Tampere University

JOHN NJUMA LIBWEA

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Baseline all-cause under-five mortality data and vaccine impact on otitis media

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DEDICATION

"The scientific man does not aim at an immediate result. He does not expect that his advanced ideas will be readily taken up. His work is like that of the planter — for the future. His duty is to lay the foundation for those who are to come and point the way."
-Nikola Tesla

This work is dedicated to

My wife - Miatta, and children - Eyole, Elliot and Esther

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ABSTRACT

Title: Prevalence of nasopharyngeal pneumococcal carriage and otitis media among Cameroonian children under-five years old in the era of the 13-valent pneumococcal conjugate vaccine: *Baseline all-cause under-five mortality data and vaccine impact on otitis media.*

Streptococcus pneumoniae has more than ninety serotypes and continue to be a major cause of childhood illnesses and deaths worldwide. Treating affected children and preventing the spread of infection is a critical public health priority especially in developing countries including Cameroon. The 13-valent pneumococcal conjugate vaccine (PCV13) was introduced to Cameroon's Expanded Programme on Immunization (EPI) in July 2011, through funding from the Global vaccine alliance initiative (GAVI, the Vaccine Alliance). PCV13 is administered to infants using the accelerated primary dose series of three doses at six, ten and fourteen weeks of age. PCV13 vaccination programme targets the prevention of paediatric invasive pneumococcal diseases and infections of the middle ear resulting from the pneumococcus. In the absence of GAVI funding, sustaining PCV13 immunization in Cameroon, as in most low-income economies, would be practically impossible. It will rely heavily on convincing policymakers of the PCV's effectiveness in reducing the burden of disease associated with pneumococcal infections as well as overall under-five morbidity and mortality.

However, studies evaluating the effectiveness of the PCV-13 against both mucosal and invasive pneumococcal diseases as well as nasopharyngeal carriage in the country are lacking. The purpose of this research project was to estimate the baseline proportion of pneumococcal associated deaths, prevalence of otitis media, and nasopharyngeal carriage of *Streptococcus pneumoniae* among children under the

age of five who were vaccinated with PCV13 versus those who were not vaccinated during the PCV13 era.

This dissertation consists of four original studies. A retrospective cohort study design was used to examine existing hospital data on the major causes of under-five mortality recorded at the infectious disease surveillance sites (hospitals) in Yaoundé, between January 2006 and December 2012 (Study I). In order to examine the prevalence of otitis media and nasopharyngeal carriage in the era of PCV13, two rounds of surveillance studies were conducted in which tympanometry data and nasopharyngeal swabs were collected from randomly selected 24 to 36 months old children living in and around Yaoundé, in 2013 and 2015, respectively. Children born between June 2010 and June 2011 (baseline data) and between June 2012 and June 2013 (comparison data), were targeted (Studies II, III and IV).

The findings from Study I (N=817) provided country-specific evidence that approximately 29% of under-five deaths between January 2006 – December 2012 were due to pneumococcal infections (including pneumonia, meningitis and sepsis). Additionally, more than 70% of children in this setting die before their second birthdays. In Study II (N=877), overall carriage and residual vaccine-type pneumococci prevalence were 62% and 18%, respectively. Furthermore, eleven of the thirteen vaccine-serotypes were still in circulation. However, the 18% residual vaccine-type pneumococci obtained in Cameroon four years after PCV13 introduction were not very different from those reported in The Gambia (13%), except that, only three vaccine-type pneumococci were identified four years after infant vaccination programme. The results of the otitis media studies were surprising. Two years after PCV13 introduction in 2013 (Study III, N=433), 9.7% of PCV13-unvaccinated children had otitis media (OM). Four years after PCV13 implementation (Study IV, N=413), 16.7% of PCV13-vaccinated children had OM. This shows an unexpectedly higher OM prevalence among the PCV13-vaccinated group compared to the PCV13-vaccinated. Additionally, PCV13 effectiveness

estimate against OM prevalence was negative 72%, four years after PCV13 infant vaccination. However, multivariate analyses did not show any statistically significant evidence that PCV13-vaccinated children in 2015 were associated with higher odds of OM compared to PCV13-unvaccinated children in 2013. Furthermore, the attributable proportion estimates which depicts the public health impact of PCV13 against OM infection was 42%; suggesting that the remainder (58%) of OM infections in the PCV13-vaccinated cohort would have still occurred even without PCV13 vaccination. However, study participants were drawn from same population overtime, and the results may be attributed to several factors including: the predominance of other pathogens and/or non-PCV13-type pneumococci as disease causing microbes or early vaccine waning effects or residual confounding. Furthermore, immunity due to natural exposure could as well be a contributing factor.

In conclusion, these findings support the need for continuous surveillance to determine the possible long-term effects of the PCV13 implementation programme in Cameroon on nasopharyngeal carriage, pneumococcal disease and pneumococcal-associated under-five mortality.

I am of the opinion that in addition to these findings, the evidence and gaps on the broader public health impact of the PCV13 implementation discussed in this thesis will support Cameroon government's policies to prioritize the continuation of PCV13 programme in the absence of GAVI funding.

TIIVISTELMÄ

Otsikko: Pneumokokin nenänielukantajuuden ja välikorvatulehduksen esiintyvyys alle viisivuotiailla kamerunilaislapsilla 13-valenttisen pneumokokki konjugaattirokotteen aikakaudella: *tutkimus alle viisivuotiaiden kuolleisuudesta ennen rokotteen käyttöönottoa ja rokotteen vaikutuksesta välikorvatulehdukseen*.

Streptococcus pneumoniaella on yli yhdeksänkymmentä serotyyppiä ja ne ovat edelleen maailmanlaajuisesti merkittävä lapsuuden infektioiden ja kuolemantapausten aiheuttaja. Tartunnan saaneiden lasten hoitaminen ja tartunnan leviämisen estäminen on kansanterveyden kannalta ensisijaisen tärkeää. 13-valenttinen pneumokokkikonjugaattirokote (PCV13) sisällytettiin Kamerunin laajennettuun rokotusohjelmaan heinäkuussa 2011, GAVI allianssin (The Global vaccine alliance initiative) rahoituksella. PCV13-rokote annetaan imeväisille nopeutettuna perusannossarjana: kolme annosta, kuuden, kymmenen ja neljäntoista viikon iässä. PCV13 rokotusohjelmalla pyritään ehkäisemään vakavia pneumokokkitauteja ja välikorvantulehduksia. Ilman GAVIn rahoitusta PCV13:sta ei olisi mahdollista pitää Kamerunin, tai muidenkaan alhaisen tulotason valtioiden, rokoteohjelmassa. Jotta rahoitusta olisi mahdollista saada täytyy poliitikoille todistaa pneumokokkirokotteen vähentävän infektioita ja vähentävän myös alle viisivuotiainen kokonaissairastavuutta ja -kuolleisuutta.

Edellisestä huolimatta, tutkimuksia, joissa arvioidaan PCV13 tehoa pinnallisiin infektioihin ja vakaviin tauteihin ja nenänielukantajuuteen, ei ole tehty. Tämän väitöskirjan tarkoituksena oli arvioida pneumokokkiin liittyvien kuolemantapausten määrää ennen rokoteohjelmaa, ja vertailla välikorvatulehduksen ja Streptococcus pneumoniae -bakteerin nenänielukantajuuden esiintyvyyttä alle viisivuotiailla rokotetuilla ja rokottamattomilla lapsilla PCV13 rokotusohjelman aikana.

Tämä väitöskirja koostuu neljästä alkuperäistutkimuksesta.

Tutkimuksessa I käytimme retrospektiivistä tutkimusasetelmaa tarkastellaksemme tietoja alle viisivuotiaiden kuolleisuuden tärkeimmistä syistä,

jotka kirjattiin tartuntatautien seurantapaikoissa (sairaaloissa) Yaoundéssa tammikuun 2006 ja joulukuun 2012 välisenä aikana (Tutkimus I). Välikorvatulehdusten ja nenänielukantajuuden esiintyvyyttä PCV13 aikakaudella tutkittiin kahdella poikkileikkaustutkimuskierroksella, joilla kerättiin tympanometriatietoja ja nenänielunäytteitä satunnaisesti valituilta 24-36 kuukauden ikäisiltä Yaondéssa asuvilta lapsilta vuosina 2013 ja 2015. Tutkimuksen kohteena olivat kesäkuussa 2010 – kesäkuussa 2011 syntyneet (rokottamattomat) ja kesäkuussa 2012 – kesäkuussa 2013 syntyneet (vertailutiedot).

Tutkimus I:n (N=817) tulokset antoivat maakohtaista näyttöä siitä, että noin 29% alle viisivuotiaiden kuolemista johtui pneumokokki-infektioista (mm. keuhkokuume, aivokalvontulehdus ja sepsis) tammikuun 2006 ja joulukuun 2012 välisenä aikana. Lisäksi yli 70 prosenttia lapsista tässä tutkimusasetelmassa menehtyi ennen toista syntymäpäiväänsä.

Tutkimuksen II (N=877) mukaan pneumokokin kokonaiskantajuus oli 62% ja rokoteserotyyppien kantajuus 18%. Lisäksi kierrossa oli edelleen yksitoista rokotteen kolmestatoista serotyypistä. Rokoteserotyyppien kantajuus (18%) oli hyvin samanlainen kuin Gambiassa raportoitu (13%), tosin Gambiassa havaittiin vain kolmea rokoteserotyyppiä neljä vuotta imeväisikäisten rokotusten alkamisen jälkeen.

Välikorvatulehdustutkimusten tulokset olivat yllättäviä. Kaksi vuotta PCV13 käyttöönoton jälkeen, vuonna 2013, (Tutkimus III, N=433) rokottamattomista lapsista 9,7 % sairasti korvatulehdusta. Neljä vuotta PCV-rokotusten aloittamisesta (Tutkimus IV, N=413) 16,7 % rokotetuista sairasti korvatulehduksen. Näin ollen korvatulehduksen esiintyvyys oli korkeampi rokotetuilla kuin rokottamattomilla. Lisäksi PCV13 arvioitu tehokkuus korvatulehdusta vastaan oli -72 % neljä vuotta PCV13 rokotusohjelman aloituksen jälkeen. Monimuuttuja-analyysi ei kuitenkaan osoittanut, että PCV13 rokotetuilla olisi vuonna 2015 ollut suurempi riski saada korvatulehdus kuin rokottamattomilla lapsilla vuonna 2013. Tulostemme mukaan PCV13 rokotteen teho korvatulehduksia vastaan olisi voinut olla 42 %, joten 58% korvatulehduksista ei olisi voitu estää. Kuitenkin tutkimuksen osanottajat oli valittu samasta väestöstä eri aikapisteissä ja tuloksiin on voinut vaikuttaa useat tekijät: muiden patogeenien/ei-rokoteserotyyppien aiheuttamat infektiot tai rokotetehon heikkeneminen ajan myötä tai jäännössekoittuneisuus. Lisäksi luonnollinen immuniteetti on voinut vaikuttaa tuloksiin.

Yhteenvetona voidaan todeta, että jatkuva seuranta on tarpeen, jotta voidaan arvioida PCV13:n pitkän aikavälin vaikutuksia nenänielukantajuuteen,

pneumokokkitauteihin ja pneumokokkiin liittyvään alle viisivuotiaiden kuolleisuuteen.

Mielestäni näiden löydösten ja tässä väitöstyössä esiin nostettujen PCV13 ohjelman kansanterveydellisten tutkimustulosten tulisi ohjata Kamerunin hallinon politiikkaa priorisoimaan PCV13 ohjelman jatkamista GAVI rahoituksen loppuessa.

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ABBREVIATIONS

AOM	Acute otitis media
OM	Otitis media
OME	Otitis media with effusion
CSOM	Chronic suppurative otitis media
IRB	Institutional Review Board
MCH	Mother and Child Center
IPD	Invasive Pneumococcal Disease
PCV	Pneumococcal Conjugate Vaccine
Sp	*Streptococcus pneumoniae*
THL	Finnish Institute for Health and Welfare
EPI	Expanded Immunization Programme
WHO	World Health Organization
VT	Vaccine serotypes
VRT	Vaccine related serotypes
NVT	Non-vaccine serotypes
PCV7	7- valent pneumococcal conjugate vaccine
PCV10	10- valent pneumococcal conjugate vaccine
PCV13	13- valent pneumococcal conjugate vaccine
RCT	Randomized control trials
SURVAC	Surveillance epidemiologigue dans l'Afrique Centrale
LMIC	Low- and middle- income countries
HIV	Human immuno-deficiency virus
NPC	Nasopharyngeal pneumococcal carriage
CoD	Cause of death
SSA	Sub-Saharan Africa
GAVI	Global Alliance for vaccine and immunizations
MDG	Millennium development goals
SDG	Sustainable development goals
PD	Prevalence difference
NC	Non-encapsulated
PCR	Polymerase chain reaction

NP	Nasopharyngeal pneumococci
CRF	Case report form
HIV	Human-immunodeficiency virus
TB	Tuberculosis
ALRI	Acute lower respiratory tract infections
ICD-10-CM	International Classification of Diseases, Tenth Revision, Clinical Modification
GAVI	Global alliance for vaccines and immunizations
MCH	Mother and child Hospital
DTP+HeB+Hib	Diphtheria, tetanus, pertussis; hepatitis B; and *Haemophilus influenzae* type b combination vaccine
USA	United States of America
STD	Sexually transmitted Disease
NPS	Nasopharyngeal samples
CoD	Causes of death
CI	Confidence interval
SPSS	Statistical Programme for Social Sciences
MEP	Middle ear pathology
MEF	Middle ear fluid
ECV	Ear can volume
POR	Prevalence odds ratio
RD	Risk difference
AP	Attributable proportion

ORIGINAL PUBLICATIONS

This dissertation is based on the following articles, which have been referenced in the text using Roman numerals I to IV.

I. Njuma Libwea J, Bebey KSR, Taku AN, Kobela M, Boula A, Sinata K-S, Koki PN. (2019) Assessing the causes of under-five mortality and proportion associated with pneumococcal diseases in Cameroon. *A case-finding retrospective observational study: 2006–2012*. PLoS ONE 14(4): e0212939. https://doi.org/10.1371/journal.pone.0212939

II. Njuma Libwea J, Gröndahl-Yli-Hannuksela K, Kobela M, Toropainen M, Nyholm O, Ndombo PK, Sinata K-S, Nohynek H, Nuorti JP, Palmu AA. Prevalence of pneumococcal nasopharyngeal colonization and serotypes circulating in Cameroonian children after the 13-valent pneumococcal conjugate vaccine introduction. Int J Infect Dis. 2020;98: 113–120. doi:10.1016/j.ijid.2020.06.048

III. Njuma Libwea J, Kobela M, Koki PN, Syrjanen RK, Huhtala H, Ninying F, Sinata K-S, Nohynek H, Nuorti JP, Palmu AA. The prevalence of otitis media in 2–3 year old Cameroonian children estimated by tympanometry. Int J Pediatr Otorhinolaryngol [Internet]. Elsevier; 2018;115(October):181–7. Available from: https://doi.org/10.1016/j.ijporl.2018.10.007

IV. Njuma Libwea J, Kobela M, Koki PN, Syrjanen RK, Huhtala H, Ninying F, Sinata K-S, Nohynek H, Nuorti JP, Palmu AA. Impact of 13-valent pneumococcal conjugate vaccines on otitis media in 2 to 3 years old Cameroonian children (*submitted to The IJID).*

1 Introduction

Pneumococcal infections continue to be a leading cause of sickness and mortality in all age groups worldwide (O'Brien et al., 2009; Wahl et al., 2018). The bacterium *Streptococcus pneumoniae* frequently colonizes the nasopharynx. However, *S. pneumoniae* colonization does not always result in serious disease (Waroux et al., 2018). Pneumococcal colonization is most often manifested as local, non-invasive diseases such as otitis media and sinusitis. They rarely enter the lungs, the bloodstream, or the meninges, where they cause pneumonia, septicemia, and meningitis, respectively (Henriques-normark & Tuomanen, 2013), a condition known as invasive pneumococcal disease (IPD). In Sub-Saharan Africa including Cameroon, pneumococcal-related infections are estimated to account for 10 - 19 % of mortality in children under-five (Cutts et al., 2005; Wahl et al., 2018). Although IPD can be treated, if it is not treated, it can become complicated, or fatal if neglected (Källander et al., 2004). As a result, it is preferable to protect the population from IPDs through vaccination (Källander et al., 2004). Despite the development of effective pneumococcal conjugate vaccines (PCV), more than two million children under-five still die each year from pneumococcal infections and sequalae, and a majority of these are reported to occur in low income settings (Wahl et al., 2018).

More than ninety pneumococci disease-causing serotypes have been identified. They have different distributions and account for different proportions of disease in different geographical areas (Esposito et al., 2007). In Cameroon, data on the incidence of pneumococcal disease and the epidemiology of circulating pneumococcal serotypes are scarce. However, Gervais et al., conducted a study prior to PCV13 introduction where they assessed the etiology of bacterial meningitis among 170 hospitalized Cameroonian children aged from 2 months to 15 years, to determine the prevailing pneumococcal serotypes (Gervaix et al., 2012). They reported that 77% (10 of the 13) PCV13 serotypes were isolated from meningitis cases including serotypes 1 (21.7%), 6A and 14 each with 6.9%, which were the most frequently isolated, as were some non-PCV13 serotypes i.e. 32A/32F (12.1%) and 23B (6.9%).

Despite the lack of complete country-specific baseline data on IPD, PCV13 was included in the Global Alliance for Vaccines and Immunization (GAVI, the Vaccine Alliance)-eligible countries' national immunization programmes (NIPs), including Cameroon (Madhi & Nunes, 2016). This was due to the World Health Organization (WHO) and GAVI's recommendations and financial support (Madhi & Nunes, 2016). PCV13 is given to infants in three doses (3+0) at six, ten and fourteen weeks at birth, without catch-up programmes for older children. PCV13 is given in conjunction with a pentavalent vaccine that protects against diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, and hepatitis B (DTP-Hib-HepB). Since 2012, EPI coverage for the 3rd dose of PCV13 has exceeded eighty percent (WHO/UNICEF, 2018). PCV-

vaccinated and PCV-unvaccinated populations experienced rapid declines in IPDs across all ages, according to reports from countries that first introduced PCV (Dagan, 2019; Izurieta et al., 2018; Olivia Cohen et al., 2017; Palmu et al., 2017; Simell et al., 2012). However, until now the impact of the PCV13 programme in Cameroon on any aetiologic outcome or carriage has not been thoroughly assessed, which may jeopardize the government's continuation of the programme when GAVI support ends.

The 7-valent pneumococcal conjugate vaccine (PCV7) formulation consisting of a solution of saccharides of the capsular antigens of Streptococcus serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM 197 protein was initially licensed in the USA in the year 2000, then in Europe in 2001 and Africa in 2009 (Loo et al., 2014; Olivia Cohen et al., 2017). Following that, 10- and 13-valent products (PCV10 [with 3 additional serotypes 1, 5, and 7F] and PCV13 [with 6 additional serotypes 1, 3, 5, 6A, 7F, and 19A], respectively, with serotypes 1 and 5 that are most prevalent in developing countries) are currently in use worldwide (Loo et al., 2014; Olivia Cohen et al., 2017). The higher valent PCVs (PCV10 & PCV13) are expected to provide broader coverage of the prevalent disease-causing serotypes in Sub-Saharan Africa (SSA). PCV13 has been available in Cameroon since July 2011 with no prior use of either PCV7 or PCV10.

PCV's efficacy in SSA was first evaluated in South Africa in a randomized control trial (RCT) with a PCV9 candidate vaccine (Klugman et al., 2003); later in the national immunization programme with PCV7, before switching to PCV13 (Nzenze et al., 2015). The PCV was evaluated in The Gambia in a RCT with a PCV9 candidate vaccine and then, in the national immunization programme with PCV7/PCV11 (Cutts et al., 2005).

The South African PCV9 trial found that vaccine serotypes reduced IPD by 83 percent in HIV-negative participants (Klugman et al., 2003). Even though the clinical trial results were on hospital-based outcomes, they provided an approximation of the vaccine's overall impact in the community. Furthermore, it supported a role for PCVs in reducing the burden of pneumococcal infections in low-resource settings, consistent with recent reports from Senegal, Mozambique and Kenya (Faye et al., 2019; Hammitt et al., 2019; Madhi & Nunes, 2016; Nhantumbo et al., 2017; Soeters et al., 2019).

In resource-limited settings like Cameroon, what commonly drives policy decisions for programme intervention like the PCV13 is the cost effectiveness analysis (CEA), compared to the broader public health value (BPHV) of such a programme (Gessner et al., 2017; Saadatian-elahi, 2016). According to Gessner et al. (Gessner et al., 2017), cost-effectiveness analysis reflects a therapeutic paradigm that is based on individual health risk/benefit assessment on prophylactic interventions. CEA has been widely used in clinical trials where efficacy and safety on etiologically-confirmed clinical outcomes are evaluated (Gessner et al., 2017). Briefly, CEA compares one intervention to another by estimating how much it costs to gain a unit of a health outcome e.g., a life year gained or a death prevented (Gessner et al., 2017). A public health model, on the other hand, goes beyond the individual level to consider the population impact of a programme intervention and represents a holistic approach to societal benefits against a variety of

outcomes. These include, among others health inequity, socio-political, developmental, educational and occupational loss, health care utilization, antimicrobial stewardship assessment, and different etiologically and non-etiologically defined clinical outcomes (Beutel, 2016; Gessner et al., 2017; Saadatian-elahi, 2016).

Vaccinations are widely regarded as one of the most successful and economical public health interventions in the history of medical sciences. The BPHV of vaccines has been repeatedly emphasized at every vaccine-preventable disease meeting, as well as in several reports, including a special issue of Health Affairs in February 2016. In summary, despite the advancements recorded, more efforts are required to include a more broadly designed assessment of the BPHV of vaccines as evidence-base for substantial recommendation (Saadatian-Elahi et al., 2016). In addition, such a framework should include avenues for analyzing unmet medical needs in order to consider vaccine development, implementation policies, vaccine licensure and financing decisions as priorities (Gessner et al., 2017; Longini et al., 2017; Saadatian-elahi, 2016). These efforts are expected to result in the establishment of an alternative framework for vaccine evaluation in an era of growing concerns about vaccine hesitancy. As reported by Gessner and colleagues, such a framework will result in a more vigorous vaccine chain and a greater understanding of vaccine value. Further, it will result to relatively low-cost, increased access to the public and a need to accept it (Gessner et al., 2017). Making these agreements is one thing; putting them into action is another, particularly in a resource-low country such as Cameroon, where community health needs outnumber available resources at any given time.

The PCV13 was introduced nation-wide and made accessible to all eligible children irrespective of their socio-economic status as opined in the BPHV concept. We estimated the baseline prevalence and impact of the PCV13 on otitis media and nasopharyngeal pneumococcal serotype distribution four years after the PCV13 vaccination programme was implemented in Cameroon in this dissertation. We also estimated the proportion of under-five deaths caused by pneumococcal infections as a baseline for future vaccine impact evaluations, and discussed some critical considerations and gaps needed for documenting the BPHV of PCV13 implementation programme in Cameroon.

2 Literature review

2.1 The microbiology of *Streptococcus pneumoniae*

Prior to its isolation and characterization in the late 1800s, the pneumococcus was a major cause of human disease for a long time (Sanders, 2002). It was previously known as *Diplococcus pneumoniae* until 1974, when the organism was renamed *Streptococcus pneumoniae* due to its ability to grow in chain-like manner in liquid media (López & Rubens, 2006). Aside from its pair-wise growth in short chains, the bacterium is described as facultatively anaerobic and a fastidious gram-positive extracellular pathogen with round colonies measuring 0.5 to 1.5mm in diameter on blood agar. On culture plates, different strain categories can be distinguished, with those with heavy encapsulation forming mucoid colonies and others forming smooth colonies or less mucous colonies (Pinto et al., 2013). During aerobic conditions, *Streptococcus pneumoniae* exhibits α-haemolytic characteristics as evidenced by an encircling greenish discoloration on colonies. Optochin sensitivity testing is the gold standard used for distinguishing the pneumococcus from other streptococci species, and the pneumococcus's solubility in bile salts is a distinguishing feature. Because some pneumococci isolates are resistant to optochin, bile solubility testing is widely regarded as the most reliable test for pneumococci identification (Pinto et al., 2013).

2.2 Nasopharyngeal pneumococcal carriage in low- and middle-income countries

Streptococcus pneumoniae is known to be a normal flora on the nasopharyngeal mucosal surface; and, contrary to previous speculation that most episodes of nasopharyngeal carriage resulted in no infections or symptoms, studies have shown evidence on the occurrence of respiratory tract symptoms (Auranen et al., 2016; Kwambana et al., 2011; Sleeman et al., 2005). The public health significance of asymptomatic pneumococcal nasopharyngeal carriage in disease occurrence (sinusitis, otitis, meningitis, sepsis or bacteraemia) is viewed as a two-edge sword. The host's nasopharyngeal pneumococci acts as both a reservoir of disease causing strains and a source for bacterial transmission in households and communities (Sleeman et al., 2005).

The prevalence of pneumococcal colonization is higher among infants of younger age, especially in the presence of pneumococcal carriers within the immediate vicinity (Dunne et al., 2016). It has been reported that children, particularly newborns, are exposed to bacteria almost immediately after birth in resource-

limited settings (Dunne et al., 2016; Usuf et al., 2018; Watson et al., 2006). This implies that almost every child has experienced at least one episode of colonization, and thus more than 70% of children are thought to be colonized by the pneumococcus at various intervals during their early years (Bogaert et al., 2004; Hill et al., 2008; Usuf et al., 2018). This continues until they reach their tenth birthdays, when the carriage prevalence may drop to around 10% (Bogaert et al., 2004; Hill et al., 2008; Usuf et al., 2018). It is suggested that in more affluent settings, the first episode of pneumococci colonization may be delayed until later in infancy (Ercibengoa et al., 2012; P. Turner et al., 2012). Furthermore, in contrast to resource-limited settings, the pneumococci colonization prevalence of about 45 percent is not reached until after 2 years of age in affluent populations (Syrjanen et al., 2001). However, by the age of five, this prevalence is reported to drop to less than 10% until adulthood, a trend similar to that observed in resource-limited populations (Hammitt et al., 2006; Loo et al., 2014).

Carriers who spread pneumococci to close contacts increase the prevalence of pneumococci carriage (Nzenze et al., 2013; Tramuto et al., 2017). The role of carriers in the transmission of pneumococci in families has been studied. It has been discovered that colonization of the newborn occurs first in families with other children, and a statistically significant relationship between the number of children in a household and the risk of colonization had earlier been reported (Tramuto et al., 2017; Waroux et al., 2018). Another risk factor associated with carriage has been reported to be daycare attendance, but this is likely to be different in resource-limited settings that lack amenities for day care centers (Chavanet et al., 2011). Additionally, it has been demonstrated that there is an association between an increase in pneumococci carriage prevalence in children and acute respiratory infections or otitis media symptoms (Auranen et al., 2016; Syrjanen et al., 2001). A child, regardless of their socio-economic status, may be exposed to multiple colonization episodes with different pneumococci strains (Murad et al., 2019).

Although antimicrobial therapy can reduce or eliminate pneumococci carriage, the effect is short-lived and results to a return to pre-antibiotic carriage prevalence only about a week later (Burr et al., 2014; Murad et al., 2019). Antibiotic use in the treatment of pneumococcal infections is strictly regulated in developed countries to limit the development of drug resistance. In contrast, the indiscriminate use of antimicrobials in most developing countries may instead promote the spread of antibiotic resistant serotypes (Vergison et al., 2010). As a result, the introduction of the pneumococcal conjugate vaccine (PCV) is a significant public health advance with the potential to reduce the transmission of pneumococci nasopharyngeal carriage, consequently limiting the spread of antibiotic resistant strains.

2.2.1 Relationship between nasopharyngeal carriage and invasive pneumococcal diseases

One major characteristic regarding the global epidemiology of pneumococcal carriage is the consistency of the dominant carriage serotypes in varying geographical settings and at different times (Weinberger et al., 2016). Studies conducted across the globe have consistently reported serogroups 6, 14, 19 and 23 to be among the four most frequent nasopharyngeal isolates from healthy children (Wangirapan et al., 2020). Although nasopharyngeal carriage remains a precursor to IPD, the global dynamics of serotype distribution maybe experiencing a shift, especially as the interpretation of serotype data from carriage studies is hampered by several constraints (Kalizang'oma et al., 2021). For example, the sensitivity of different detection methods varies from one serotype to another (Olwagen et al., 2017). Also, approximately 30% of all carriers harbour several pneumococci serotypes simultaneously, but it is not always certain which serotypes are dominant, thus bringing serotype isolation based on the method of choice (Ndlangisa et al., 2018). More so, the duration of colonization varies by serotype and by age of the host, which could vary significantly (Khan & Pichichero, 2014).

Based on the aforementioned technical and methodological disparities, to my opinion, it is certain that the quantification on the relationship between carriage and serotype propensity to disease must be carefully considered. However, there are certain serotypes that are rarely found in carriage but are found in IPD e.g., serotype 1 was reported to cause IPD within the African meningitis belt but was rarely observed in carriage among carriage isolates (Chaguza et al., 2022; Kwambana-Adams et al., 2016). Such observations are indicative of those serotypes with high invasive-disease attack rates; and this may raise the question on how such highly invasive serotypes are maintained in the community if they are rarely found in carriage (Weinberger et al., 2016)?

