introduction, our data suggest a definitive additional benefit of vaccination. In age groups enriched for vaccine age-eligible children where there have been high rates of vaccine uptake, IPD incidence declined faster and was lower following vaccine introduction than anticipated from secular trends alone.

Though this work provides a robust estimate of vaccine impact, there were several limitations. While we were not able to correct for episodes of concurrent bacteraemia and meningitis, we suspect this number is small and consistent over time; therefore, this limitation is unlikely to introduce substantial bias. Broken down by bacteraemia and meningitis, the observed longitudinal patterns parallel the overall results (Supplementary Figure S5.2). Given the retrospective, observational nature of these analyses, there is no information available on vaccine history for any of the sampled children. While we had considered using age strata as a reflection of the probability of children having received the vaccine, further age stratification of the infant group would not have been informative, primarily because the resulting sample sizes would have been too small. As shown in Supplementary Table S5.5, in the post-PCV group (2014-2018), there were 18 children aged <1 year with VT results. Among these, only two were <8 weeks of age. A vaccine with high uptake and even moderate efficacy within a population can undermine the ability to assess its effectiveness in individual recipients using a case-control design. The estimated vaccine uptake of 60% among controls for the purpose of sample size calculation was too conservative, given Malawi's successful rapid PCV13 roll-out and high coverage.

The smaller sample size challenged the analytical power of the matched case-control study, which requires discordance in coverage. A successful vaccine decreases the risk of disease and this, together with the long-standing gradual declines in IPD incidence, made it difficult to recruit sufficient vaccine age-eligible cases of VT IPD for the case-control study.[30] The resulting insufficient recruitment of cases led to the case-control study being underpowered. In populations with high vaccine coverage, there is always a concern about the comparability of unvaccinated individuals to those who are vaccinated. Notwithstanding these limitations, the observed impact and effectiveness are consistent in suggesting substantial reductions in VT IPD risk in Malawi among vaccine-eligible age groups.

5.5 Conclusions

In Malawi, even with pre-existing declines in IPD, the introduction of a 3+0 PCV13 infant schedule has led to a substantial reduction in IPD among vaccine age-eligible children. Yet, despite high vaccination coverage and adequate time since PCV introduction, indirect protection among adolescent and adult disease has been more muted, especially in contrast to that seen in high-income countries. A trend of decreasing IPD incidence before PCV introduction further underlines the need for a strategy of implementing multiple public health interventions to significantly reduce disease burden. Nonetheless, as improvements in available vaccine interventions arise, including alternative schedules and extended-spectrum vaccines at reduced costs, consideration should be given to evaluating PCV vaccination among high-risk populations, including HIV-infected adults, in settings with high FOIs and disease burdens.

5.6 Data availability

The data supporting the findings of this study has been deposited in the supplementary material and in a Figshare data repository (doi: 10.6084/m9.figshare.14098709).

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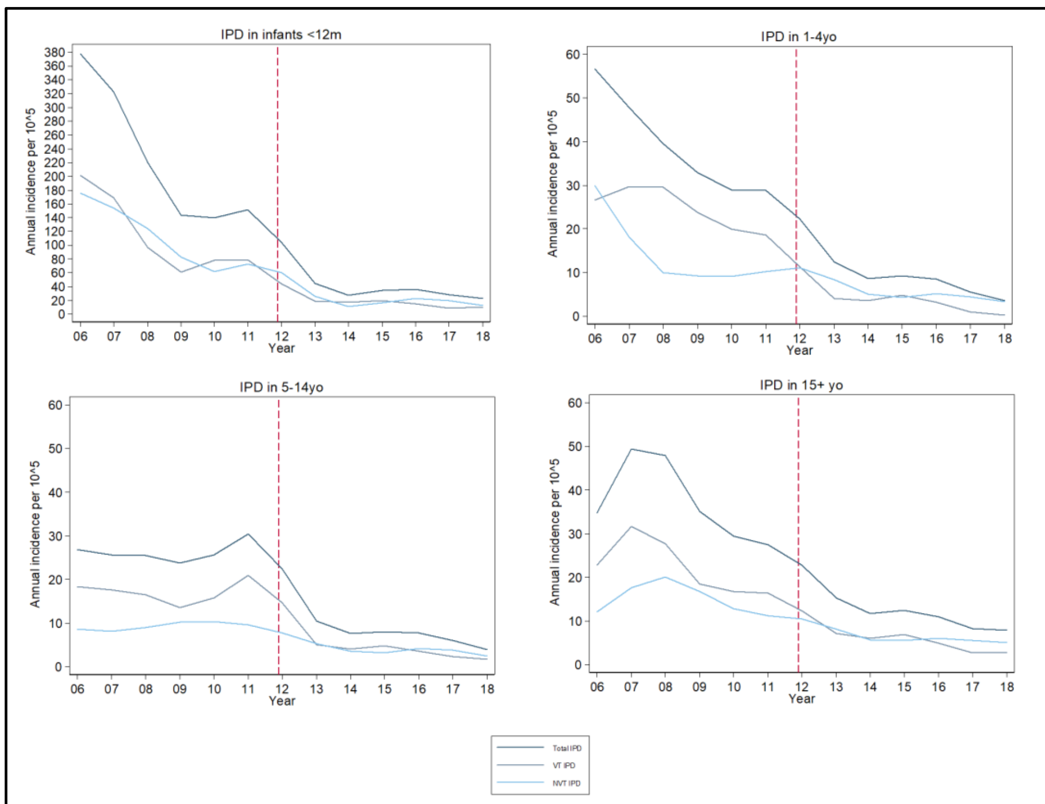
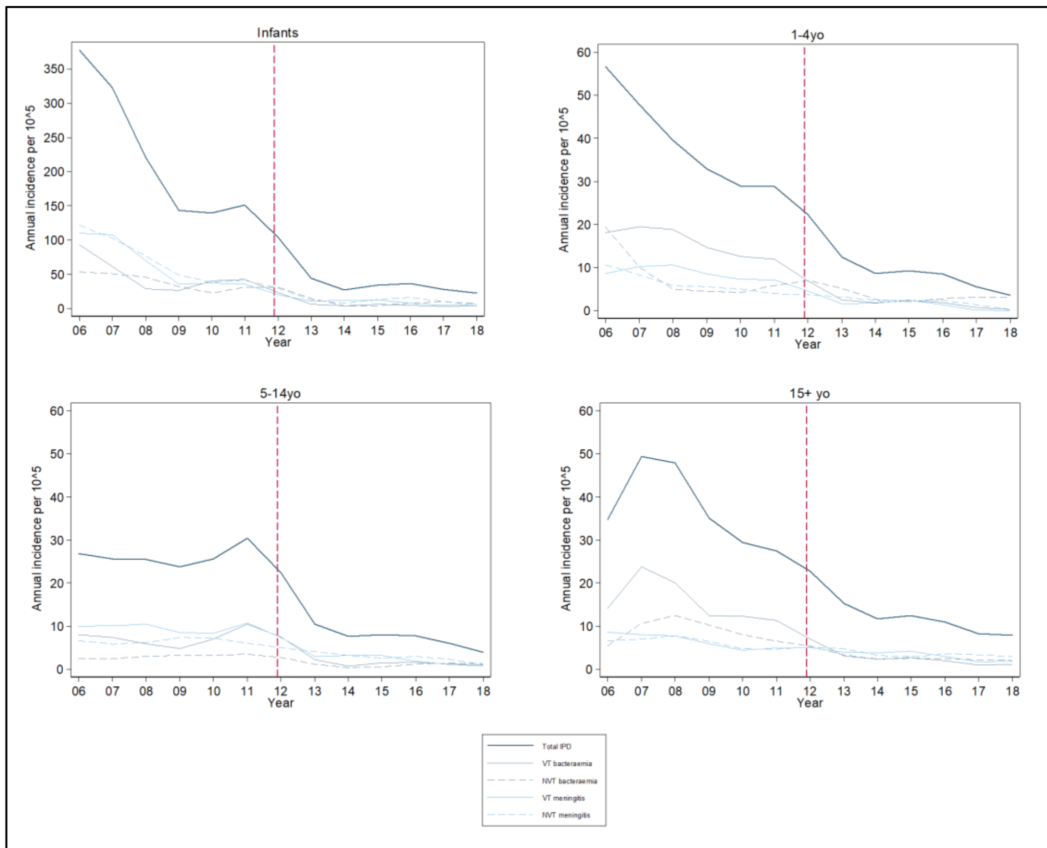
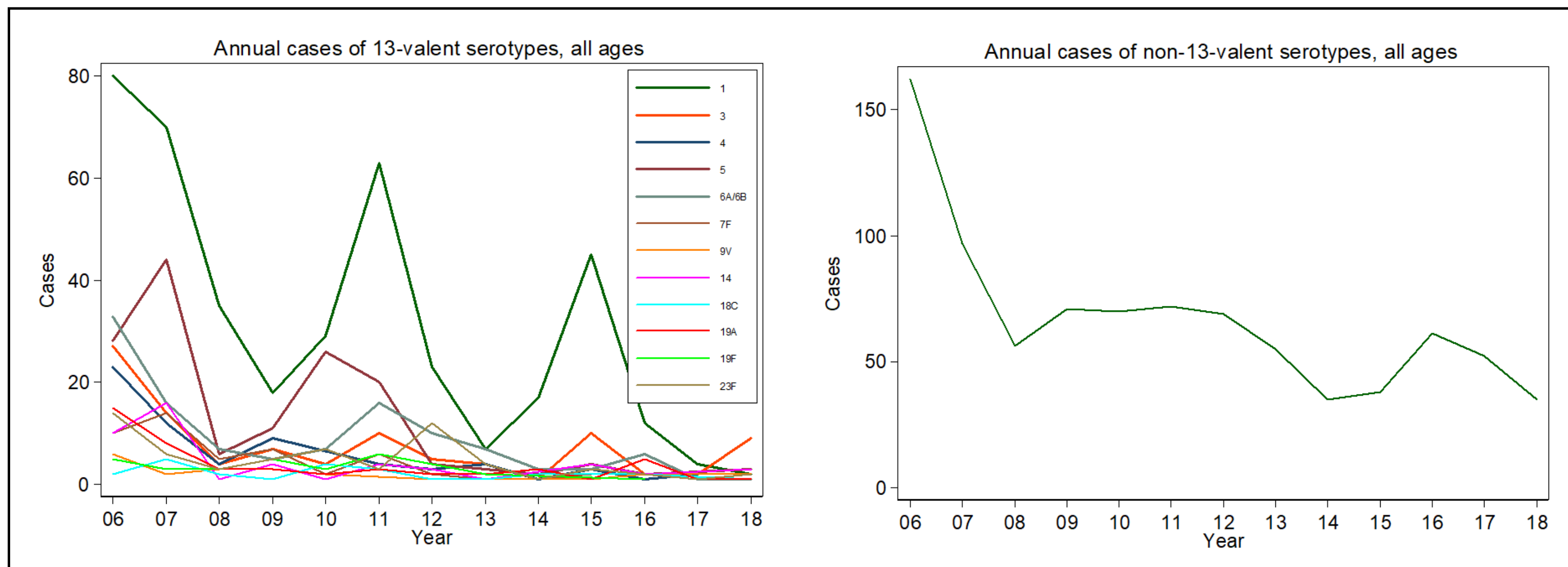
5.8 Supplementary material



Supplementary Figure S5.1. Three-year moving average smoothed incidence per 100 000 age-specific population of IPD in Blantyre, Malawi, 1 January 2006 through 31 December 2018. Dashed red line indicates introduction of 13-valent pneumococcal conjugate vaccine. Note: Y-axis in infant panel differs to other panels. IPD, invasive pneumococcal disease; VT, vaccine serotype; NVT, non–vaccine serotype



Supplementary Figure S5.2. Three-year moving average smoothed incidence per 100 000 age-specific population of pneumococcal bacteraemia and meningitis, Blantyre, Malawi, 1 January 2006 through 31 December 2018. Dashed red line indicates introduction of 13-valent pneumococcal conjugate vaccine. IPD, invasive pneumococcal disease; VT, vaccine serotype; NVT, non–vaccine serotype



Supplementary Figure S5.3. Serotype-specific cases by year, Blantyre, Malawi, 1 January 2006 through 31 December 2018

5.8.1 Serotyping results

5.8.1.1. All ages, specific vaccine-serotype data and grouped non–vaccine-serotype data

Supplementary Table S5.1. Frequency of serotypes stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	80	70	35	18	29	63	23	7	17	45	12	4	2	405
3	27	14	4	7	4	10	5	4	1	10	2	2	9	99
4	23	12	4	9	0	4	3	4	1	3	1	2	0	66
5	28	44	6	11	26	20	4	3	2	4	2	0	3	153
6A	15	5	3	1	2	7	6	3	2	1	4	1	1	51
6A/6B	0	0	0	2	2	1	0	1	0	0	0	0	0	6
6B	18	11	4	2	3	8	4	3	1	2	2	0	0	58
7F	10	14	5	7	2	6	2	1	1	2	2	2	2	56
9V	6	2	3	3	2	0	1	1	1	1	2	2	2	26
14	10	16	1	4	1	4	3	1	0	4	2	0	3	49
18C	2	5	2	1	4	3	1	1	2	0	2	0	1	24
19A	15	8	3	3	2	3	2	2	3	1	5	1	1	49
19F	5	3	3	5	3	6	0	2	0	0	1	0	0	28
23F	14	6	3	5	7	3	12	4	1	3	2	1	2	63
Total VT	253	210	76	78	87	138	66	37	32	76	39	15	26	1133
NVT	162	97	56	71	70	72	69	55	35	38	60	52	35	872
Recovered	415	307	132	149	157	210	135	92	67	114	99	67	61	2005
Unrecovered	49	52	117	47	17	15	15	5	5	10	3	2	5	342

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.2. Prevalence of VT as a proportion of total VT IPD isolates