A likely pathway is that these serotypes are for example, transmitted directly from persons (carriers) with pneumococcal pneumonia through coughing /droplets (Henriques-normark & Tuomanen, 2013). Pneumococcal carriage studies of patient contacts have reported that patients with pneumonia are highly susceptible in transmitting infections to their close contacts (Neal et al., 2020; Qian et al., 2022). Moreover, the association of highly invasive serotypes with outbreaks of pneumococcal meningitis provides more evidence to the hypothesis of person-to-person transmission (Kwambana-Adams et al., 2016). Thus, suggesting that carriage levels of serotypes 1 and 5 gets temporarily higher specifically in children with severe IPD in LMICs (Nzenze et al., 2015). As observed in most LMICs, cases of disease due to highly invasive serotypes are sporadic, and often results to epidemics or outbreaks, yet difficult to identify the primary source (index case) of infection (Kwambana-Adams et al., 2016). An intriguing aspect is that, a subset of the population becomes chronic carriers of invasive serotypes, especially amongst patients with chronic bronchitis and/or persons who are convalescent (Weil-Olivier et al., 2012). Probably, and as recent evidence show, even low carriage levels of highly invasive serotypes mainly among patients is sufficient to

maintain those serotypes in residual carriage among the general population in the era of PCV (Chan et al., 2021).

Invasiveness or invasive disease potential, is a measure or the ability of the pneumococcus to progress from nasopharyngeal carriage to invasive disease in humans (Yildirim et al., 2017). Studies have demonstrated that strains with different multi-locus sequence typing (MLST) genotypes but expressing the same serotype had the same invasiveness (Dong et al., 2017). This suggests that the capsular serotype maybe more important than the genotype in the ability of the pneumococcus to cause invasive diseases (Merit, 2010).

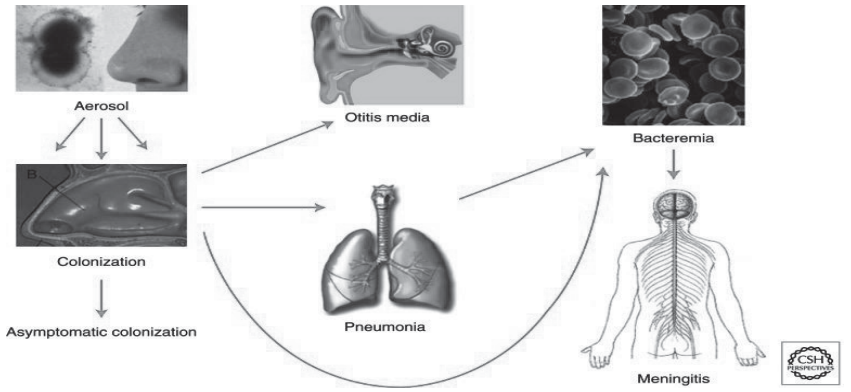
Further, pneumococcal serotypes differ in invasiveness and that serogroups and serotypes 1, 4, 5, 7, 14 and 18C were commonly associated with invasive disease, whereas serogroups and serotypes 3, 6A, 6B, 15, 19 and 23 are associated commonly with carriage (del Amo et al., 2014; Song et al., 2013). In addition, a considerable inverse relationship between invasiveness and carriage prevalence have been reported i.e., the most invasive serotypes were the least commonly isolated in carriage and vice-versa (Weinberger et al., 2016). This suggest that, if invasiveness is primarily a function of capsular expression, then repopulating the nasopharyngeal niche with serotypes of low-level invasiveness by using multivalent vaccines may produce considerable decline in IPD incidence (Scelfo et al., 2021).

Moreover, mathematical models and other studies of carriage that examine serotype competition in the presence of serotype-specific vaccines suggest that the pneumococcal population in the nasopharynx will shift toward non-vaccine serotypes overtime (Bosch et al., 2016; Ojal et al., 2017; Paulo & Sá-Leão, 2017). Hence, elucidating that even serotypes with low level of invasiveness could and do result to a substantial portion of invasiveness (IPD) when such serotypes are highly prevalent within residual carriage in the population (Chan et al., 2021). Additionally, serotype replacement in the nasopharynx which has been reported following the widespread implementation of PCVs globally, should not necessarily lead in commensurate invasive disease replacement in the general population, except that the replacing serotypes are as invasive as the original serotypes (Weinberger et al., 2011).

2.2.2 Pneumococci infections and pneumococcal diseases

Nasopharyngeal colonization is a precursor to pneumococcal infections and is transmitted from person to person via droplets/aerosols (Henriques-normark & Tuomanen, 2013). Despite advances in antimicrobial therapies, invasive pneumococcal diseases (IPD) in children are a major cause of morbidity and mortality worldwide. It is believed that almost every child has at least one episode of nasopharyngeal colonization before the age of three, though only a small number of children develop invasive disease (Simell et al., 2012; Syrjanen et al., 2001).

The bacteria's pathophysiology begins with their entry into the nasal cavity and nasopharyngeal epithelial cells, where they either colonize it or gain access deep into other organs or via the bloodstream into the lungs or bronchi. It is suggested that the bacteria enters the bloodstream and/or crosses the blood-brain barrier (Figure 1), causing meningitis (Henriques-normark & Tuomanen, 2013). After colonization, invasive disease can spread from a respiratory focus, as seen in meningitis or pneumonia, or from an unknown focus to the central nervous system, pleural fluid or elsewhere (Henriques-normark & Tuomanen, 2013). Regardless of the route, the main clinical syndromes are related to the disease onset as either bacteraemia or contiguous dissemination from the nasopharynx to mucosal surfaces of the lungs, resulting in pneumonia (Kishaba, 2016; Minovi & Dazert, 2014). Despite advances in understanding the pathogenesis of IPD, the actual disease prevalence in children around the world remains unknown. It ranges from less than 100 cases per 100,000 children in affluent communities like in Finland to more than 1000 per 100,000 in resource-limited settings like the Australian Aborigines (Greenwood, 1999; Palmu et al., 2015). However, in most of Sub-Saharan Africa (SSA) the disease prevalence is exacerbated by an increasing prevalence of the human immunodeficiency virus (HIV) and poor living conditions (Shabir et al., 2000). Additionally, incidence rates have been reported to be higher among immigrants from low-resource settings living in developed countries, as well as African-American children in the United States (CDC, 2010; Wenger et al., 2010).



Pneumococci are aerosol-borne pathogens that live in the nasopharynx. This interaction almost always results in clearance and serotype-specific immunity. In children, the progression to otitis media is very common. Invasive disease involves the spread of bacteria to the lungs and bloodstream. Meningitis is the most serious complication. This pattern of invasive steps is shared by the three major bacterial pathogens of children (pneumococcus, Haemophilus*, and Meningococcus) and is driven by a fundamental interaction between innate invasion and innate immunity* (Henriques-normark & Tuomanen, 2013).

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Figure 1. Flow diagram outlining stages in the progression of pneumococcal diseases

2.2.3 The definition of otitis media and epidemiology in low- and middle-income countries

Most often, pneumococci carriage is asymptomatic and causes no harm. Pneumococci, on the other hand, cause a wide range of diseases, including IPD, otitis media and sinusitis (Rovers, 2008). Otitis media, particularly in its acute form, is one of the most common illnesses requiring medical attention in children under the age of five worldwide (Leach et al., 2014; Monasta et al., 2012; Verhoeff et., 2006). Otitis media is defined as an inflammation of the middle ear (Rovers, 2008). It could be classified as acute otitis media, otitis media with effusion or chronic suppurative otitis media based on its presentation, associated symptoms, and duration of symptoms (Gotcsik, 2012).

Definitions:

Otitis Media (**OM**) is defined as any active disease process in the middle ear, including acute otitis media (AOM), otitis media with effusion (OME) and chronic suppurative otitis media (CSOM) or probable result of previous disease process, such as tympanic membrane dry perforation (WHO, 2004).

Acute Otitis Media (AOM) is an acute upper respiratory tract infection that affects the respiratory mucosa of the middle ear cleft. AOM is a frequent illness in children under-five, and it is defined by the presence of a tympanic membrane that is visibly abnormal in terms of colour, position, or mobility, indicating middle ear effusion, as well as at least one of the following acute infection symptoms or signs: Fever, earache, irritability, diarrhea, vomiting, acute otorrhea unrelated to otitis externa, and other respiratory infection symptoms (Lieberthal et al., 2013).

Otitis Media with Effusion (OME): This is the most recurrent type of otitis media and is defined as a condition in which there is a thick or sticky fluid behind the eardrum in the middle ear but no signs or symptoms of active infection (Monasta et al., 2012).

Chronic suppurative otitis media (CSOM): This is the most severe form of OM and is more common in developing countries. It is typically defined as chronic otorrhea (lasting more than 6-12 weeks) caused by a perforated tympanic membrane with persistent drainage from the middle ear (WHO, 2004).

OM can originate from a bacterium or virus, and infection is strongly associated with eustachian tube structure and function impairment. At first, the middle-ear space has a negative air pressure in comparison to the surrounding environment (Gotcsik, 2012). This pressure is relieved on a regular basis by opening the eustachian tube (e.g., during the processes of yawning and chewing). Failure to achieve this pressure equalization results in inflammation, which is referred to as OM (Karppinen et al., 2019; Rovers, 2008). Although most of the disease and sequalae are easily diagnosed and managed in developed countries, this is not the situation in resource-constrained settings such as Cameroon. In most resource-constrained settings, AOM is usually unnoticed and diagnosed, and as a result, children who are infected remain unidentified and

do not get treated at this early stage. Diagnosis of OM depends on typical signs and symptoms, e.g., acute ear pain and bulging of the tympanic membrane for AOM and hearing loss for OME. Diagnosis of OM could be made using otoscopy, tympanometry and/or audiometry (Adeyi et al., 2010). Nonetheless, OME which can manifest as CSOM, as well as complications resulting to tympanic membrane perforations, can persist in early childhood and have a negative impact on children's development (Adeyi et al., 2010).

2.3 The leading causes of death among children under the age of five in low- and middle-income countries

According to a 2015 report by Li Lui and colleagues of the Child Health Epidemiology Reference Group (CHERG) coordinated by WHO pneumonia, diarrhea and malaria continue to be the main causes of death in children under-five globally (Liu et al., 2015). The majority of the over 9.2 million under-five deaths which occur each year were caused by preventable conditions that could be treated with simple healthcare interventions (Liu et al., 2015). Many of these deaths occurred in low- and middle-income countries (LMIC), particularly in SSA and South Asia, where the child mortality rates average 175 per 1000 live births (compared to 6 per 1000 in industrialized countries) (Liu et al., 2015). More so, the majority of these under-five deaths are associated to a lack of access to healthcare facilities, poor hygiene and sanitation, contaminated water and food insecurity, and low levels of education and information (Institut Nationale de la Statistique, 2011).

2.3.1 *Streptococcus pneumoniae's* role in under-five mortality in low- and middle-income countries

Despite reported decreases in overall mortality rates, *S. pneumoniae* remains one of the leading causes of illness and death in children under the age of five, mostly in low- and middle-income countries, with serious disease resulting from pneumonia, meningitis and sepsis (O'Brien et al., 2009; Wahl et al., 2018). In a 2015 publication, Liu and colleagues (Liu et al., 2015), supported previous reports that pneumonia burden from children in South American and the Caribbean was lower than those from South-east Asia and SSA (Table 1).

The pneumonia disease burden and death vary across and within regions, despite its global prevalence. As previously stated, children under the age of five account for more than 70% of all pneumonia cases in SSA and Southeast Asia (Liu et al., 2015). Unfortunately, this is the most exposed and affected age group that is getting little of the desired treatment, despite advances in preventative and management strategies (Zar et al., 2013). In the last two decades, pneumonia mortality in children dropped to approximately 800,000 cases per year, with most deaths occurring in 15 countries from South-east Asia and SSA (Kulkarni et al., 2022; Walker et al., 2013). With recent advances in medical care, including the widespread

implementation of protein-polysaccharide conjugate vaccines against Haemophilus influenzae type B and *Streptococcus pneumoniae*, implementation of case-management algorithms and better prevention and treatment of comorbidities such the human-immunodeficiency virus (HIV), further drops in pneumonia disease burden are expected (Zar et al., 2013).

Table 1. The regional distribution of pneumonia as a leading cause of death in children under the age of five (in percentages).

Region	Pneumonia	Complications of neonatal infection (primarily from sepsis/meningitis)
Eastern Asia	14 %	2%
South-Eastern Asia	16 %	7%
Southern Asia	14 %	9%
Sub-Saharan Africa	16 %	6%
Caucacus & Central Asia	16 %	6%
Northern Africa	12 %	6%
Oceania	17 %	7%
Western Asia	13 %	7%
Latin America & Caribbean	12 %	7%
Developed Regions	5 %	3%

Source: Adapted from Liu et al., 2015: Causes of child deaths in 2013 by MDG region (Liu et al., 2015)

Generally, in many resource-constrained settings, diagnosing pneumonia through radiological diagnosis of the chest and/or laboratory examinations are not readily available in the suburbs , though this may be functional at referral hospitals in the big cities (Karppinen et al., 2019). It is however, challenging to ascertain the aetiology of pneumonia in the absence of reliable diagnostic tests in resource-limited settings. But, there are also heightened expectations that the increasing coverage of the 10- or 13-valent pneumococcal conjugate vaccines in LMICs may contribute in reducing the pneumonia burden (Madhi et al., 2016; Zar et al., 2013).

Despite this, a study on the assessment of the major reasons on why children die in Cameroon's northern regions found that, malaria and upper respiratory infections were the most frequently reported causes of death among children under-five (Einterz & Bates, 2011). Although global under-five mortality rates have decreased since the 1990 baseline, there is still work to be done (Liu et al., 2015). Vaccinations, adequate nutrition, and increased education will all help to reduce child mortality. This justifies in Cameroon's Health Sector Strategic plan (HSS) for 2001 – 2015 (revised in 2016) the urgency to introduce new vaccines by the end of 2011 including the pneumococcal conjugate vaccine and vaccines against rotaviruses implicated in diarrhea infections (MINSANTE, 2016).

2.4 Justification for the anticipated impact of the introduction of pneumococcal conjugate vaccines in low- and middle-income countries

The PCVs (PCV7 and later, PCV10 and PCV13) were developed primarily to protect against *Streptococcus pneumoniae*-caused paediatric pneumococcal infections. Before the approval of the first conjugate vaccines against pneumococcal infections in 2000, the pneumococcal polysaccharide vaccine (PPV) was the only licensed vaccine against the disease (Esposito et al., 2007). The capsular polysaccharide (PS) of the pneumococcus is a fundamental building block of both the PPV and PCV. The PPV, on the other hand, is derived from the refinement of pure PS, whereas the PCV is obtained following conjugation of the PS to a protein carrier (Turner et al., 2017). The formulation of pneumococcal vaccines is based on the most prevalent pathogenic serotypes of the over ninety different serotypes (disease-causing strains). As a result, PPV branded as Pneumovax23™ or Pneumo23™ is constituted of 2 micrograms of the purified PS from each of the 23 selected serotypes of the pneumococcus that have been linked to more than 90% of severe pneumococcal infections and mortality worldwide (Geno et al., 2015).

The first conjugate vaccine Prevnar™ or Prevenar™, (PCV7) was authorized in the USA in 2000 and contained serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) fused with a *diphtheria toxin* CRM_{197} carrier protein that had been genetically detoxified (Grabenstein & Klugman, 2012). PCV-10, which is conjugated to protein D (NTHi protein D), diphtheria toxoid, and tetanus toxoid carriers consist of serotypes 1, 4, 5, 6B, 7F 9V, 14, 18C, 19F and 23F, and PCV-13, which contains serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F, are the two PCVs currently in use (WHO, 2019). Serotypes 1 and 5 of PCV10 and PCV13 are the most common in developing countries (Esposito et al., 2007). The conjugate vaccines are expected to ensure a maximum coverage of the prevalent serotypes in SSA (Shabir & Nunes, 2016). It is also anticipated that, these higher valent-PCVs will lead to reductions in the level of disability, sickness and deaths caused by pneumococcal infections, especially in resource-poor areas (Shabir & Nunes, 2016). It was due to this anticipation that, Cameroon included the PCV-13 into its Expanded Programme on Immunization (EPI) for childhood vaccinations in July 2011.

The PCV formulations that are currently available are preferable to the PPVs (Esposito et al., 2007; Grabenstein & Klugman, 2012; WHO, 2019). The PCVs are also highly immunogenic in all age groups, especially for infants for whom PPV23 fails. The vaccine has also shown significant reductions in vaccine-type pneumococcal nasopharyngeal carriage and IPD in resource-limited settings, which is consistent with findings from developed economies (Shabir A Madhi & Nunes, 2016; von Gottberg et al., 2014). It also has a very significant and beneficial impact on herd immunity, conferring approximately 90% protection against invasive diseases (Shabir A Madhi & Nunes, 2016; von Gottberg et al., 2014).

2.4.1 Relationship between pneumococcal conjugate vaccine coverage and carriage

As earlier mentioned, the widespread use of PCVs has resulted to a considerable reduction in the burden of IPD both to the vaccinated and unvaccinated populations across all ages (WHO, 2019). These reductions have mainly been driven through both the direct and indirect effects (herd protection). Although the indirect effects of the PCV form an integral component of its broader vaccine impact, including its cost-effectiveness and other factors (see details in discussion section), the role of vaccination coverage needs to be careful considered (Chan et al., 2021).

Since PCV implementation in the year 2000, questions on what level of vaccine uptake are needed for optimal protection and herd protection have not been clearly addressed (Chan et al., 2021). With the changing epidemiology of the pneumococcus in the era of PCV immunization amidst concerns of serotype replacement and vaccine hesitancy; understanding the relationship between PCV coverage, carriage and invasive disease is vital. Recent considerations for the optimal control of vaccine-type IPD are focused either on shifting from the primary accelerated 3+0 to a 2+1 dose-schedule (for developing countries) or reducing the number of PCV doses from 3 to 2 (for mature PCV programmes like in most of Western countries) (Flasche et al., 2015; O'Brien, 2018; Yang et al., 2019). Whether this reflects the context of the LMICs or Western countries, the ultimate goal is that of sustaining the overall vaccine effects globally (Flasche et al., 2015).

Previous studies have suggested that high levels of PCV coverage (at least 58 to 75%) were required to achieve a substantial indirect protection (Tsaban & Ben-Shimol, 2017). However, this was contradicted with recent findings from Australia where the association between population PCV7 coverage and indirect protection against IPD and pneumonia hospitalization among undervaccinated children was studied (Chan et al., 2021). In this study a fully PCV-vaccinated child was defined as having received an adequate number of doses to develop a protective immune response against vaccine-type IPD at least 14 days prior to onset of any study outcome i.e., 2 or more PCV doses administered at less than 12 months of age or at least 1 PCV dose administered after the age of 12 months. While undervaccinated children were referred to those who have received less than the adequate number of PCV (either PCV7 or PCV13) including children who were never PCV-vaccinated (Chan et al., 2019). In their findings, Russel and colleagues demonstrated a statistically significant non-linear relationship between PCV coverage and the incidence of severe invasive disease as well as with pneumonia hospitalization due to vaccine serotypes among undervaccinated children (Chan et al., 2021). Additionally, they reported substantial indirect effects at relatively low levels of PCV coverage i.e., with 50% and 90% PCV7 uptake among children under-five years old prevented over 72% and 95% of PCV7-type severe invasive disease, respectively (Chan et al., 2021). Furthermore, they showed that with 50% and 90% PCV7 uptake, over 33% and 51% of all-cause pneumonia hospitalization among undervaccinated children may be prevented, respectively (Chan et al., 2021) In summary, these findings

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demonstrated that reduced dose schedules have the potential to substantially reduce programme cost while maintaining overall vaccine effects, as long as indirect effects are sustained. The public health implications from these findings will be wider, especially for LMICs including Cameroon (as presented in the discussion section).

2.4.2 Replacement of pneumococcal serotypes, direct and indirect effects of conjugate vaccines

The use of conjugate vaccines has resulted in significant improvements in global public health. However, there are concerns that these benefits will quickly be eroded as a result of the so called "serotype replacement" phenomenon (Weinberger et al., 2011). This phenomenon refers to an increased transmission of non-vaccine type (NVT) pneumococci as a result of the elimination of vaccine-serotypes (VT) that compete with them for colonization of their host (Weinberger et al., 2011). PCV13 only targets thirteen of the more than ninety known pneumococcal serotypes. Vaccination not only protects against VT-IPD, but it also prevents the transmission of VT pneumococci in carriage and thus eliminates them from the population (Geno et al., 2015). Apart from other bacteria, it is reported that VT- and NVT-pneumococci are said to compete in the human nasopharynx (Dunne et al., 2012). Therefore, the elimination of VT pneumococci provides an opportunity for the NVT pneumococci to benefit from the absence of VT pneumococci to increase the prevalence of NVT pneumococci.

The terminology of the term *effect of an exposure* differs per disciplinary field. In epidemiology, it depicts the amount of change (increase or decrease) in a population disease (or any other outcome) caused by an exposure. However, in vaccinology, it basically consists in vaccination – where, subjects are exposed to a specific vaccine administered with a given schedule at a specific time (Rothman, Greenland, & Lash, 2011). This implies, a vaccination programme may also reduce the risk of disease by reducing transmission in the entire population, including the unvaccinated. Therefore, the effect of the programme would be more than the sum of the effects of vaccination on those vaccinated, due to this indirect effect. As such, in a population in which there is a vaccination programme, the entire population is exposed to the effect of the programme, even if only a fraction is vaccinated (Hanquet et al., 2013).

The direct effect of a vaccination programme represents the difference between the outcome in the subjects receiving the vaccine and what the outcome would have been if the subjects had not been vaccinated, all other things remain equal (Shim & Galvani, 2012). The direct effect is estimated by comparing the vaccinated and unvaccinated groups from the same population who were both exposed to the same vaccination programme (Hanquet et al., 2013).

The indirect effect of a vaccination programme on an individual refers to the difference between what the outcome is in the individual not being vaccinated in a community with the vaccination programme and what the outcome would have being in the individual, again not being vaccinated, but in a comparable

community with no vaccination programme (Lefebvre et al., 2015). Briefly, it is the effect of the vaccination programme on an individual who personally was not vaccinated (Shim & Galvani, 2012). The indirect protection in this thesis reflects to the population-level effects of widespread vaccination because of reduction in the transmission of VT pneumococci (Hanquet et al., 2013). Indirect effects can be calculated from the difference in the degree of protection that unvaccinated individuals receive in the presence versus the absence of a vaccine programme (Shim & Galvani, 2012).

The overall effect of a vaccination programme is the the effect of the vaccination programme in the entire population, including vaccinated and unvaccinated. Whereas, the total effect is the combined effect of being vaccinated and being in a population with a vaccination programme i.e., the sum of the direct and indirect effects (Hanquet et al., 2013).

Moreover, other concepts including vaccine efficacy, vaccine effectiveness and vaccine impact are important terminologies used in this thesis. Vaccine efficacy is the proportional reduction of infection in a vaccinated group compared with an unvaccinated group under optimal conditions such as a randomized controlled trial (Crowcroft & Klein, 2018). A vaccine's efficacy is measured in a controlled clinical trial and is based on how many people who got vaccinated developed the outcome of interest (oftenly, disease) compared with how many people who got the placebo (dummy vaccine) developed the same outcome (Shim & Galvani, 2012). Whereas, vaccine effectiveness is the proportional reduction of infection in a real-world scenario delivered with normal storage and administration processes to an unselected population (Crowcroft & Klein, 2018). It is similar to vaccine efficacy except that it is a measure of how well a vaccine works in real life i.e., it is calculated using data from the population once a vaccine is approved and in use (Shim & Galvani, 2012). Meanwhile, the impact of a vaccine (vaccination programme like the PCV13), could be defined using the prevented fraction among exposed i.e., the proportion of potential cases among exposed that is prevented by the beneficial exposure (Hanquet et al., 2013). It is measured by comparing populations with and without a vaccination programme, mostly in the same population before and after vaccination (Shim & Galvani, 2012) i.e., by comparing the risks or rates in the population with a programme to the baseline risk in a reference population without a programme (Hanquet et al., 2013).

2.4.3 Impact of pneumococcal conjugate vaccines on carriage in low- and middle-income countries

There is an increasing body of evidence elucidating the major impact of the PCVs on pneumococcal carriage and, as a result, on pneumococcal diseases in LMIC (Grabenstein & Klugman, 2012; Klugman et al., 2003; Nzenze et al., 2013; Roca et al., 2015). Following earlier reports from clinical trials and subsequent observational studies in The Gambia, the United States, Canada, and South Africa, which showed that

vaccine-type pneumococci decreased in both carriage and disease after PCV infant vaccination, the PCV programme was implemented into national immunization schemes in LMIC (Madhi, 2013). In 1996 and 1999, the first PCV studies in Africa enrolled children from The Gambia and South Africa, measuring the safety, immunogenicity, and impact on carriage of a 9-valent investigational PCV and PCV7 given at 6, 10 and 14 weeks after birth (Effua et al., 2014). The researchers reported that, when compared to PCV-unvaccinated children, the proportion of vaccine-type pneumococci prevalence was approximately 50% lower in PCV-vaccinated children at 9 months of age (Effua et al., 2014). Additionally, drops in vaccine-type pneumococci among PCV-vaccinated infants have been reported to be associated with reductions in vaccine-type carriage among the general population in age groups that were not initially targeted for PCV (Madhi et al., 2016).

PCV implementation has been carried out in LMIC using a variety of immunization plans, dosing regimens and diverse age groups within the population. As a result, PCV evaluation has been conducted in the absence of a standardized criterion. However, its impact has been associated to a decrease in vaccine-type pneumococci and a simultaneous increase in non-vaccine type pneumococci in carriage (Madhi et., 2016). This replacement by non-vaccine serotypes has resulted to no change in the overall prevalence of pneumococcal carriage (Weinberger et al., 2011).

In both vaccinated and control villages, a Gambian clinical trial found a significant reduction in vaccine-type carriage prevalence across all age-groups (Roca et al., 2013). In that study, PCV7 was given to children below 30 months of age and to those born during the trial in all study villages. Villages were randomized (older children and adults) to receive one dose of PCV7 (11 vaccinated villages) or meningococcal serogroup C conjugate vaccine (10 control villages) (Roca et al., 2013). The findings demonstrated that community-wide vaccination with older subjects improves the PCV effect in breaking the transmission of vaccine-type pneumococci in a resource-limited setting with a high vaccine serotype prevalence (Madhi et al., 2016).

Annual cross-sectional studies were conducted in Kenya two years before and two years after the introduction of PCV10 in 2011. The researchers reported a 64% reduction in vaccine-type pneumococci among children aged 5 years within two years after PCV10 introduction, as well as a 66% reduction in vaccine-type carriage among older subjects (Hammitt et al., 2014). PCV10 was administered in a three-dose primary schedule at 6, 10 and 14 weeks after birth. Additionally, children aged 12 to 59 months were given up to two PCV10 doses as part of an outreach campaign. These reductions in vaccine-type colonization were first observed one year after PCV10 introduction in all study age groups, demonstrating significant indirect effects when less than 70% of subjects aged 5 years were fully vaccinated (Hammitt et al., 2014).

The results of PCV impact evaluation on carriage were also reported in a South African study conducted after PCV7 was introduced in 2009 (Nzenze et al., 2013) . PCV7 was administered to infants following a 2-dose primary series at 6 and 14 months, as well as a boaster dose at 40 weeks of age, with no catch-up schedules for older infants (Nzenze et al., 2013). Two cross-sectional studies on carriage found

reductions in PCV7 serotype colonization when less than 52% of the targeted population (infants <1-year-old) was fully vaccinated, less than 2 years after PCV7 implementation. This confirmed previous reports that children under the age of two, are the immediate source of pneumococci transmission in communities (Nzenze et al., 2013). Furthermore, the findings from the South African study mirrored a decrease in vaccine-type invasive pneumococcal disease (IPD) observed in a related study conducted in the country (von Gottberg et al., 2014). In addition, after switching from PCV7 to PCV13 in 2011, a subsequent South African study extended PCV impact evaluation to address effects against carriage among uninfected- and infected-HIV mother-child pairs (Nzenze et al., 2015). Within one year of the transition period, PCV13-carriage decreased by 68%. Furthermore, there was a 66% reduction of vaccine-type pneumococci among HIV-uninfected mothers and a 37% decrease in HIV-infected mothers (Nzenze et al., 2015).

In Brazil, 7 years after PCV10 was introduced into the NIP, VT colonization among toddlers decreased significantly to a residual level, along with significant serotype replacement by novel serotypes not present in any current conjugated pneumococcal vaccine as well as serotype 19A (Brandileone et al., 2016). Similar findings were reported from Fiji, where direct and indirect effects on pneumococcal carriage following PCV10 led to reductions in pneumococcal disease, including among infants who were too young to be vaccinated (Dunne et al., 2018). However, in the aforementioned studies, decreases in vaccine-type pneumococci were accompanied by increases in non-vaccine type carriage (Hammitt et al., 2014; Madhi et al., 2016; Nzenze et al., 2015; Roca et al., 2015). More so, while the PCV had a positive effect in these African-based studies, a nation-wide azithromycin campaign (in The Gambia) and changes in HIV drug management programme (in South Africa) are suggested to have influenced the outcome (Nzenze et al., 2015; Roca et al., 2015).

2.4.4 Impact of pneumococcal conjugate vaccines on pneumonia in low- and middle-income countries

Pneumococcal diseases are among the most important vaccine-preventable causes of childhood mortality, accounting for over 40% of the more than 500,000 reported annual deaths in African children under the age of five (Madhi et al., 2016). However, in most developing countries including Cameroon, the PCV was introduced in response to the WHO and GAVI recommendations and financial support, respectively, without any specific-pneumonia baseline disease burden (CDC, 2010; WHO/UNICEF, 2013). The WHO decision point for PCV inclusion in national immunization programmes was primarily based on infant mortality rates above 50/1000 live births, a gross national per capita income of less than one thousand US dollars based on the 2003 World bank estimates, more than 10% pneumococcal-associated under-five deaths and > 1% HIV/AIDS infection prevalence in adults aged 15 to 49 years (Madhi et al., 2016)

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Additionally, substantial evidence have been reported in reductions of IPD rates and hospitalizations for pneumonia in children under the age of five, following the licensing and implementation of the first conjugate vaccines (PCV7) in the year 2000 (Jayasinghe et al., 2017; Levy et al., 2020).

Furthermore, the findings supported previous reports on the efficacy of PCV against pneumonia in two independent RCTs which were carried out in The Gambia and South Africa using a 9-valent PCV investigational vaccine (Cutts et al, 2005; Klugman et al., 2003). Based on WHO criteria for chest radiography interpretation, the reported vaccine efficacy (VE) against radiologically confirmed pneumonia (CXR-AC) was estimated to be between 20% and 37% (Cutts et al, 2005; Klugman et al., 2003). However, in the South African trial the VE did not demonstrate a decrease in CXR-AC in children infected with HIV who had been vaccinated with PCV9 (Klugman et al., 2003; Mackenzie et al., 2014). There was however a 15% decrease in hospitalization for all-cause pneumonia among these children (Mackenzie et al., 2014). More so, the vaccine attributable reduction (per 100.000 vaccinated children) for clinical pneumonia hospitalization was nearly 9-times higher in children infected with HIV than in those who were not (Klugman et al., 2003). Additionally, in the South African trial, lower respiratory tract infections (LRTI) were responsible for 66% of all deaths, and there were fewer overall deaths (5% difference) among PCV9 recipients, but this was not statistically significant (Klugman et al., 2003). A sixteen percent VE in all-cause mortality was reported in both the Gambia and South African trials (Mackenzie et al., 2014). Researchers in Malawi assessed the impact of the PCV13 on clinically diagnosed severe or very severe pneumonia. In this study, PCV13 resulted in a 33% decrease in pneumonia hospitalization two years after its introduction, with 50% of the target population fully immunized (Mccollum et al., 2017).

2.4.5 Impact of pneumococcal conjugate vaccines on otitis media in low- and middle-income countries

There is paucity of data on the impact of the PCV on otitis media in LMIC, where the majority of disease burden and sequelae are reported (Vergison et al., 2010). A large proportion of research in LMIC has been concentrated on the effects of vaccines on pneumonia, carriage and IPD (Grant et al., 2016; Hammitt et al., 2014; Klugman et al., 2003; Madhi et al., 2016; Roca et al., 2013). Previous research has shown that PCV vaccinations given to infants prior to their first episodes of infections of the middle ear may have some health benefits (Fortanier et al., 2019).

Studies from the United States, Australia and Europe have reported reductions in overall AOM episodes and severe complications after the introduction of various PCV formulations (PCV7, PCV10 or PCV13) into national immunization programmes (Ben-Shimol et al., 2014; Fletcher & Fritzell, 2012; Lau et al., 2015; Leach et al., 2014). In some studies, these reductions outperformed the vaccine effect observed in randomized clinical trials (Eskola et al., 2001; Kilpi et al., 2003). Data from the United States on trends

before and after PCV13 implementation revealed that OM hospital visits among American Indians and Alaska Natives children under-five declined by one third from 2003 – 2005 to 2010 – 2011, to a rate similar to the United States general population <5 years (Singleton et al., 2018). However, a slight increase in OM incidence was observed in children aged 3 and 6 years (Vojtek et al., 2017). These variations suggested that, in addition to PCV-induced immune pressure, other factors were likely influencing the epidemiology of otitis media infections (Vojtek et al., 2017).

Studies on the aetiology of AOM in LMICs are uncommon because tympanocentesis is not routinely performed in clinical practice, particularly in SSA (Karppinen et al., 2019). Since the introduction of the PCV, estimates based on other reports have provided evidence of AOM disease caused by pneumococcus and other bacteria (Fortanier et al., 2019; Vojtek et al., 2017). As a result, middle ear fluid (MEF) from children with spontaneous purulent ears and tympanostomy tube placement or MEF samples isolated through tympanocentesis from children with serious or recurrent AOM are regarded as optimal (Lieberthal et al., 2013; Schilder et al., 2016). Researchers in Finland (FinOM study) reported that, in addition to non vaccine serotypes, vaccine-type pneumococci as bacterial causes of OM might be replaced by other microbes such as *H. influenzae* and *M. catarrhalis* following PCV immunization (Eskola et al, 2001). Similar findings have been reported in the United States, United Kingdom, Israel and Australia (Ben-Shimol et al., 2014; Kaur et al., 2017; Lau et al., 2015; Leach et al., 2014).

Therefore, it appears that routine PCV vaccination causes changes in bacterial nasopharyngeal carriage (Kaur et al., 2017). Subsequently, this causes changes in bacterial pathogens that cause ear infections, including an increase in non-vaccine type pneumococci and non-pneumococcal microbes (Dagan et al. 2013; Kaur et al., 2017). PCV significantly reduces the prevalence of pneumococcal AOM, which should enhance the development of novel vaccines that will elucidate long-term protection against otitis media (Fortanier et al., 2019).

3 Aims of the study

The general aim of this dissertation was to estimate the baseline proportion of pneumococcal associated deaths, otitis media prevalence, nasopharyngeal carriage of *Streptococcus pneumoniae* and the impact of PCV13 on otitis media prevalence among children under the age of five in Cameroon.

Sub-studies were conducted to address the following specific aims:

I. To estimate the proportion of under-five hospital-registered mortality caused by pneumococcal diseases in Cameroon prior to the implementation of the PCV13 programme (Study I)
II. To assess and compare the prevalence of nasopharyngeal pneumococcal carriage (NPC) among PCV13-unimmunized and PCV13-immunized children aged 2 to 3 years in Cameroon after PCV-13 introduction (Study II).
III. To estimate the prevalence of otitis media in Cameroonian children aged 2 to 3 years who were not immunized against PCV13 (Study III).
IV. To assess the impact of PCV13 on otitis media in Cameroonian children aged 2 to 3 years (Study IV)

4 Materials and Methods

4.1 PCV13 inclusion in Cameroon's infant vaccination schedule

PCV-13 arrived in Cameroon in July 2011 and followed the routine infant vaccination calendar for diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae type b* combination vaccine (DTP-HepB+Hib). It is administered using the primary accelerated series at 6, 10 and 14 weeks of age, respectively, for the three doses to infants under a year old. No booster doses of the PCV13 are given and no catch-up schedules to older infants were planned.

4.2 Study area, designs, subjects, and data collection

4.2.1 Study setting

Cameroon, a Central African Country with a population of over 25 million people, is located at the entrance to the Gulf of Guinea, in the middle of latitudes 2° and 13° North of the equator and longitudes 9° and 16° East towards the Greenwich meridian. It has a surface area of 475.440 Km² and resembles a triangle in shape, standing at a height of approximately 1,200 km and a width of 800 km. Following the 1996 constitutional amendments, it was restructured into ten regions, each region having a regional capital. One of these regional capitals, Yaoundé, also serves as the country's national and political capital. Cameroon's main climatic zones consist of the warm desert and warm semi-arid climate in the north, tropical savanna climate in the centre, and monsoon and equatorial climate in the south as well as along the coast.

The study sites are located in Cameroon's capital city covered a radius of about 80 kilometers. According to the 2010 National Population Census, there were over 3.5 million people living within Yaoundé and its suburbs and 18% of the population were children aged from 2 to 3 years. The sites were chosen because they are part of a network of healthcare establishments termed the infectious disease sentinel surveillance sites. These include four rural health districts, in addition to the Cite Verte Health District (urban) which is host to the Mother & Child Reference Hospital (MCH). Using the health map, sites were divided into 40 blocks (clusters), and at least one public or private health institution was in each cluster.

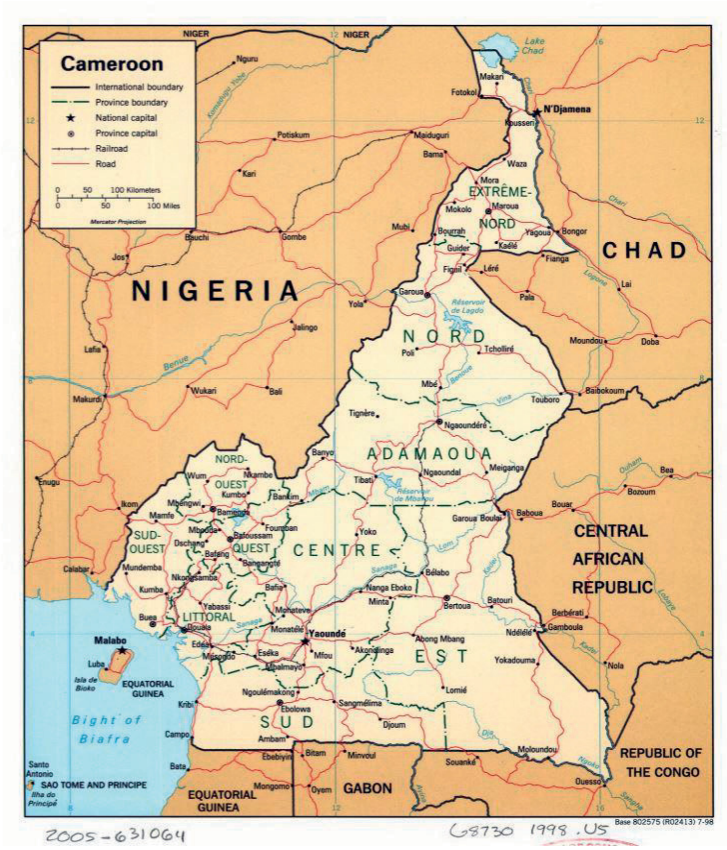

Figure 2. Map of Cameroon depicting regional capitals, including Yaoundé, where study sites were located, as well as border countries

4.2.2 Study population

This consisted of all children under the age of 60 months who were entitled to benefit from Cameroon's national childhood immunization scheme from 2006 to 2012 for the baseline mortality data, and from 2011 to 2015 for the PCV13 era. The total study sample size was over 2800 subjects drawn from a source population of approximately one million children aged 1 to 59 months, and more than 500 subjects were sampled in each sub-study.

4.2.2.1 Study-specific population for studies II, III, IV

We targeted children born between June 2010 and June 2011 (baseline data) and between June 2012 and June 2013 (comparison data). Cameroon has an annual birth cohort of approximately 856000 according to the 2016 report of The Vaccine Alliance – GAVI (www.gavi.org/country/cameroon). Selection of subjects

was done systematically following the WHO cluster sampling method and guided by the inclusion and exclusion criteria. The starting household within the cluster was selected after spinning a pen, usually at a central location in the community, and selection of participants was done randomly after every 10th home within a cluster. One participant was selected per home even if two or more were eligible (in such an event, selection was with respect to birth order); and twenty-five children were enrolled per "cluster".

4.2.2.2 Inclusion and exclusion criteria for Studies II, III and IV

Those included in these studies were children aged from 24 to 36 months, residing in the study area for at least six months, availability of parental signed consent and subjects who were PCV-unvaccinated (for the baseline group in 2013). In 2013, enrolment was restricted to those who had not received any doses of the PCV as was confirmed from child's vaccination card or registers. Children had to have at least two documented doses of PCV13 in order to be eligible for inclusion in the comparison group in 2015.

4.2.3 Study designs and data collection

All studies used an observational study design, with cross-sectional design for Study II and retrospective cohort design for Studies III & IV in which questionnaires were used during parental interviews to collect clinical, demographic, and household information, and a retrospective cohort design for hospital-based data collection in Study I.

4.2.3.1 Pneumococcal-associated mortality (Study I)

The data on the leading causes of death among children under the age of five between January 1st 2006, and December 31st, 2012 were obtained using a retrospective cohort design. The study sites were those involved in infectious disease surveillance at Yaounde's Mother and Child reference hospital. A quasi-random sampling technique was used in which cases were selected after every twentieth-page from the annual registers and the personal identifiers of each case was then extracted, and used to search for the full patient's record/medical file which were saved in the hospitals. We accessed and reviewed hospital records for all under-five deaths, and estimated the proportion associated with all-cause under-five mortality (including pneumococcal infections) from January 2006 through December 2012.

Furthermore, in an attempt to get a vivid understanding of the real-world potential effects of the PCV13 on pneumococcal-associated mortality in Cameroon, the outcomes were modelled into two time-periods

i.e., 1st January 2006 – 30th June 2011 (pre-PCV13 period) and from 1st July 2011 – 31st December 2012 (post-PCV13 period). Additionally, for analytical purposes year 2011 was divided into two time frames to correspond with the period before and after PCV13 introduction i.e., 1st January 2011 – June 30th 2011(within the pre-PCV13) and from 1st July 2011 – 31st December 2011 (within post-PCV13). Moreover, some of the post-PCV13 introduction period was included because we had considered the administrative PCV13 vaccination coverage for 2011 and 2012 which averaged below 55% (World Health Organization, 2018); and assumed that herd effects should not have strongly been implanted. This was especially true as on consulting with the hospitals' death counts, pneumococcal-related causes of death (including sepsis, meningitis and pneumonia) in the early-PCV13 years were still high or similar to pre-PCV13 period compared to late PCV13 years i.e., from 2013 upwards.

To address issues with probable multiple causes of death, a tabulation system was established using the WHO recommended criteria for medical certification of deaths, in which the causes of deaths are outlined in a sequential format (WHO, 2016). This format begins with the immediate direct cause of death through the intermediate causes, while the underlying cause of death is registered in the lowest line of Part I of the medical certificate of cause of death (134). However, due to the retrospective nature of the study and our inability to determine the exact sequence of causality, we have reported only the primary cause of death as stated in the medical record/death declaration form by the attending clinicians (who had multiple years of experience in tropical medicine). Secondly, although we have gone further to classify causes of death using the ICD-10-CM codes, our final determination of cause of death was dependent solely on what was clinically reported. For example, in a case of sepsis with no laboratory confirmation, we reported and coded it as sepsis from unspecified origin and this was same with pneumonia or the other causes of death.

The cause of death as described in hospital registers was classified using the ICD-10-CM codes. Detailed socio-demographic information was retrieved and entered into structured forms designed for the study, which were further elaborated in death declaration forms. Using hospital-based ICD-10-CM list diagnoses, mortality proportions resulting from each disease were calculated as a fraction of the totality of deaths that could be attributed to specific conditions. Due to the complexities of defining infections with pneumococcal aetiology, the cases of death were classified in this study as:

a) Clinical pneumococcal infections:

i. Acute Lower Respiratory Tract Infection (ALRI): Pneumonia diagnosed as according to medical declaration as cause of death (i.e., any ALRI or pneumonia); or

ii. Clinically severe pneumonia: Death due to coughing or difficulty to breathe as symptoms of admission for a child aged 1–59 months living in the study area. This could be accompanied by any danger signs (including either with a respiratory rate of over 40 beats/minute, or temperature of more than 38.5^{o}C, or when the child refuses to feed, or vomits, and/or lower chest in-drawing).

b) Infections with laboratory confirmation: This includes;

i. Invasive pneumococcal disease cases which were culture-positive,

ii. Antigen test-positive or polymerase chain reaction (PCR) invasive pneumococcal disease

c) Infections with radiological confirmation

i. Pneumonia with radiological confirmation i.e., Chest X-ray Community Acquired Pneumonia consistent with endpoint consolidation

ii. Pneumonia with radiological confirmation i.e., Chest X-ray Community acquired Pneumonia showing any abnormal radiological images.

N.B: Clinical infections with suspected pneumococcal aetiology that did not meet these criteria, were classified as non-confirmed pneumococcal disease (unspecified).

d) Causes of death with non-pneumococcal aetiology: These included non-pneumococcal biologically related causes of death among children under-five, such as tuberculosis (TB), human immuno-deficiency virus (HIV) or malaria among others.

e) Injury-related deaths: These were deaths of children under-five not due to biological causes e.g., injuries or accidents.

In the final analysis, pneumococcal deaths include a combination of sepsis, pneumonia and meningitis. Moreover, there were sixteen cases with complete missing data points and these were excluded for not meeting the study inclusion criteria. Briefly, the classification above was meant to capture at least one of medically declared causes of death with or without laboratory confirmation. Further, the sentinel surveillance system had contact information for parents/guardians who were contacted via phone calls to first express our sympathy and kindly asked if they could provide us with some clinical and/or demographic information of the deseased children. This was successful in the majority of cases, and as such bias from missing information was minimized.

4.2.3.2 Nasopharyngeal sample collection and laboratory methods (Study II)

Study II was conducted concurrently with the otitis media studies (III & IV). In this study, two rounds of surveys were carried out at the start of March through July: in 2013 (PCV13-unimmunized), and in 2015 (PCV13-immunized) in which nasopharyngeal (NP) specimens were obtained from children aged 2 to 3 years. The World Health Organization (WHO) systematic cluster sampling technique was used, in which NP swabs were obtained from eligible subjects and processed in accordance with WHO recommendations (Satzke et al., 2013). Pathogens were identified using standard bacterial culture techniques. *S. pneumoniae* was isolated using gentamicin-blood agar plates and optochin susceptibility testing was used for identification.

Serotyping was performed via sequential multiplex polymerase chain reaction (PCR), and verified using the Quellung test, if needed.

The initial intention in Study II was to draw comparison between the 2013 and 2015 NP samples, but this was hampered by transportation delays resulting in unintentional subjection to inappropriate temperatures (≤ -20°C against the recommended -70°C) which may have jeopardized the viability of the 2013 samples. Hence, vaccine effectiveness analysis on carriage could not be performed.

4.2.3.3 Otitis media assessment (Studies III & IV)

Studies III & IV were carried out using community-based retrospective cohort study designs. Two rounds were required: the first for children who had not been vaccinated from March to June of 2013 (baseline data), and the second for PCV13-vaccinated children 2 years later in 2015. The prevalence of otitis media (OM) was estimated using tympanometry for otitis media with effusion (OME) and by clinical inspection for chronic suppurative otitis media (CSOM). Using a Welch Allyn with Siegel's speculum, pneumatic otoscopy was done and many of the subjects had cerumen stacked in the middle ear, but we lacked sufficient material to clean earwax at field conditions. Therefore, AOM evaluation could not be done. Tympanometry was performed using the middle ear analyzer Grason Stadler tympanometer (GSI-38 Autotymp, Grason-Stadler Inc., Milford, NH, USA). Tympanograms were recorded with a 226Hz probe tone with a pressure varying from +200 deca Pascals (daPa) to -400 daPa in a time of 7 seconds. We did not perform tympanometry on draining ears.

Study-specific case report forms (CRF) were used for parental interviews to obtain socio-demographic and clinical history of study subjects. The CRF were structured to obtain a history of the presenting/previous symptoms from within the last six months, to capture primary symptoms of any respiratory infections including clinical diagnosis of otitis media. The CRF were also used to gather data on demographic characteristics, parental smoking status, and source of household cooking, duration of breastfeeding, antibiotic use, family socio-economic status and number of children under 18 years living in household; besides the number of children sleeping in the same bedroom. Questionnaires (CRFs) were administered in either English or French depending on the language preference of the respondent.

Middle ear statuses were categorised as: (a) Healthy; (b) Otitis media with effusion (OME); (c) otitis media with effusion (OME); (d) dry perforations and scarred tympanic membranes; and (e) Chronic suppurative otitis media (CSOM).

4.2.4 Rationale for selecting participants in Studies II, III and IV

One of the pitfalls of the PCV implementation programme in resource-low settings including Cameroon was the absence of country-specific pneumococcal disease burden baseline research data; as other criteria

were used by WHO and GAVI to select eligible countries to prioritize vaccine funding (Shabir A Madhi & Nunes, 2016). This entails, assessing vaccine impact in most of LMICs is dependent primarily on using administrative or hospital data as baseline. The PCV13-unvaccinated children (as well as the PCV13-vaccinated) in our study were randomly selected from within the general population (although a few parents declined the participation of their children for undisclosed reasons), and as such should share same differences or similarities.

The assumption was that both the selected children and the general population were equally exposed to same level of infection or carriage i.e., there was random mixing in the community, and that the vaccinated and unvaccinated were equally exposed to infection and that pockets of high vaccination coverage may not be present which may have reduced exposure to infection.

Hence, our rationale of using 2-3-year-old PCV13-unvaccinated children was to serve as a proxy to disease baseline prevalence considering PCV13 coverage in the early years (2011-2012) averaged <55% (WHO, 2018), based on administrative data (which were advised to be interpreted with caution). Although recent studies have demonstrated that even at 50% PCV coverage important herd effects could be achieved (Chan et al., 2021), the dynamics on disease burden and transmission maybe totally different in the context of Cameroon.

More so, the proxy-baseline carriage data in 2013 were somehow flawed due to storage under inappropriate temperatures (≤-20^{o}C against the recommended -70^{o}C) during the cause of transportation in a European port of entry for over two months. This may have compromised the viability of some pathogens, especially those of *S. pneumoniae* (Kaijalainen & Palmu, 2015; Satzke et al., 2013), hence to avoid selection bias they were not used for comparison with the 2015 survey. Another consideration was that as the PCV programme in Cameroon matures overtime, the dynamics too will change and the herd effects will be widely spread then. In view of this, there are plans to conduct broad-based population studies including adult (mother)-infant pairs as well older children to effectively assess the long-term impact of the PCV13 on the epidemiology of carriage and disease.

4.3 Questionnaires

4.3.1 The content of questionnaires used for Study I

The principal causes of under-five mortality in the country have never been assessed and there is no baseline data for vaccine effectiveness evaluation as is the case with the pneumococcal conjugate vaccine introduced in July 2011. The goal was to access and identify all cases of under-five deaths in the study area

within the study population and define the most probable causes of under-five deaths based on available data sources. The study-specific case report form below was used to capture relevant information.

CASE REPORT FORM: Mortality study **CRF code No.**

a) **Data sources**

1. Hospital registers
2. Vital registration systems (municipal and population offices)
3. Others: _____ (state, please)

b) **Subject identification**

Subject number in the study_____
Forename _____
Surname _____
Place of residence _____
Date of Birth _____
Parents' names/contact details_____
Gender _____
Date of Death_____
Hospital issued from_____
Hospitalised on: _____
Left hospital on: _____
Outpatient: _____

Place of death: hospital/home/other (if other specify: _____)

Death declaration based on:

i) Diagnostic data obtained during hospitalization; ii) autopsy; iii) verbal autopsy iv) other (specify: _____)

j) The primary cause of death as written in the death declaration: *(tick all that apply in table 1 below)*

Section 1: Cause of Death

Disease	Not diagnosed	Primary cause of death (tick only one)	Contributing to death (tick all that apply)	Clinically diagnosed	laboratory-confirmed disease	Unknown
Malaria						
Diarrhoea						
Measles						
Accident						
Meningitis						
HIV						
TB						
STDs						
Ischemic heart disease						
Conditions originating from perinatal period						
Nervous system						

conditions						
Diabetes mellitus						
Mastoiditis						
Conditions originating from perinatal period						
External causes						
Other causes (specify)						
Pneumonia						
Disease not specified or cause of death not specified						

Section 2: For pneumococcal cases (*Yes, clinical disease*)

	1. No	2. Yes	3. Unknown /Missing
Cough/ difficult breathing			
Respiratory of rate ≥ 40/minute			
Temperature >38.5°C			
Refusing to feed			
Vomiting/lower chest in-drawing			

Section 3: For pneumococcal cases **(***Yes, laboratory-confirmed disease***)**

	1. No	2. Yes	3. Unknown /Missing
Culture positive IPD			
Culture-negative PCR/antigen positive IPD			
Radiologically confirmed pneumonia			

4.3.2 The content of questionnaires used for Studies II, III and IV

The carriage and otitis media studies were conducted simultaneously during two rounds of survey in 2013 and 2015, respectively using the same set of questionnaires with details below.

Carriage and Otitis Media Studies: Cameroon Case Report Form Version 3.0, 09.04.2015

Study nurse completes the form by interviewing the parents. From response lists, check that one response that applies best unless otherwise stated. For other questions, use the provided space to write out the replies.

Child identifiers

1. Identification number (6 digits = 5 digits + check/Control digit) _____ ___
2. Initials (first 2 letters of the first Christian name and first 2 letters of the family name _____ __

3. Date of visit ____. ____._____

4. Place of visit

1. ="Study clinic" 2. ="Home" 3. ="Other"

Enrolment and Consent:

5. Has one of the parents/guardians their consent to participate either verbatim or signed form?

1No (If **no**, the child cannot be enrolled and no further questions will be asked)
2 Yes (if yes, give the informed consent date ____. ____._____)

Inclusion criteria

6. Age 2 to 3 years

1. No 2. Yes

7. At least one parent with the capacity to communicate in French or English

1. No 2. Yes

*If the response to any of the questions was **"No"** the child cannot be enrolled and no further questions will be asked.*

Exclusion criteria

8. Commercial/ PCV vaccination administered

1.No 2. Yes

9. Study-related PCV vaccination administered (open or blind)

1. No 2. Yes

10. Antimicrobial treatment within 4 weeks (the child can be enrolled later)

1. No 2. Yes

*If the response to any of the questions was **"Yes"** the child cannot be enrolled and no further questions will be asked.*

INTERVIEW QUESTIONS FOR THE PARENT(S)

Family information

11. How many adults (18 years or more) live with the child in the same household? _____

12. How many children (less than 18 years) live in the same household in addition to this child? _____

Parental smoking and source of cooking

13. Does any member of your family smoke regularly?

1. No 2. Yes 3. Unknown

14) If yes, does any family member smoke indoors in your residence?

1. No 2. Yes 3. Unknown

15) Wood/coal as source of cooking at home

1. No 2. Yes 3. Others

Following questions from the child enrolled in the carriage study only.

Antibiotics:

16) Has the child received antibiotics during the last three months (only systemic antibiotics are mentioned here)?
1 No
2 Yes, but the treatment was stopped 1 to 2 months ago
4 Yes, but the treatment was stopped 2 to 3 months ago
9 Unknown

Respiratory infections:

17) Has your child had any symptoms of respiratory infection during the past four weeks, for example runny nose, cough or throat pain or fever over 38 degrees C.
1 No
2 Yes, the child is currently symptomatic
3 Yes, but the child has been symptomless for 1-7 days
4 Yes, but the child has been symptomless for 8-14 days
5 Yes, but the child has been symptomless for 15-28 days
9 Unknown

18. Child enrolled in carriage study	19. What is the birth month and year of the child?	20.Daycare/nursery/school	21. What is the group size?	22. Has the child been immunized with pneumococcal conjugate vaccine or participated in a study in which the child may have received PCV	**23.** When was the first dose of the vaccine given? (MM/yyyy)
1st child: Yes		1="No" 2="Family day care" 3="Day care center" 4="Preschool" 6="School" 7="Other" 9="Unknown"		1="No PCV given" 2="UMV in the PMI clinic (free of charge)" 3="Prevenar commercial" 4="Synflorix commercial 6="Other study vaccine" 7="Unknown"	
2nd child: No		1="No" 2="Family day care" 3="Day care center" 4="Preschool" 6="School" 7="Other" 9="Unknown"		1="No PCV given" 2="UMV in the PMI clinic (free of charge)" 3="Prevenar commercial" 4="Synflorix commercial 6="Other study vaccine" 7="Unknown"	
3rd Child: No		1="No" 2="Family day care" 3="Day care center" 4="Preschool" 6="School" 7="Other" 9="Unknown"		1="No PCV given" 2="UMV in the PMI clinic (free of charge)" 3="Prevenar commercial" 4="Synflorix commercial 6="Other study vaccine" 7="Unknown"	

Nasopharyngeal Samples (NPS):

NPS sample (mixed in STGG, stored at -20°C within 8 hours)
1="Sample taken according study protocol"
2="Not obtained, but was tried for"
3="Parents refused"
4="No sample for some other reason" (specify _____)
9="Unknown"

Tympanometry performed:

1="Sample taken according study protocol"
2="Not obtained, but was tried for" (specify _____)
3="Parents refused"
9="Unknown"

Sample sticker series number: (affix sticker here →_____)

4.4 Statistical analyses

4.4.1 Descriptive and multivariate analyses for the baseline data

4.4.1.1 *S. pneumoniae*-related causes of death in children under the age of five (Study I)

For the all-cause under-five mortality study, included were cases of children aged from 1 to 59 months (for analytical purposes, 29 days = 1 month), and whose deaths were registered between January 1, 2006, and December 31, 2012. Excluded were deaths that occurred during the first 28 days i.e., four weeks from birth (neonatal period). The follow-up period was from January 1, 2006 to 31st December, 2012. Therefore, the entry point into the study was the date of birth (age in months) of each case recorded at admission, and the follow-up period ended at the reported date of death. The determination of the cause of death (CoD) was dependent on medical report and the International Classification of Diseases and Clinical Modifications 10th revision (ICD-10-CM) codes (ICD10Data.com 2017 edition) were used to reclassify the various CoD. The age-stratified proportions of deaths due to pneumococcal infections and other pathogens were calculated by dividing the total number of deaths retained by the number of each specific CoD. We used the Kaplan-Meier method to calculate the median ages of death for both males and females. The data was analyzed using the SPSS 25.0 software package, and 95 percent confidence intervals (CI) for estimates were calculated.