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	31.6	33.3	46.1	23.1	33.3	45.7	34.9	18.9	53.1	59.2	30.8	26.7	7.7	35.8
3	10.7	6.7	5.3	9.0	4.6	7.3	7.6	10.8	3.1	13.2	5.1	13.3	34.6	8.7
4	9.1	5.7	5.3	11.5	0.0	2.9	4.6	10.8	3.1	4.0	2.6	13.3	0.0	5.8
5	11.1	21.0	7.9	14.1	29.9	14.5	6.1	8.1	6.3	5.3	5.1	0.0	11.5	13.5
6A	5.9	2.4	4.0	1.3	2.3	5.1	9.1	8.1	6.3	1.3	10.3	6.7	3.9	4.5
6A/6B	0.0	0.0	0.0	2.6	2.3	0.7	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.5
6B	7.1	5.2	5.3	2.6	3.5	5.8	6.1	8.1	3.1	2.6	5.1	0.0	0.0	5.1
7F	4.0	6.7	6.6	9.0	2.3	4.4	3.0	2.7	3.1	2.6	5.1	13.3	7.7	4.9
9V	2.4	1.0	4.0	3.9	2.3	0.0	1.5	2.7	3.1	1.3	5.1	13.3	7.7	2.3
14	4.0	7.6	1.3	5.1	1.2	2.9	4.6	2.7	0.0	5.3	5.1	0.0	11.5	4.3
18C	0.8	2.4	2.6	1.3	4.6	2.2	1.5	2.7	6.3	0.0	5.1	0.0	3.9	2.1
19A	5.9	3.8	4.0	3.9	2.3	2.2	3.0	5.4	9.4	1.3	12.8	6.7	3.9	4.3
19F	2.0	1.4	4.0	6.4	3.5	4.4	0.0	5.4	0.0	0.0	2.6	0.0	0.0	2.5
23F	5.5	2.9	4.0	6.4	8.1	2.2	18.2	10.8	3.1	4.0	5.1	6.7	7.7	5.6
Total VT	100	100	100	100	100	100	100	100	100	100	100	100	100	100

IPD, invasive pneumococcal disease; VT, vaccine serotype

Supplementary Table S5.3. Prevalence of VT as a proportion of total (VT+NVT) IPD isolates

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	19.3	22.8	26.5	12.1	18.5	30.0	17.0	7.6	25.4	39.5	12.1	6.0	3.3	20.2
3	6.5	4.6	3.0	4.7	2.6	4.8	3.7	4.4	1.5	8.8	2.0	3.0	14.8	4.9
4	5.5	3.9	3.0	6.0	0.0	1.9	2.2	4.4	1.5	2.6	1.0	3.0	0.0	3.3
5	6.8	14.3	4.6	7.4	16.6	9.5	3.0	3.3	3.0	3.5	2.0	0.0	4.9	7.6
6A	3.6	1.6	2.3	0.7	1.3	3.3	4.4	3.3	3.0	0.9	4.0	1.5	1.6	2.5
6A/6B	0.0	0.0	0.0	1.3	1.3	0.5	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.3
6B	4.3	3.6	3.0	1.3	1.9	3.8	3.0	3.3	1.5	1.8	2.0	0.0	0.0	2.9
7F	2.4	4.6	3.8	4.7	1.3	2.9	1.5	1.1	1.5	1.8	2.0	3.0	3.3	2.8
9V	1.5	0.7	2.3	2.0	1.3	0.0	0.7	1.1	1.5	0.9	2.0	3.0	3.3	1.3
14	2.4	5.2	0.8	2.7	0.6	1.9	2.2	1.1	0.0	3.5	2.0	0.0	4.9	2.4
18C	0.5	1.6	1.5	0.7	2.6	1.4	0.7	1.1	3.0	0.0	2.0	0.0	1.6	1.2
19A	3.6	2.6	2.3	2.0	1.3	1.4	1.5	2.2	4.5	0.9	5.1	1.5	1.6	2.4
19F	1.2	1.0	2.3	3.4	1.9	2.9	0.0	2.2	0.0	0.0	1.0	0.0	0.0	1.4
23F	3.4	2.0	2.3	3.4	4.5	1.4	8.9	4.4	1.5	2.6	2.0	1.5	3.3	3.1
Total VT	61.0	68.4	57.6	52.3	55.4	65.7	48.9	40.2	47.8	66.7	39.4	22.4	42.6	56.5

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.4. Prevalence of NVT as a proportion of total (VT+NVT) IPD isolates

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
NVT	39.0	31.6	42.5	47.6	44.6	34.3	51.1	59.8	52.2	33.3	60.6	77.6	57.4	43.5

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.1.2. Infants <1 year old

Supplementary Table S5.5. Frequency of serotypes, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	2	2	0	0	1	1	0	0	1	2	0	0	0	9
3	2	0	1	0	0	1	0	0	0	3	0	0	0	7
4	0	1	0	0	0	2	0	0	0	0	0	0	0	3
5	7	13	0	3	4	7	1	0	1	0	1	0	2	39
6A	2	1	0	0	0	1	0	0	1	0	0	0	0	5
6B	3	1	1	0	0	2	1	2	0	0	1	0	0	11
7F	2	1	0	1	0	1	0	0	0	0	0	0	0	5
9V	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	3	1	0	0	1	0	1	0	0	1	0	1	8
18C	1	0	1	0	0	1	0	0	1	0	1	0	0	5
19A	1	2	0	0	0	0	0	0	0	0	0	0	0	3
19F	2	1	0	0	0	1	0	0	0	0	1	0	0	5
23F	2	1	0	0	4	0	3	0	0	0	0	0	1	11
Total VT	24	26	4	4	9	18	5	3	4	5	5	0	4	111
NVT	23	18	8	6	4	17	10	4	1	5	5	8	2	111
Recovered	47	44	12	10	13	35	15	7	5	10	10	8	6	222
Unrecovered	13	8	12	0	0	2	5	2	1	0	2	0	1	46

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.6. Prevalence of VT as a proportion of total VT IPD isolates, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	8.3	7.7	0.0	0.0	11.1	5.6	0.0	0.0	25.0	40.0	0.0	0.0	0.0	8.1
3	8.3	0.0	25.0	0.0	0.0	5.6	0.0	0.0	0.0	60.0	0.0	0.0	0.0	6.3
4	0.0	3.8	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7
5	29.2	50.0	0.0	75.0	44.4	38.9	20.0	0.0	25.0	0.0	20.0	0.0	50.0	35.1
6A	8.3	3.8	0.0	0.0	0.0	5.6	0.0	0.0	25.0	0.0	0.0	0.0	0.0	4.5
6B	12.5	3.8	25.0	0.0	0.0	11.1	20.0	66.7	0.0	0.0	20.0	0.0	0.0	9.9
7F	8.3	3.8	0.0	25.0	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5
9V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	11.5	25.0	0.0	0.0	5.6	0.0	33.3	0.0	0.0	20.0	0.0	25.0	7.2
18C	4.2	0.0	25.0	0.0	0.0	5.6	0.0	0.0	25.0	0.0	20.0	0.0	0.0	4.5
19A	4.2	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7
19F	8.3	3.8	0.0	0.0	0.0	5.6	0.0	0.0	0.0	0.0	20.0	0.0	0.0	4.5
23F	8.3	3.8	0.0	0.0	44.4	0.0	60.0	0.0	0.0	0.0	0.0	0.0	25.0	9.9
Total VT	100	100	100	100	100	100	100	100	100	100	100	0	100	100

IPD, invasive pneumococcal disease; VT, vaccine serotype

Supplementary Table S5.7. Prevalence of VT as a proportion of total (VT+NVT) IPD isolates, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	4.3	4.5	0.0	0.0	7.7	2.9	0.0	0.0	20.0	20.0	0.0	0.0	0.0	4.1
3	4.3	0.0	8.3	0.0	0.0	2.9	0.0	0.0	0.0	30.0	0.0	0.0	0.0	3.2
4	0.0	2.3	0.0	0.0	0.0	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
5	14.9	29.5	0.0	30.0	30.8	20.0	6.7	0.0	20.0	0.0	10.0	0.0	33.3	17.6
6A	4.3	2.3	0.0	0.0	0.0	2.9	0.0	0.0	20.0	0.0	0.0	0.0	0.0	2.3
6B	6.4	2.3	8.3	0.0	0.0	5.7	6.7	28.6	0.0	0.0	10.0	0.0	0.0	5.0
7F	4.3	2.3	0.0	10.0	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3
9V	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	0.0	6.8	8.3	0.0	0.0	2.9	0.0	14.3	0.0	0.0	10.0	0.0	16.7	3.6
18C	2.1	0.0	8.3	0.0	0.0	2.9	0.0	0.0	20.0	0.0	10.0	0.0	0.0	2.3
19A	2.1	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4
19F	4.3	2.3	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0	10.0	0.0	0.0	2.3
23F	4.3	2.3	0.0	0.0	30.8	0.0	20.0	0.0	0.0	0.0	0.0	0.0	16.7	5.0
Total VT	51.1	59.1	33.3	40.0	69.2	51.4	33.3	42.9	80.0	50.0	50.0	0.0	66.7	50.0

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.8. Prevalence of NVT as proportion of total (VT+NVT) IPD isolates, stratified by year

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
NVT	48.9	40.9	66.7	60.0	30.8	48.6	66.7	57.1	20.0	50.0	50.0	100.0	33.3	100.0

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.1.3. Children 1 to 4 years old

Supplementary Table S5.9. Frequency of serotypes, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	1	4	2	2	1	8	1	1	0	4	0	0	0	24
3	1	0	0	0	0	0	0	0	0	1	0	0	0	2
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	4	2	1	2	1	0	1	1	1	0	0	0	14
6A	0	0	1	0	0	2	2	1	0	0	1	0	0	7
6A/6B	0	0	0	1	0	1	0	0	0	0	0	0	0	2
6B	3	0	0	0	2	1	0	0	0	0	0	0	0	6
7F	1	0	0	1	0	1	0	0	0	0	0	0	0	3
9V	1	0	0	0	0	0	0	0	0	1	1	0	0	3
14	0	2	0	1	0	0	2	0	0	0	0	0	0	5
18C	0	1	0	0	1	1	0	0	0	0	0	0	0	3
19A	0	1	0	0	0	0	0	0	0	0	0	0	0	1
19F	0	1	0	2	0	1	0	0	0	0	0	0	0	4
23F	1	1	1	3	1	0	1	1	1	1	1	1	0	13
Total VT	9	14	6	11	7	16	6	4	2	8	3	1	0	87
NVT	15	5	2	4	5	5	11	8	5	3	8	4	4	79
Recovered	24	19	8	15	12	21	17	12	7	11	11	5	4	166
Unrecovered	6	5	12	4	3	1	2	0	1	1	1	1	0	37

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.10. Prevalence of VT as proportion of total VT IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	11.1	28.6	33.3	18.2	14.3	50.0	16.7	25.0	0.0	50.0	0.0	0.0	0.0	27.6
3	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	0.0	0.0	0.0	2.3
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	11.1	28.6	33.3	9.1	28.6	6.3	0.0	25.0	50.0	12.5	0.0	0.0	0.0	16.1
6A	0.0	0.0	16.7	0.0	0.0	12.5	33.3	25.0	0.0	0.0	33.3	0.0	0.0	8.0
6A/6B	0.0	0.0	0.0	9.1	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3
6B	33.3	0.0	0.0	0.0	28.6	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.9
7F	11.1	0.0	0.0	9.1	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4
9V	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	33.3	0.0	0.0	3.4
14	0.0	14.3	0.0	9.1	0.0	0.0	33.3	0.0	0.0	0.0	0.0	0.0	0.0	5.7
18C	0.0	7.1	0.0	0.0	14.3	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4
19A	0.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
19F	0.0	7.1	0.0	18.2	0.0	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6
23F	11.1	7.1	16.7	27.3	14.3	0.0	16.7	25.0	50.0	12.5	33.3	100.0	0.0	14.9
Total VT	100	100	100	100	100	100	100	100	100	100	100	100	0	100

IPD, invasive pneumococcal disease; VT, vaccine serotype

Supplementary Table S5.11. Prevalence of VT as a proportion of total (VT+NVT) IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	4.2	21.1	25.0	13.3	8.3	38.1	5.9	8.3	0.0	36.4	0.0	0.0	0.0	14.5
3	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	1.2
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	4.2	21.1	25.0	6.7	16.7	4.8	0.0	8.3	14.3	9.1	0.0	0.0	0.0	8.4
6A	0.0	0.0	12.5	0.0	0.0	9.5	11.8	8.3	0.0	0.0	9.1	0.0	0.0	4.2
6A/6B	0.0	0.0	0.0	6.7	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2
6B	12.5	0.0	0.0	0.0	16.7	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6
7F	4.2	0.0	0.0	6.7	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8
9V	4.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	9.1	0.0	0.0	1.8
14	0.0	10.5	0.0	6.7	0.0	0.0	11.8	0.0	0.0	0.0	0.0	0.0	0.0	3.0
18C	0.0	5.3	0.0	0.0	8.3	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8
19A	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
19F	0.0	5.3	0.0	13.3	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4
23F	4.2	5.3	12.5	20.0	8.3	0.0	5.9	8.3	14.3	9.1	9.1	20.0	0.0	7.8
Total VT	37.5	73.7	75.0	73.3	58.3	76.2	35.3	33.3	28.6	72.7	27.3	20.0	0.0	52.4

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.12. Prevalence of NVT as proportion of total (VT+NVT) IPD isolates, stratified by year

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
NVT	62.5	26.3	25.0	26.7	41.7	23.8	64.7	66.7	71.4	27.3	72.7	80.0	100.0	47.6

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.1.4. Children 5 to 14 years old

Supplementary Table S5.13. Frequency of serotypes, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	8	7	4	2	7	21	4	0	6	8	4	1	2	74
3	0.0	0.0	0.0	1	0.0	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4
4	3	0.0	0.0	2	0.0	0.0	2	0.0	1	1	0.0	2	0.0	11
5	0.0	2	0.0	0.0	4	2	2	1	0.0	0.0	0.0	0.0	0.0	11
6A	3	0.0	0.0	0.0	0.0	1	2	0.0	0.0	0.0	0.0	0.0	0.0	6
6B	0.0	1	0.0	0.0	0.0	2	1	0.0	1	0.0	0.0	0.0	0.0	5
7F	0.0	2	1	1	0.0	1	1	0.0	1	0.0	2	2	0.0	11
9V	0.0	0.0	0.0	0.0	1	0.0	1	0.0	1	0.0	1	0.0	0.0	4
14	0.0	2	0.0	1	0.0	1	0.0	0.0	0.0	1	0.0	0.0	0.0	5
18C	0.0	0.0	1	0.0	1	0.0	1	0.0	0.0	0.0	0.0	0.0	1	4
19A	2	0.0	0.0	0.0	0.0	1	1	0.0	0.0	0.0	1	0.0	0.0	5
19F	0.0	0.0	3	3	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7
23F	2	0.0	1	0.0	0.0	3	3	0.0	0.0	0.0	0.0	0.0	0.0	9
Total VT	18	14	10	10	14	35	18	1	10	10	8	5	3	156
NVT	9	6	5	9	13	12	10	9	8	4	11	10	3	109
Recovered	27	20	15	19	27	47	28	10	18	14	19	15	6	265
Unrecovered	5	5	21	7	1	4	4	0.0	0.0	1	0.0	0.0	1	49

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.14. Prevalence of VT as a proportion of total VT IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	44.4	50.0	40.0	20.0	50.0	60.0	22.2	0.0	60.0	80.0	50.0	20.0	66.7	47.4
3	0.0	0.0	0.0	10.0	0.0	8.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
4	16.7	0.0	0.0	20.0	0.0	0.0	11.1	0.0	10.0	10.0	0.0	40.0	0.0	7.1
5	0.0	14.3	0.0	0.0	28.6	5.7	11.1	100.0	0.0	0.0	0.0	0.0	0.0	7.1
6A	16.7	0.0	0.0	0.0	0.0	2.9	11.1	0.0	0.0	0.0	0.0	0.0	0.0	3.8
6B	0.0	7.1	0.0	0.0	0.0	5.7	5.6	0.0	10.0	0.0	0.0	0.0	0.0	3.2
7F	0.0	14.3	10.0	10.0	0.0	2.9	5.6	0.0	10.0	0.0	25.0	40.0	0.0	7.1
9V	0.0	0.0	0.0	0.0	7.1	0.0	5.6	0.0	10.0	0.0	12.5	0.0	0.0	2.6
14	0.0	14.3	0.0	10.0	0.0	2.9	0.0	0.0	0.0	10.0	0.0	0.0	0.0	3.2
18C	0.0	0.0	10.0	0.0	7.1	0.0	5.6	0.0	0.0	0.0	0.0	0.0	33.3	2.6
19A	11.1	0.0	0.0	0.0	0.0	2.9	5.6	0.0	0.0	0.0	12.5	0.0	0.0	3.2
19F	0.0	0.0	30.0	30.0	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5
23F	11.1	0.0	10.0	0.0	0.0	8.6	16.7	0.0	0.0	0.0	0.0	0.0	0.0	5.8
Total VT	100	100	100	100	100	100	100	100	100	100	100	100	100	100

IPD, invasive pneumococcal disease; VT, vaccine serotype

Supplementary Table S5.15. Prevalence of VT as a proportion of total (VT+NVT) IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	29.6	35.0	26.7	10.5	25.9	44.7	14.3	0.0	33.3	57.1	21.1	6.7	33.3	27.9
3	0.0	0.0	0.0	5.3	0.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
4	11.1	0.0	0.0	10.5	0.0	0.0	7.1	0.0	5.6	7.1	0.0	13.3	0.0	4.2
5	0.0	10.0	0.0	0.0	14.8	4.3	7.1	10.0	0.0	0.0	0.0	0.0	0.0	4.2
6A	11.1	0.0	0.0	0.0	0.0	2.1	7.1	0.0	0.0	0.0	0.0	0.0	0.0	2.3
6B	0.0	5.0	0.0	0.0	0.0	4.3	3.6	0.0	5.6	0.0	0.0	0.0	0.0	1.9
7F	0.0	10.0	6.7	5.3	0.0	2.1	3.6	0.0	5.6	0.0	10.5	13.3	0.0	4.2
9V	0.0	0.0	0.0	0.0	3.7	0.0	3.6	0.0	5.6	0.0	5.3	0.0	0.0	1.5
14	0.0	10.0	0.0	5.3	0.0	2.1	0.0	0.0	0.0	7.1	0.0	0.0	0.0	1.9
18C	0.0	0.0	6.7	0.0	3.7	0.0	3.6	0.0	0.0	0.0	0.0	0.0	16.7	1.5
19A	7.4	0.0	0.0	0.0	0.0	2.1	3.6	0.0	0.0	0.0	5.3	0.0	0.0	1.9
19F	0.0	0.0	20.0	15.8	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6
23F	7.4	0.0	6.7	0.0	0.0	6.4	10.7	0.0	0.0	0.0	0.0	0.0	0.0	3.4
Total VT	66.7	70.0	66.7	52.6	51.9	74.5	64.3	10.0	55.6	71.4	42.1	33.3	50.0	58.9

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.16. Prevalence of NVT as a proportion of total (VT+NVT) IPD isolates, stratified by year

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
NVT	33.3	30.0	33.3	47.4	48.1	25.5	35.7	90.0	44.4	28.6	57.9	66.7	50.0	41.1

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.1.5. Participants aged ≥15 years

Supplementary Table S5.17. Frequency of serotypes, stratified by year

VT	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	11	47	23	13	14	31	17	6	10	26	8	3	0	209
3	2	12	2	5	4	6	5	4	1	6	2	2	9	60
4	3	6	3	6	0	2	1	4	0	1	1	0	0	27
5	3	19	4	6	14	8	1	1	0	3	0	0	1	60
6A	0	3	2	1	1	2	1	2	1	1	3	1	1	19
6A/6B	0	0	0	1	2	0	0	1	0	0	0	0	0	4
6B	0	8	1	2	0	3	2	1	0	2	1	0	0	20
7F	2	6	3	4	2	3	1	1	0	1	0	0	2	25
9V	0	2	3	1	1	0	0	1	0	0	0	2	2	12
14	1	8	0	1	1	2	0	0	0	3	1	0	2	19
18C	0	3	0	1	1	0	0	1	1	0	1	0	0	8
19A	2	4	2	3	2	2	1	2	3	1	4	1	1	28
19F	0	1	0	0	1	2	0	1	0	0	0	0	0	5
23F	3	2	0	1	2	0	5	3	0	1	1	0	1	19
Total VT	27	121	43	45	45	61	34	28	16	45	22	9	19	515
NVT	19	54	39	43	36	32	35	34	19	24	36	29	26	426
Recovered	46	175	82	88	81	93	69	62	35	69	58	38	45	941
Unrecovered	6	27	57	31	11	6	3	3	3	6	0	0	3	156

NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S5.18. Prevalence of VT as a proportion of total VT IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	40.7	38.8	53.5	28.9	31.1	50.8	50.0	21.4	62.5	57.8	36.4	33.3	0.0	40.6
3	7.4	9.9	4.7	11.1	8.9	9.8	14.7	14.3	6.3	13.3	9.1	22.2	47.4	11.7
4	11.1	5.0	7.0	13.3	0.0	3.3	2.9	14.3	0.0	2.2	4.5	0.0	0.0	5.2
5	11.1	15.7	9.3	13.3	31.1	13.1	2.9	3.6	0.0	6.7	0.0	0.0	5.3	11.7
6A	0.0	2.5	4.7	2.2	2.2	3.3	2.9	7.1	6.3	2.2	13.6	11.1	5.3	3.7
6A/6B	0.0	0.0	0.0	2.2	4.4	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.8
6B	0.0	6.6	2.3	4.4	0.0	4.9	5.9	3.6	0.0	4.4	4.5	0.0	0.0	3.9
7F	7.4	5.0	7.0	8.9	4.4	4.9	2.9	3.6	0.0	2.2	0.0	0.0	10.5	4.9
9V	0.0	1.7	7.0	2.2	2.2	0.0	0.0	3.6	0.0	0.0	0.0	22.2	10.5	2.3
14	3.7	6.6	0.0	2.2	2.2	3.3	0.0	0.0	0.0	6.7	4.5	0.0	10.5	3.7
18C	0.0	2.5	0.0	2.2	2.2	0.0	0.0	3.6	6.3	0.0	4.5	0.0	0.0	1.6
19A	7.4	3.3	4.7	6.7	4.4	3.3	2.9	7.1	18.8	2.2	18.2	11.1	5.3	5.4
19F	0.0	0.8	0.0	0.0	2.2	3.3	0.0	3.6	0.0	0.0	0.0	0.0	0.0	1.0
23F	11.1	1.7	0.0	2.2	4.4	0.0	14.7	10.7	0.0	2.2	4.5	0.0	5.3	3.7
Total VT	100	100	100	100	100	100	100	100	100	100	100	100	100	100

IPD, invasive pneumococcal disease; VT, vaccine serotype

Supplementary Table S5.19. Prevalence of VT as a proportion of total (VT+NVT) IPD isolates, stratified by year

Serotype	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
1	23.9	26.9	28.0	14.8	17.3	33.3	24.6	9.7	28.6	37.7	13.8	7.9	0.0	22.2
3	4.3	6.9	2.4	5.7	4.9	6.5	7.2	6.5	2.9	8.7	3.4	5.3	20.0	6.4
4	6.5	3.4	3.7	6.8	0.0	2.2	1.4	6.5	0.0	1.4	1.7	0.0	0.0	2.9
5	6.5	10.9	4.9	6.8	17.3	8.6	1.4	1.6	0.0	4.3	0.0	0.0	2.2	6.4
6A	0.0	1.7	2.4	1.1	1.2	2.2	1.4	3.2	2.9	1.4	5.2	2.6	2.2	2.0
6A/6B	0.0	0.0	0.0	1.1	2.5	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.4
6B	0.0	4.6	1.2	2.3	0.0	3.2	2.9	1.6	0.0	2.9	1.7	0.0	0.0	2.1
7F	4.3	3.4	3.7	4.5	2.5	3.2	1.4	1.6	0.0	1.4	0.0	0.0	4.4	2.7
9V	0.0	1.1	3.7	1.1	1.2	0.0	0.0	1.6	0.0	0.0	0.0	5.3	4.4	1.3
14	2.2	4.6	0.0	1.1	1.2	2.2	0.0	0.0	0.0	4.3	1.7	0.0	4.4	2.0
18C	0.0	1.7	0.0	1.1	1.2	0.0	0.0	1.6	2.9	0.0	1.7	0.0	0.0	0.9
19A	4.3	2.3	2.4	3.4	2.5	2.2	1.4	3.2	8.6	1.4	6.9	2.6	2.2	3.0
19F	0.0	0.6	0.0	0.0	1.2	2.2	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.5
23F	6.5	1.1	0.0	1.1	2.5	0.0	7.2	4.8	0.0	1.4	1.7	0.0	2.2	2.0
Total VT	58.7	69.1	52.4	51.1	55.6	65.6	49.3	45.2	45.7	65.2	37.9	23.7	42.2	54.7

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

Supplementary Table S 5.20. Prevalence of NVT as a proportion of total (VT+NVT) IPD isolates, stratified by year

	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	Total
NVT	41.3	30.9	47.6	48.9	44.4	34.4	50.7	54.8	54.3	34.8	62.1	76.3	57.8	45.3

IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.1.6. All ages, grouped vaccine-serotype and non–vaccine-serotype data

Supplementary Table S5.21. Frequency of VT and NVT IPD stratified by year*

	Infants			**1-4 years old**			**5-14 years old**			**≥15 years old Adolescents and adults**			**All ages**		
Year	**NVT**	**VT**	**Total**	**NVT**	**VT**	**Total**	**NVT**	**VT**	**Total**	**NVT**	**VT**	**Total**	**NVT**	**VT**	**Total**
2006	41	42	83	33	20	53	14	29	43	14	29	43	102	120	222
2007	30	44	74	10	28	38	11	25	36	11	25	36	62	122	184
2008	29	14	43	8	25	33	14	29	43	14	29	43	65	97	162
2009	17	11	28	8	23	31	18	21	39	18	21	39	61	76	137
2010	9	19	28	9	13	22	17	17	34	17	17	34	52	66	118
2011	24	26	50	9	27	36	18	53	71	18	53	71	69	159	228
2012	17	8	25	15	8	23	15	26	41	15	26	41	62	68	130
2013	5	4	9	8	4	12	9	1	10	9	1	10	31	10	41
2014	1	5	6	6	2	8	8	11	19	8	11	19	23	29	52
2015	6	6	12	3	9	12	4	11	15	4	11	15	17	37	54
2016	7	6	13	9	3	12	12	8	20	12	8	20	40	25	65
2017	8	0	8	5	1	6	10	5	15	10	5	15	33	11	44
2018	2	5	7	4	0	4	4	4	8	4	4	8	14	13	27
Total	**196**	**190**	**386**	**127**	**163**	**290**	**154**	**240**	**394**	**154**	**240**	**394**	**631**	**833**	**1464**

*VT and NVT corrected for proportion serotype. IPD, invasive pneumococcal disease; NVT, non–vaccine serotype; VT, vaccine serotype

5.8.2 Incidence rate ratio data for invasive pneumococcal disease

Supplementary Table S5.22. Incidence rate ratio of IPD using empirically observed data, 1 January 2006 through 31 December 2018

		2006-2011		2012-2013		2014-2018		2014-2018 vs 2006-2011		
Serotype	Age group	Cases*	Incidence†	Cases	Incidence	Cases	Incidence	IRR‡	95% CI	p-value
All IPD	Infants	306	226.6	34	62.0	45	29.1	0.54	0.29-1.01	0.05
	Children 1-4 years	213	39.4	35	15.9	42	6.8	0.58	0.30-1.12	0.11
	Children 5-14 years	265	27.3	51	13.4	76	6.7	0.26	0.12-0.54	<0.001
	Persons ≥15 years	902	36.7	178	18.8	266	10.0	0.53	0.29-0.99	0.05
VT IPD	Infants	156	115.5	12	21.9	22	14.2	0.62	0.32-1.22	0.17
	Children 1-4 years	136	25.2	12	5.5	15	2.4	0.24	0.10-0.58	0.002
	Children 5-14 years	174	17.9	27	7.1	39	3.4	0.21	0.08-0.56	0.002
	Persons ≥15 years	539	21.9	85	9.0	121	4.5	0.54	0.23-1.25	0.15
NVT IPD	Infants	150	111.1	22	40.1	24	15.5	0.53	0.23-1.22	0.13
	Children 1-4 years	77	14.3	23	10.5	27	4.4	1.78	0.64-4.96	0.27
	Children 5-14 years	92	9.5	24	6.3	38	3.4	0.31	0.10-1.00	0.05
	Persons ≥15 years	363	14.8	93	9.8	145	5.4	0.50	0.20-1.26	0.14

IRR 95% confidence intervals based on raw data were calculated using the [. ci] Stata command.*Cases of VT and non-VT IPD corrected for proportion serotyped. †Incidence per 100 000 age-specific population. ‡Negative binomial regression of locally weighted incidence, adjusted for year. IPD, invasive pneumococcal disease, IRR, incidence rate ratio; CI, confidence interval; VT, vaccine serotype; NVT, non–vaccine serotype

Chapter 6. A pragmatic health centre-based evaluation comparing the effectiveness of a PCV13 schedule change from 3+0 to 2+1 in a high pneumococcal carriage and disease burden setting in Malawi: a study protocol

N.B., Associated published peer-reviewed article: ***Swarthout TD**, *Ibarz-Pavon A, Kawalazira G, Sinjani G, Chirombo J, Gori A, Chalusa P, Bonomali F, Nyirenda R, Bulla E, Brown C, Msefula J, Banda M, Kachala J, Mwansambo C, Henrion MY, Gordon SB, French N, Heyderman RS. A pragmatic health centre-based evaluation comparing the effectiveness of a PCV13 schedule change from 3+0 to 2+1 in a high pneumococcal carriage and disease burden setting in Malawi: a study protocol. BMJ Open. 2021 Jun 17;11(6):e050312. doi: 10.1136/bmjopen-2021-050312. PMID: 34140345; PMCID: PMC8212416.