4.4.1.2 Estimates of the baseline prevalence of otitis media

We adapted a version of Liden and Jerger's categorization criteria (Table 2) in the interpretation of tympanograms to assess middle ear status in both Studies III (baseline data) and IV (post-PCV13 data) (Palmu et al., 2002).

In this categorization, the presence of middle ear fluid (MEF) is depicted from a flat, 'type B' tympanogram, and tympanograms with curve types A, As, C or Cs represented the absence of MEF (Studies III & IV). Tympanic membrane (TM) perforations i.e., type P tympanograms were interpreted from 'flat curves' having high external ear canal volumes (ECV >1.0cm^3). We interpreted as failed (type F tympanogram) curves which had invalid peaks because of artifacts or instability of the child, as well as those with ECV <0.3cm^3 without any recording of a standard curve.

Table 2. Categorization criteria used for reporting tympanograms in this thesis (Palmu et al., 2002)

Curve Type	Criteria	Clinical Presentation
A	TPP ≥ -100daPa, SAA ≥ 0.2 cm^3	Middle ear pressure (MEP) is normal, static admittance is normal and there is no MEF
B	Flat curve; ECV = 0.3 to 1.0; no values for TPP	This is consistent with Middle ear pathology (MEF)
C	TPP < -100daPa, SAA ≥ 0.2 cm^3	MEP is significantly negative, static admittance is normal, and there is no MEF
As	TPP≥ -100daPa, SAA ≤ 0.2 cm^3	Reduced admittance, MEP is normal, and there is no MEF
Cs	TPP< -100daPa, SAA ≤ 0.2 cm^3	Reduced admittance, lower MEP, and no MEF
F	Erroneous peaks (no distinct curves) or ECV < 0.3 in the absence of a distinct curve	Failed tympanogram, unstable child during procedure, or probe in contact with ear canal or ear wax
P	No Peak (or flat curve); ECV>1.0	Perforated Tympanic Membrane

SAA= Static acoustic admittance; TPP= Tympanometry peak pressure; daPa= deca-pascals; MEF=Middle ear fluid; ECV = Ear canal volume. The difference between A and As (and C & Cs) at SAA=0.2cm3 was dependent on the curve's graphical representation. When the curve crossed the lower limit of the graphic normal box, it was labeled as A (or C, depending on the TPP); A, As, C, Cs = Healthy ears; B= Diseased ear, F=Failed tympanogram; P=Perforation.

However, during otoscopy we discovered that most of the children had ear wax that had accumulated in their middle ears which could occlude the ear canal and, produce a flat tympanogram curve i.e., 'false type B'. This issue was resolved by examining how the ECV measurements were distributed between the different tympanogram types. Initially, we had interpreted most of the flat tympanograms as 'type B' which had lower ECV values i.e., 0.3cm^3 and 0.4cm^3 in contrast to other tympanogram types, and these differences in the mean ECV values of flat and 'Other type' tympanograms were statistically significant. This difference, to our reasoning, was most likely caused by occlusion from ear wax, which resulted in flat tympanograms observed in most of the children. In view on how the ECV values in 'Other type'

tympanograms with discernible curves (A, As, C and Cs) were distributed, we observed that ninety percent of these fell between the 0.5cm^3 to 1.2cm^3 ECV categories, and ten percent fell between 0.3cm^3 and 0.4cm^3 ECV range. The original number 'type B' tympanograms having low ECV values of 0.3cm^3 and 0.4cm^3 were factored to follow a similar ECV distribution for the set categories of 0.3cm^3 and 0.4cm^3 i.e., only 10% of type B tympanograms with ECV values of 0.3cm^3 and 0.4cm^3 were true positives (Study III).

We used multivariate logistic regression to look for associations between OM and selected previously identified potential confounders (Simões et al., 2015). Gender, age, number of siblings who were sleeping in the same bedroom, number of siblings living in same household aged under 18 years, history of previous otitis media, breastfeeding, antibiotic use, parental socio-economic and smoking statuses, and type of household cooking fuel were considered. The inclusion of covariates in the multivariate analyses was limited to a 5% level of statistical significance. The strength of the associations between otitis media and covariates was assessed using prevalence odds ratios (PORs) and ninety-five percent confidence intervals (95%Cs). The statistical software programme SPSS 25.0 version was used for the analyses.

4.4.2 Descriptive and multivariate analyses for the post-PCV13 effects

4.4.2.1 Prevalence of nasopharyngeal pneumococcal carriage after PCV-13 introduction (Study II)

Following previous reports, our assumption was that the colonization prevalence of any pneumococcus in circulation in the population was estimated at sixty percent (Gervaix et al., 2012); total vaccine-type pneumococci proportion was over thirty-seven percent, i.e., 37.2 percent out of all subjects, and the expectation was that, following vaccination there will be at least a 50% decrease in vaccine-type carriage i.e. from 37.2 percent to 18.6 percent.

For sample size estimations, we used the website https://statpages.info/proppowr.html. To achieve 80% power, 101 subjects per group was the minimum sample size needed. However, to get a higher statistical power, we sampled more subjects i.e., 198 in 2013 and 689 in 2015. We examined how pneumococcal serotypes were distributed after PCV13 introduction and the χ^2-test was used to compare differences between the PCV13-immunized and PCV13-unimmunized groups. The level of statistical significance was set at <0.05. All analyses were performed using the statistical software package SPSS version 25.0.

4.4.2.2 Estimates of the impact of PCV13 on the prevalence of otitis media (Study IV)

We earlier stated that, AOM prevalence in the baseline survey was not reported because many of the ears were occluded in the otoscopy and we did not have enough materials at the time to clear earwax, but this was corrected in the 2015 survey. As a result, in the vaccine effectiveness (VE) analysis (Study IV), the focus is solely on CSOM and OME, which were evaluated using the same criteria in both surveys. In Study IV, we compared the prevalence of OM in PCV13-vaccinated children who had received at least two doses of PCV13 in 2015 to that of children who had not received PCV13 vaccination in 2013. In addition, we explored associations between OM prevalence and potential risk factors. To compare differences in OM prevalence of baseline characteristics between PCV13-vaccinated and PCV13-unvaccinated groups, the Chi-square test was used. Variables with p-values of 0.05 were entered iteratively into multivariate logistic regression analyses models. Potential predictors found in the first model were entered into a second model to obtain adjusted prevalence odds ratios (PORs). At a statistical significance level of 5%, we calculated PORs and risk difference (RD) with their 95%CI.

The attributable proportion (AP) also described as the attributable risk percent (ARP) was used to quantify the public health impact of the PCV13 on OM in the study population overtime. In imputing the AP, two assumptions were considered; (1) that the two groups were comparable and the occurrence of OM in the unexposed group represents the baseline or expected risk of the disease, (2) if the risk of OM in the exposed group is higher than risk of OM in the unexposed group, then the difference can be attributed to the exposure (PCV13). AP was estimated as (Risk in the PCV13-vaccinated group – Risk in the PCV13-unvaccinated group) divided by the (Risk in the PCV13-vaccinated group) x100%.

Additionally, VE was estimated by comparing the OM outcomes in the two groups i.e., VE was calculated as 1- RR {ARV ÷ ARU}x100% (where ARV = attack rate in the vaccinated cohort, ARU = attack rate in the unvaccinated cohort and RR = Relative Risk). Using the sample size calculator (http://www.raosoft.com/samplesize.html), we estimated to sample at least 384 participants during each study period, for each Studies III and IV, respectively. Further, weighted avaerages were computed using administrative vaccination coverage figures for 2013 and 2015, respectively. SPSS 25.0 statistical software was used to conduct the statistical analyses.

4.5 Ethical considerations

The studies in this dissertation were approved by the National Ethics Committee of Cameroon, and the Gynaecology, Obstetric and Paediatric Hospital of Yaoundé institutional review boards (IRB). This was part of a larger study protocol titled "Estimating the burden of pneumococcal disease and estimating the impact

of the 13-valent pneumococcal conjugate vaccine (PCV13) inclusion into Cameroon's childhood immunization programme". We obtained from parents signed informed consent forms or consent was given orally.

5 Results

5.1 Baseline data

5.1.1 The proportion of under-five mortality caused by pneumococcal diseases (Study I)

During our initial baseline under-five mortality study, we collected data which covered all-cause under-five mortality from January 2006 to December 2012. Over 1800 deaths among children under the age of five were reported among the approximately 85000 hospital visits and admissions recorded during the study period (Study I). Eight hundred and seventeen patient files containing clinical and laboratory data were chosen at random for review. According to our age-stratified analysis of the baseline study on all-cause under-five mortality (Table 3), 29.3% of the children died from pneumococcal-associated infections including meningitis, sepsis and pneumonia.

Table 3. Proportions of the leading causes of death among children under the age of five in Yaoundé, Cameroon, from 2006 to 2012 (N = 817)

CoD	Age group (months)					All
	1 - 11	12 - 23	24 - 35	36 - 47	48 - 59	
	(N= 368)	(N = 187)	(N=104)	(N= 55)	(N= 103)	(N= 817)
	%	%	%	%	%	% (95%CI)
Malaria	11.7	22.5	21.2	32.7	17.5	17.5 (15.0 - 20.3)
Pneumococcal meningitis	11.7	10.2	16.3	5.5	7.8	11.0 (09.0 - 13.4)
Sepsis$^{\beta}$	13.0	11.2	6.7	0.0	5.8	10.0 (08.1 - 12.3)
S. pneumoniae*	9.8	7.0	8.7	3.6	3.9	8.3 (06.5 - 10.4)
Malnutrition¥	7.6	12.8	7.7	0.0	3.9	8.3 (06.5 - 10.4)
Diarrhoea	8.7	5.3	4.8	1.8	2.9	6.2 (04.7 - 08.1)
Other CoD	37.5	31.0	34.6	56.4	58.3	38.6 (35.2 - 42.0)

*CoD = Causes of death; N = number; % = percentage; CI = Confidence Interval; * = S. pneumoniae as the cause of disease classified elsewhere; ¥= Protein-calorie malnutrition not specified; β = Sepsis from unspecified cause (likely due to poorly treated invasive disease)*

As shown in Figure 3, there were no remarkable variations in the percentages of pneumococcal-associated deaths in year 2011 vs 2012 covering the immediate years pre-PCV13 and post-PCV13. However, in the first six months of 2011 (within the pre-PCV13 era) and last six months of 2011 (within the post-PCV13 era), a stark difference could be observed in the percentages of pneumococcal-associated deaths as well as with the other causes of death. But this maybe due to secular trends rather than immediate vaccine impact, following sensistivity analysis.

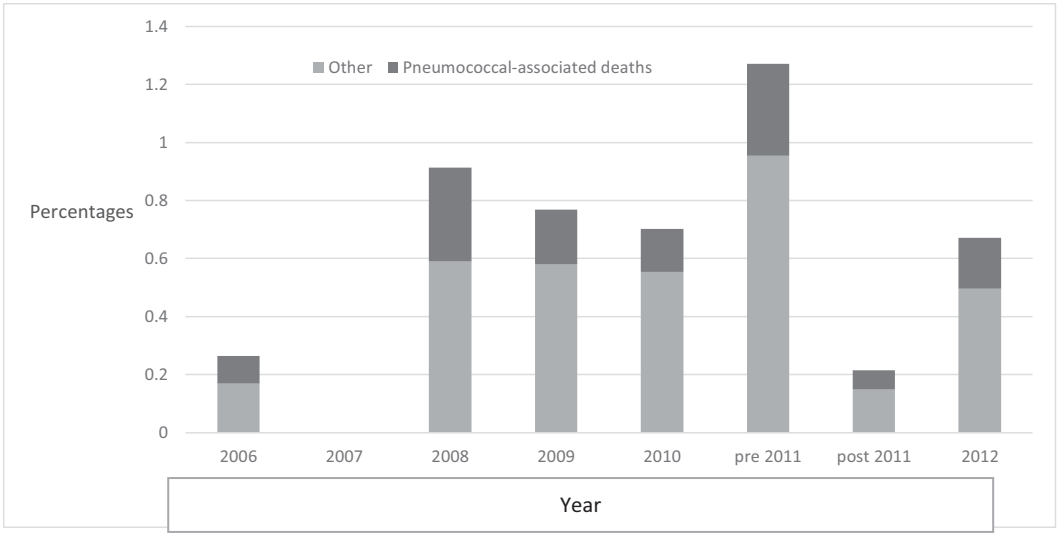

Figure 3. Percentages of pneumococcal-associated deaths and other causes of death among 1- to 59-month-old children registered at the infectious disease sentinel surveillance hospitals in Yaoundé, 2006 – 2012).

5.1.2 Prevalence of otitis media in children aged 2 to 3 years in Cameroon (Study III)

We enrolled 529 PCV13-unvaccinated children aged from 24 to 36 months in this study, and 82% (433) had a tympanogram measurement. Electrical power outages were the primary cause of missing tympanometry data. The baseline characteristics of the participants are shown in Table 4. Forty percent of subjects were sleeping alone and the remainder shared their bedrooms with at least one other sibling. Also, majority of subjects were living in same household with at least one sibling who was ≤18 years old. Day care amenities are uncommon in this setting for this age group so all children were enrolled during home visits.

Table 4. Baseline characteristics and Otitis Media (OM) prevalence among 2 to 3 years old children in Yaoundé, Cameroon, N= 433

Characteristics	N (%)	Prevalence of OM (%)	p-value
Gender of child			
Male	241 (55.7)	22/241 (9.1)	0.653
Female	192 (44.3)	20/192 (10.4)	
Age (group) of child in months			
24 to 29	157 (36.3)	15/157 (9.3)	
30 to 35	110 (25.4)	10/110 (9.1)	0.948
36	166 (38.3)	17/166 (10.2)	
No. of siblings sleeping in same bedroom ≤18years			
Alone	172 (39.7)	16/172 (9.3)	
One	143 (33.0)	13/143 (9.1)	0.949
Two	84 (19.4)	9/96 (10.7)	
≥ Three	34 (07.9)	4/34 (11.8)	
No. of siblings living in same home ≤18years			
One	77 (17.8)	11/77 (14.3)	0.214
Two	100 (23.1)	11/100 (11.0)	
≥ Three	256 (59.1)	20/256 (7.8)	
History of previous otitis media			
No	346 (79.2)	29/346 (8.4)	0.065
Yes	87 (20.8)	13/87 (14.9)	
Breastfeeding period			
≤ 6months or not breastfed	38 (8.8)	3/38(7.9)	
≤12 months	182 (42.0)	15/223 (6.7)	0.554
>12 months	213 (49.2)	24/213 (11.3)	
Antibiotic use			
No	150 (34.6)	13/150 (8.7)	0.597
Yes: with / without medical report	283 (65.4)	29/283 (10.2)	
Noticed any current URT symptoms			
No	344 (79.4)	28/344 (8.1)	**0.001**
Yes	49 (11.3)	13/49 (26.5)	
Unknown	40 (09.1)	1/40 (2.5)	
Parental educational level (SES1)			
≤ Primary school	148 (34.2)	14/148 (9.5)	
≥ Secondary school	214 (49.4)	25/214 (11.7)	0.183
≥ University Education	71 (16.4)	3/71 (4.2)	
Parental occupational/income level (SES2)			
No Education	212 (49.0)	17/212 (8.0)	
No Education, some income	93 (21.5)	8/93 (8.6)	
Some Education	11 (02.5)	1/11 (9.1)	0.201
Higher Education	57 (13.2)	5/57 (8.8)	
Student and others	60 (13.9)	11/60 (18.3)	
Parental smoking status	374 (86.4)	36/374 (9.6)	0.896
Non-smokers	59 (13.6)	6/59 (10.2)	
Smokers			
Using wood/cool as household cooking fuel			
No	128 (29.6)	10/128 (7.8)	0.390
Yes	305 (70.4)	48/305 (10.5)	

N = Number; % = percentage; OM = Otitis Media; URTI = upper respiratory tract infection; SES = Socio-economic status

Forty percent of these children were reported to be sleeping alone, while the rest had a common bedroom with one or more siblings. More than 90% of the parents said their children were staying at home with at least one 18-year-old sibling.

The overall baseline prevalence of otitis media was 9.7 percent (42/433) among children who were found with at least a single form of OM or its sequalae. This included 3 (0.7%) of the children having unilateral CSOM, 7 (1.6%) with bilateral OME and 31 (7.2%) were diagnosed with unilateral OME and 1 (0.2) of them had unilateral dry tympanic membrane perforation (ECV >1.0 cm^3). However, a statistically significant relationship was found between OM and the parentally reported symptoms of upper respiratory tract infections (URTI) variable (Table 4). When this sole predictor was modelled in a univariate analysis (Table not shown), the statistically significant relationship was maintained between OM and "parentally reported symptoms of URTI"; prevalence odds ratio (POR) = 4.1; 95% CI = 1.9 – 8.6; p= 0.001).

5.2 PCV13 effects following its introduction

5.2.1 PCV13's impact on pneumococcal nasopharyngeal carriage in children (Study II)

We collected data from 887 children ranging in age from 24 to 36 months (including 198 children not vaccinated with PCV13 in 2013 and, 689 PCV13 vaccinated children in 2015). The prevalence of *S. pneumoniae* was 57.6% (114/198) in the 2013 baseline group, and the respective prevalence of NVT, VRT and VT pneumococci were 27.8%, 3.5% and 21.2%. The prevalence of nasopharyngeal pneumococci carriage in PCV13-vaccinated children in 2015 was 61.8% (426/689), and the prevalence of VT, VRT and NVT pneumococci was 18.0%, 5.4% and 31.2%, respectively. From 540 pneumococcal isolates, 39 different serotypes were identified, and no statistically significant difference was found in the serotype spectrum between vaccinated and unvaccinated children (Figure 4).

In the baseline group, the most common serotypes were 15B/C (9.1%, 18/198), 19F (7.1%, 14/198), and 23F (5.1%, 10/198). Whereas the most frequent serotypes in 2015 were 15B/C (7.3%, 50/689) and 19F (4.5%, 31/689). In addition, out of the 13 vaccine serotypes, eleven were found in the vaccinated group. Vaccine serotypes 4 and 5 were only found in the PCV13 vaccinated children with respective percentages of 0.5% and 0.2%; whereas vaccine serotypes 1 and 7F, as well as the cross-reactive 6C, were not found in either groups (Figure 4).

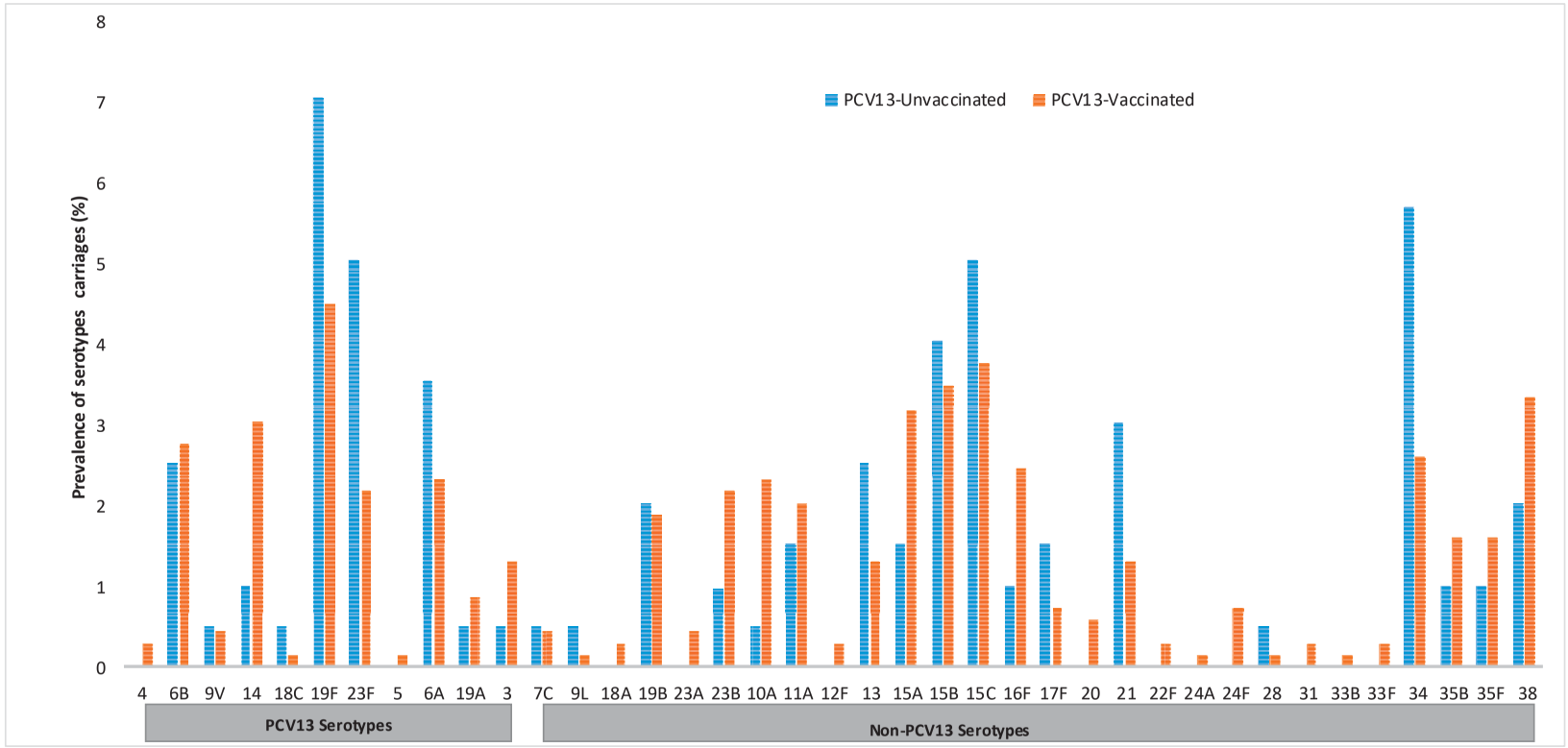

Figure 4. Prevalence of pneumococci carriage serotypes found in 2013 (PCV13-unvaccinated, n=198) and in 2015 (PCV13-vaccinated, n=689) groups of children aged 24 to 36 months in Yaounde, Cameroon.

5.2.1.1 Isolation and identification of pneumococci from nasopharyngeal samples

As previously reported (Kaijalainen and Palmu, 2015), nasopharyngeal samples were cultured for the isolation of *S. pneumoniae*. After thawing and passage through a vortex rotator for thorough mixing, 10 µl of each specimen was pipetted and inoculated on 5% sheep blood agar plate (for semi-quantitative evaluation of the growth) and 5% sheep blood agar + 2.5 µl/ml of gentamicin for isolation of *S. pneumoniae*. The plates were incubated in 5% carbon dioxide atmospheric conditions at 35 °C for 18 – 20 hours. If no colonies appeared, the incubation was continued for up to 48 hours. Pneumococci were identified from both plates by their morphological α-hemolytic characteristics and by optochin sensitivity testing. From each sample, if several suspected pneumococcal colonies appeared, up to four colonies were confirmed and stored. The isolates were stored in 10% skimmed milk-glycerol and sent in batches on dry ice for serotyping at the bacteriology laboratory of THL, in Helsinki, Finland.

5.2.1.2 Serotyping of pneumococcal isolates

In accordance with a previously validated typing scheme (Siira et al., 2012), based on sequential multiplex polymerase chain reaction (mPCR) supplemented with Quellung test (when needed), pneumococcal isolates were serotyped. In mPCRs, a primer pair targeting the pneumococcal specific cpsA locus was used as an internal control. The species of cpsA negative, Omni serum negative suspected non-encapsulated (NC) pneumococci were verified by lytA PCR. Serotypes were categorized as vaccine types (VT: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F), vaccine-related serotypes (VRT: 6C, 7A, 7B, 7C, 9A, 9N, 9L, 18A, 18B, 18F, 19B, 23A, and 23B), non-vaccine types (NVT) and NC pneumococci. Serotypes 15B and 15C were reported together as 15B/C because of the reversible capsule switching between these serotypes (Van Selm et al., 2003). To assess for co-colonization by multiple pneumococcal serotypes, randomly selected duplicate pneumococcal isolates obtained from 13.5% (n = 70) of the swabs (15/108 isolates obtained from 2013 swabs and 55/406 isolates obtained from 2015 swabs) were also serotyped. Although more colonies were available for testing, we could only swab for 70 due to limited resources.

5.2.2 PCV13 effects on otitis media in children aged 2 to 3 years (Study IV)

As shown in Figure 5, a total of 846 children from aged 24 to 36 months were enrolled in our post-PCV13 assessment of otitis media prevalence (Study IV). These included 433 children from the 2013 baseline survey (who had not received PCV13 vaccination) and 413 in 2015 comparison group (PCV13 vaccinated children). Among the PCV13-unvaccinated children, 42/433 (9.7%) were diagnosed with OM or its complications, compared to 69/413 (16.7%) for PCV13-vaccinated children (PD = 7% [95%CI: 2.5 to 11.6], p=0.003). This included 3/433 (0.7%) children identified with unilateral CSOM in the baseline survey

in 2013, compared to 9/413 (2.2%) of CSOM subjects in the PCV13-vaccinated group in 2015 (PD 1.5% [95%CI: -0.2 to 3.5], p= 0.067). Bilateral OME was diagnosed in 7/433 (1.6%) of unvaccinated PCV13 children and 12/413 (2.9%) of vaccinated PCV13 children (PD =1.3% [95%CI: -0.8 to 3.6%], p=0.201). The proportions of children with unilateral OME in the PCV13-unvaccinated group was 31/433 (7.2%) compared to 48/413 (11.6%) in the PCV13-vaccinated group (PD = 4.4% [95%CI: 0.5 to 8.4], p=0.028). No statistically significant difference was found in the proportions of subjects who had unilateral dry tympanic membrane perforation i.e., with ECV >1.0 cm^3 between the two groups, 0.2% and 0.0%, respectively (PD = 0.2% [95%CI: -0.7 to 1.2], p=0.365).

According to crude estimates from logistic regression analyses, PCV13-vaccinated children were significantly associated with more OM (POR = 1.76[95%CI: 1.12 to 2.68], p = 0.013) than PCV13-unvaccinated children (Table 5). Further, a negative 72% vaccine effectiveness (VE) estimates were obtained for PCV13 against OM prevalence, four years after PCV13 infant vaccination. This suggested that the vaccine was not working as expected i.e., likely to cause more OM infections amongst the vaccinated than in the vaccinated group. However, in the multivariate analyses (after controlling for significant predictors in the univariate model including symptoms of URTI and previous history of OM), there was no evidence that vaccinated children in 2015 were associated with higher odds of OM compared to unvaccinated children in 2013, adjusted POR = 1.50 [95%CI: 0.84 to 2.67], p = 0.171 (Table 6).

More so, the attributable proportion (AP), was estimated at 42% i.e., 42% of OM infection among the PCV13-vaccinated group might be attributable to PCV13 vaccination. This implies, the remainder (58%) of OM infections in the vaccinated group would have still occurred even without the vaccine.