*Joint first author

Chapter introduction: In chapters 3 to 5, we report—based on highly accurate laboratory techniques (chapter 4)—a less-than-optimal impact of the 13-valent pneumococcal vaccine (PCV13) on reducing both vaccine-serotype nasopharyngeal carriage (chapter 3) and vaccine-serotype–associated invasive pneumococcal disease (chapter 5). These findings have prompted recommendations to further evaluate alternative vaccine implementation strategies, including alternative schedules. One such schedule could include the World Health Organization–approved 2+1 schedule, a three-dose schedule that includes two primary doses and one booster dose. Chapter 6 presents the protocol for the PAVE study, a pragmatic health centre–based evaluation comparing impact of a PCV13 schedule change from Malawi's current 3+0 schedule (one dose each at 6, 10, and 14 weeks of age) to 2+1 (one dose at each 6 weeks, 14 weeks, and 9 months of age) in Blantyre District, Malawi.

Abstract

Introduction: *Streptococcus pneumoniae* (the pneumococcus) is commonly carried as a commensal bacterium in the nasopharynx but can cause life-threatening disease. Transmission occurs via human respiratory droplets, and interruption of this process provides herd immunity. A 2017 World Health Organization consultation on optimization of the impact of pneumococcal conjugate vaccines (PCVs) highlighted a substantial research gap in investigating why the impact of PCVs in low-income countries has been lower than expected. Malawi introduced the 13-valent PCV (PCV13) into the national Expanded Programme on Immunization in 2011, using a 3+0 schedule (three primary doses

and zero booster doses). With evidence of greater impact of a 2+1 schedule (two primary doses plus one booster dose) in other settings, including South Africa, Malawi's National Immunizations Technical Advisory Group is seeking adequate evidence of the superiority of a 2+1 schedule to inform vaccine policy.

Methods: In this pragmatic health centre–based evaluation comparing the impact of a PCV13 schedule change from 3+0 to 2+1 in Blantyre District, Malawi, 20 government health centres will be randomly selected. Ten health centres will implement a 2+1 schedule, and 10 will continue with the 3+0 schedule. Health centres implementing 3+0 will collectively serve as the direct comparator to determine if the 2+1 schedule provides superior direct and indirect protection against pneumococcal carriage. Pneumococcal carriage surveys will evaluate carriage prevalence among children 15 to 24 months old, randomized at the household level, and schoolgoers 5 to 10 years of age, randomly selected from school registers. Carriage surveys will be conducted 18 and 33 months following 2+1 implementation.

Analysis: The primary end point is powered to detect an effect size reflecting a 50% reduction in vaccine-serotype carriage among vaccinated children 15 to 24 months old, with expected 14% and 7% vaccine-serotype carriage prevalences in the 3+0 and 2+1 arms, respectively.

6.1 Introduction

Streptococcus pneumoniae (the pneumococcus) is commonly carried as a commensal bacterium in the nasopharynx but can cause life-threatening disease. Infections due to the pneumococcus are estimated to be responsible for approximately 300 000 deaths worldwide, with one-third of these occurring among children <5 years of age and with the greatest burden in low- and middle-income countries.[1,2]

S. pneumoniae has 100 known serotypes, with nasopharyngeal (NP) carriage as a prerequisite for the development of disease but also a key process for developing natural immunity.[3] Transmission occurs largely via human respiratory air droplets. With serotype-specific differences, carriage duration decreases with age, lasting from 2 weeks in adults up to 4 months in children.[4,5] Pneumococcal carriage prevalence is age dependent, peaking among children <5 years old. Pneumococcal carriage prevalences reported in sub-Saharan Africa are among the highest described: up to 28% among adults and in excess of 80% among children <5 years old, resulting in high transmission rates.[6-14].

In high-income countries, routine administration of pneumococcal conjugate vaccines (PCVs) through infant immunization schedules has contributed to a rapid decline of vaccine-serotype (VT) invasive pneumococcal disease (IPD) in both vaccinated and unvaccinated populations.[15-23] PCVs protect vaccinated individuals (direct protection) against pneumococcal disease and carriage. The resulting reduction in carriage also interrupts transmission from vaccinated individuals to the unvaccinated population (indirect protection). The resulting herd immunity effect has been a major contributor to the success of vaccination programmes.[24-26] The added cost-effectiveness and vaccine impact gained through indirect protection have been key drivers of vaccination policy in these settings.[27,28] The impact of PCVs on pneumococcal carriage continues to be considered a viable end point in vaccine licensure evaluations.[29]

Studies undertaken prior to PCV introduction in The Gambia, Kenya, Mozambique, Malawi, and South Africa reported VT carriage prevalences ranging from 28% to nearly 50% among children <5 years old.[30-33] While PCV introduction in African countries has resulted in substantial direct effects in terms of reducing the risk of VT IPD,[34] pneumonia, and all-cause mortality[35] among vaccinated children,[10,11,36] the impact of PCV on VT carriage has been markedly less than that observed in high-income countries. Although Kenya,[12] The Gambia,[13] Mozambique,[14] and South Africa[31] have reported VT carriage reductions, carriage prevalence remains higher than expected, and

serotype replacement (i.e., increased non-VT [NVT] carriage prevalence associated with decreased VT carriage) is increasing.[6,37] As the effect of PCV on carriage is considered an indicator of vaccine impact,[29] it remains uncertain whether PCV introduction in sub-Saharan Africa will achieve the sustained direct or indirect protection necessary to reduce pneumococcal carriage to levels sufficient to interrupt transmission and disease.[38]

Currently, the World Health Organization (WHO) recommends the implementation of PCVs using either a 3+0 schedule (three primary doses, most commonly at 6, 10, and 14 weeks of age) or a 2+1 schedule (two primary infancy doses at 6 and 14 weeks of age and one booster at 9 months of age or after the first year of life). The WHO further recommends that the decision on which schedule to use be based on the epidemiology of disease in the local setting.[39] While both schedules have been shown to be effective in reducing VT disease and VT carriage, there have been no direct comparisons of vaccine impact on carriage in a high-burden setting.[40]

A 2017 WHO technical expert consultation on optimization of PCV impact highlighted a substantial research gap in investigating why the impact of PCVs in low-income countries has been less than expected, underlining the need to define an optimal PCV schedule that will maximize the benefits of pneumococcal immunization in such settings.[41] The PCV Review of Impact Evidence (PRIME), commissioned to supplement the WHO expert consultation, is a systematic review of available evidence on PCV effectiveness and impact on NP carriage, disease, and mortality, as well as on PCV immune responses. The review includes evidence from research studies published from January 2010 through December 2016 recorded in 14 databases. Those attending the 2017 WHO technical expert consultation gave head-to-head studies of 2+1 vs 3+0 vaccine schedules the greatest research priority.[42] Countries with high pneumococcal disease and carriage burdens, such as Malawi, could implement 2+1 schedules quickly, with limited logistical or financial demands, providing a booster dose at the time of the first measles vaccine at 9 months of age.

Malawi introduced the 13-valent PCV (PCV13) into the national Expanded Programme on Immunization (EPI) in November 2011, using a 3+0 schedule (with the vaccine administered at 6, 10, and 14 weeks of age), with a three-dose catch-up vaccination campaign among all infants <1 year of age. This introduction has been highly successful, with field studies showing an EPI vaccine coverage exceeding 90%.[43,44] Similar to other settings, Malawi's vaccine introduction resulted in an approximately 70% reduction in IPD among PCV-vaccinated children[34] and an estimated 35% fall in all-cause mortality.[35]

However, high levels of residual VT carriage persist in Malawi among vaccinated children up to 8 years after the introduction of the vaccine.[45] As presented by Lourenço et al,[46] a high force of infection in settings such as Malawi contribute significantly to a 3+0 schedule achieving only a short duration of VT carriage control among infants. Though a 2+1 schedule, as implemented in South Africa, may improve colonization control, this remains unproven in other African settings such as Malawi.

In this context, the Malawi Ministry of Health (MoH) and the National Immunizations Technical Advisory Group (NITAG) are now seeking evidence of adequate superiority of a 2+1 vaccine schedule to inform a change to Malawi's current EPI schedule. To this end, a pragmatic health centre–based evaluation comparing the current 3+0 schedule with a 2+1 schedule will be implemented in Blantyre District, southern Malawi. Two pneumococcal carriage surveys, conducted 18 and 33 months following the implementation of the 2+1 schedule, will have the objective of comparing the 3+0 and 2+1 schedules in terms of their carriage-reducing effects among healthy children and evaluate their potential to enhance herd immunity.

6.2 Methods

6.2.1 Study design

The study is a pragmatic health centre–based randomized evaluation of the direct effect of a 2+1 PCV13 schedule on pneumococcal carriage on vaccinated infants and the indirect effect on vaccine-ineligible children and high-risk adults.

6.2.2 Study setting

The study will be conducted in Blantyre District, southern Malawi. The District is 240 km^2. Healthcare is delivered through a network of private and government hospitals and 28 government primary health centres, where EPI vaccinations are administered. Queen Elizabeth Central Hospital (QECH) is the government referral hospital providing free medical care to the 1.3 million urban, peri-urban, and rural residents of Blantyre District. Children <5 years old account for 16% of the district's total population.[47] Health centres cover a fixed geographic area (hereafter referred to as 'clusters'), and study sampling will be undertaken within these clusters (Figure 6.1).

6.2.3 Study questions

We will investigate whether a 2+1 PCV13 schedule (two primary doses at 6 and 14 weeks of age plus 1 booster dose at 9 months of age), compared with the current 3+0 schedule (three primary doses at 6, 10, and 14 weeks of age with no booster), is superior at reducing

VT carriage prevalence among vaccinated children and, consequently, creating a superior herd effect among older children and HIV-infected adults. To assess the effect of a district-wide change from 3+0 to 2+1, this study will address two specific research questions: Firstly, will a 2+1 schedule provide enduring vaccine-induced protection against pneumococcal VT carriage into the second year of life? Secondly, will the 2+1 vaccine schedule generate stronger herd protection and result in decreased VT carriage among older children and unvaccinated high-risk adults in the general population?

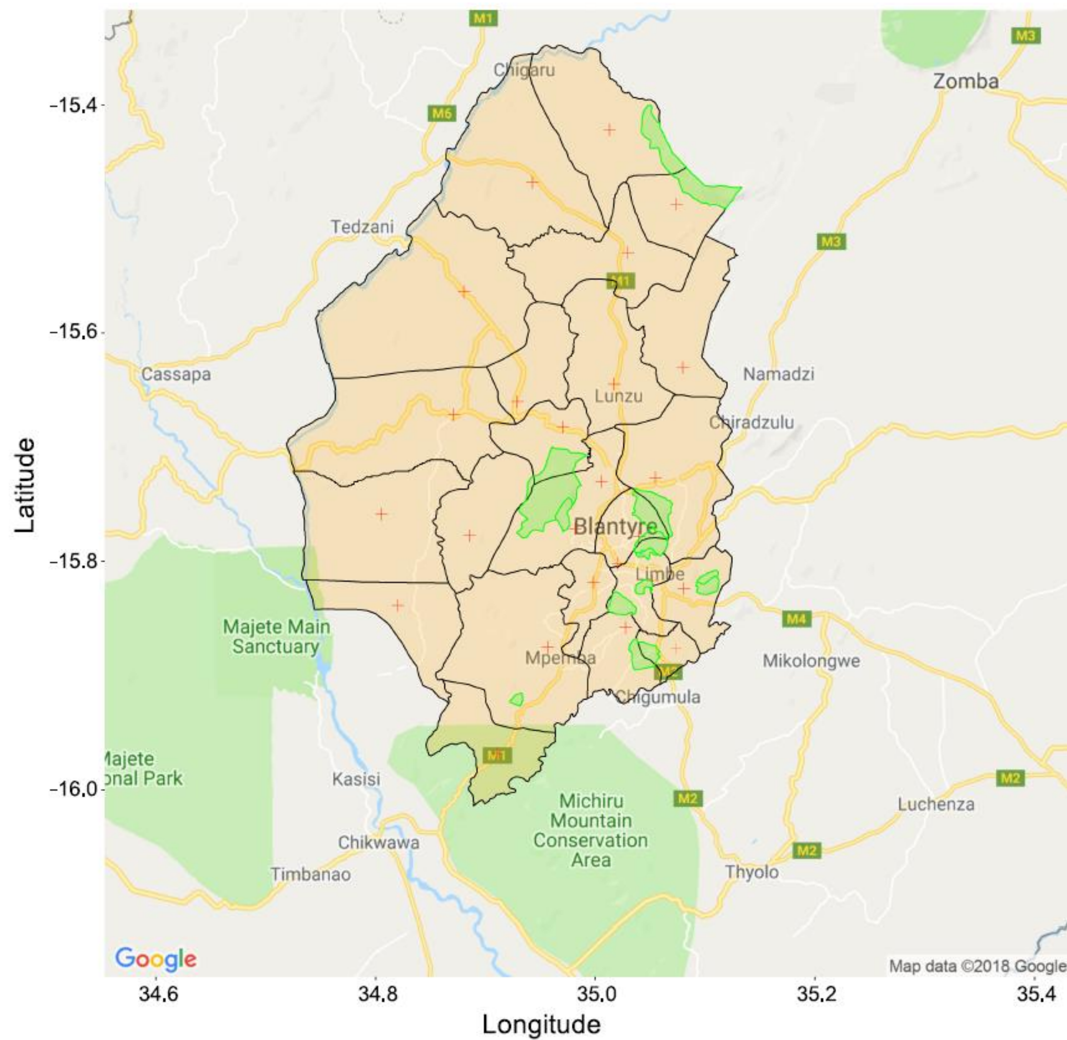


Figure 6.1. Map of Blantyre District with borders of health centre catchment areas. Red crosses (+) indicate locations of health centres offering vaccination trough the Expanded Programme on Immunization. Areas shaded in green are not inhabited (including mountains, industrial zones, and other regions administratively declared not for habitation).