Table 5. Baseline characteristics and clinical outcomes of PCV13-vaccinated (N = 413) and PCV13-unvaccinated (N=433) children aged 2 to 3 years' children screened for otitis media (OM) in Yaoundé, Cameroon

Characteristics/ Clinical outcomes	PCV13-unvaccinated		PCV13-vaccinated		Prevalence difference in % [95%CI]	ΩP-value
	N	%	N	%		
Otitis Media status						
Healthy children	391	90.3	344	83.3	7.0% [2.5 to 11.6]	**0.003**
Otitis cases	42	9.7	69	16.7		
Gender of child						
Male	241	55.7	212	51.3	4.4% [-2.3 to 11.6]	0.1999
Female	192	44.3	201	48.7		
Age (group) of child in months						
24 to 29	157	36.3	154	37.3	1.0% [-5.5 to 7.5]	0.7632
30 to 36	176	63.7	259	62.7		
No. of children living in same home ≤ 18 years						
One	77	17.8	52	12.6	5.2% [0.34 to 10.02]	**0.0356**
Two	100	23.1	99	24	0.9% [-4.8 to 6.6]	0.7579
≥ Three	256	59.1	262	63.4	4.3% [-2.3 to 10.8]	0.1998
No. of siblings sleeping in same bedroom ≤ 18 years						
Alone	172	39.7	37	9.0	30.7% [25.2 to 36.0]	**< 0.0001**
One	143	33.0	65	15.7	17.3% [11.6 to 22.9]	**< 0.0001**
Two	84	19.4	140	33.9	14.5% [8.6 to 20.3]	**< 0.0001**
≥ Three	34	7.9	171	41.4	33.5% [28.0 to 38.8]	**< 0.0001**
History of previous otitis media						
No	346	79.9	374	90.6	10.7% [6.0 to 15.4]	**< 0.0001**
Yes	87	20.1	39	9.4		
Breastfeeding period						
≤ 6months or not breastfed	38	8.8	21	5.1	3.7% [0.2 to 7.2]	**0.0350**
≤ 12 months	182	42.0	149	36.1	5.9% [-0.7 to 12.4]	0.0790
> 12 months	213	49.2	243	58.8	9.6% [2.9 to 16.2]	**0.0051**
Antibiotic rational usage						
No	150	34.6	23	5.6	29.0% [24.0 to 34.0]	**<0.0001**
Yes: with /without medical report	283	65.4	283	94.4		
Noticed any current upper respiratory tract symptoms						
No	344	79.4	374	90.8	11.4% [6.6 to 16.1]	**<0.0001**
Yes	49	11.3	33	8.0	3.3% [-0.7 to 7.3]	0.1050
Unknown	40	9.2	5	1.2	8.0% [5.1 to 11.2]	**<0.0001**
Parental educational level (SES)						
≤ Primary school	183	45.5	147	34.3	11.2% [4.6 to 17.7]	**0.0009**
≥ Secondary school	179	44.5	211	49.2	4.7% [-2.0 to 11.4]	0.1711
≥ University Education	40	10.0	71	16.6	6.6% [2.0 to 11.2]	**0.0046**
Parental smoking status						
Non-smokers	374	86.4	358	86.7	0.3% [-4.3% to 4.9]	0.8983
Smokers	59	13.6	55	13.3		
Using wood/coal as household cooking fuel						
No	128	29.6	102	24.7	4.9% [-1.1 to 10.8]	0.1096
Yes	305	70.4	311	75.3		

PCV13 = 13-valent pneumococcal conjugate vaccines; N=number; Ω = In bold denotes p-values less than 0.05; SES = Socio-economic status; % = percent; CI = Confidence Interval

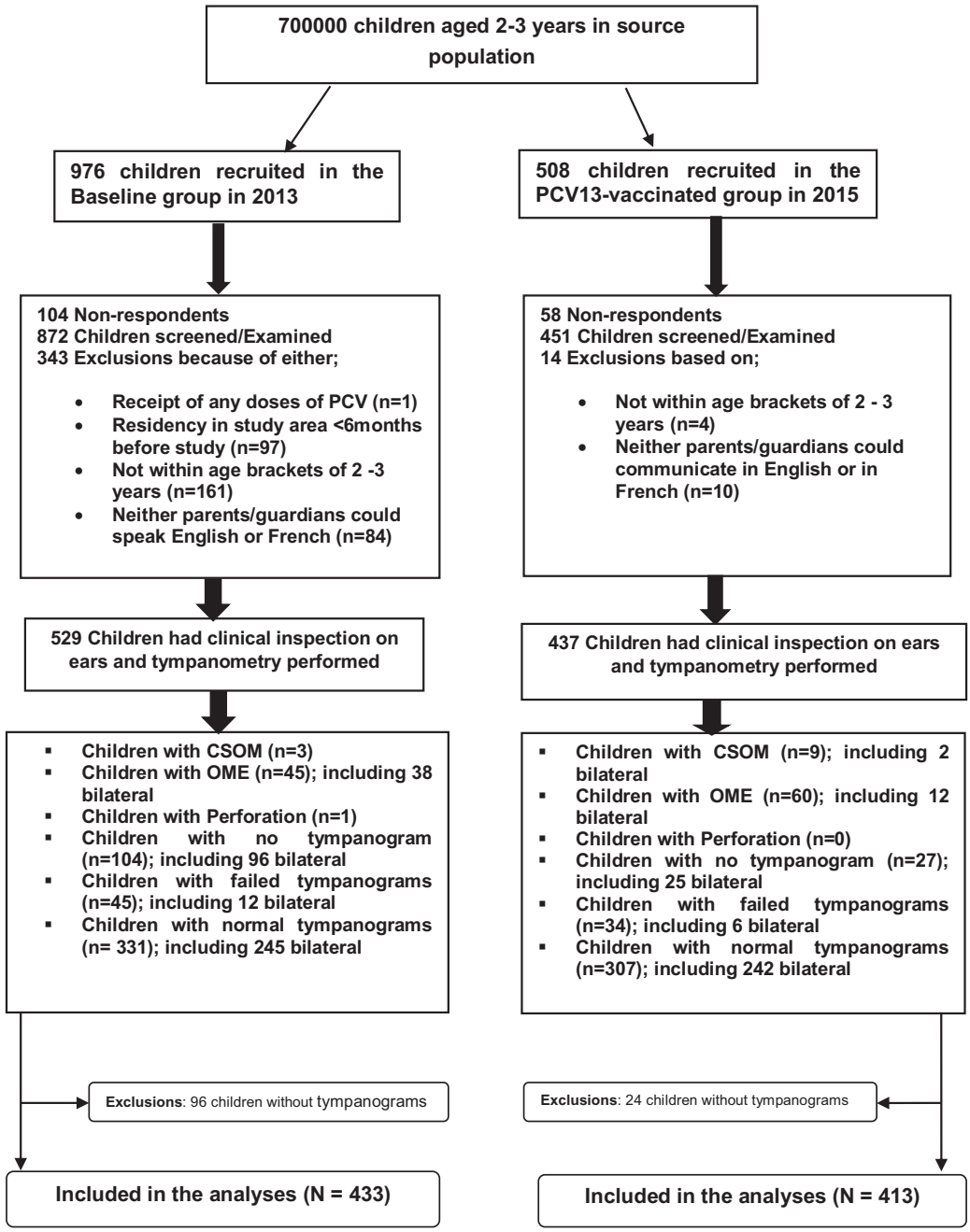

Figure 5. Flow chart showing the selection of children aged 2 to 3 years for the assessment of PCV13 impact on otitis media in Cameroon [*CSOM = Chronic suppurative otitis media; OME = Otitis media with effusion; n= number of subjects with specific outcomes; PCV13 = 13-valent pneumococcal conjugate vaccine.*]

Table 6. Risk factors for otitis media (OM) in PCV13-vaccinated (N = 402*) and PCV13-unvaccinated (N=429*) in children aged 2 to 3 years in Yaoundé, Cameroon

Characteristics/Clinical outcome	OM Prevalence N (%)	Univariate analyses			Multivariate analyses♣		
		POR	95%CI	P-value	aPOR	95%CI	P-value
Vaccine cohort							
PCV13-Unvaccinated (2013)	38/429 (8.7)	1.0			1.0		
PCV13-Vaccinated (2015)	56/402 (14.4)	1.76	1.124 - 2.677	0.013	1.50	0.84 - 2.67	0.171
Age (group) of child in months							
24 to 29	39/307 (12.7)	1.0	0.543 - 1.295	0.427			
30 to 36	57/524 (10.9)	0.43					
No. of persons living in same household ≤18 years							
One	16/126 (12.7)	1.0		0.945			
Two	24/193 (12.4)	0.98	0.496 - 1.921	0.576			
≥ Three	56/512 (10.9)	0.84	0.466 - 1.528				
No. of siblings sleeping in same bedroom							
Alone	19/206 (9.2)	1.0					
One	23/205 (11.2)	1.24	0.655 - 2.361	0.505			
Two	27/218 (12.4)	1.39	0.748 - 2.588	0.297			
≥ Three	27/202 (13.4)	1.52	0.815 - 2.829	0.188			
Previous otitis media history							
No	74/710 (10.4)	1.0			1.0		
Yes	22/121 (18.2)	1.91	1.134 - 3.216	0.015	1.43	0.66 - 3.09	0.368
Breastfeeding period							
≤ 6months or not breastfed	5/56 (8.9)	1.0					
≤12 months	39/327 (11.9)	1.38	0.520 - 3.671	0.517			
>12 months	52/448 (11.6)	1.34	0.511 - 3.508	0.552			
Antibiotic use when child is sick							
No	13/172 (7.6)	1.0					
Yes: with / without medical report	83/659 (12.6)	1.76	0.957 - 3.245	0.069			
Noticed any current symptoms of URTI							
No	76/706 (10.8)	1.0			1.0		
Yes	19/79 (24.1)	2.63	1.487 - 4.633	0.001	2.15	0.95 - 4.90	0.067
Unknown	1/45 (2.2)	0.19	0.026 - 1.387	0.101	0.22	0.03 - 1.66	0.143
Parental educational level (SES)							
≤ Primary education	39/327 (11.9)	1.0					
≥ Secondary education	47/393 (12.0)	1.01	0.638 - 1.577	0.989			
≥ Tertiary Education	10/111 (9.0)	0.73	0.352 - 1.518	0.401			

URTI = Upper respiratory tract infection; PCV13= 13-valent pneumococcal conjugate vaccine; N= number; %= percentage; CI= confidence interval; ♣= OR was adjusted for symptoms of URTI and previous OM history; POR= Prevalence odds ratio; aPOR= Adjusted POR; *=subjects with ECV values < 0.5 cm3 with tympanograms type B initially adjusted for were excluded in logistic regression analyses (The observed differences in the ECV values suggested that many occluded ears were initially misinterpreted as 'type B'. We corrected this by converting the majority of these 'type B' to 'type F' tympanograms based on the ECV distribution in normal tympanograms)

6 Discussion

6.1 Summary of main findings

The finding that infectious diseases such as malaria and pneumococcal infections (including meningitis, pneumonia and sepsis), in addition to malnutrition were among the leading causes of death (CoD) in our all-cause under-five mortality data (Study I) is significant, but expected from this type of setting. This is because approximately 72% of these deaths were reported to have happened within the first two years of life. Because these infectious diseases are either preventable or treatable, expanding measures to reduce their spread will improve both treatment and disease prevention strategies (Mccollum et al., 2017). However, in our analysis of the causes of under-five deaths, we recognize that more than one pathogen may have played a role in a specific case, it is common for patients to harbour concomitant infections, particularly in resource-constrained settings as has been reported previously (Bang & Bang, 1992). In this study, we only reported one CoD as the primary CoD which was initially declared medically. However, in using a single CoD, we do not ignore the possible contributions of other underlying comorbidities on the cause-specific deaths to the total under-five mortality rate, that witnessed a decrease from 146/1000 live births in 2001 to 122 /1000 live births in 2011 (Institut Nationale de la Statistique, 2011).

Our findings of an 18% residual vaccine type pneumococci carriage among PCV13-vaccinated children, as well as that, eleven PCV13-serotypes were still circulating in the population four years after the vaccine was introduced is also very important. This might represent a continuous transmission of residual vaccine-type pneumococci in circulation and may lead to a high force of infection, thereby ensuring children remain at risk of infection and disease (Study II). Moreover, pneumococcal carriage is a necessary step in the pathogenesis for pneumococcal disease (Nzenze et al., 2015). The public health implication of this result is that, the PCV13 programme is likely not working as ideally as might be expected by sufficiently breaking the chain of transmission of VT pneumococci.

The 2.3% of significant middle ear disease obtained within the 9.7% OM prevalence from the baseline study provides first country-specific data for Cameroon. This was used for assessing PCV13 effectiveness against otitis media disease and complications, four years after PCV13 introduction in 2011. However, our findings showed that otitis media prevalence was higher (16.7%) in the post-vaccine data (Study IV) than in the baseline prevalence data, resulting to a negative 72% vaccine effectiveness. Also, our findings suggest that 42% of OM infection among the PCV13-vaccinated

group might be attributable to PCV13 vaccination. These were unexpected findings, implying that either PCV13 was unable to elicit sustained antibody response or that other pathogens and/or non-PCV13 serotypes than the residual vaccine-type pneumococci were contributing more to OM disease. However, there was no evidence that PCV13-vaccinated children had more OM than the unvaccinated. These results should be interpreted with caution as samples were not obtained to determine the microbiology of OM disease causing serotypes. Hence, direct evidence of disease replacement of vaccine types by non-vaccine type pneumococci or other bacteria could not be ascertained. Also, only PCV13-vaccinated children were selected in 2015 among the general population. However, the estimated weighted average for the general population was 14.7% OM prevalence, which was close to that reported for the PCV13-vaccinated children (16.7%).

6.2 Methodological aspects: Strengths and limitations of the study

6.2.1 Strengths of the study

A retrospective cohort study design was used in Study I to assess data from hospital registers collected at the surveillance sites for infectious disease located at the mother and child reference hospital in Yaoundé, Cameroon. Accurate vital registration statistics are a perennial problem in most of resource-constrained settings where high-quality data are required for assessing disease prevalence and estimating the impact of interventions such as vaccines (Omore, 2020; Perry et al., 2005). As a result, no irrational evaluations from caregivers or unrestricted algorithms were required for the determination of the primary cause of death when using this methodology. Thus, high-quality data could be collected under difficult conditions using this approach, especially in resource-limited settings. As such, challenges on quantification (measurement) bias which is too often encountered when using the verbal autopsy technique were compromised. More so, given that access to hospitals has been made available to all members of the community, the methodology we have used could be applied in monitoring potential changing trends in mortality rates across the country.

Our baseline and post-PCV13 otitis media prevalence studies (Studies III & IV) used tympanometry, an objective and reliable method for detecting middle ear effusion, and provided reliable estimates for middle ear statuses in children aged 2 to 3 years (Palmu et al., 2002; Puhakka et al., 2014). Tympanometry is a dependable technique that could improve data quality and accurate measurement of OME and diagnosis in Cameroon and other low- and middle-income countries (LMIC). Besides, the method we have deployed is not influenced by sicknesses that lead to health care visits or healthcare seeking attitudes. Our ability to measure for the first time the impact of PCV13 on the local burden of OM (CSOM & OME), which could result to serious hearing impairments in children, is particularly noteworthy (Fletcher & Fritzell, 2012; Taylor et al., 2012;

Vergison et al., 2010). This was accomplished despite enormous challenges, which is a significant strength from the standpoint of a developing country. Additionally, the use of specific endpoints that reflect local standards of care emphasizes the importance of the study. Furthermore, the collection of data from communities to assess OM statuses demonstrates the public health impact of vaccines. Hence, these findings served as a useful baseline when assessing the effect of PCV13 programme implementation against OM and its sequelae (excluding AOM), in related settings using a similar methodology. Additionally, in Studies II, III & IV, the WHO cluster sampling technique was used in which participants were systematically recruited within the same communities using the same procedures, including sample collection period and age cohort. This has the potential to generate reliable estimates, and the results could be generalizable and simple to interpret.

6.2.2 Limitations of the study

There are some flaws in each of the studies. First, in the all-cause under-five mortality data (Study I), only the primary cause of death (CoD) was considered as initially determined by a physician, although it is obvious that a chain of underlying comorbid conditions may have contributed to a cause-specific death especially in resource-limited settings (Bang & Bang, 1992). The main shortcoming was not being able to determine the exact sequence of events that lead to a specific cause of death. Nonetheless, CoD was clinically determined by clinicians with extensive experience in tropical and paediatric medicine. Additionally, the methodology that was utilized did not rely on the subjective opinions from caregivers to determine the primary CoD. This reduces the challenges of measurement bias that have been reported with the verbal autopsy technique.

Second, the main caveat in our carriage data (Study II) was the inappropriate storage (-20^{0}C) of the 2013 batch of nasopharyngeal samples outside of the required temperatures of -70^{0}C at a port of entry in Europe for more than two months. These inappropriate laboratory temperatures of sample storage may have distorted the viability of some pneumococci species (Kaijalainen & Palmu, 2015; Satzke et al., 2013). This notwithstanding, some differences were observed in the prevalence of other bacteria, particularly non-typeable *H. influenzae,* although in both study periods the overall pneumococcal carriage prevalence was almost similar. This implies that our baseline carriage prevalence and the relative reductions were most likely underestimated or compromised, which explains why we did not use them for comparison with the post-vaccine data. Furthermore, we employed a novel technique whereby PCV impact was evaluated from a baseline of 24- to 36-month-old PCV13-unimunized children, almost two years after PCV13 implementation nation-wide. As a result, the 2013 baseline group was most likely benefiting from early indirect effects due to routine childhood vaccination (Roca et al., 2011). However, PCV13 coverage was not high enough (mean

uptake was <55%) during the early years following its introduction i.e., in 2011 and 2012 (WHO, 2018).

Furthermore, our baseline and post-vaccine otitis media data (Studies III & IV) were in some ways limited. AOM findings obtained via pneumatic otoscopy, for example, were not reported here because they were not diagnosed in the same way in both the baseline and post-vaccine groups. Most children in the 2013 baseline group had considerable cerumen accumulation, and we were not in possession of appropriate materials in the field to clean cerumen, so data was sparse (Study III), but this problem was resolved with the 2015 comparison group (Study IV). Additionally, tympanometry requires an airtight seal between the probe and the external auditory canal, which can be difficult in children who refuse to cooperate (Palmu et al., 2002). Because of this, misclassification bias may occur, resulting in difficulties to distinguish 'type B' tympanograms from those of 'type F' or 'type P', since all of them graphically produce 'flat' curves. The differences noticed with the ear canal volume (ECV) values indicate that there was an initial misclassification with most of the ears having occlusion as 'type B'. This was adjusted by converting most of the misclassified curves to 'type F' curves in accordance with the distribution of ECV in normal tympanogram types, following sensitivity analysis. In this analysis, all tympanograms with resulting 'flat curves' i.e., 'Type B', 'Type P' and 'Type F' (see Table 2) were excluded to determine the distribution of ECV values in other type tympanograms (A, As, C and Cs), with discernible curves. We noticed that 90% of these curves fell in the ECV categories from 0.5cm^3 to 1.2cm^3, and 10% fell between 0.3cm^3 and 0.4cm^3 ECV categories (Studies III & IV). It was in this respect that the adjustment was done i.e., of all flat curves, only 10% with lower ECV values were true positive "Type B". In addition, because we did not examine the microbiology of OM disease causing serotypes, direct evidence of disease replacement of vaccine types by non-vaccine type pneumococci or other bacteria could not be determined.

6.3 Comparison and interpretation of main findings to previous reports

6.3.1 Comparison of baseline findings

6.3.1.1 The leading causes of death among children under the age of five and proportion of deaths associated with pneumococcal diseases (Study I)

High proportions of under-five deaths due to treatable or preventable infections and protein-calorie malnutrition may jeopardize already achieved progress in many LMICs toward reducing under-five deaths by two-thirds from the 1990 baseline level. According to our findings, approximately 70% of the reported deaths in children under the age of five occurred during their first two-years of life and pneumococcal infections accounted for over 29% of deaths. This is consistent with earlier trends reported on the geographical distribution of causes of under-five mortality worldwide (Liu et al.,

2015). Our findings, however, differ in some ways from those reported previously in the Northern region of Cameroon (Einterz & Bates, 2011) and other parts of SSA (Sacarlal et al., 2009). In these studies, the proportion of under-five deaths caused by non-communicable diseases such as cancers and diseases arising from perinatal conditions was rarely reported. Instead, there was an overemphasis on communicable diseases. In our study, however, it was clear that these conditions contributed to approximately 15% of all-cause under-five mortality (Study I). In addition, our study found that 29.3% of under-five deaths were caused by pneumococcal infections, compared to 23.1% reported for the regions in SSA (Figure 6). Moreover, another study on the regional and global CoD revealed that in 2009 communicable diseases are the primary cause of 50% of all-cause mortality among children from SSA (Mathers et al., 2009), a similar trend observed in our study. This knowledge is critical for prioritizing the most effective interventions for children of this age group.

Further, although the PCV13-associated reduction in childhood pneumonia mortality were not yet strongly evident in our study, such benefits have been reported to occur in similar settings in LMIC and followed simultaneous improvements in the overall level of nutrition, hygiene, education and healthcare (Chan et al., 2019; Mccollum et al., 2017; Schuck-paim et al., 2019).

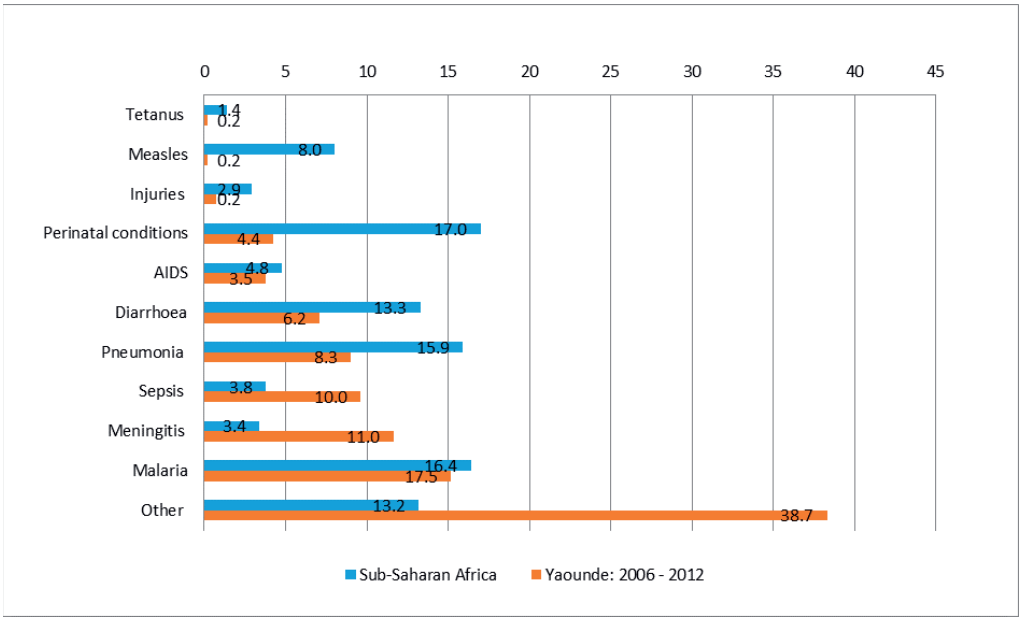

Figure 6. The leading causes of deaths among children under the age of five in Yaoundé, Cameroon, in comparison with percentages from the Sub-Saharan African region (Study I)

6.3.1.2 Prevalence of otitis media in children who were not vaccinated with PCV13 (Study III)

According to our findings, 2.3% of children aged 2 to 3 years who were not vaccinated with PCV13 developed severe middle ear disease. These findings are compatible with those from previous studies which used different methodological approaches in assessing OM prevalence than in our study (Adeyi et al., 2010; Chadha et al, 2014; Daly et al., 2010; Gultekin et al., 2010; Simões et al., 2015). Previous studies have frequently used hospital-based data rather than that from communities and, their focus has been on the prevalence of OM and its association with potential risk factors within the first 24 months of life or early infancy to the early teen years (Lieberthal et al., 2013; Simões et al., 2015). In addition to data paucity on OM prevalence in SSA, such age disparities may jeopardize extensive comparisons with our findings. A Nigerian study targeting children aged from 0 to 12 years on OM epidemiology within communities found that OM prevalence was highest (22.5%) among those aged 1 to 4 years (Amusa et al., 2005). This is approximately 2.3 times higher than the overall OM prevalence (9.7%) found in our study. Another Nigerian study which enrolled day-care and non-day-care children aged from 6 to 24 months, reported an OM prevalence of 37.7% and 43.7%, respectively (Asoegwu et al., 2013).

Moreover, two other studies conducted in Turkey and Saudi Arabia that assessed OME prevalence among children in primary schools reported a prevalence of 10% and 7.5%, respectively (Erdivanli et al., 2012; Humaid A-HI et al., 2014). These findings were supported by those from studies in remote communities in Australia, where CSOM prevalence of 15% was reported among 709 children aged 6 to 30 months from the Aborigines (Morris & Leach, 2009). Thus, in resource-limited communities, there is a high OM prevalence among children particularly in first years of life. Therefore, an extensive literature on OM epidemiology in Cameroon and other resource-constrained countries is important for public health planning.

In a resource-limited settings, parents and caregivers must be aware of the risk factors associated with OM prevalence in order to respond to prevention and health promotion efforts (oZBAY, 2015). In Study III, the relationship between the prevalence of OM and parentally reported symptoms of upper respiratory tract infections (URTI) was found to be statistically significant. This was within our expectations because, children with prevailing URTI symptoms are known to be more at risk of contracting OM than others (Morris & Leach, 2009; oZBAY, 2015). Several other factors, including breastfeeding, the number of siblings in the household, and passive smoking, have been reported previously to be statistically significantly associated to OM (Erdivanli et al., 2012; Macintyre et al., 2010; Simões et al., 2015), but this was not found in our study.

6.3.2 Comparison of post-PCV13 findings

6.3.2.1 Effects of PCV13 on pneumococcal nasopharyngeal colonization (Study II)

Previous research on the effects of pneumococcal conjugate vaccines (PCV) on nasopharyngeal carriage found significant reductions in the proportions of vaccine serotype (VT) pneumococcal carriage across all populations following PCV vaccination (Brandileone et al., 2016; Collins et al., 2017; Flasche et al., 2011; Grant et al., 2016; Hammitt et al., 2014; Nzenze et al., 2013; Palmu et al., 2017; Roca et al., 2013). In our study, we found an 18% prevalence of residual pneumococci vaccine serotypes four years after PCV13 implementation using the primary EPI accelerated dose series given at six, ten and fourteen weeks of age. According to our data, approximately a 62% prevalence of pneumococcal colonization was found among the PCV13 immunized children. Additionally, out of the thirteen PCV13 serotypes, 11 of these were still circulating up to four years after the PCV13 vaccination programme was implemented, despite an annual vaccine uptake of more than 80%.

However, in our data the 18% residual carriage of VT-pneumococci was not very different from some results obtained in other African studies (Table 7). In The Gambia a thirteen percent VT-pneumococci proportion was reported in a study involving children aged below 30 months, four years after PCV7 implementation, with about 90% vaccine uptake (Roca et al., 2015). In Kenya, a nineteen percent VT-pneumococci carriage proportion in children under-five was reported two years after PCV10 implementation (Hammitt et al., 2014), when vaccine coverage was approximately 80%.

Similar findings have been reported previously in which the impact of the PCV have been evaluated in programmes that have used a catch-up schedule for older populations or not. In Kenyan (Hammitt et al., 2014) and the Gambian (Roca et al., 2015, 2011) studies, PCV implementation was rolled out along with a catch-up programme. Whereas, in South Africa, there were no catch-up campaign, but a thirty-two percent residual VT-pneumococci carriage proportion was observed across all study age groups including those which were not initially targeted (Nzenze et al., 2013). This was accomplished under two years after PCV7 implementation, with just over 50% of the children targeted had been completely vaccinated using a primary two-dose series given at 6 and 14 weeks of age, accompanied with a booster dose at 9 months (Nzenze et al., 2013).

The dramatic decreases in PCV7-, PCV10- or PCV13-serotypes reported in the Gambian (Roca et al., 2015, 2011) South African (Nzenze et al., 2013) and Kenyan studies (Hammitt et al., 2014), among both immunized and unimmunized population are attributed to the indirect and direct effects of the PCV. This is due to decrease in transmission of vaccine serotypes and their acquisition, rendering children less exposed to subsequent vaccine-type pneumococci colonization (Madhi et al.,

2016). However, such findings could not yet be deduced among the immunized group in our study because of an appropriate baseline data, four years after PCV13 vaccination. This notwithstanding, an 18% residual VT carriage may result to a high force of infection, suggesting that the indirect and direct effects following PCV13 vaccination have not led to the anticipated low levels of vaccine-type transmission in the population. The absence of a booster dose and lack of catch-up programmes for older children, coupled with vaccine waning effects are possible contributors, considering that children are vaccinated using an accelerated primary dose series (3+0 schedule) early in infancy. There are recent reports from some SSA countries suggesting that a switch to a 2+1 schedule whereby the middle dose is given between the ages of 9 and 12 months, concomitantly with the vaccine against measles could prolong the duration of immunity (Hammitt et al., 2019; Madhi et al., 2016; Soeters et al., 2019).

6.3.2.2 Isolation of multiple-serotype colonization (Study II)

Multiple serotypes were found in 11.4% (8/70) of the swabs tested and a single serotype in the rest of the 62/70 (88.6%) swabs. In three cases, the first isolate was serotype 3 and the second isolate non-encapsulated (NC) pneumococcus. The other double serotype findings were 21/19B, 19F/9N, 14/6B, 19F/33F and 15C/33B. However, due to limited resources only 13.5% (70/514) of the second isolates could be randomly screened for co-colonization.