6.2.3.1 Primary objective

The primary objective of the study is to evaluate the direct effect of a 2+1 PCV13 vaccination schedule on VT pneumococcal carriage among children aged 15 to 24 months, 3 years after introducing the 2+1 schedule. This objective will answer the question of whether the 2+1 schedule induces a more enduring direct protection against pneumococcal VT carriage into the second year of life, compared with that observed with a 3+0 schedule.

6.2.3.2 Secondary objectives

The secondary objectives of the study are to evaluate, 3 years after introducing the 2+1 schedule, (1) the indirect effect of a 2+1 schedule on VT pneumococcal carriage among children aged 5 to 10 years (PCV age-ineligible at time of implementing the 2+1 schedule); (2) the indirect effect of a 2+1 vaccination schedule on VT pneumococcal carriage among HIV-infected adults 18 to 40 years old and on antiretroviral therapy (ART).

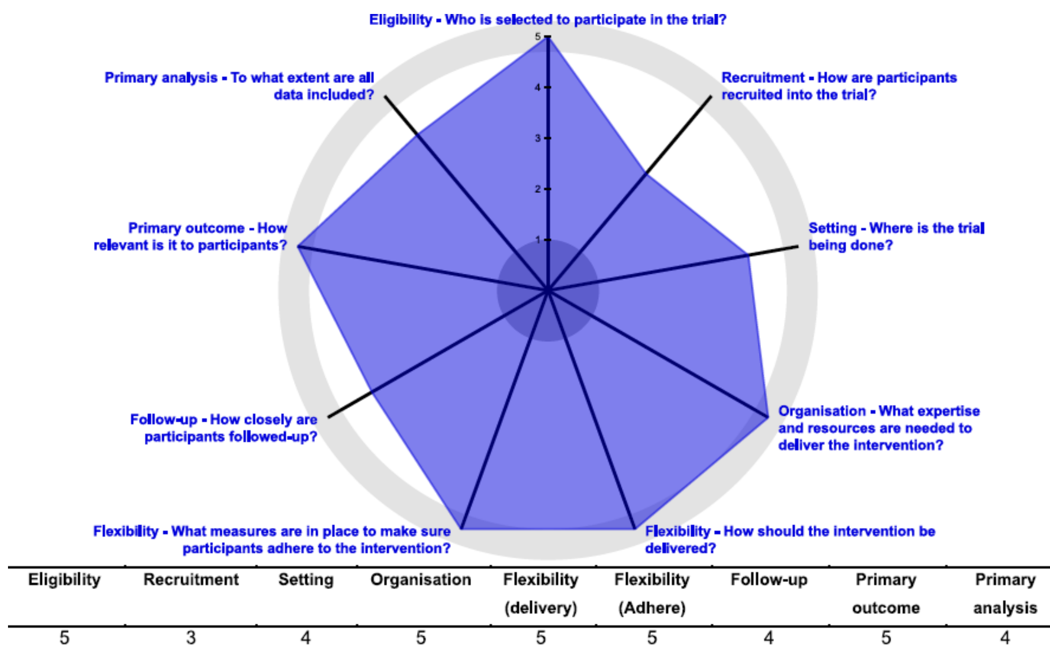
To address these questions, the Malawi MoH and Blantyre District Health Office (DHO) will randomly select 10 health centres (among a total 28 in Blantyre District) in which the routine PCV13 schedule will be switched to a 2+1 schedule. An additional 10 health centres will be randomly selected to continue with the current 3+0 schedule but will collectively serve as the direct comparator for evaluating the effectiveness of the 2+1 schedule.

6.2.4 A pragmatic study design

This switch to a 2+1 schedule is an initiative led by the MoH and will be implemented within the scope of the routine EPI, subject to EPI standard procedures for delivery, monitoring, and performance assessment. All vaccination activities will be implemented, as per routine practices, by MoH EPI vaccinators. The MoH will monitor the completeness of dosing following standard reporting practices within the scope of the EPI. Routine study activities throughout the duration of the 3-year study period will include research nurses providing support and guidance to the EPI vaccination teams through weekly site visits. Additionally, research enumerators will monitor patient-retained health passports of a representative sample of vaccine recipients to confirm that they are receiving the correct vaccine schedule (2+1 or 3+0) assigned to the catchment population of their respective health centres.

The pragmatic design of this study has been assessed through the PRECIS-2 tool (PRagmatic-Explanatory Continuum Indicator Summary 2).[48] This tool was developed

to help trialists make design decisions consistent with the intended purpose of their trial. To facilitate domain discussion and consensus, PRECIS-2 assesses pragmatic designs through nine domains—eligibility criteria, recruitment, setting, organization, flexibility (delivery), flexibility (adherence), follow-up, primary outcome, and primary analysis—scored from 1 (very explanatory) to 5 (very pragmatic). The report from this tool is in the format of a wheel (Figure 6.2). The PRECIS-2 results for this study (mean score, 4.4; range, 3-5) indicate that it is largely a pragmatic randomized study undertaken in the 'real world' with usual care and that it is intended to help support a decision on whether to deliver an intervention.



Eligibility	Recruitment	Setting	Organisation	Flexibility (delivery)	Flexibility (Adhere)	Follow-up	Primary outcome	Primary analysis
5	3	4	5	5	5	4	5	4

Figure 6.2. The PRagmatic-Explanatory Continuum Indicator Summary 2 (PRECIS-2) wheel with study-specific scores.

6.2.4.1 A 3+1 schedule during the early initiation period

To ensure the 2+1 schedule is implemented efficiently, and to limit the risk of confusion during the transition to the 2+1 schedule (including not deferring PCV13 vaccination to a later visit), a 3+1 schedule will be implemented during the first several months at health centres implementing the 2+1 schedule. At these health centres, children who have received either their 10-week or 14-week PCV13 dose before 2+1 implementation will receive a dose at 14 weeks (third PCV13 dose) and 40 weeks (fourth PCV13 dose). Approximately 6 months after 2+1 implementation, all first-contact vaccine visits (after 2+1 implementation) will be for the scheduled visit at 6 weeks of age, and the 3+1 schedule will no longer be required.

6.2.4.2 Carriage surveys and sampling frame

The study will include two cross-sectional carriage surveys, implemented 18 and 33 months after the switch to 2+1. Carriage surveys will be conducted using well-established methods implemented extensively in the setting,[45,46] allowing comparability of study results to previous carriage surveys conducted in the area. The sampling frame is illustrated in Figure 6.3.

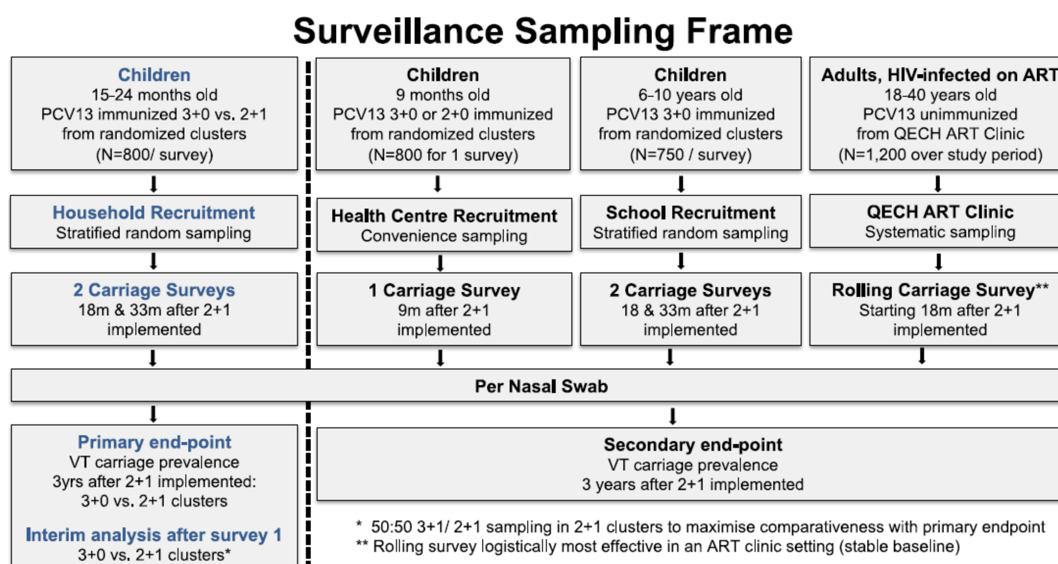


Figure 6.3. Carriage surveillance sampling frame. The black dashed vertical line separates primary (left of dashed line) from secondary study objectives. QECH, Queen Elizabeth Central Hospital; PCV, pneumococcal conjugate vaccine; ART, antiretroviral therapy

Sampling will include: (1) children 15 to 24 months of age who will have received PCV13 (confirmed by reviewing patient-retained health passports) with either the 2+1 or the 3+0 schedule. Evaluation within this age group will allow us to evaluate for our primary objective: the direct effect of vaccination on carriage. Measuring the direct effect of vaccination on carriage reduction within any age group is challenging, given the difficulty of disentangling the contributions of the direct vs indirect effects of vaccination. However, health centres in this study were randomly selected to maximize homogeneity between study arms, including in terms of routine EPI coverage (and, thus, indirect effects associated with PCV13). As such, we largely attribute the reduction in VT prevalence in the 2+1 arm to a direct effect of the booster dose.

Within each of the catchment areas of the 20 participating health centres, we will apply the random walk method to systematically identify households with eligible children. An 'egg and yolk' strategy will be applied, defining two geographic sampling perimeters around

each health centre. Sampling will prioritize those living within the geographic perimeter closest to the health centre. If recruitment targets are not met, sampling will move into the second geographic perimeter. This strategy will maximize the likelihood of recruiting children who have received their vaccines at the selected health centre, minimizing recruitment from buffer zonal borders and, therefore, minimizing risk of contamination (i.e., recruitment of children receiving a PCV13 vaccine schedule different than the schedule assigned to the child's local health centre).

(2) Children 5 to 10 years of age, vaccinated with 3+0 PCV13 schedule. These children, having received PCV13 in infancy, will be recruited from government schools. Evaluation within this age group will allow us to address a secondary objective: evaluating the indirect effect of a 2+1 schedule on VT pneumococcal carriage among children aged 5 to 10 years, the majority of whom will have received PCV13 in infancy using a 3+0 schedule. Six schools will be selected, three located centrally in each of the 3+0 and 2+1 clusters. Children will be chosen at random from school registers. This component will allow for the evaluation of indirect effects of the 2+1 schedule compared with the 3+0 schedule among children with waning vaccine-induced immunity.

(3) Adults 18 to 40 years of age, living with HIV and taking ART, with no history of receiving PCV13. Evaluation within this group will allow us to address the secondary objective of evaluating the indirect effect of a 2+1 schedule on VT pneumococcal carriage among HIV-infected adults 18 to 40 years old on ART—a population at high risk of pneumococcal disease. Participants will be recruited from the QECH Lighthouse ART Clinic in Blantyre.

6.2.4.3 Safety: carriage prevalence among children receiving two vs three primary doses

To assess the differential effects of three vs two primary vaccine doses in the first year of life, an additional carriage survey will be implemented at health centres approximately 9 months after 2+1 implementation. A convenience sample of children aged 9 months will be recruited at health centres providing either the 2+1 or 3+0 schedule. This will allow for an evaluation of carriage prevalence among children receiving three (according to the 3+0 schedule) vs two (according to the 2+1 schedule and presenting for their booster dose) primary doses. Additionally, this additional carriage survey contributes to evaluating the coverage of the 2+1 vaccination schedule and the proportion of children receiving all three PCV13 doses at health centres providing the 2+1 schedule.

6.2.4.4 Inclusion and exclusion criteria

The inclusion criteria for all individuals to be enrolled include permanent residence in Blantyre District. For children 15 to 24 months of age (assigned to either the 2+1 or 3+0 schedule), additional inclusion criteria are as follows: age 15 to 24 months, parent/legal guardian providing written informed consent, evidence (recorded in health passport) of having received a full schedule of PCV13. For children 5 to 10 years of age, the additional inclusion criteria are: age 5 to 10 years old, parent/legal guardian providing written informed consent, providing written informed assent (if the child is aged ≥8 years), and either verbal or documented evidence of having received PCV13. For adults, the additional inclusion criteria are: age 18 to 40 years, providing written informed consent, being HIV infected, and receiving ART.

Among children 9 months of age, recruited as part of the safety component, the additional inclusion criteria are: age 9 months, parent/legal guardian providing written informed consent, and evidence (health passport documentation) of having received either a full 3+0 PCV13 vaccine schedule (at health centres implementing 3+0) or both primary doses of PCV13 at approximately 6 and 14 weeks of age (at health centres implementing 2+1).

The exclusion criteria for all screened individuals consist of the following: ongoing tuberculosis treatment at the time of screening, hospitalization for pneumonia within 14 days prior to study screening, and terminal illness. The exclusion criteria for all children are as follows: parental/legal guardian not providing consent, not providing assent (for children aged ≥8 years), and having received antibiotic treatment within 14 days prior to study screening. The exclusion criteria for adults are as follows: not providing written informed consent or prior vaccination with a pneumococcal vaccine.

6.2.4.5 Intervention

The intervention will consist of two carriage surveys conducted 18 and 33 months after the 10 randomized health centres switch from a 3+0 to a 2+1 PCV13 schedule. In both surveys, a single NP swab will be collected from each participant. Following previously described WHO-recommended procedures,[45,49] these will be collected and taken to the laboratory at the Malawi-Liverpool-Wellcome Trust Clinical Research Programme in Blantyre for the isolation and characterization of *S. pneumoniae*. VT pneumococcal carriage will be determined by latex agglutination. Samples from participants with confirmed

pneumococcal carriage will be sent to the UK for assessment of multiple-serotype carriage (genomic microarray) and for whole-genome sequencing (WGS).

6.2.4.6 Expected outcomes

The primary end point of this study will be the difference in VT pneumococcal carriage prevalence among children aged 15 to 24 months, comparing those vaccinated with PCV13 in either a 2+1 or 3+0 schedule, 3 years after implementing the switch to a 2+1 schedule. Additionally, the study will evaluate four secondary outcomes: (1) the difference in VT carriage prevalence among children 5 to 10 years old, 18 months and 33 months after 2+1 implementation; (2) VT carriage prevalence among HIV-infected adults aged 18 to 40 years and receiving ART at the time of sampling; (3) VT carriage prevalence among infants aged 9 months, who will have received three primary doses (at 3+0 health centres) or two primary doses (at 2+1 health centres) prior to the booster dose, 9 months after the implementation of the 2+1 schedule; and (iv) prevalence of multiple-serotype carriage, 18 and 33 months after 2+1 implementation.

6.2.4.7 Patient and public involvement statement

Before the development of this protocol, key stakeholders were informed of the study, including the personnel at the study sites (i.e., selected health centres) and members of their surrounding communities (i.e., catchment areas), the Blantyre DHO, the MoH, and the Ministry of Education. We actively sought and incorporated input from these stakeholders into the study objectives and overall design. Community sensitization will be further strengthened through a community advisory board.

6.2.4.8 Informed consent process

The study will only recruit children whose parents/legal guardians have the capacity to provide informed consent; similarly, among adults, only those who can provide consent will be eligible. Minors ≥8 years of age will be required to provide informed assent in addition to their parents/legal guardians providing informed consent. Participants will receive both verbal and written information about the study and will be given the opportunity to ask questions and express their doubts and concerns before accepting to take part. They will also be given time to reflect before they come to a decision. An informed consent and/or assent form will be signed and dated by the participant and a member of the research team. The participant will keep a copy of the document, and a second copy will

be kept in the study file with the principal investigator based in Blantyre. Participants will be informed of their right to withdraw consent at any point until the study ends, without the need to provide a reason and without penalty.

6.2.5 Statistical methods

6.2.5.1 Study power and sample size calculation

The primary end point is powered to detect an effect size reflecting a 50% reduction in VT carriage, with expected 14% and 7% VT carriage prevalences among vaccinated children 15 to 24 months old in the 3+0 and 2+1 arms, respectively. Sample sizes were calculated based on a power of 80% and a statistical significance of 0.05. The calculations accounted for household similarities using an intraclass correlation coefficient (ICC) of 0.005 (based on previous experience in this setting) and adjusted for a design effect of 1.21 (dependent on both ICC and cluster size). The minimum sample sizes needed to achieve the necessary power under these assumptions are shown in Table 6.1.

Table 6.1. Sample size estimations

ICC	p1	p2	Power	Alpha	DE	n, per arm	Clusters, per arm	Clusters, total	n, Total
0.005	0.14	0.07	0.8	0.05	1.20	358	9	18	720
0.005	0.14	0.07	0.8	0.05	1.21	400	10	20	800
0.005	0.14	0.07	0.8	0.05	1.22	366	9	18	810
0.005	0.14	0.07	0.8	0.05	1.25	373	8	16	800
0.005	0.14	0.07	0.8	0.05	1.27	381	7	14	770
0.005	0.14	0.07	0.8	0.05	1.30	388	7	14	840
0.010	0.14	0.07	0.8	0.05	1.39	417	11	22	880
0.010	0.14	0.07	0.8	0.05	1.44	432	10	20	900
0.010	0.14	0.07	0.8	0.05	1.49	447	9	18	900
0.010	0.14	0.07	0.8	0.05	1.54	462	9	18	990
0.010	0.14	0.07	0.8	0.05	1.59	477	8	16	960
0.015	0.14	0.07	0.8	0.05	1.59	475	12	24	960

DE, design effect; ICC, intraclass correlation coefficient

To assess the primary and secondary end points, (1) one child 15 to 24 months of age (2+1 or 3+0) will be recruited from each of 40 households randomly selected within each of the 20 clusters. This equals a total 800 vaccinated children per survey (1600 total for two surveys). (2) 125 children will be recruited from each of the six schools per survey. This equals a total 750 children per survey (1500 total). (3) A total of 1200 HIV-infected adults will be recruited from the QECH ART clinic over the course of the 3-year study period. (4)

A total of 800 children 9 months of age will be recruited from the vaccination centres 9 months after the schedule change (800 total).

6.2.5.2 Data collection, management, and anonymization procedures

Demographic and relevant medical history data will be collected using password-protected electronic data capture. Each participant will be assigned a unique participant identification number (PID) at recruitment. This PID will be used for all datasheets and files, and it will be linked to the laboratory data; hence, only anonymized data will be used for the analysis. Fully anonymized data will be uploaded daily to a secured on-site server, which is backed up daily to both local and off-site facilities. A logbook containing identifiable information (including names) will be kept separate in a secured location by an authorized member of the study team and will only be accessed by authorized study members. This will allow the study team to recover any missing epidemiological information later (e.g., missing vaccination dates) and to facilitate any participants who choose to withdraw consent at any time.

6.2.5.3 Statistical analysis

Continuous variables will be summarized as means and standard deviations, or medians and interquartile ranges if the distribution is skewed. Categorical variables will be summarized by frequency distributions. The direct effect of the PCV13 schedule change on VT carriage will be evaluated by comparing VT carriage prevalence among children aged 15 to 24 months residing in the recruitment clusters of health centres randomized to the 2+1 schedule with the prevalence among children in the same age group in recruitment clusters of health centres randomized to the 3+0 schedule. . The indirect effects of a booster dose on children 5 to 10 years old and HIV-infected adults will be investigated by comparing VT carriage prevalences among these groups in the recruitment clusters of health centres randomized to the 3+0 schedule with carriage prevalences of corresponding groups in the recruitment clusters of health centres randomized to the 2+1 schedule. Additionally, the data obtained in this study will be compared with those obtained from previous carriage surveys to ascertain any changes in VT carriage prevalence before and after the PVC13 schedule change. Statistical tests will be selected depending on the data distribution patterns. Potential confounders and sources of interaction (including age, gender, and health centre) will be identified by testing the associations between variables and VT carriage and included in the multivariable models when $p<0.1$. Sensitivity analyses will include assessing the impact of the following factors on VT prevalence and on the VT distribution: (1) receiving only one, only two, or all three PCV doses; (2) having document-

confirmed PCV vaccination; or (3) schedule adherence to within 2 weeks of each scheduled dose.

6.2.6 Data monitoring external advisory group

The implementation of the study protocol will be reviewed and monitored by an external advisory group, providing oversight of study activities and advising the study team at the times of the interim and final analyses. The group will include experts from the Kamuzu University of Health Sciences (formerly the University of Malawi College of Medicine), the London School of Hygiene & Tropical Medicine, and the Medical Research Council.

6.2.7 Interim analysis and changes to public health policy

Upon completion of the first carriage survey, an interim analysis will be implemented using data obtained on the VT carriage prevalence among vaccinated children 15 to 24 months of age. These results will be used to consider possible adaptations before the second community carriage survey. Three possible scenarios are considered: (1) if convincing evidence of a major change in carriage prevalence is demonstrated, a plan for immediate action to move to change the schedule and adapt year-3 sampling will be discussed with the MoH; (2) if no major change in carriage prevalence is identified, the study will continue as planned; (3) if carriage prevalence has fallen by more than 30% but does not meet the primary threshold of 50%, the second carriage survey will be brought forward by 6 months. Decisions will be reached in consensus between investigators, the expert advisory board, and the MoH.

6.2.8 Dissemination policy and plans

Study results will be shared with local stakeholders and published in peer-reviewed journals. Partial results and interim analyses will be shared with the Malawi MoH and other relevant policymakers and decision-making stakeholders. Partial and final findings will be presented at relevant international conferences and meetings. Copies of all published materials and reports will be shared with the research ethics committees and collaborators. We will return to the community partners and work with the community advisory board to report and further disseminate our results to those communities in which we worked.

6.2.9 Data availability

Data used in analyses for reported findings of this study will be made publicly available on Figshare (https://figshare.com), an online open-access repository.

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Chapter 7. Conclusions and further work

In this thesis, I have combined epidemiology and modelling to describe the impact of the 13-valent pneumococcal conjugate vaccine (PCV13) on pneumococcal carriage and invasive pneumococcal disease (IPD) in Blantyre, Malawi, an urban sub-Saharan setting. This work aimed to evaluate, in a setting with high pneumococcal carriage and high HIV seroprevalence, the impact of a three-primary-dose PCV13 schedule on (1) reducing vaccine-serotype (VT) carriage and disease and (2) serotype replacement. The work focused on at-risk populations, including (1) PCV13-vaccinated children, in whom vaccine-induced immunity begins to wane after the first year of life; (2) PCV-unvaccinated children, with no vaccine-induced direct protection and potentially limited indirect protection; and (3) HIV-infected adults on antiretroviral therapy (ART), who do not routinely receive pneumococcal vaccination and are at high risk for developing IPD. In this chapter, I review the findings of this thesis in the context of this aim and suggest directions for future work.

7.1 Summary of findings

In chapter 3, I report on a population-based assessment of pneumococcal carriage that surveyed potential reservoir populations from 3.6 to 7.1 years after the introduction of routine PCV13 in Malawi. Despite evidence of a reduction in VT carriage over the study period, there was high persistent residual carriage among PCV-vaccinated children (16.1% relative reduction from 19.9%-16.7%), PCV-unvaccinated (older, age-ineligible) children (40.5%; 26.4%-15.7%) and HIV-infected adults on ART (41.4%; 15.2%-8.9%). To minimize confounding, adjusted prevalence ratios (aPRs; adjusted for age) were calculated on a narrower age range within each study group (Table 3.2). Among children 3 to 5 years old (PCV vaccinated), the aPRs over the 3.5-year study were 0.919 (95% confidence interval [CI], 0.845-0.999; p=0.047) for VT carriage and 0.978 (95% CI, 0.944-1.013; p=0.208) for non-VT (NVT) carriage. Among children 6 to 8 years old (PCV unvaccinated), the aPRs were 0.919 (95% CI, 0.845-0.999; p=0.047) for VT carriage and 0.978 (95% CI, 0.944-1.013; p=0.208) for NVT carriage. Among HIV-infected adults on ART, the aPRs were 0.831 (95% CI, 0.735-0.938; p=0.003) for VT carriage and 0.963 (95% CI, 0.895-1.036; p=0.307) for NVT carriage. Despite a statistically significant relative reduction in VT prevalence over the 3.5-years, residual carriage was higher than what has been reported in high-income settings.

Non-linear regression analysis, with carriage data censored below 3.6 years of age, was used to investigate the individual probability of VT carriage as a function of a child's age. The probability of VT carriage declined with age at similar rates for both vaccinated and unvaccinated children (Figure 3.4). The estimated population-level half-lives (derived from individual carriage probability data) of VT carriage prevalence among vaccinated and unvaccinated children were 3.34 years (95% CI, 1.78-6.26 years) vs 3.26 years (2.42-4.38 years), respectively. It is proposed that a comparable VT half-life at 3.6 years of age is due to a limited role of vaccine-induced immunity that started to wane, perhaps within the first 6 to 12 months of life.[1,2] However, of particular interest was the fact that the population-averaged effect of not receiving PCV more than doubled (β=2.15 [95% CI, 1.47-2.83]) an individual's probability of VT carriage at 3.6 years of age (i.e., age below which carriage data were censored; Figure 3.4 and Table 3.3). From this, it appears that vaccine-induced protection, despite evidence of early waning of vaccine-induced immunity, does provide a longer-term benefit to this population in providing a lower VT carriage setpoint at an age when naturally acquired and herd immunity begin to play greater roles in controlling pneumococcal carriage. A lower carriage setpoint among vaccinated children is of benefit in both direct protection and in reducing transmission.

In chapter 5, recognizing that carriage is a prerequisite for disease, we evaluated the impact of Malawi's 2011 introduction of PCV13 on disease, examining its impact on VT and NVT IPD among vaccine age-eligible and vaccine age-ineligible children and adults. We conducted prospective surveillance for IPD from which we derived incidence rates to infer vaccine impact (population reduction in disease incidence).

In a setting where the IPD incidence was already declining, we used robust long-term, hospital-based surveillance to show a substantial additional decline in VT IPD incidence following PCV13 introduction. Compared with the vaccine-era counterfactually predicted VT IPD incidence (i.e., in the hypothetical absence of the vaccine), there was a substantial additional decline in VT IPD incidence following PCV13 introduction among children in both the 1- to 4-year (74%; 95% CI, 70%-78%) and 5- to 14-year (79%; 95% CI, 76%-83%) age categories. Importantly, we also report a more modest, non-significant decline in VT IPD incidence among infants <1 year old (38%; 95% CI, 37%-40%) and persons aged ≥15 years (47%; 95% CI, 44%-51%). In terms of absolute IPD case numbers from 2006 to 2018, there was a reduction in all age groups in both VT and NVT IPD. When aggregating all ages, for example, there was a reduction in VT IPD from 120 confirmed IPD cases in 2006 to 13 in 2018. Similarly, the NVT IPD frequency decreased from 102 to 14.

Chapter 5 also reports on a case-control study of vaccine effectiveness (VE) conducted between 7 October 2011 and 27 June 2016. Conditional logistic regression of PCV13 receipt among VT IPD patients and their matched controls yielded an odds ratio of 0.19 (95% CI, 0.02-1.74; p=0.14), equivalent to a VE of 80.7% (95% CI, −73.7 to 97.9). However, as has been observed elsewhere,[3] the power of the case-control analysis was undermined by high levels of vaccine uptake (the CIs are wide and include 1). The VE point estimate derived from the case-control analysis suggests a level of protection among vaccine age-eligible children comparable with other post-introduction studies in similar populations.[4,5]

Given the importance of this work (i.e., evaluating PCV impact on VT pneumococcal carriage and disease) to informing national and international vaccine policy, it is critical to implement robust and site-appropriate techniques to assess the serotype distribution associated with pneumococcal colonization and disease. Chapter 4 describes, in the context of the PCVPA (Pneumococcal Carriage among Vulnerable Populations in Africa) study (reported in chapter 3), a high level of concordance between three serotyping methods commonly used during ongoing routine pneumococcal surveillance activities in our lab: latex agglutination, DNA microarray, and serotyping by sequencing with PneumoCaT (Pneumococcal Capsular Typing; https://github.com/phe-bioinformatics/PneumoCaT; Public Health England, London, UK). The work also addresses factors for researchers, reference laboratories, and policymakers to consider when deciding which assay to implement in their local settings. While the latex agglutination serotyping method was better suited for the Malawi setting, in terms of costs and operational investment (i.e., implementation and maintenance), there was also high concordance in terms of serotype detection between the three methods analysed. Concordance values were 90.7% (95% CI, 89.0%-92.2%) between latex and PneumoCaT, 95.2% (95% CI, 93.9%-96.3%) between latex and microarray, and 96.6% (95% CI, 95.5%-97.5%) between microarray and PneumoCaT.