Table 7. Summary of a few African-based studies on the effect of pneumococcal conjugate vaccine (PCV) immunization on nasopharyngeal colonization

Country	Study design (reference)	Schedule/Age	PCV formulation/ inclusion	Age group at swabbing	Years after vaccination/ Catch-up	VT detected post-vaccination	% of VT-carriers before vaccination	% of VT-carriers after vaccination	Vaccine uptake (3PCV doses)	VT-pneumococci proportion after vaccination
Cameroon	Cohort (Njuma Libwea et al., 2020)	3+0 dose-schedule administered at 6, 10, 14 weeks of age	PCV13; 2011	24 to 36 Mo	Four years; no catch-up campaigns	11 out of 13	21.2%	18%	23% in 2011, 84% in 2012, 88% in 2013, 87% in 2014, 88% in 2015	29.1% (18/61.8)
Kenya	CSS (Hammitt et al., 2014)	3+0 dose-schedule administered at 6,10 and 14 weeks of age	PCV10; 2011	<60 Mo	Two years; catch-up in children <5years old	6 out of 10	34%	13%	32% in 2009, 94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	19.1% (13/68)
Gambia	RCT (Roca et al., 2011)	3+0 dose-schedule administered at 2, 3 and 4 months of age	PCV7; 2009	30 to < 59Mo	Two years; 1 dose of PCV given to subjects > 30 months old	3 out of 7	50%	13.3%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	17.3% (13.3/76.7)
Gambia	RCT (Roca et al., 2013)	3+0 dose-schedule administered at 2, 3 and 4 months of age	PCV7; 2009	30 to < 59Mo	Four years; 1 dose of PCV given to subjects > 30 months old	Unspecified, but the VRT 6A prevalence decreased	50%	8.9%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	13.3% (8.9/67)
South Africa	CSS (Nzenze et al., 2013)	2+1 dose-schedule administered in two primarily doses at 6, 14 and a booster at 40weeks	PCV7; 2009	< 24 Mo	Two years; no catch-up campaign (switching in May 2011 to PCV13).	7 out of 7; and following the switch to PCV13 it was 11 out of 13	45.1%	23.5%	12% in 2009, 86% in 2010, 51% in 2011	32.0% (23.5/73.4)

VT= Vaccine type; PCV7, PCV10 or PCV13 = 7, 10 or 13-valent pneumococcal conjugate vaccines; VT-pneumococci proportion after vaccination = VT-pneumococci prevalence divided by overall-pneumococci prevalence after vaccination; % = percent; CSS = Cross-sectional study; Randomized control trial = RCT; Vaccine related type = VRT; Months = Mo

6.3.2.3 Effects of PCV13 on the prevalence of otitis media (Study IV)

The prevalence of OM was found to be higher in PCV13 vaccinated children than in those who were not vaccinated in our study. Our VE estimate of PCV13 against OM prevalence, four years after PCV13 infant vaccination was negative 72%. This may seem paradoxical in that the vaccine is not working as expected, contrary to the findings of the experimental trials which demonstrated vaccine efficacy (Fireman et al., 2003; Juhani et al., 2001), or observational studies in Israel/UK (Ben-Shimol et al., 2014; Lau et al., 2015) which have reported near-elimination of VT in AOM children. However, multivariate regression analyses showed that there was no evidence PCV13-vaccinated children were more likely to get OM than the PCV13-unvaccinated.

Additionally, the estimated attributable proportion (AP) was 42% i.e., 42% of OM infection among the PCV13-vaccinated group might be attributable to PCV13 vaccination. The remainder (58%) of OM infections in the vaccinated group would have still occurred even without the vaccine. I will interpret these excess OM infections among the vaccinated as probably resulting from other pathogens than *S. pneumoniae* or originating from non-PCV13 serotypes.

Several factors could explain the findings of our study. After the second year of life, the impact of PCV on OM is thought to decrease (Sáez-Llorens et al., 2017). This is consistent with our findings, in which children aged 24 to 36 months had been vaccinated within their first 6 months of life. Also, vaccine waning effects with increasing age may be heavily implicated, especially since the PCV13 is being rolled out using the 3+0 EPI accelerated primary dose-schedule with no booster (Tregnaghi et al., 2014). However, this contradicts findings from the Finnish OM trial, in which no vaccine waning effects were observed with increasing age, despite the fact the children were older (4 to 5 years of age) than those in our data (Kilpi et al., 2003).

Furthermore, an increase in pneumococci that were not included (NVT-pneumococci) in the PCV13 could be contributing to increased OM. We assessed nasopharyngeal carriage (NPC) in the same age groups from same population in Cameroon and found a slight increase in NVT-pneumococci NPC but a significant increase in *Moraxella catarrhalis* and *Haemophilus influenzae* NPC (Study II). This could indicate a shift in the aetiology of OM in children, as pathogens other than non-PCV13 pneumococci maybe contributing to more OM (Aguilar et al., 2009). This finding is consistent with the findings of an Australian community cross-sectional study of indigenous children under the age of 36 months. Subjects vaccinated with the 10-valent pneumococcal *Haemophilus influenzae* protein-D conjugate vaccine (PHiD-CV10) had lower CSOM than those vaccinated with PCV7, but this was accompanied by an increase in OME (Leach et al., 2014).

Besides, differences in the patho-physiology of AOM (e.g., swollenness or blockage of the eustachian tubes, could result in mucus formation and exposure to a microbe, and subsequently ear infection) compared to that of IPD, such as bacteraemia and meningitis, may be a contributing factor (Fireman et al., 2003; Eskola et al, 2001; Kilpi et al., 2003). OM disease's polymicrobial nature may also play a role. Protection against pneumococcal OM alone, even if obtained with high efficacy, is thought to have a limited impact on the overall burden of OM disease (Kilpi et al., 2003). More so, since early pneumococcal AOM is thought to cause inflammation and damage to the middle ear and eustachian tube mucosa, this suggests that PCV may not be protective against OME/CSOM (Schilder et al., 2016). This predisposes infants to future OM episodes (Schilder et al., 2016). It is unclear, however, whether the infants were exposed to OM episodes prior to vaccination. But, it has been documented that when compared to children from more affluent communities, children in resource-limited settings are most likely to experience bacterial nasopharyngeal colonization almost immediately after birth (Kwambana et al.,

2011). PCV vaccination prior to this cascade of patho-physiological events may play a significant role in preventing recurrent and complicated infections in otitis-prone children. However, once this chain of events has begun, PCV vaccination is thought to have little or no effect (Veenhoven et al., 2003).

6.4 Interpretation of the findings

As per the finding of our all-cause under-five mortality survey (Study I), malnutrition and preventable infectious diseases were among the leading causes of death within the study population. A similar observation that 45% of under-five child deaths in the last three decades have been caused by infectious diseases, was reported recently (Liu et al., 2015; Troeger et al., 2018). Additionally, our findings revealed that 72% of these deaths had occurred during the first two years of life, with pneumococcal infections accounting for over 29% of these deaths. These findings are consistent with previous reports indicating that the majority of under-five deaths in LMICs occur before children reach the age of five (Liu et al., 2015). More specifically, the younger the child, the greater the risk of death from an infectious disease (Liu et al., 2015).

According to our baseline data, the prevalence of otitis media is usually high in resource-limited settings, particularly in early childhood. Moreover, the data presented in this dissertation indicated that children with current upper respiratory tract symptoms were more susceptible to infection than others. These findings support previous research suggesting that affected children may suffer from long-term hearing loss and delayed development as a result of otitis media (Capra et al., 2000). This could result in significant socio-economic losses for families and the healthcare systems, especially in low and middle income countries (Capra et al., 2000). The frequent occurrence of OM in

children under-five years of age and the associated direct and indirect cost is a heavy toll to both parents and the healthcare providers (Capra et al., 2000). Even when parents do not seek medical care for a child, they may still need to be absent from work to care for them (Rovers, 2008). In some countries, costs related to parental loss of productivity have been reported to represent a 50% average relative to the total costs of OM, depending on country, age, and number of previous episodes, accounting for a substantial volume of disability-adjusted life years (DALYs) (Capra et al., 2000).

Our carriage survey data from Study II revealed that four years following the implementation of infant PCV13 vaccination in Cameroon, there was an 18% residual VT-pneumococci in carriage among vaccinated children in 2015. Additionally, eleven of the 13 vaccine serotypes remained in circulation. Thus, suggesting a very high force of infection with VT pneumococci in this setting. However, there is evidence suggesting a non-linear relationship between carriage and disease (Chan et al., 2021; Ojal et al., 2017; Simell et al., 2012). Before and after studies on the impact of PCV have reported substantial decrease in IPD to almost total elimination of VT pneumococci, whereas carriage has decreased substantially but remained at below 10% in SSA nations such as Malawi and Kenya (Bar-Zeev et al., 2021; Ojal et al., 2017). However, it remains unclear whether residual VT carriage in these settings does not result in disease as current IPD surveillance programmes indicate (Bar-Zeev et al., 2021; Ojal et al., 2017). But in resource-low settings IPD surveillance programmes lack the capacity to fully monitor or surveil pneumonia appropriately (Dunne et al., 2018; Roca et al., 2013). Where appropriate surveillance systems are lacking as in Cameroon, carriage studies have shown to be a cheaper alternative in assessing such changes (Chan et al., 2019; Dunne et al., 2018), but this too could not be performed consistently in all LMICs.

With the 18% residual VT pneumococci observed in our carriage study, it remains to be understood if this translates directly to disease. As earlier

mentioned, the widespread use of PCVs has resulted to a considerable reduction in the burden of IPD both to the vaccinated and unvaccinated populations across all ages (WHO, 2019). These reductions have mainly been driven through both the direct and indirect effects (herd protection). Although the indirect effects of the PCV form an integral component of its broader vaccine impact, understanding the relationship between PCV coverage, carriage and invasive disease is vital (Chan et al., 2021). Previous studies have suggested that high levels of PCV coverage (of at least 58 to 75%) were required to achieve a substantial indirect protection (Tsaban & Ben-Shimol, 2017). However, this was contradicted with recent findings from Australia where the association between population PCV7 coverage and indirect protection against IPD and pneumonia hospitalization among undervaccinated children was studied (Chan et al., 2021). In their findings, Russel and colleagues demonstrated a statistically significant non-linear relationship between PCV coverage and the incidence of severe invasive disease and pneumonia hospitalization due to vaccine serotypes among undervaccinated children (Chan et al., 2021).

According to other studies, younger children are the main reservoirs and primary source of transmission of the pneumococci (Ben-Shimol et al., 2014; Kwambana et al., 2011). Hence, contrary to what we found in our study, PCV infant vaccination has been reported to result in significant reductions or near elimination of vaccine serotypes (Ben-Shimol et al., 2014; Kwambana et al., 2011). These findings suggest that Cameroon's vaccination programme, which uses the accelerated primary dose schedule, is not yet providing adequate protection against vaccine serotype transmission. It also suggests that there may be programmatic issues with the programme. Recent considerations for the optimal control of vaccine-type IPD are focused either on shifting from the primary accelerated 3+0 to a 2+1dose-schedule (for developing countries) or reducing the number of PCV doses from 3 to 2 (for mature PCV programmes

like in most of Western countries) (Soeters et al., 2019; Tsaban & Ben-Shimol, 2017). Hence, with the goal to sustaining the overall vaccine effects, this is an important public health consideration for Cameroon (Chan et al., 2021).

Moreover, we noticed that more otitis media with effusion cases occurred during the post-vaccine survey than during the baseline survey in our OM data. A similar finding was made in a study on Aboriginal children in Australia where children vaccinated with PCV10 had a higher OME prevalence compared to those vaccinated with PCV7 (Leach et al., 2014). This reaffirms the hypothesis that either PCV13 was unable to elicit sustained antibody response or that other pathogens than the vaccine-type pneumococci were responsible for more OM disease in our study (Leach et al., 2014).

6.5 Public Health implications of the study

The research topic is important for national development because vaccinations reduce high infant mortality rates, which are extremely valuable tools in sustainable development. However, there was an 18% residual carriage of VT-pneumococci reported four years after PCV13 infant vaccination, suggesting a possible high force of infection. Such findings support the need for continuous surveillance so as to determine the possible long term effects of the PCV13 implementation programme in Cameroon on nasopharyngeal carriage and pneumococcal disease. Further, these findings may be used as a benchmark for assessing the effects of the PCV13 on overall morbidity and mortality following PCV13 infant vaccination.

Such data which aid in gaining a better understanding of country-specific causes of death, are especially scarce in resource-constrained settings because of inadequate vital registration systems. In understanding the aetiology of the main causes of under-five deaths in our study and with lessons drawn from

Ethiopia (Tadesse et al., 2015), we recommend possible steps Cameroon could take to meet the MDG4 /SDG3 targets. The MDG4 captioned "Reduce child mortality", has a target of reducing the under-five mortality rate by two-thirds from the 1990 baseline level. Whereas, SDG 3 aspires to ensure health and well-being for all, including a bold commitment to end the epidemics of AIDS, tuberculosis, malaria and other communicable diseases by 2030. These recommendations include;

- Increase in health funding/budget to facilitate the amelioration of health infrastructure and workforce through an enhanced community-based framework;
- Establishing a sustainable multi-sectoral approach which integrates child health, nutrition and social welfare policies; and programmes for food security and safety, upgrading water, hygiene and sanitation systems;
- Political commitment such as the enforcement on legislation on civil status registration systems including human resource training and mobilisation at the community level;
- Important to reorient targeted strategies, programmes and interventions by critically analysing evidence on the epidemiology of child deaths in the country;
- The need for healthcare administrators to recognize what worked well in their respective communities and use lessons learned in combination with the MDGs to address challenges in future health interventions, and outline roadmaps to achieve specific indicators.

In addition, the public health value derived from these studies is consistent with the SDGs. The thesis provided additional information on the changing trends in the epidemiology of vaccine and non-vaccine serotypes following the implementation of PCV13 programme. Such findings provide decision-makers

and other national and international partners with information about the societal needs that the vaccination programme is expected to address.

Furthermore, ear infections can cause hearing loss and even deafness, jeopardizing the affected person's ability to communicate in social environments. However, the effects on PCV13 were not yet evident in the current study, and this could be as a result of confounding due to unknown factors or the predominance of non-vaccine type pneumococci as causative pathogens in otitis or waning vaccine effects. Therefore, further research is needed to assess these findings and potential causes, as well as to monitor the long-term impact of PCV13 programme implementation on the epidemiology of pneumococcal diseases, including otitis media in African settings. The scientific documentation of the vaccine programme's impact is extremely important for the pursuit of better global health. This is especially relevant to support the continuation of the vaccination programme with government funding when the GAVI support eventually ends.

6.6 Beyond vaccine effectiveness: The broader public health value of the PCV13 in Cameroon

Most countries have frequently relied on vaccine effectiveness (VE) analysis to assess the value of any vaccine-related health intervention. This tool's contextualization into a country's health expenditure budget is critical (Verguet, Kim, & Jamison, 2016). According to our findings (Study II), the PCV13 impact evaluation, which revealed an 18% residual VT pneumococci carriage prevalence, was conducted primarily on subjects aged 24 to 36 months, and over a short period of time. This could jeopardize vaccine effects that are not immediately apparent such as the effects of PCV13 on pneumonia (a major cause of most severe and fatal pneumococcal disease), IPD, and all-cause mortality in children under the age of five. These are important public health

issues for the country. In Study I, we presented data on the proportion of deaths caused by pneumococcal infections. However, there is a paucity of country-specific data on the impact of the PCV on any syndromic aetiology, particularly pneumococcal pneumonia. However, the PCV has previously been shown to be effective against bacteraemic pneumonia in an African setting (Shabir A Madhi & Nunes, 2016). This should provide Cameroon with important lessons to draw from on the beneficial effects of the PCV13 against bacteraemic pneumonia, which is yet to be determined in the country. Therefore, the PCV13 has a public health value in terms of reducing serious causes of morbidity and mortality caused by *Streptococcus pneumoniae.* Despite this, there are ongoing challenges in establishing reliable, valid, and complete data on vaccination statuses, in addition to those on vital registrations (Study I). This requires immediate attention because it is a critical component of assessing the PCV13 programme's overall public health impact.

Beyond effectiveness and impact studies, the PCV13 should bring a broader public health value in Cameroon. Its social impact should indirectly contribute to the promotion of health equity, the preservation of public goods, and the stimulation of social integration (Beutel, 2016). In Cameroon, PCV13 vaccination may not be sufficient to eliminate social differences. However, its public health impact could be seen in bridging the inequality gap by providing most children (especially those who miss routine vaccination) with access to these lifesaving vaccines via free mass vaccination campaigns activities (Li et al., 2018; Saadatian-Elahi et al., 2016). This is particularly relevant in Cameroon, a culturally diverse country with over 200 ethnic groups expressing a wide range of social and health concepts. My personal experience shows that when it comes to healthcare, especially when it involves children, people come together. The PCV13 implementation programme has resulted in a massive level of socio-cultural cohesion, the direct impact of which cannot be quantified.

PCV13 was implemented nationwide, which should help to bridge the gap in pneumococcal disease infections rates between people in the lower and upper socio-economic levels of the society. This is critical because most of the population are dependent on less than two United Sates dollars (US $2) per day, and healthcare costs are borne primarily through out-of-pocket individual payments (Study III). Hence, the large-scale implementation of the PCV13 programme is an essential tool in the country's advocacy for health equity and social integration.

However, there is a major impediment to addressing the issue of intergenerational health equity. As previously reported, the benefits, risks, and opportunity costs of a vaccination programme are not always evenly distributed across different age groups and generations (Williams, 1997). Annual influenza vaccination in children, for example, greatly reduces the risk of influenza in all age groups. Additionally, there is the case of childhood varicella-zoster virus vaccination, which reduces chickenpox in children but increases shingles in adults and the elderly (Ogunjimi et al., 2013). Nonetheless, the PCV's mode of action differs from that of either the influenza or the varicella vaccinations. Infant PCV13 vaccination prevents the spread of vaccine-type pneumococci in the community and provides herd protection to both the vaccinated and unvaccinated populations across all ages (WHO, 2019). Herd protection is an asset to anyone at any time. And, as Jeroen Luyten, puts it, "we've all been children and we expect to grow old at some point in time, and because we can't be certain about our protection in the lifetime in between, we all benefit from herd immunity at different stages in our lives" (Beutel, 2016). Hence, the PCV13 herd effect provides an important public health benefit to the entire society.

6.7 Research gaps: Future considerations and recommendations

Every year, approximately half a million children under-five deaths occur as a result of pneumococcal infections worldwide (Liu et al., 2015; Wahl et al., 2018). Besides, nasopharyngeal colonization is known to be a prerequisite for invasive pneumococcal disease (Nzenze et al., 2015). As a result, our data on the epidemiology of nasopharyngeal serotypes provide important information for depicting pneumococcal disease control, prevention, and antimicrobial resistance in the country. Thus, continuous monitoring of prevalent serotypes and antimicrobial resistance is an important tool for assessing the long-term impact of the pneumococcal conjugate vaccines.

We addressed some methodological gaps and exploited approaches needed for consideration in assessing the broader public health impact of the PCV13 in this thesis, but there are other windows of opportunity that future studies could investigate, such as:

1. More research is needed to fill the knowledge gap of PCV evaluation on the incidence in children of all-cause pneumonia hospitalizations among PCV-vaccinated and PCV-unvaccinated age groups. In South Africa, for example, the vaccine preventable disease incidence (vaccine attributable risk reduction) per 100.000 vaccinated children for clinical pneumonia hospitalization was approximately 9-fold higher among HIV-infected (2.302) than among HIV-uninfected children (267). More so, a 16% reduction in all-cause under-five mortality was reported in the Gambia PCV9 efficacy trial.
2. Further, IPD and AOM studies should be rigorously conducted using both hospital-based and population-based approaches.
3. Ongoing surveillance and prospective impact assessment studies are required to determine whether there is replacement disease by non-

vaccine serotypes which could jeopardize early gains associated with the country's PCV13 immunization programme.

4. Additionally, studies on bacterial nasopharyngeal carriage in PCV13-vaccinated and PCV13-unvaccinated cohorts of infants and adults (mothers) pairs or different strata of the populations are required to understand important epidemiologic trends as well as the development of indirect effects usually required to compare the long term impact of the PCV13 both on nasopharyngeal colonization and IPD.
5. Also, because of possible vaccine waning effects after the first year of life (Fortanier et al., 2019), and an increasing OM prevalence among the PCV13-vaccinated supports recommendation that shifting to a 2 +1 schedule in which the third dose is given at 9- to 12-months of age may be more effective (Soeters et al., 2019). Therefore, country-specific studies to support evidence on this are needed.

7 Summary and Conclusions

Pneumococcal diseases are the leading estimated cause of infections and death in children under-five, globally. The development of the pneumococcal conjugate vaccines (PCV) remains a major advancement in global child health. However, about a million children under-five years still die from pneumonia annually, with two-thirds of these occurring in developing countries. The works presented in this thesis informs policy to the understanding of the country-specific causes of death and the proportion attributed to the pneumococcal infections, the prevalence of otitis media and nasopharyngeal pneumococcal carriage in the era of the 13-valent pneumococcal conjugate vaccines in Cameroon.

The three main research questions addressed were:

1. What is the proportion of under-five hospital-registered mortality caused by pneumococcal diseases in Cameroon prior to the implementation of the PCV13 programme? Here, the results presented in this thesis provides country-specific evidence that pneumococcal infections accounted for 29.3% of under-five deaths within the study period. Additionally, more than 70% of the deaths were reported to have occurred within the first 2 years after birth. This observation is consistent with previous reports from other developing countries.
2. What is the prevalence of nasopharyngeal pneumococcal carriage among PCV13-unimmunized and PCV13-immunized children aged 2 to 3 years in Cameroon after PCV-13 introduction? The results showed that four years after PCV13 infant vaccination, the carriage prevalence of residual vaccine-type pneumococci was 18%. In addition, the overall

NPC prevalence of pneumococci among the vaccinated children was 62%. Moreover, four years after beginning of PCV13 only three of the 13 VT were isolated in carriage among the vaccinated in The Gambia, compared to eleven in Cameroon. This suggest that the 18% residual VT carriage prevalence among vaccinated children in 2015 may be contributing to high disease burden e.g., in OM but we lack data on the microbial aetiology of OM to confirm these findings.

3. What is the prevalence of otitis media (OM) in Cameroonian children aged 2 to 3 years who have been vaccinated with PCV13 compared to those not immunized with PCV13? Here, our results were surprising, with PCV13-vaccinated children in 2015 having 16.7% OM disease prevalence compared to 9.7% among PCV13-unvaccinated children. VE was negative 72%. But there was no evidence that PCV13-vaccinated children carried more OM infections than the PCV13-unvaccinated. These findings could be explained by predominance of other pathogens and non-PCV13-type pneumococci as disease causing microbes among PCV13-vaccinated children. Additionally, these results are consistent with waning vaccine immunity among the vaccinated children and the probable absence of any indirect effects. Furthermore, natural immunity could as well be implicated.

In summary, the inclusion of the PCV13 into the EPI in Cameroon in July 2011 through GAVI funding was a welcome agenda. However, this agenda is incomplete because PCV13 impact has only been assessed on nasopharyngeal pneumococcal carriage and otitis media (Studies II & IV). The results obtained revealed an 18% residual VT pneumococci carriage prevalence compared to results from similar settings e.g., 13% in the Gambia.

Possible explanations for the differences in residual VT-pneumococci carriage prevalence may include lower vaccine effects, age at sampling,

variations in the nasopharyngeal microbiome and/or secular trends. For instance, changes in HIV treatment regimens in South Africa and a community-wide azithromycin campaign in the Gambia are thought to have influenced the high vaccine effects in these countries (Hammitt et al., 2014, Roca et al., 2013, Roca et al., 2011).

In the absence of GAVI funding, the continuation of PCV13 immunization in Cameroon, as in most of low-income economies, may be difficult. It will mostly depend on convincing policymakers of the PCV's effectiveness in decreasing the burden of disease associated with pneumococcal infections, as well as overall morbidity and death among children under the age of five.

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PUBLICATION I

Assessing the causes of under-five mortality and proportion associated with pneumococcal diseases in Cameroon. A case-finding retrospective observational study: 2006–2012

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PLOS ONE

RESEARCH ARTICLE

Assessing the causes of under-five mortality and proportion associated with pneumococcal diseases in Cameroon. *A case-finding retrospective observational study: 2006–2012*

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Abstract

Background

Vital registration data outlining causes of deaths (CoD) are important for a sustainable health system, targeted interventions and other relevant policies. There is data paucity on vital registration systems in developing countries. We assessed the leading causes and proportions of under-five deaths, and particularly those related to pneumococcal infections in Yaoundé, Cameroon, using hospital registration data.

Methods

A retrospective case-finding observational study design was used to access and identify data on 817 death cases in children under-five years of age recorded in health facilities in Yaoundé, within the period January 1, 2006 and December 31, 2012. Patients' files were randomly selected and needed information including demographic data, date of admission, clinical and laboratory diagnosis, principal and/or underlying causes of death were abstracted into structured case report forms. The International Classification of Diseases and Clinical Modifications 10th revision (ICD-10-CM) codes (ICD10Data.com 2017 edition) were used to classify the different CoD, retrospectively. Ascertainment of CoD was based on medical report and estimates were done using the Kaplan-Meier procedure and descriptive statistics.

Results

Of the 817 death records assessed, malaria was the leading CoD and was responsible for 17.5% of cases. Meningitis was the second largest CoD with 11.0%; followed by sepsis

(10.0%), *Streptococcus pneumoniae* infections (8.3%), malnutrition (8.3%), gastro-enteritis / diarrhoea (6.2%), anaemia (6.1%) and HIV (3.5%), respectively.

Conclusion

The main CoD in this population are either treatable or vaccine-preventable; a trend consistent with previous reports across developing countries. Besides, the health effects from non-communicable infections should not be neglected. Therefore, scaling-up measures to reduce causes of under-five deaths will demand sustainable efforts to enhance both treatment and disease prevention strategies, to avoid a decline in the progress towards reducing under-five deaths by 2/3 from the 1990 baseline.

Background

Accurate civil registration systems are essential in the documentation on the distribution of CoD in children as well as in the general population. This is important in the planning of sustainable health policies and needed interventions in concordance with the millennium development goal (MDG 4) [1], with a target of reducing by two-thirds between 1990 and 2015 the under-five mortality rate [2]. We have exceeded the year 2015 deadline and globally, reports suggest the number of under-five deaths dropped from 12.7 million in 1990 to 6.3 million in 2013 [3]. However, most of the 6.3 million reported under-five deaths occurred in developing countries, with porous data on child health and mortality [4]. In these countries, reaching the MDG4 target will require an acceleration of essential, effective and affordable interventions against diarrhea, sepsis, the human immuno-deficiency virus / acquired immuno-deficiency syndrome (HIV/AIDS), malaria and pneumonia including improved nutrition and vaccines access.

The reduction of under-five mortality remains a major priority in developing countries, considering the high number of deaths resulting from preventable conditions [5]. However, to achieve the MDG4 target, reliable data are needed on under-five mortality to guide health planners and to scale up prevention and treatment strategies [5,6].

Acute Lower Respiratory Infections (ALRI) account for over 6.0% of morbidity and mortality of children worldwide, with *Streptococcus pneumoniae* reported as one of the principal causes of illness and death in children younger than five years of age [2]. About 75% of all cases occur in only 15 countries, with Sub-Saharan Africa and Southeast Asia representing the vast majority of cases [7]. Pneumococcal pneumonia is more frequent than can be confirmed by positive blood cultures and up to half of pneumonia deaths in children is attributed to pneumococcus [8]. In the absence of recent research data in the country, up to 19% of deaths in children under-five years old, have been estimated to result from pneumococcal infections in Cameroon with a total under-five mortality rate of 84 /1000 live births [9–11]. Addressing major risk factors such as malnutrition, breastfeeding and indoor air pollution are essential in prevention of pneumonia, but vaccination remains the cornerstone [12].

In this paper, we present data on hospital-based case finding of CoD in children abstracted into structured case report forms (CRF) in four health districts including the mother and child hospital (MCH) in Yaoundé, Cameroon. The MCH is one of the largest children's hospitals in the country and it is accessible and affordable to all strata of the population. It keeps records of hospital visits, admissions, and deaths, in addition to specific clinical, laboratory and serotype data on invasive diseases including *Haemophilus influenzae* and *Streptococcus pneumoniae*. It

has a capacity of 300 beds and had over 85000 admissions with 1816 reported under-five deaths between January 2006 and the end of December 2012.

The principal causes of under-five mortality in the country have not been assessed and there is no baseline data for vaccine effectiveness evaluation, as is the case with the pneumococcal conjugate vaccine introduced in July 2011. Our primary goal was to access and identify all cases of under-five deaths in the study area and define the most probable causes of under-five deaths based on available data sources. Secondarily, we estimated the proportion of deaths possibly or definitely due to pneumococcus. Moreover, it is expected the findings may be a useful baseline with information on disease pattern needed to re-scale appropriate public health targets and indicators to measure their progress and achievements in the country, with respect to the MDG4.

Material and methods

Study design and study site

We applied a retrospective case-finding observational study design using hospital registration data from the infectious disease surveillance sites hosted at the MCH in Yaoundé, Cameroon (Fig 1). As earlier described [13], the sites involved hospitals in both an urban and rural/semi-urban zones around Yaoundé, with a population of over 3.5 million inhabitants out of which 18% are children under-five [14].

In 2013, we conducted a sample survey on over 1800 cases of death registered within the MCH, representing less than 2% of an estimated 117000 expected deaths in Yaoundé [9], among children aged under-five between 2006 and 2012. Trained study personnel randomly selected 817 of these cases and information on the socio-demographic and CoD were extracted from medical reports and keyed into structured case report forms (CRF). This facilitated the identification of the direct, intermediate and underlying causes of CoD from medical reports. Besides, it avoided duplicate or repeated counting of cases from the different hospital registers. Ascertainment of CoD was based on medical declaration and the International Classification of Diseases and Clinical Modifications 10th revision (ICD-10-CM) codes were used to retrospectively classify the different CoD.

Ethical considerations

Death registration data for the general population are held at Civil Status Registries (CSR) in municipalities and local population offices in each municipality in Cameroon, and permission for their use was obtained from the National Ethics Committee (CNE) No. 234/CNE/SE/2012, written and signed on May 2, 2012.(*Because we needed to effectively link patients' records in registers to their respective files and to avoid duplicate entries, they were accessed unencoded. All patient records abstracted into CRF were later encoded and analysed anonymously)*. This study was approved as one of the specific objectives in a broader protocol entitled "Estimating pneumococcal disease burden and evaluating the impact of introducing the pneumococcal conjugate vaccine (PCV13) into the Expanded Programme of Immunization in Cameroon."