Though latex agglutination is highly accurate and requires the least input (expertise and resources) for implementation and analysis, it is not optimal for a fully nuanced surveillance of vaccine impact. The primary limitations of latex agglutination are its challenges in detecting multiple-serotype carriage and serotypes in low relative abundance. Though significantly more costly than latex agglutination, microarray has the advantage of reporting multiple-serotype carriage, including differentiation of all 100 serotypes and the relative abundance of each serotype detected. This allows microarray to detect VT pneumococcus carried at low relative abundance, often undetected by purely

microbiological methods, such as latex agglutination. In this work, by detecting additional VT pneumococcus carried in low relative abundance (median, 8%), microarray increased VT detection by 31.5% (all ages aggregated) compared with latex serotyping. This was entirely due latex reporting NVT for a sample that also carried VT in low relative abundance. Among samples with multiple-serotype carriage, latex identified the dominant serotype in 85.3% of samples. Despite the overall increase in detection of VT carriage, the proportion of individual VT serotypes detected did not differ significantly when comparing microarray with latex.

Chapter 6 presents the protocol for the PAVE study, a pragmatic study that begins to evaluate alternative strategies to improve the impact of PCV in Malawi and similar settings. The PAVE study was designed to evaluate the impact of an alternative schedule for PCV13 administration within Malawi's current Expanded Programme on Immunization, comparing the impact of a 2+1 schedule vs the current 3+0 schedule in terms of their pneumococcal carriage–reducing effects. The study design was assessed through the PRECIS-2 (PRagmatic-Explanatory Continuum Indicator Summary 2) tool, developed to evaluate a study's pragmatic design through nine domains, each weighted equally and scored from 1 (very explanatory) to 5 (very pragmatic). The PRECIS-2 results (mean score, 4.4; range, 3-5) for the PAVE study indicate that it is a largely pragmatic randomized study undertaken in the 'real world' with usual care and that it is intended to help support a decision on whether to deliver an intervention. The pragmatic design includes strong collaboration with the Malawi Ministry of Health, leveraging and strengthening existing infrastructure to ensure that such an evaluation is feasible and that it will contribute to fulfilling the national research agenda. This design has several advantages, including better alignment with the Malawi national research agenda, greater opportunities for capacity strengthening, and lower study costs.

The PAVE study was a response to a 2017 World Health Organization (WHO) technical expert consultation on optimization of PCV impact. The consultation highlighted a substantial research gap in investigating why the impact of PCVs in low-income countries has been less than expected, underlining the need to define an optimal PCV schedule that will maximize the benefits of pneumococcal immunization in such settings.[6] Those attending the 2017 WHO technical expert consultation gave head-to-head studies of 2+1 vs 3+0 vaccine schedules the greatest research priority.[7] Countries with high pneumococcal disease and carriage burdens, such as Malawi, could implement a 2+1 schedule quickly, with limited logistical or financial demands, providing a booster dose at the time of the first measles vaccine at 9 months of age.

7.2 Limitations

The limitations of the individual studies contributing to this thesis are reported in the Discussion sections of the respective chapters. Some recurring themes can be identified, relating to the study designs, analyses, sampling frames, laboratory analyses, and confounding of other public health initiatives.

All epidemiological studies presented here are observational studies. No randomized controlled trials (RCTs) were conducted as part of this PhD thesis. Observational studies are more prone to bias than RCTs because treatment is not provided at random. RCTs could have been used to give us a more robust estimate of the impact of PCV13 vaccination on pneumococcal carriage and IPD. For instance, a cluster-RCT design could have been implemented before the national roll-out of PCV13 administration to investigate changes in pneumococcal carriage among vaccinated individuals. However, there are limitations to implementing RCTs to answer these questions. The pneumococcus first colonizes the nasopharynx and then only occasionally spreads to the surrounding tissues to cause mucosal disease or a disseminated invasive disease. This kind of pathogenesis has implications for vaccine efficacy studies with disease end points. Efficacy studies would need to include much larger study samples over longer periods to find out whether the vaccine protects against IPD, for example. Furthermore, though a similar approach was used in recent and ongoing evaluations of the RTS,S malaria vaccine, this would have been deemed to be unethical given the widespread routine use and proven high efficacy of PCV13.

Carriage and disease surveillance studies, as reported in chapters 3 and 4, respectively, are often conducted over relatively short time frames to facilitate a better understanding of long-term temporal trends. For this reason, statistical analyses can be limited in their ability to disentangle the effects of calendar time and age since vaccination. Regarding the chapter 3 carriage prevalence results based on rolling cross-sectional surveys, there are multiple causes contributing simultaneously to the change in VT prevalence over time. These include (1) the age structure of the groups, (2) the proportion of PCV-vaccinated children within each group, and (3) the indirect effects associated with cumulative population uptake. While the analysis described in chapter 3 worked to control for these factors, it is important to acknowledge that it is not feasible to fully disentangle these concurrent influences.

It is also possible that readjustment of carriage dynamics (VT and NVT prevalence, as well as serotype-specific trends) occurred between PCV introduction and the first carriage survey.

As reported elsewhere,[8,9] a major challenge in field-based surveillance studies is ascertainment of vaccine coverage. Measurement of vaccination status depends predominantly on health passports, with limited capacity for verification of either recorded or reported vaccination status. However, our reported high coverage was concordant with vaccine coverage data reported by other studies in Malawi, and any misclassification is, therefore, likely to be small and would not significantly change the findings.

Although there are data from before the PCV era originating from elsewhere in Malawi, there are no equivalent historical carriage data for urban settings in Malawi derived from the same sampling frame used in chapter 3. However, this does not detract from the finding of high levels of residual VT carriage in these reservoir populations. Given evidence that more sensitive serotyping methods that detect multiple serotype carriage (e.g., by DNA microarray) will increase VT carriage estimates, our carriage prevalence data likely underestimate the true residual VT prevalence levels.

7.3 Conclusions and future research priorities

Ultimately, the primary measure of success in any vaccine programme is reduction in disease burden. While data presented in this thesis indicate persistent carriage, there is evidence of reduction in carriage and, more importantly, significant reductions in IPD. In terms of absolute IPD case numbers from 2006 through 2018, for example, there were reductions in all age groups in both VT and NVT IPD. Although NVT IPD events now outnumber VT IPD events, the absolute numbers of such events are low, and the overall burden of all-cause IPD has declined.

It is challenging to use observational data to attribute vaccine causality to PCV-era declines in carriage and IPD incidence. The duration and scale of the downward trend in IPD incidence in Malawi before vaccine introduction is an important finding and missing from many studies in Africa. While declines in all-serotype IPD were observed well before PCV13 introduction, the data suggest a definitive additional benefit of vaccination. However, it is likely that several concurrent non-vaccine interventions also contributed, including health-improving measures, such as availability of ART, rotavirus vaccination, improved control of malaria, and improved nutrition security. The cumulative impact of these public health measures on reducing the overall burden of IPD caused by any

pneumococcal serotype underlines the fact that no one public health intervention (including vaccines) is adequate to significantly reduce disease burden. Rather, it is optimal to implement a strategy involving multiple public health interventions, including a pneumococcal vaccine that provides both direct and indirect protection.

We have worked to develop methodologies to mitigate the identified challenges and weaknesses associated with using observational data to attribute vaccine causality. For the PAVE study (described in chapter 6), for example, we have outlined a pragmatic cluster randomized design in an attempt to increase methodological control. Our conceptualization of the study design was largely influenced by our acknowledgement that it is difficult to control for several confounding variables, and we have set out to control such factors from the beginning of the PAVE study. Though it will retain some weaknesses (including the risk of irregular clusters and contamination), the PAVE study design will improve the monitoring of these factors and benefit future investigations of the relative benefits of alternative vaccine schedules.

Alternative methodological approaches to further avoid the limitations of observational data could include annual serological assessments. This could be informative while (1) avoiding the often-inevitable weaknesses of observational work in collecting robust data (as discussed above) and (2) limiting the costs involved in managing the large teams required for ongoing community surveillance based on nasopharyngeal carriage endpoints. We have recently undertaken serological surveillance in Blantyre, showing clear vaccine-induced, serotype-specific anticapsular immuloglobulin G (IgG) antibody profiles according to age groups. [10] Such approaches, exemplifying efficiency in leveraging for annual surveillance of multiple vaccine-preventable diseases, should be considered as a way forward for serosurveillance.

In light of these findings, I highlight several priority areas for further research. Nasopharyngeal samples collected for this PhD thesis are being used in several collaborations with national and international partners. Though outside the scope of this thesis, whole-genome sequencing was performed on samples collected. This has led to two ongoing projects: (1) a report on the complete genome sequence of *Streptococcus pneumoniae* serotype 1 and (2) work to show that, beyond serotype replacement, there are marked changes in *S. pneumoniae* carriage population genetics characterized by metabolic genotypes with distinct virulence gene profiles and antimicrobial resistance profiles. Carriage data have also been shared with the global PCV carriage impact project: “Pneumococcal carriage serotype replacement post–PCV immunization – a global

evaluation (P-CARRIAGE). This is a global initiative to gather and analyse the global evidence base on the impact of PCV7, PCV10, and PCV13 on serotype-specific carriage.

7.3.1 Pneumococcal vaccination for high-risk adult populations

It is possible that the improved survival of HIV-infected persons on ART contributes to residual VT pneumococcal carriage and transmission in the community, further reducing potential indirect vaccine effects. Adults at particularly high risk of pneumococcal colonization and disease (including those living with HIV) or those whose pneumococcal acquisition is derived from other adults, may benefit less from an infant schedule.[11] Given the higher risk of IPD, ongoing burden of pneumococcal pneumonia,[12] and the evidence that PCV protects HIV-infected adults from recurrent VT pneumococcal infections,[13] targeted vaccination benefitting this at-risk population should be reconsidered in this context. Certainly, in settings similar to Malawi, there is a dearth of robust cost-benefit data on PCV introduction within an adult population that would inform national vaccine policy. A systematic review by Cafiero-Fonseca et al[14] further highlights this need for more robust empirical accounting of the full benefits of adult pneumococcal vaccination. Moreover, within the paucity of studies capturing broad health gains and healthcare cost savings, a small proportion has considered additional benefit categories, such as productivity gains.

7.3.2 Maternal vaccination

The PCVs have now been rolled out internationally, including in an increasing number of low- and middle-income countries. PCVs have been highly effective at preventing VT carriage and disease among infants. However, a window of susceptibility remains before the first vaccination at approximately 6 weeks of age.[15] Whether receiving PCV under a 3+0 or a 2+1 schedule, infants are not directly protected against pneumococcal disease—as a result of active immunization—until approximately 2 weeks after the primary schedule has been completed. In evaluating the impact of PCV13 on the incidence of IPD among infants less than 90 days of age in Malawi, Koenraads et al[16] found that VTs were the main cause of IPD among neonates and young infants, both before and after PCV13's introduction. Herd protection against IPD in newborns, before the age of first vaccination, has been demonstrated in high-income settings.[17] Further strategies need to be considered and further evaluated to protect this vulnerable population, including maternal or neonatal immunization.

7.3.3 Global serotype-specific sentinel surveillance

Serotype-specific surveillance for IPD is essential for assessing the impact of PCV10 and PCV13. At present, the role of carriage studies in evaluating vaccine impact and monitoring serotype replacement is not well defined. The global pneumococcal research community should work to systematically implement a coordinated sentinel surveillance system. A number of initiatives to gather and review global data on the impact of PCV on carriage and IPD have highlighted the dearth of such data, especially from low- and middle-income countries. These include (1) the Pneumococcal Serotype Replacement and Distribution Estimation (PSERENADE) project, aimed at evaluating global evidence to estimate the impact of PCV10 and PCV13 by age, product, schedule, and syndrome and (2) the PCV Review of Impact Evidence (PRIME),[7] a systematic review of the available evidence on PCV effectiveness and impact on nasopharyngeal carriage, disease, and mortality, as well as on PCV immune response.

Further complicating the review of global pneumococcal data is the inability to aggregate some multisite data due to methodological differences, especially in terms of serotyping. Within the context of a coordinated sentinel surveillance system, there needs to be either an agreed-upon protocol for sample collection and serotyping or systematic means to bridge results from different methods.

7.3.4 Alternative vaccine strategies

As presented by Lourenço et al,[18] a high force of infection in settings like Malawi limits a 3+0 schedule to achieving only a short duration of VT carriage control among infants. A 2+1 schedule, as deployed in South Africa, may improve colonization control. While it remains unproven in other African settings, the 2+1 schedule is now being evaluated in Mozambique and Malawi, as described in chapter 6 of this thesis. Given the likely importance of an early reduction in transmission intensity to maintain a reduced carriage prevalence, a catch-up-campaign with booster doses over a broader age range (e.g., <5 years of age) may also be required. Although Gavi has considerably reduced PCV costs for low-income countries,[19,20] vaccine impact must be optimized (particularly the indirect effects) to achieve financial sustainability. Therefore, alternative schedules and vaccine introduction approaches in high-pneumococcal-carriage, high-disease-burden countries should be revisited through robust evaluation rather than through programmatic change without supporting evidence.

7.3.5 Serology and waning vaccine-induced immunity

Vaccine-induced immunity reduces the risk of VT carriage in children; however, in the context of a high residual force of infection, this impact is limited by pneumococcal recolonization and rapid waning of vaccine-induced mucosal immunity. A population-level pneumococcal serosurvey in Blantyre, Malawi, demonstrated serotype-specific VT profiles with rapid waning of IgG subsequent to a vaccine-induced peak and before a more gradual increase at later ages.[10] The serotype-specific proportion of samples attaining vaccine-induced IgG levels protective against both carriage and disease (0.35 µg/mL) ranged from 33% to 69%. The age at waning IgG nadir ranged from 11 to 27 months, with IgG population-averaged nadir estimates below CoPc for all serotypes. Therefore, we need to better understand the relative impact of waning vaccine-induced immunity, indirect vaccine protection, and naturally acquired immunity on VT carriage in the 5 years after vaccination.

In conclusion, the impact of PCV on reducing VT carriage and disease in Malawi is suboptimal compared with that achieved in high-income settings with alternative WHO-approved strategies. These findings, based on robust laboratory and analytical methods, underline the importance of national and regional surveillance to measure vaccine impact and inform public health policy.

7.4 References

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Chapter 8. Appendices

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Appendix A. STROBE Statement (PAVE study, chapter 6) – Checklist of items that should be included in reports of cross-sectional studies

	Item No	Recommendation	Page in Protocol
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Title: Page 1 Abstract: Page 2, lines 13-14
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	What will be done: Page 2, lines 14-21 What will be found: Page2, lines 22-24
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 5, line 103-116 Page 6, lines 132-134
Objectives	3	State specific objectives, including any prespecified hypotheses	Introduction: Page 6, lines 137-140 Methods: Page 7, line 169-181
Methods			
Study design	4	Present key elements of study design early in the paper	Page 7, line 142-145
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 6, lines 134-140 Page 7, line 147-155 Page 9, lines 226-230
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	Participant eligibility: Page 11, lines 277-301 Participant selection: Page 9, lines 236-246 Page 10, lines 253-257 Page 10, lines 263-264
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 12, lines 314-325 Page 14, line 381-386
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Vaccine schedule received: Page 8, line 198-201 Vaccination status: Page 9, line 233-234
Bias	9	Describe any efforts to address potential sources of bias	Page 8, lines 183-188 Page 9 line 236-246 Page 10, lines 254-257
Study size	10	Explain how the study size was arrived at	Page 12, lines 336-344
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 13, lines 369-380 Groupings: Page 9, lines 233-236 Page 10, lines 248-253 Page 10, lines 259-263

Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 12, line 369-386
		(b) Describe any methods used to examine subgroups and interactions	Page 14, line 381-383
		(c) Explain how missing data were addressed	Page 13, lines 361-366
		(d) If applicable, describe analytical methods taking account of sampling strategy	Not applicable
		(e) Describe any sensitivity analyses	Page 14, line 383-386
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Not applicable for a protocol manuscript
		(b) Give reasons for non-participation at each stage	Not applicable for a protocol manuscript
		(c) Consider use of a flow diagram	Not applicable for a protocol manuscript - Will be included in post-implementation manuscript
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	Not applicable for a protocol manuscript
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable for a protocol manuscript
Outcome data	15*	Report numbers of outcome events or summary measures	Not applicable for a protocol manuscript
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Not applicable for a protocol manuscript
		(b) Report category boundaries when continuous variables were categorized	Not applicable for a protocol manuscript
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable for a protocol manuscript
Other analyses	17	Report other analyses done - e.g., analyses of subgroups and interactions, and sensitivity analyses	Not applicable for a protocol manuscript
Discussion			
Key results	18	Summarise key results with reference to study objectives	Not applicable for a protocol manuscript
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Not applicable for a protocol manuscript
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Not applicable for a protocol manuscript
Generalisability	21	Discuss the generalisability (external validity) of the study results	Not applicable for a protocol manuscript
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 22, lines 646-654

*Give information separately for exposed and unexposed groups.

Appendix B. List of manuscripts arising from PhD activities involving pathogens other than *Streptococcus pneumoniae* (¥ = Joint senior author)

1. [¥]Mandolo J, Msefula J, Henrion MYR, Brown C, Moyo B, Moyo-Gwete T, Makhado Z, Ayres F, Kalata N, Muula A, Kwatra G, Moore PL, French N, Heyderman RS, **Swarthout TD**[¥], Jambo KC. Dynamics of SARS-CoV-2 exposure in Malawian blood donors: a retrospective seroprevalence analysis between January 2020 and February 2021. medRxiv 2021.08.18.21262207 [Preprint]. 2021 [posted 2021 Aug 22; cited 2021 Nov 7]: [24 p.]. doi: 10.1101/2021.08.18.21262207. BMC Med. Forthcoming [2021 Aug, favourable reviews].

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4. Stockdale AJ, Meiring JE, Shawa IT… **Swarthout TD,** et al. Evaluation of hepatitis B virus (HBV) epidemiology, vaccine impact and treatment eligibility: a census-based community serological survey in Blantyre, Malawi. J Infect Dis. Forthcoming [accepted 2021 Sep].

Appendix C. Approved versions of the case report forms (CRFs), participant information sheets, and informed consent forms (ICFs) for the PCVPA Study, as reported in chapter 3

Study ID No. Label	Household ID No. Label	Lab ID No. Label

	Today's date (dd-mmm-yyyy)	|___|___| - |___|___|___| - 2017
1	What is the child's date of birth? (dd-mmm-yyyy) (For unknown, enter 99 for day; UNK for month; 9999 for year)	|___|___| - |___|___|___| - |___|___|___|___|
	If date of birth unknown, what is the age?	

Inclusion Criteria

2	Has the child received one or more doses of the PCV vaccine? *(PCV vaccine given to infants at 6, 10 and 14 weeks of age.)*	**No$_0$ Yes$_1$ UNK$_{88}$**
3	Are you able to confirm PCV vaccination status by HP or other document? • **If yes to Q2**, does parent/ guardian have the child's HP and does it show date of at least one dose PCV13? • **If no to Q2**, does parent/ guardian have the child's HP and does it show no dates or record of PCV13 received? **Note:** Children 4-8 weeks of age and PCV13-unvaccinated must have evidence of having received BCG or Polio-0 birth dose in own or parent's health passport but no evidence of having received PCV13.	**No$_0$ Yes$_1$ UNK$_{88}$**
4	Is the child healthy?	**No$_0$ Yes$_1$ UNK$_{88}$**

Exclusion Criteria

7	Has the child ever tested positive for HIV?	**No$_0$ Yes$_1$ UNK$_{88}$**
8	Has the child received any antibiotics within the previous 14 days?	**No$_0$ Yes$_1$ UNK$_{88}$**
9	Is the child currently on TB treatment?	**No$_0$ Yes$_1$ UNK$_{88}$**
10	Has the child been hospitalized for pneumonia within the previous 14 days?	**No$_0$ Yes$_1$ UNK$_{88}$**
11	Does child have any gross respiratory tract pathology? (example: respiratory tract Kaposi's sarcoma)	**No$_0$ Yes$_1$ UNK$_{88}$**
12	Does child have a terminal illness? (example metastatic malignancy, terminal AIDS)	**No$_0$ Yes$_1$ UNK$_{88}$**

lealth Passport

13	Does parent/guardian have the child's HP with them at the time of recruitment?	No$_0$ Yes$_1$
	Is child eligible? • Infant 4-8 weeks: Must be unvaccinated • 18 weeks - 4 years old: Must be vaccinated • 5 years old and born after 31 September 2011: Must be vaccinated • 5 years old and born before 01 October 2011: Must be **NOT** vaccinated **Note:** If no, stop interview.	**No$_0$ Yes$_1$**

Preliminary Data

14	Consent obtained?	**No$_0$ Yes$_1$**
16a	Scan barcode for Participant ID	
	Enter Participant ID (PCV 4 S - ###)	**PCV - 4 - H - __ __ __**
16b	Is this the first child you are recruiting from this household?	**No$_0$ Yes$_1$**
17	Has this child been previously recruited into this study at any time? (Note: A participant can be recruited into study more than once)	**No$_0$ Yes$_1$**
18	If yes, Scan barcode for previous Participant ID	
	If yes, enter previous Participant ID (UNK if unknown)	**PCV**
19	Scan the barcode for Household ID	
	Enter the Household ID (HH - # - ###)	**HH - 4 - __ __ __**

Child Characteristics

20	Gender	**Male$_1$ Female$_2$**

21	Location of recruitment	**Home$_2$**

Zones (if recruited from home)

25	If Home, what Zone?	1	Ndirande	
		2	Likhubula	
		3	Chilomoni	|___|
26	Cluster name:			

he following questions are about vaccines your child may have received as part of the routine EPI

'accine status

		Vaccines received (Circle answer)			Date of Vaccination (dd-mmm-yyyy)
27a	BCG	**No$_0$ Yes$_1$ UNK$_{88}$**	bcg	27b	|___|___| - |___|___|___| - |___|___|___|___|
28a	Polio-0	**No$_0$ Yes$_1$ UNK$_{88}$**	pol0	28b	|___|___| - |___|___|___| - |___|___|___|___|
29a	Polio-1	**No$_0$ Yes$_1$ UNK$_{88}$**	pol1	29b	|___|___| - |___|___|___| - |___|___|___|___|
30a	Polio-2	**No$_0$ Yes$_1$ UNK$_{88}$**	pol2	30b	|___|___| - |___|___|___| - |___|___|___|___|
31a	Polio-3	**No$_0$ Yes$_1$ UNK$_{88}$**	pol3	31b	|___|___| - |___|___|___| - |___|___|___|___|
32a	DPT-1	**No$_0$ Yes$_1$ UNK$_{88}$**	dpt1	3	|___|___| - |___|___|___| - |___|___|___|___|
33a	DPT-2	**No$_0$ Yes$_1$ UNK$_{88}$**	dpt2	3	|___|___| - |___|___|___| - |___|___|___|___|
34a	DPT-3	**No$_0$ Yes$_1$ UNK$_{88}$**	dpt3	3	|___|___| - |___|___|___| - |___|___|___|___|
35a	Measles 1	**No$_0$ Yes$_1$ UNK$_{88}$**	measl1	3	|___|___| - |___|___|___| - |___|___|___|___|
36a	Measles 2	**No$_0$ Yes$_1$ UNK$_{88}$**	measl2	3	|___|___| - |___|___|___| - |___|___|___|___|
37a	PCV-1	**No$_0$ Yes$_1$ UNK$_{88}$**	pcv1	3	|___|___| - |___|___|___| - |___|___|___|___|
38a	PCV-2	**No$_0$ Yes$_1$ UNK$_{88}$**	pcv2	3	|___|___| - |___|___|___| - |___|___|___|___|
39a	PCV-3	**No$_0$ Yes$_1$ UNK$_{88}$**	pcv3	3	|___|___| - |___|___|___| - |___|___|___|___|
40a	RV-1	**No$_0$ Yes$_1$ UNK$_{88}$**	rv1	4	|___|___| - |___|___|___| - |___|___|___|___|
41a	RV-2	**No$_0$ Yes$_1$ UNK$_{88}$**	rv2	4	|___|___| - |___|___|___| - |___|___|___|___|

42	Take an image of the vaccination page of Health passport	**Not Done$_0$ Done$_1$**

Household information

The following questions will be about the house you live in, including who lives in your home and location
We are not going to visit your home at any time.

52	GPS coordinates	Lat |__|__|__|.|__| / Long |__|__|__|.|__|		
53	If no GPS coordinates entered, why have you not used ePAL to record the location of the participant's household?	**Participant does not want to show house location**	**Participant cannot locate house**	**Other**

54	How many bedrooms in the main house?	
55	Number of adults (16+ years of age) living in main house? (living in main house during at least the past 1 month)	
56	Number of children 5-15 years of age living in main house, including recruited child? (living in main house during at least the past 1 month)	
57	Number of children 0-4 years of age living in main house? (living in main house during at least the past 1 month)	
58	Amongst the total children in the household, how many were born after 1 October 2011? (Including recruited child if born after 1 October 2011)	

Household PCV13 Vaccination (amongst children in household that were born after 1 October 2011)
Child -1 (child 1 is recruited child if born after 1 October 2011)

59a	Are you able to report if child-1 received PCV-1, PCV-2, and PVC-3?	**No$_0$ Yes$_1$**

	PCV 1	**Yes**	**No**	**Unknown**
	PCV 2	**Yes**	**No**	**Unknown**
	PCV 3	**Yes**	**No**	**Unknown**
60a	Did parent/guardian have a HP or other document for you to confirm/review this information?			**No**$_0$ **Yes**$_1$

61a	Does anybody in the household smoke tobacco (cigarettes, pipes, or cigars)?	**No**$_0$ **Yes**$_1$
61b	If yes, how many cigarettes per week?	

The following questions ask about the type of house you live in.

62a	Exterior walls of house	1 Burnt brick 2 Unburnt brick 3 Pounded thick mud 4 Plastered thin mud 5 Bamboo 6 Grass or no walls 7 Iron sheets 8 Concrete blocks 99 Other, specify	
62b	If Other in 62a, specify		
63	Roof of house	1 Grass or leaves 2 Grass+Iron sheets 3 Grass+plastic sheet 4 Iron sheets or tiles	
64	Quality / state of the roof (Poor roof = leaking roof)	1 Good 2 Poor	
65a	Floor inside house (Record floor of living room if more than one interior floor type)	1 Mud 2 Concrete/ cement 3 Tiles 99 other, specify below:	
65b	If Other in 65a, specify		
66	Toilet facilities	0 None 1 Simple pit latrine 2 VIP 3 Water toilet	
67	Source of electricity	1 Escom 2 Solar 3 None	
68	Does house have glass windows?		**No**$_0$ **Yes**$_1$
69	Source of household drinking water	1 Tap to house 2 Shared communal tap 3 Bore hole 4 Covered well 5 Open well 6 River	

The following questions will be about some possessions you may have. We are not able to give you any of these items, even if you report not having them.

Are you comfortable answering questions about items owned by people in your household?	**No**$_0$ **Yes**$_1$

Possessions. Does anyone in the household possess any of the following working items?

70	Watch or clock (working)	**No**$_0$ **Yes**$_1$	watch	79	Mattress	**No**$_0$ **Yes**$_1$
71	Radio (working)	**No**$_0$ **Yes**$_1$	radio	80	Bed	**No**$_0$ **Yes**$_1$
72	Bank account (or bank book)	**No**$_0$ **Yes**$_1$	bank	81	Bicycle	**No**$_0$ **Yes**$_1$
73	Charcoal iron	**No**$_0$ **Yes**$_1$	ironc	82	Motorbike (working)	**No**$_0$ **Yes**$_1$
74	Sewing machine (working)	**No**$_0$ **Yes**$_1$	sew	83	Car (working)	**No**$_0$ **Yes**$_1$
75	Mobile phone (working)	**No**$_0$ **Yes**$_1$	mobil	84	Television (working)	**No**$_0$ **Yes**$_1$
76	Tape/CD player (working)	**No**$_0$ **Yes**$_1$	cd	85	Refrigerator (working)	**No**$_0$ **Yes**$_1$
77	Fan, electric (working)	**No**$_0$ **Yes**$_1$	fanelec	86a	Other electric items	**No**$_0$ **Yes**$_1$
78a	Mosquito net (number)	**No**$_0$ **Yes**$_1$	netyn	86 b	If other working electrical items, specify:	
78b	Number of mosquito nets		netnum			

Sample Collection

87a	NP swab taken?	**No**$_0$ **Yes**$_1$
87b	If no swab was collected, specify why not.	
87c	Was the sample you collected 'adequate'? (Adequate means swabbed passed to back of nasopharynx for at least 3 seconds)	**No**$_0$ **Yes**$_1$
87d	Is there nasal mucus on swab?	**No**$_0$ **Yes**$_1$

Scan/enter the Lab barcode	BVY -	
Form completed by (Enumerator Code):	Code	|___|___|___|
Form completed by:	Signature	