Data sources for death identification

It is mandatory in the country that, in the case of death in a hospital or other medical institution or in a prison, the head of the establishment must declare the death within fifteen days [15]. The certification by medical personnel of the CoD is an essential step in the series of processes in the construction of vital registration data. In this setting, the customary practice in the registration of deaths commences with the issuance of a death declaration form (DDF) to

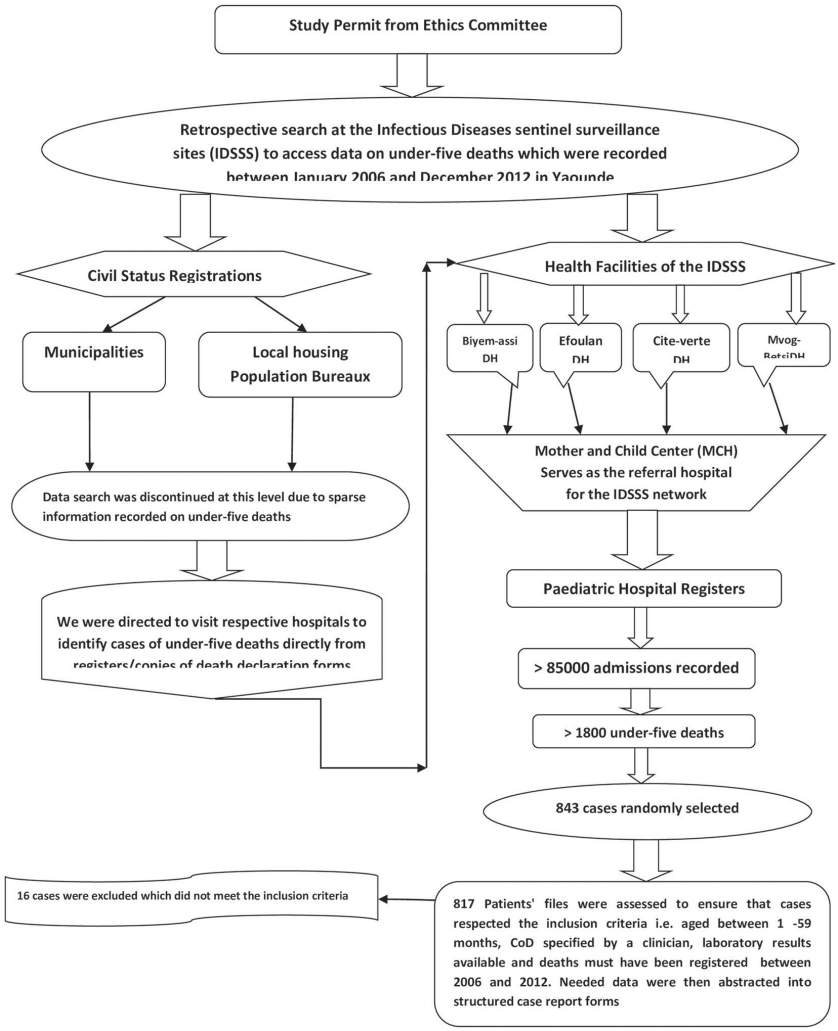

Fig 1. Flow chart on data identification and collection processes. N.B: For the sample size estimation, we assumed that, 18% of study population (630000) were children under-five years old; a = 0.05; power = 80%, and proportion of death cases with missing data on cause-specific death = 10%. Using the computer-based Creative Research Systems Survey software (http://www.surveysystem.com), we assumed a desired confidence level of 95% and a confidence interval of 4% units on each side; the estimated sample size for this study is 600 deaths. Therefore, a minimum of 660 cases of death in children aged 29days to 59 months was targeted as the sample size in this study. DH = District Hospital; ICD-10-CM = the International Classification of Diseases and Clinical Modifications 10th revision.

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the family of the deceased by a qualified medical staff. The family is expected to transmit the DDF to the municipality for registration and be issued a death certificate. However, this last step is hardly respected and this generates an inherent problem with the quality of data in vital registrations systems witnessed in most of resource-low settings. The recommended practice is

to respect the World Health Organisation's (WHO) criteria for medical certification; where the CoD are outlined in a sequential format beginning with the direct CoD through the intermediate causes, while the underlying CoD is registered in the lowest line of Part I of the medical certificate of cause of death [10].

The data search was limited only to hospital registers because we could not find sufficient information on under-five deaths from the civil status registers (vital registration systems) in municipalities and local population offices. Generally, typical hospital setting in the country consists of independent units and wards such as the female, male, paediatric, HIV/AIDS and oncology wards. Each keeps a register of patients' medical records (usually manual but in cities both manual and electronic formats maybe available). Our search to identify under-five hospital deaths relied principally on inpatient paediatric hospital registers, as well as on other outpatient and emergency units registers (Fig 1). This included all causes of hospital documented under-five deaths within the study period. From these, further verifications were made for any cases of death with pneumococcal aetiology.

Case definitions

The CoD as described in hospital registers were retrospectively coded using the ICD-10-CM codes. Cause-specific mortality proportions were derived as the fraction of total deaths possibly associated to specific conditions, using hospital-based ICD-10-CM list diagnoses. Here, owing to the complexity associated with defining infections with pneumococcal aetiology, we limit our perspectives only on pneumococcal-related causes of deaths defined as follow:

A): Clinical pneumococcal infections

1. ALRI: Diagnosis of pneumonia based on medical declaration as cause of death (i.e. any Acute Lower Respiratory Tract Infection (ALRI) or pneumonia including all diagnosis of ALRI); or
2. Clinically severe pneumonia: Death resulting from cough or difficult breathing as admission symptoms for child 1–59 months old residing in study area,

And with either

- A respiratory rate $\geq$ 40/minute, or temperature >38.5˚C, or refusing to feed, or vomiting and/or lower chest in-drawing

B): Laboratory confirmed infections

- Culture-positive invasive pneumococcal disease
- Culture-negative polymerase chain reaction (PCR) or antigen test positive invasive pneumococcal disease

C): Radiologically confirmed infections

- Radiologically confirmed pneumonia i.e. Chest X-ray-Community Acquired Pneumonia compatible with endpoint consolidation
- Radiologically confirmed pneumonia i.e. Chest X-ray-Community Acquired Pneumonia with any radiologic abnormality

Clinical cases of pneumococcal infections, which did not meet these criteria, were considered as non-confirmed pneumococcal diseases (unspecified).

D): Causes of deaths not related to pneumococcus

These included other biologically related causes of under-five deaths apart from those due to pneumococcal infections e.g. Tuberculosis (TB), HIV or malaria and others.

E): Deaths due to injuries

This consisted of under-five deaths with non-biological causes such as those resulting from injuries or accidents.

Statistical considerations

Data analysis. Cases included in this analysis were children aged between 29 days and 59 months (for uniformity we have used 29 days = 1 month, since no cases were registered as 29 or 30 days old), whose deaths were recorded between 1st January 2006 and 31st December 2012 in the study area. Deaths occurring in the neonatal period (i.e. 28 days following birth) were excluded. The date of birth (age in months) was considered as the entry point to the study in 2006. Follow-up time was up to 2012, which was the end of the study period, or at time of death reported. The proportion and causes of deaths were estimated from data collected on the total deaths using descriptive measures and Kaplan-Meier method. Data has been analysed using the software package SPSS 25.0 version.

Results

Distribution and main causes of death in children under-five years old

A total of 817 randomly selected cases of under-five deaths from hospital registers that were recorded between January 2006 to December 2012 in Yaoundé were assessed. The median ages at death were 11months for males and 15 months for females (Fig 2).Table 1 shows the age groups and distribution of the principal causes of death. The most vulnerable were those in the age group 1–11 months old, harbouring 45% of all-cause mortality. Malaria (17.5%) was the leading CoD followed by meningitis (11.0%), sepsis (10.0%), and pneumococcal diseases (8.3%).

Causes of death registered with corresponding ICD-10-CM codes

Table 2 shows a summary distribution of the 817 cases of under-five deaths classified by ICD-10-CM coding. Communicable infections resulting from infectious and parasitic diseases, respiratory infections, nutritional deficiencies and perinatal conditions were accountable for most deaths (71.5%). Non-communicable infections including those from malignant disorders, cardiovascular disorders and congenital abnormalities contributed for some 11.6%, anaemia contributed to 6.2% of deaths and injuries were responsible for 0.2%.

Comparison of leading CoD in our study with estimates from Sub-Saharan Africa (SSA)

In Fig 3, are presented percentages of cause-specific deaths of children age 1–59 months obtained in our study compared to estimates from the SSA region [3]. Meningitis and sepsis were contributing more cause-specific deaths in our study population compared to the rest of the sub-region. The statistics were similar for malaria and HIV/AIDS. Some positives are observed with a decline in CoD resulting from tetanus, measles and perinatal conditions in our study. Deaths attributed to other and ill-defined causes were as much as threefold higher in our study compared to that of the entire SSA region [3].

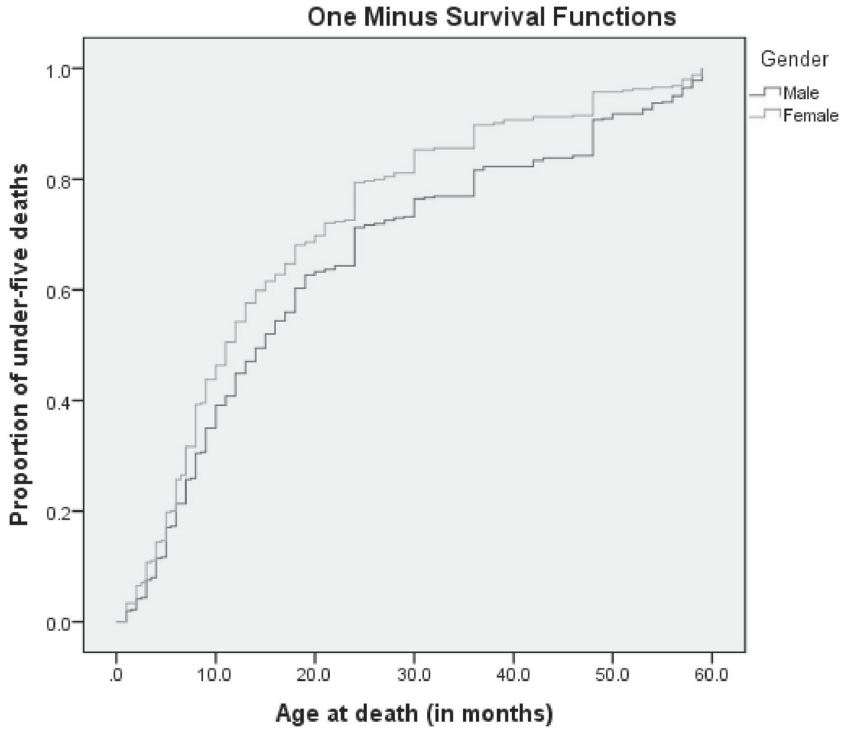

Fig 2. Cumulative proportion of age at death by gender among children under-five years registered at the Infectious Diseases Surveillance Sites in Yaoundé, Cameroon: 2006–2012.

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Clinical and laboratory diagnosed pneumococcal disease associated cause of death

Table 3 presents the distribution of clinically diagnosed and laboratory confirmed pneumococcal disease associated CoD. Overall, more than 55% of cases (n = 158) diagnosed with clinically

Table 1. Distribution of the main CoD in children 1–59 months old in Yaoundé, 2006–2012 (N = 817).

CoD	Age groups					
	1–11	12–23	24–35	36–47	46–49	All
	(N = 368)	(N = 187)	(N = 104)	(N = 55)	(N = 103)	(N = 817)
	%	%	%	%	%	% (95%CI)
Malaria	11.7	22.5	21.2	32.7	17.5	17.5 (15.0–20.3)
Meningitis	11.7	10.2	16.3	5.5	7.8	11.0 (09.0–13.4)
Sepsis	13.0	11.2	6.7	0.0	5.8	10.0 (08.1–12.3)
Pneumonia	9.8	7.0	8.7	3.6	3.9	8.3 (06.5–10.4)
Malnutrition	7.6	12.8	7.7	0.0	3.9	8.3 (06.5–10.4)
Diarrhoea/gastro-enteritis	8.7	5.3	4.8	1.8	2.9	6.2 (04.7–08.1)
Others	37.5	31.0	34.6	56.4	58.3	38.6 (35.2–42.0)

CoD = Causes of death; N = number; % = percentage; CI = Confidence Interval

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Table 2. List of ICD-10-CM codes collected for this study and percent of diagnostic CoD in children 1–59 months old in Yaoundé, 2006–2012.

No.	ICD-10-CM* code	Clinical diagnosis	%
1.	B54	Unspecified malaria	17.5
2.	G00.1	Pneumococcal meningitis	11.0
3.	A41.9 / B96.29	Sepsis	10.0
4.	B95.3 / J13	*Streptococcus pneumoniae* as the cause of diseases classified elsewhere	8.3
5.	C83.7/C95/D17.9/R59.1	Malignancy / Tumours/ Generalized lymph nodes	8.2
6	E46	Unspecified protein-calorie malnutrition	8.3
7.	K52.1/K52.89/R19.7	Gastro-enteritis / Diarrhoea	6.2
8.	D61.2	Aplastic anaemia due to other external agents	6.1
9.	P96.89	Other specified conditions originating in the perinatal period	4.4
10.	B20	Human immunodeficiency virus (HIV) disease	3.5
11.	A15.5	Tuberculosis of larynx, trachea and bronchus	2.3
12.	I25.5	Ischemic heart diseases	2.3
13	D57.1	Sickle-cell disease without crisis	1.1
14.	T14.90	Injury	0.2
15	R99/A35/B05.9/K75.9	Ill-defined and unknown cause of mortality and others	10.0

*ICD codes specific for diseases and compatible with clinical diagnosis as primary cause of death used in the study; No. = Serial number based on leading cause of death; S = Streptococcus; % = percent

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severe pneumonia had been coughing, had a respiratory rate $\geq$ 40/minute and a body temperature of over 38˚C. Seventeen percent of these were confirmed as culture-positive invasive pneumococcal disease, and 73.4% were confirmed as antigen test-positive. Only 1.3% of these had a radiological confirmation.

Discussion

This study aimed to determine the leading CoD in children aged 1–59 months in the MCH and health facilities within the infectious disease surveillance network in Yaoundé, Cameroon, using hospital-based registers. Vital statistics regarding the CoD among children based on death certificates are porous in resource-limited settings like Cameroon, which remain amongst the highest contributors to the number of under-five reported deaths [16]. In this study, we have assessed only about 2% of deaths of an estimated 117000 deaths expected to have occurred within the study period in Yaoundé [9], justified by our intention to capture CoD with clinical and laboratory-culture specific data to depict deaths from pneumococcal related aetiology. Thus, we could not ascertain that these deaths were representative of all deaths within this age group at that period of time, but we remain optimistic they mirrored the major causes of morbidity and mortality in this community since they were medically declared. Based on the data we assessed, infectious diseases and malnutrition were the leading CoD in children aged 1–59 months within the study period in this population. Additionally, 71.5% of the deaths occurred within the first 24 months of life.

However, our findings are in agreement with previous reports on the trends in the geographical distribution of CoD in the under-fives globally [16,17], although these studies had explored various methodologies than what we have used. This is partly due to the absence of a standardized universal algorithm to measure CoD.

In a related study on the causes and circumstances of death in a district hospital in Kolofata, Northern Cameroon between 1993 to 2009; malaria (15.9%), infectious diseases including

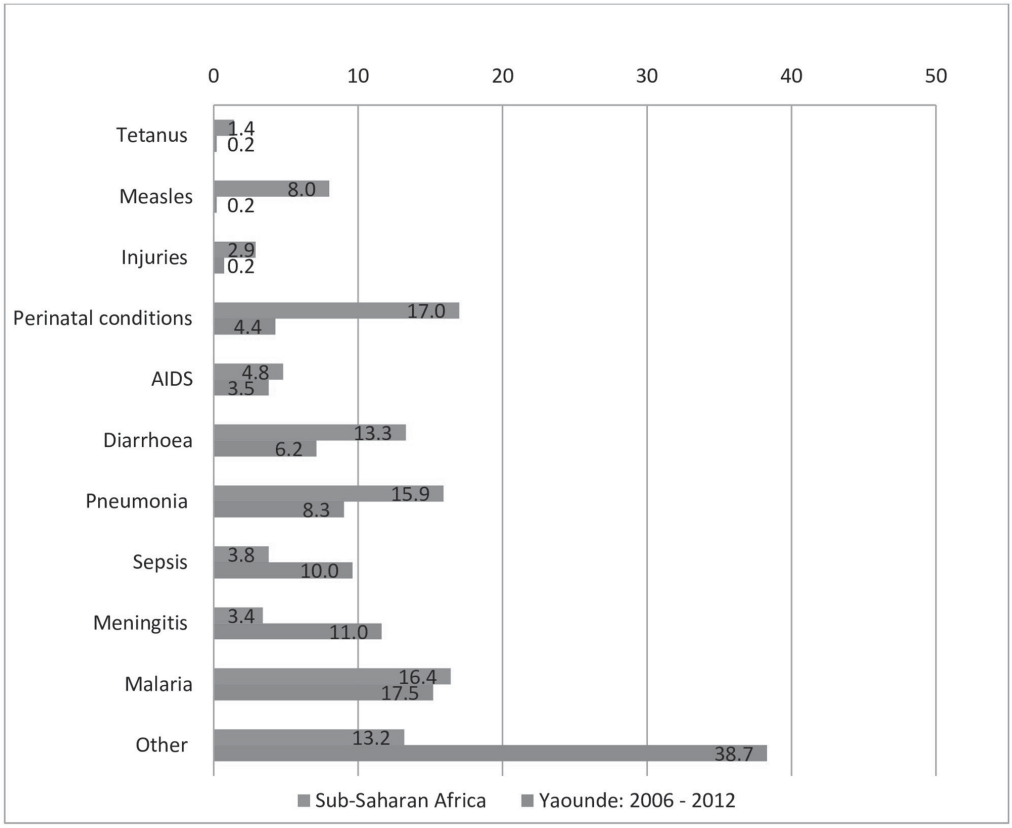

Fig 3. Frequent causes of deaths in our study with comparative percentages for the Sub-Saharan Africa region.

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ALRI (15.1%) and diarrhoeal diseases (13.3%) were the leading CoD in children before the age of 5 years [18], a similar trend to that obtained in our study. The Kolofata study included 1281 inpatient deaths of which 46.9% were deaths before the age of five years. Although there was a decline in the under-five mortality rate in the country from 144/1000 live births reported in 2004 to 122/1000 live births in 2011 [9], country-specific data on the major causes of death are scarce [18]. Vital statistics with notifications on CoD in children are not maintained in Cameroon, despite legislative provisions. However, the 2011 Cameroon Demographic Health Survey (CDHS) documented important information on symptoms and other characteristics leading to the death for children aged under-five years old nationwide [9]. Based on the CDHS data, ALRI, particularly those with pneumococci aetiology had 7% prevalence in Yaoundé, similar to the 8.3% we have obtained but differ with the 16% reported for the SSA region where diarrhoea (21%), malaria (30%), malnutrition (33%) and anaemia (60%) were the predominant CoD in children aged under-five years (Fig 3).

In the CDHS questionnaires, to ascertain pneumococcal-related causes of deaths caretakers were asked the following; *if the child was coughing in the last two weeks prior to death and if*

Table 3. Distribution of pneumococcal disease associated deaths by study case definition in children 1–59 months old in Yaoundé, between January 2006 to December 2012 (N = 158).

Diagnostic algorithm	YES		NO		UNKNOWN/ MISSING	
	n	%	n	%	n	%
Clinically severe pneumonia						
Cough	89	56.3	16	10.1	53	33.5
Respiratory rate ≥ 40/minutes	119	75.3	12	7.6	27	17.1
Temperature > 38˚C	114	72.2	24	15.2	20	12.7
Refusing to feed	62	39.2	25	15.8	71	44.9
Vomiting /lower chest in-drawing	57	36.1	6	3.8	95	60.1
Laboratory confirmed pneumococcal disease						
Culture positive IPD*	27	17.1	4	2.5	127	80.4
Culture negative PCR♠/ antigen test positive IPD	116	73.4	10	6.3	32	20.3
Radiologically confirmed infection						
X-ray CAP♣ with endpoint consolidation	2	1.3	1	1.6	155	98.1
X-ray CAP without endpoint consolidation	0	0	0	0	0	0

*Invasive pneumococcal disease;

♠ = polymerase chain reaction;

♣ = Community acquired pneumonia; N (n) = number; % = percentage.

Endpoint consolidation refers to the presence of alveolar or pleural effusion on chest X-ray

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yes, *was the cough accompanied with difficult and fast breathing prior to death*? This reflects the present case definition on pneumococcal related deaths we have adopted in this study, in addition to laboratory confirmation (Table 3).

In both the CDHS and Kolofata studies, malaria and diarrhoea were the primordial causes of death in children of the age groups 1–11 months and 12–23 months old than were deaths from pneumococcal diseases. In our study, 68% of deaths also occurred within similar age groups i.e. the first 24 months of life. This, according to the CDHS 2011 report may be explained by the period when the child (at ≥ 6 months) begins to receive other nutritional components than breast milk. The latter age group also corresponds to the age when the children begin to exploit their environment, and as such exposes them to contamination with pathogens. Another contributing factor on the prevalence of diarrhoea is that of the non-availability of potable water source. Based on the CDHS 2011 report, 19% of children from homes with a potable water source were exposed to diarrhoea infection as compared 24% in those from homes without potable water within this population.

Further, related studies conducted in other parts of Africa demonstrated that communicable infections including malaria, diarrhoea, respiratory tract infections, HIV/AIDS, TB and fever of unspecified origin accounted for over 50% of death in children before their first birthdays [19–23]. More so, a study on the global and regional CoD reported in 2009 that an estimated one in two deaths in children from SSA is attributed to communicable diseases as the primary cause [24].

In our study, malaria due to *Plasmodium falciparum* was the most important contributor to under-five deaths. As many as 18 of a hundred children from this population died from malaria. This is surprising when we consider the huge investments on malaria control programs in the country, just as in most of SSA [25]. Reducing the proportion of deaths due to malaria relies partly on the preventive and treatment regimens currently in use [25]. With support from the Global Fund for Health, the government of Cameroon besides the distribution

of long-lasting insecticide-treated mosquitoes bed nets to expectant mothers, is implementing the use of artemisinin-based combination therapy (ACT) as first-line malaria treatment in replacement of chloroquine [9,18]. We have limited understanding on this disproportional trend between malaria as the primordial CoD against on-going treatment and preventive measures being implemented for two decades rolling. However, there is a hypothesis that increasing resistance to antimalarial therapies and their improper use are contributing factors [9,26].

Bacterial meningitis (pneumococcal meningitis), was the second highest CoD, and occurred mostly in the age groups 1–11 and 24–35 months. The implication of the pneumococcus as the predominant organism causing bacterial meningitis had earlier been reported [27–30]. We were not surprised with this finding since the country is situated along the "African meningitis belt", and shares a long and porous border with Nigeria, where meningococcal disease incidence is high [31]. With the advent of the monovalent serogroup A meningococcal conjugate vaccine (MenAfriVac) in the country since 2009, other pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* are reported to cause more infections than was previously thought [9,31].

Septicaemia with ICD-10-CM codes *A41.9* and *B96.29* was responsible for 10.0% of deaths and is seen as another major contributor to under-five deaths in this community. Most of the sepsis resulted from bacterial infections with different aetiologies acquired during the neonatal period; subsequently leading to the hospitalisation and death of the children in their early infancy. This finding aligns with previous reports that, in a malaria endemic region like Cameroon, affected children have an increased risk to die from septic shock resulting secondarily from severe anaemia and/or bacteraemia [32,33]. More so, sepsis is described as a cascade of diseases involving a systemic inflammatory response syndrome (SIRS) in the midst of infection, intensifying in septic shock to cardiovascular and organ system dysfunction [34]. Some authors have attributed this systemic organ dysfunction as the last inflammatory stage for a majority of infectious disease-related paediatric deaths globally [34–36]. This phenomenon is consistent with our findings in which a myriad of infections resulting from HIV/AIDS (3.5%), *Streptococcus pneumoniae* (8.3%), malignancies (8.2%), ischemic heart diseases (2.3%), conditions from perinatal period (4.4%) and aplastic anaemia due to other external agents (6.1%) could be important underlying contributors to under-five deaths, although they were recorded as individual CoD (Table 2). We also recognize that multiple diagnostic CoD are a possibility, especially in a remote setting where patients present with concomitant infections as previously reported [37]. We have reported just a single CoD in this study based on the primary CoD with respect to medical declaration. However, to use a single CoD as we have done turned not to underestimate the contributions of the individual causes to the overall under-five mortality rate which witnessed a decline from 146/1000 live births in 2001 to 122/100 live births in 2011 [9].

Pneumonia due to *Streptococcus pneumoniae* (8.3%) was the fourth most important CoD, with most of the deaths occurring in children aged between 1–11 months. This observed aged pattern of pneumococcal related deaths highlights the importance of the pneumococcus as a serious health challenge to children in SSA including Cameroon [1,38].

Malnutrition related deaths which accounted for 8.3% overall CoD in our study, are a baseline to causes of child death in Africa [39]. In our data, chronic malnutrition usually protein-calorie malnutrition (with ICD-10-CM code: *E46*), is translated by a small height-for-age index which corresponds to growth retardation in children [9]. There are some variations in the prevalence of malnutrition by age, which is highest in the age group 12–24 months old (Table 1). We lack direct answers on why this form of malnutrition was predominant since our study was not designed to address risk factors for malnutrition. However, according to the CDHS 2011 survey this may be because of inadequate food intake and / or attributed to recurrent or relatively long periods of infectious diseases [9]. More so, for children with such

growth retardation it is evidenced that after the age of two years, there is a limited chance to ameliorate their growth with any nutritional intervention [40]. The height-for-age index is a measure of the long-term effect of malnutrition and is a useful indicator of the environmental, developmental and socio-economic level of a given population [40]. Thus, ameliorating the challenges of malnutrition should lead to a reduction in the number of under-five deaths in this population, and subsequently an improvement in their living standards.

Diarrhoea associated CoD was another predominant contributor to under-five mortality in the study area with 6.2% of all deaths. Majority of the deaths occurred between the age group 1–11 months. This is lower than the 13.3% prevalence reported in the Kolofata study in Northern Cameroon [18]. In a related study in Haiti, diarrhoea was found to be responsible for up to 60% of deaths among those in the age bracket 1–11 months old [4]. Hospital-based diarrhoea associated CoD is reported to vary in SSA from between 2% to about 40%, with most of the deaths occurring before the children reach their first birthdays [41]. However, the 6.2% prevalence obtained in our study shows a decline in diarrhoeal associated CoD when compared with the 13% aggregated data obtained from SSA region (Fig 3).

In our data, 8.2%, 6.1%, 4.4% and 2.3% of deaths were respectively, due to malignancies/tumours/generalized lymph nodes, anaemia, and diseases originating from perinatal conditions and tuberculosis (Table 2). The contributions of non-communicable diseases including malignancies/tumours and/or generalized lymph nodes to under-five deaths in the Yaoundé study area should not be undermined. Previous studies on causes of under-five mortality in SSA have scarcely highlighted the role of malignancies and related CoD, as there is too much focus on communicable infections [1,18,34,35,38].

Strength and limitation

There are some shortcomings in this study. First, we have only considered a single CoD as ascertained by a clinician, despite the understanding that in many resource-low settings a cascade of infections may be involved in a specific CoD [37]. Our main difficulty was the inability to ascertain the exact order in sequence of events leading to a specific CoD. However, the advantage here is that clinicians with long years of experience in tropical and paediatric medicines clinically validated the CoD. In addition, the methodology we used did not require subjective assessments from caretakers or open-ended algorithms in the determination of the primary CoD. Hence, compromising issues on measurement bias as it is common with the verbal autopsy technique. Further, we could not incorporate the verbal autopsy technique that should have addressed some of the deaths, which occurred outside the hospital settings, even if this too is plagued with inherent limitations. More so, our methodology may be useful to monitor possible changes in mortality trends considering that access to the hospitals were opened to everyone in the communities.

Recommendations and conclusion

The analyses and estimates we have presented are to support a better understanding on country-specific CoD in children under-five years old in a resource-limited setting, where vital registration system is weak to provide valid estimates on CoD distribution among the populations. The current estimates on the CoD we have reported are consistent with diagnoses based on medical declaration as ascertained by clinicians. Such estimates and information are needed for the planning of child health priorities, possible intervention and subsequently, a baseline for evaluation studies. In understanding the aetiology of the main primary causes of under-five mortality in our study, we from this basis suggest possible steps needed to reach the MDG4 objectives (Box 1).

Box 1. Recommendations on some essential points on reaching the MDG4 goal in Cameroon

- Increase in health funding/budget to facilitate the amelioration of health infrastructure and workforce through an enhanced community-based framework
- Establishment of a sustainable multi-sectoral approach to integrate child health, nutrition and social welfare policies and programmes for poverty reduction, agricultural improvements, food security and safety; upgrading of water, hygiene and sanitation systems.
- The experience of Ethiopia [42] that was used as a case study to the 2015 MDG countdown could be successfully applied to similar settings in Cameroon.
- Maternal factors such as education, dietary intake and nutritional status are strong determinants to child health outcomes, which should be carefully considered.
- There is an eminent risk of an increase of the 'triple burden of disease' i.e. the concomitant high burden of infectious diseases, malnutrition and non-infectious diseases. Appropriate cost-effective measures need to be established to counter this trend.
- Health administrators need to recognize what worked well and use lessons from the MDGs to address challenges in future health interventions, and outline roadmaps to achieve specific indicators
- Necessity to re-orientate targeted strategies, interventions and programmes by critically analysing evidence on the epidemiology of causes of death.
- Restructuring of health investment and financing mechanisms as an essential part of health intervention.
- Educating the public to follow-up with the required rules so that proper registry can be built for future generations
- Political commitment, such as the enforcement on the legislation of civil status registration systems and human resource training and mobilisation at the community level.

Our findings demonstrate that the main CoD among the inpatient deaths assessed in these hospitals are either treatable or vaccine-preventable. This trend is consistent with previous reports across developing countries. Therefore, scaling-up measures to reduce causes of under-five deaths will demand sustainable efforts to enhance both treatment and disease prevention strategies.

Supporting information

S1 Dataset.
(SAV)

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PUBLICATION II

Prevalence of pneumococcal nasopharyngeal colonization and serotypes circulating in Cameroonian children after the 13-valent pneumococcal conjugate vaccine introduction.

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Prevalence of pneumococcal nasopharyngeal colonization and serotypes circulating in Cameroonian children after the 13-valent pneumococcal conjugate vaccine introduction

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ABSTRACT

Background: Streptococcus pneumoniae remains a major contributor to childhood infections and deaths globally. In Cameroon, the 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in July 2011, using a 3-dose Expanded programme on immunization (EPI) schedule administered to infants at 6, 10 and 14 weeks of age. To evaluate PCV13 effects, we assessed pneumococcal nasopharyngeal colonization and serotype distribution among Cameroonian children after PCV13 introduction.

Methods: Nasopharyngeal (NP) swabs were collected from eligible children aged 24–36 months in two cross-sectional surveys conducted from March to July: in 2013 (PCV13-unvaccinated), and in 2015 (PCV13-vaccinated). Using a systematic World Health Organization (WHO) cluster coverage sampling technique in 40 communities, NP swabs collected were processed following WHO recommendations. Standard bacterial culture techniques were used for the isolation of *S. pneumoniae* from gentamicin-blood agar plates and identification using optochin susceptibility testing. Serotyping was performed using sequential multiplex polymerase chain reaction, supplemented with Quellung test.

Results: Among the PCV13-vaccinated children, overall pneumococcal carriage prevalence was 61.8% (426/689) and PCV13 vaccine-type carriage prevalence was 18.0% (123/689). Eleven out of the 13 vaccine serotypes were detected in the vaccinated children. The most common serotypes were 19F (4.5%, 31/689) and 15B/C (7.3%, 50/689).

Conclusion: In Cameroon, four years after infant vaccination nearly all of the PCV13-serotypes continued to circulate in the population. This suggests that the direct and indirect effects of the vaccination programme have not resulted in expected low levels of vaccine-type transmission. Continuous monitoring is needed to assess the long term effects of the PCV13 on nasopharyngeal carriage and disease.

Introduction

Streptococcus pneumoniae with its over ninety serotypes remains a major contributor to childhood infections and deaths globally (Neves et al., 2013; CDC, 2006). Its reported burden of morbidity and associated mortality is striking mostly in children in resource-low settings, like Cameroon, who are most often exposed to nasopharyngeal colonization (NP) shortly after birth

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(Kwambana et al., 2011). NP colonization is a prerequisite for the development of pneumococcal disease and a reservoir for pneumococcal transmission in the community (Sleeman et al., 2005). The ecological habitat of *S. pneumoniae* and other bacteria including *Haemophilus influenzae, Moraxella catarrhalis* and/or *Staphylococcus aureus* is the nasopharynx from where they can spread into the lower respiratory tract or the bloodstream and cause severe infections such as meningitis, sepsis or pneumonia (Bogaert et al., 2004; Simell et al., 2012).

There is a paucity of data in most low-income countries on the incidence of pneumococcal infections and other invasive diseases, and the epidemiology of circulating pneumococcal serotypes in either infant or adult populations (Kwambana et al., 2011; von Gottberg et al., 2014; Grant et al., 2016; Hammitt et al., 2014; Nzenze et al., 2013). However, a study on the epidemiologic pattern of bacterial meningitis and pneumococcal serotype distribution in Cameroon two years before vaccination reported the predominance of *S. pneumoniae* as the major pathogen among 170 hospitalized children with meningitis aged 2 months to 15 years (Gervaix et al., 2012). The prevalence of pneumococcal nasopharyngeal carriage in any healthy population in Cameroon has not been documented.

The main reservoir and primary transmitters of the pneumococci are thought to be young children (Kwambana et al., 2011). To understand the epidemiology and monitor the effects of the PCV programmes, the pneumococcal nasopharyngeal carriage (NPC) in children has often been used to assess the impact of the PCV on vaccine-type carriage and replacement of vaccine-types with non-vaccine serotypes (Weinberger et al., 2011). The reduction in vaccine-type carriage decreases the transmission of the vaccine-type disease. Serotype replacement, on the other hand, can reduce the positive benefits of PCV when the non-vaccine types have increased both in carriage and disease as has been previously reported (von Gottberg et al., 2014; Weinberger et al., 2011; Mackenzie et al., 2016; Hill et al., 2008). Therefore, it is essential to evaluate the effects of PCV13 on NP carriage after vaccine introduction, especially in a low-income country like Cameroon where carriage prevalence and disease incidence are expected to be high in younger children (Cutts et al., 2005; Hill et al., 2008). Further, implementation of PCV programmes need to be monitored continuously by an appropriate surveillance system, in order to assess impact of vaccination and epidemiological shifts in disease potentially related to it (Dunne et al., 2018). Where appropriate surveillance systems are lacking as in Cameroon, carriage studies have shown to be a cheaper alternative in assessing such changes (Roca et al., 2013). We assessed NPC and serotype distribution in PCV13-unvaccinated and PCV13-vaccinated children four years after PCV13 introduction.

Methodology

Ethical consideration

The Institutional Review Boards (IRBs) of the Cameroon National Ethics Committee and the Yaoundé Gynaecology, Obstetric and Paediatric Hospital, approved the study. Signed informed consent forms were obtained from all parents who agreed for their children to participate in the study.

Pneumococcal conjugate vaccination implementation in Cameroon

In Cameroon, the 13-valent PCV was introduced in July 2011, using a 3-dose EPI schedule administered to infants at 6, 10 and 14 weeks of age. There were no catch-up schedules for older children. The PCV13 was included into the EPI based on recommendations and financial support from WHO and the Global Vaccine Alliance initiatives (GAVI) (WHO, 2019), respectively, without any baseline data on NP carriage prevalence and invasive pneumococcal disease (IPD) incidence in Cameroon. Since 2011, EPI uptake for the third dose of diphtheria, pertussis and tetanus combination vaccine (DPT3) has been over 80% (World Health Organization, 2018); suggesting a high uptake of childhood immunization programmes in Cameroon.

Study design

Two cross-sectional surveys were conducted to collect NP swabs from children aged 24–36 months old, from March through July in 2013 (PCV13-unvaccinated) and 2015 (PCV13-vaccinated), in Yaoundé, Cameroon.

Characterization of the study population and sites

The study sites and population have been described previously (Libwea et al., 2018). Briefly, they involved localities situated within an 80 km radius from Yaoundé, Cameroon's capital city. Yaoundé and its surroundings harbour a population of over 3.5 million, out of which 18% are children aged under- five years, based on 2010 National Population Census. The sites were chosen as they constitute a group of health institutions described as the invasive disease sentinel surveillance. Sites are partitioned into 40 communities (clusters) using the health map and with each cluster hosting at least one health center/clinic, either public or private.

Inclusion and exclusion criteria

Subjects included in the study were children aged 24–36 months old; PCV-unvaccinated (for the 2013 group) and PCV-vaccinated (for the 2015 group); residing in the study area within the last 6 months prior to sampling and had a signed parental consent form. Children who did not show proof to have received at least one dose of PCV13 were excluded in the 2015 round. The proportion of children who had received 3 complete PCV13 doses was 92.5% (637/689) and for 2PCV13 doses was 7.5% (52/689). In both rounds, subjects with severe illnesses (e.g. malaria, sickle cell conditions or infectious diseases) or on antimicrobial treatment at the time of sampling were excluded.

Enrolment of subjects

In both surveys and in each study cluster, the first home to begin subject enrolment was selected randomly depending on the direction of pointer after spinning a pen at a central location e.g. church premises or market square. Subsequent subjects were enrolled through the WHO lot quality clustered sampling technique after every 10th home to provide representation within a cluster, and twenty-five children were targeted per cluster, as earlier described (Libwea et al., 2018).

Upon recruitment, parents were asked to bring their children to the study clinics where a pneumococcal nasopharyngeal sample was collected. For three successive days prior to and including the day of sampling, a community facilitator together with the study personnel with authorisation from local leaders made a public announcement to the community to inform or remind parents about the study.

Data collection procedure

Study personnel provided oral information and obtained informed consent from parents. Eligible subjects were then sampled at the study clinics and standard case report forms were

used to document demographic and clinical information following parental/caretaker interview, and a NP swab was collected from the child.

Pneumococcal nasopharyngeal samples and laboratory procedures

In accordance with WHO guidelines (Satzke et al., 2013), trained study personnel collected deep nasopharyngeal swabs by means of a sterile, flexible aluminum shaft and a dry cotton-wool tip inserted through the nasal cavity reaching the posterior nasopharyngeal area until some resistance was felt. The swab stayed fixed for a couple of seconds before being slowly withdrawn. NP samples were then inoculated immediately into vials containing 1.0 ml of skim milk–tryptone–glucose–glycerol (STGG) transport medium and placed in a cold box prior to transfer to the Mother and Child Hospital (MCH) bacteriology laboratories (a radius-distance of about 80 km from the study sites), within 8 h of collection according to WHO recommendations (Satzke et al., 2013). Inoculated vials were stored at −70 °C until they were transported in dry ice containing boxes. The 2013 samples were transported from Cameroon first to the National Institute for Health and Welfare (THL), Oulu, Finland and the 2015 samples to THL, Helsinki, Finland before they were all transported to the bacteriology laboratories at the Institute of Biomedicine, Research Center for Cancer, Infections and Immunity, University of Turku, Finland, for bacterial isolation. However, during shipment from Cameroon to Finland, the 2013 specimens were delayed for over two months at a port of entry in Germany and were reportedly stored at −20 °C.

Isolation and identification of pneumococci from nasopharyngeal samples

As previously reported (Kaijalainen and Palmu, 2015), in 2017 nasopharyngeal samples were cultured for the isolation of *S. pneumoniae.* After thawing and passage through a vortex rotator for thorough mixing, 10 μl of each specimen was pipetted and inoculated on 5% sheep blood agar plate (for semi-quantitative evaluation of the growth) and 5% sheep blood agar + 2.5 μl/ml of gentamicin for isolation of *S. pneumoniae*. The plates were incubated in 5% carbon dioxide atmospheric conditions at 35 °C for 18–20 h. If no colonies appeared, the incubation was continued for up to 48 h. Pneumococci were identified from both plates by their morphological α-hemolytic characteristics and by optochin sensitivity testing. From each sample, if several suspected pneumococcal colonies appeared, up to four colonies were confirmed and stored. The isolates were stored in 10% skimmed milk-glycerol and sent in batches on dry ice for serotyping at the bacteriology laboratory of THL, in Helsinki, Finland.

Serotyping of pneumococcal isolates

In accordance with a previously validated typing scheme (Siira et al., 2012), based on sequential multiplex polymerase chain reaction (mPCR) supplemented with Quellung test, when needed, pneumococcal isolates were serotyped. In mPCRs, a primer pair targeting the pneumococcal specific *cpsA* locus was used as an internal control. The species of cps*A* negative, Omni serum negative suspected non-encapsulated (NC) pneumococci were verified by *lytA* PCR. Serotypes were categorized as vaccine types (VT: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F), vaccine-related serotypes (VRT: 6C, 7A, 7B, 7C, 9A, 9N, 9L, 18A, 18B,18F, 19B, 23A, and 23B), non-vaccine types (NVT) and NC pneumococci. Serotypes 15B and 15C were reported together as 15B/C because of the reversible capsule switching between these serotypes (Van Selm et al., 2003). To assess for co-colonization by multiple pneumococcal serotypes, randomly selected duplicate pneumococcal isolates obtained from 13.5% (n = 70) of the swabs (15/108 isolates obtained from 2013 swabs and 55/406 isolates obtained from 2015 swabs) were also serotyped. Although more colonies were available for testing, we could only swab for 70 due to limited resources.

Statistical analyses

The intention of the study was to compare 2013 and 2015 NP samples but this was hampered by transport delays and subsequent high temperature exposure which might have compromised the 2013 samples. Based on literature, we assumed the estimated carriage prevalence of any pneumococcus circulating in the population to be around 60% (Gervaix et al., 2012); the proportion of vaccine-type pneumococci out of all: 62%, i.e. 37.2% out of all subjects, expected reduction of carriage of vaccine-type is

Table 1
Comparison of baseline characteristics of 887 children 2–3 years old sampled in 2013 (PCV13-unvaccinated, *n* = 198) and in 2015 (PCV13-vaccinated, *n* = 689) for nasopharyngeal specimens collected in Yaoundé, Cameroon.

Characteristics	PCV13-unvaccinated Cohort (2013) N (%)	PCV13-vaccinated cohort (2015) N (%)	p-Value*
Gender			
Male	117 (59.1)	372 (54.0)	0.204
Female	81 (40.9)	317 (46.0)	
No. of children <18years old in household			
One	66 (33.3)	101 (14.7)	
Two	56 (28.3)	175 (25.4)	<0.0001
≥Three	76 (38.4)	413 (59.9)	
Respiratory symptoms within last 30 days prior to sampling			
No	18 (9.6)	186 (27.0)	
Yes, but the child has been symptomless for 1–7 days	155 (78.3)	433 (62.8)	
Yes, but the child has been symptomless for 8–14 days	8 (4.1)	19 (2.8)	<0.0001
Yes, but the child has been symptomless for 15–28 days	13 (6.6)	9 (1.3)	
Unknown	3 (1.5)	42 (6.1)	
Antimicrobial treatment within last 3 months prior to sampling			
No	168 (84.8)	563 (81.7)	
Yes but treatment stopped 1–2 months ago	8 (4.0)	35 (5.1)	0.59
Yes but treatment stopped 2–3 months ago	22 (11.1)	91 (13.2)	
Source of cooking fuel (wood)			
Yes	147 (74.2)	511 (74.2)	0.983
No	51 (25.8)	178 (25.8)	

* p-Value estimates reflecting the difference in pneumococcal carriage status were computed using the Pearson Chi-square Test.

at least 50% i.e. from 37.2% to 18.6% after vaccination. Using the online tool http://statpages.org/proppowr.html, the minimum sample size for this study was 101 subjects per group to achieve 80% power. However, to increase the study statistical power, more subjects were sampled in 2015. We assessed pneumococci serotype distribution after PCV13 implementation and used the χ^2-test to compare differences between the PCV13-vaccinated and PCV13-unvaccinated groups (Tables 1 and 2). Statistical significance level was set at 5%. The statistical software package SPSS version 25.0 was used for analyses.

Results

Demographic and risk factors associated with pneumococcal carriage in 2015 compared with 2013

Of the over 1250 potentially eligible subjects contacted in both surveys, 887 were enrolled (71%). Altogether, 94 subjects were excluded in the final analyses either because they were not within the age range of 24–36 months or not residing in the study areas. The remainder were excluded because of lack of signed parental consent (n = 183) or due to severe illnesses (n = 54) or because they were on antimicrobial treatment at the time of sampling (n = 32). Nasopharyngeal swabs were collected from a total of 887 children aged 24–36 months, 198 in 2013 (PCV13-unvaccinated) and 689 in 2015 (PCV13-vaccinated), from March through June of each year. The demographic and risk factor variables are shown in Table 1. The vaccine uptake in 2015 was high (88%) for the complete 3 PCV13 doses received (Table 3).

Table 2
Prevalence of pneumococcal serotypes in the unvaccinated (2013) and vaccinated (2015) children 2–3 years old in Yaoundé, Cameroon.

Serotype	Unvaccinated group		Vaccinated group	
	N = 198 (%[a])		N = 689 (%)	
	N	%	n	%
Vaccine-serotypes				
1	–	–	–	–
3	1	0.5	9	1.3
4	–	–	2	0.3
5	–	–	1	0.2
6A	7	3.5	16	2.3
6B	5	2.5	19	2.7
7F	–	–	–	–
9V	1	0.5	3	0.4
14	2	1.0	22	2.8
18C	1	0.5	1	0.2
19A	1	0.5	6	0.9
19F	14	7.1	31	4.5
23F	10	5.1	15	2.2
Sub-total	42	21.2	124	18.0
Vaccine-related serotypes				
7C	1	0.5	3	0.4
9L	1	0.5	1	0.2
18A	–	–	2	0.3
19B	4	2.0	13	1.9
23A	–	–	3	0.4
23B	1	0.5	15	2.2
Sub-total	7	3.5	37	5.4
Non-vaccine serotypes				
15A	3	1.5	22	3.0
15B/C	18	9.1	50	7.3
21	6	3.0	9	1.3
34	6	3.0	18	2.6
38	4	2.0	23	3.3
OTHERS[b]	18	11.6	93	14.2
Sub-total	55	27.8	215	31.2
NC	10	5.1	50	7.3
Total	114	57.6	426	61.9

[a] Prevalence of serotype-specific carriage (Number of serotype detected divided by total number of samples); NC = non-encapsulated pneumococci; % = percent; n = number.
[b] In the NVT category, only the most prevalent serotypes have been presented individually. The rest for analytical purposes were grouped as "OTHERS". However, all individual serotypes are outlined in Figure 1.

Pneumococcal carriage and serotype prevalence

A total of 540 primary pneumococcal isolates were initially isolated from the 887 NP samples. The overall prevalence of *S. pneumoniae* in the PCV13-unvaccinated group was 57.6 % (114/198); VT, VRT and NVT pneumococci prevalence were 21.2%, 3.5% and 27.8%, respectively. The overall nasopharyngeal *S. pneumoniae* carriage prevalence for the PCV13-vaccinated children was 61.8% (426/689). The prevalence was 18.0%, 5.4% and 31.2% for VT, VRT and NVT pneumococci, respectively. Overall, 39 different serotypes (Figure 1) were identified from the 540 pneumococcal isolates and there were no major shifts in the serotype distribution between the vaccinated and unvaccinated groups. Additionally, 6.8% (60/887) were non-encapsulated (5.1% (10/198) in the PCV13-unvaccinated and 7.3% (50/689) in the survey). In the unvaccinated, the most frequent serotypes were 19F (7.1%, 14/198), 23F (5.1%, 10/198) and 15B/C (9.1%, 18/198). In the vaccinated, the most common serotypes were 19F (4.5%, 31/689) and 15B/C (7.3%, 50/689); and 11 of 13 vaccine serotypes were detected (Table 2). Serotypes 4 and 5 were only detected post-PCV13 introduction (0.5% and 0.2%, respectively) and this was probably due to differences in number of samples obtained between the two periods and/or storage of baseline samples under temperatures not suitable for pneumococci viability, whereas vaccine serotypes 1 and 7F or the cross-reactive 6C were not detected in either groups. Further, other microbes including *H. influenzae, S. aureus, M. catarrhalis* and beta-hemolytic Streptococci were detected (Figure 2).

Detection of multiple-serotype colonization

Multiple serotypes were detected in 11.4% (8/70) of the swabs tested and a single serotype in the rest of the 62/70 (88.6%) swabs. In three cases, the first isolate was serotype 3 and the second isolate non-encapsulated (NC) pneumococcus. The other double serotype findings were 21/19B, 19F/9N, 14/6B, 19F/33F and 15C/33B. However, due to limited resources only 13.5% (70/514) of the second isolates could be randomly screened for co-colonization.

Discussion

PCV13 infant vaccination in Cameroon using a 3 + 0-dose EPI-schedule at 6, 10 and 14 weeks of age resulted in an 18% prevalence for vaccine-type pneumococci and the overall pneumococcal carriage prevalence among the vaccinated children was 61.8%. Further, eleven of the thirteen vaccine serotypes were detected among vaccinated children in 2015, four years after vaccine introduction.

Reductions in vaccine-type pneumococci carriage have previously been demonstrated elsewhere among the vaccinated and the unvaccinated populations following PCV vaccination (Grant et al., 2016; Hammitt et al., 2014; Nzenze et al., 2013; Palmu et al., 2017; de Cunto Brandileone et al., 2016; Collins et al., 2017; Roca et al., 2011; Flasche et al., 2011). In comparison to other studies conducted in Africa (Table 3), four years after PCV7 implementation in The Gambia, a 13.3% reduction in VT-pneumococci proportion was observed among children in the 2–5 year age group, with an over 90% vaccine uptake (Roca et al., 2013). In Kenya where the impact of PCV10 was assessed two years after its introduction, a 19.1% reduction in VT-pneumococci proportion in children younger than five years was reported (Hammitt et al.,

Table 3
Studies evaluating the impact of pneumococcal conjugate vaccine (PCV) immunization on nasopharyngeal carriage in Cameroon and other African countries.

Country	References/study design	Schedule/age	PCV formulation/ inclusion	Age group at swabbing	Years after vaccination/catch-up	VT detected post-vaccination	% of VT-carriers before vaccination	% of VT-carriers after vaccination	Vaccine uptake (3PCV doses)	VT-pneumococci proportion after vaccination
Cameroon	Current study/cross-sectional	3 + 0 schedule given at 6, 10, 14 weeks of age	PCV13; 2011	24–36 months	4 years; no catch-up campaigns	11 out of 13	21.2%	18%	23% in 2011, 84% in 2012, 88% in 2013, 87% in 2014, 88% in 2015	29.1% (18/61.8)
Kenya	Hammitt et al. (2014)/ cross-sectional	3 + 0 Schedule given at 6,10 and 14 weeks of age	PCV10; 2011	<5 years old	2 years; catch-up in children <5years old	6 out of 10	34%	13%	32% in 2009, 94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	19.1% (13/68)
Gambia	Roca et al. (2011)/ randomized control trial	3 + 0 schedule given at 2, 3 and 4 months of age	PCV7; 2009	2.5 to <5 years	2 years; 1 dose of PCV given to subjects >30 months old	3 out of 7	50%	13.3%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	17.3% (13.3/76.7)
Gambia	Roca et al. (2013)/ randomized control trial	3 + 0 schedule given at 2, 3 and 4 months of age	PCV7; 2009	2.5 to <5 years	4 years; 1 dose of PCV given to subjects >30 months old	Not specified, but the cross-reactive 6A prevalence decreased	50%	8.9%	94% in 2010, 93% in 2011, 98% in 2012, 96% in 2013	13.3% (8.9/67)
South Africa	Nzenze et al. (2013)/ cross-sectional	2 + 1 schedule given as two primarily doses at 6, 14 and a booster at 40weeks	PCV7; 2009	<2 years	2 years; no catch-up campaign (switched in May 2011 to PCV13).	7 out of 7; and after the switch to PCV13 it was 11 out of 13	45.1%	23.5%	12% in 2009, 86% in 2010, 51% in 2011	32.0% (23.5/73.4)

VT = vaccine type; PCV7, PCV10 or PCV13 = 7, 10 or 13-valent pneumococcal conjugate vaccines; VT-pneumococci proportion after vaccination = VT-pneumococci prevalence divided by overall-pneumococci prevalence after vaccination; % = percent.

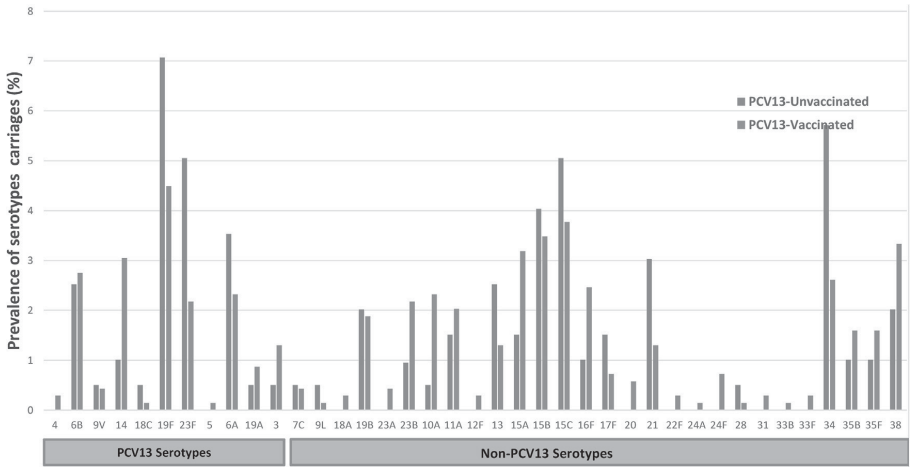

Figure 1. Serotype-specific prevalence among pneumococci carriage isolates in 2013 (PCV13-unvaccinated, n = 198) and in 2015 (PCV13-vaccinated, n = 689) cohorts of 2–3 years old children in Yaounde, Cameroon.

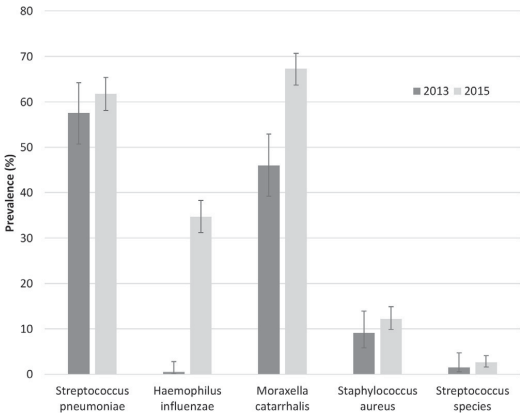

Figure 2. Prevalence of bacterial species isolated from NP swabs in 2013 (PCV13-unvaccinated) and 2015 (PCV13-vaccinated) cohorts of 2–3 years old children in Yaounde, Cameroon.

2014), with about 80% vaccine uptake. Additionally, results on the impact of PCV have been similar in studies which have either involved catch-up programmes for older populations or not. In both the Kenyan and Gambian studies, PCV implementation was rolled-on along with a catch-up plan. Whereas, in South Africa, without a catch-up campaign a 32% reduction in VT-pneumococci proportion was noted in all study age groups, including unvaccinated children aged 2–5 years. This was achieved within two years following PCV7 introduction and when only about 51% of the targeted infant population were fully immunized with a primary 2 dose series at 6 and 14 weeks after birth, followed by a booster dose at age 9 months (Nzenze et al., 2013).

The significant reductions in PCV7/PCV10/PCV13-serotypes observed in studies from the Gambia (Roca et al., 2011; Roca et al., 2015), South Africa (Nzenze et al., 2013) and Kenya (Hammitt et al., 2014) in both vaccinated and unvaccinated children are reported to have resulted from the direct and indirect effects of the PCV following a reduced transmission and acquisition of vaccine serotypes, which made children less vulnerable to subsequent carriage of vaccine-type pneumococci (Hammitt et al., 2014; Madhi, 2013). This finding was not clearly observed in our vaccinated cohort four years after PCV13 implementation. It suggests that the direct and indirect effects of the PCV13 have not resulted in expected low levels of vaccine-type transmission in our setting. This suggests that the Cameroon EPI schedule has not been able to block the transmission of vaccine type circulation. Absence of a booster PCV13 dose and waning effects may have contributed to the lower impact on carriage in this age group, as the vaccine is given on a 3 + 0 schedule early in infancy (6, 10 and 14 weeks of age). Recent data from Burkina Faso and Kenya, in addition to earlier reports from South Africa, suggest a switch to a 2 + 1 schedule in which the middle dose is given at 9–12 months along with the measles vaccine could lead to a longer duration of protection (Hammitt et al., 2019; Kambire et al., 2016; Madhi and Nunes, 2016).

Pneumococcal carriage is most prevalent in children, and its progression to disease has been reportedly influenced by predisposing risk factors such as overcrowding and upper respiratory tract symptoms (Auranen et al., 2016). Further, the risk of carriage acquisition has been demonstrated to be almost three times higher in association with the onset respiratory infection compared to health (Auranen et al., 2016). There were significant differences in the number of household children and in the different classes of respiratory symptoms among the children vaccinated and those not vaccinated (Table 1). These suggest that the unchanged overall pneumococcal carriage prevalence obtained in our study may have been influenced by children living in homes with one or more children under 18 years, and/or with respiratory symptoms, who were more likely to carry and transmit pneumococci than those without.

In summary, a considerable effect on VT-pneumococci colonization with vaccine uptake in the range of 50 to >90%, 1.5–4 years post-PCV introduction has generally been observed in these African studies, with or without catch-up campaigns. The Kenyan and Gambian randomized control trials and follow-up ecological studies reported VT-pneumococci proportions of 19.1%, 17.3% and 13.3% after the vaccine introduction, respectively (Hammitt et al., 2014; Roca et al., 2013; Roca et al., 2011); compared to the 29.1% obtained in our study. However, in a South African study (Nzenze

et al., 2013), VT-pneumococci proportion was higher (32%), but this may be as a result of only 51% of eligible subjects having completed the schedule of 3 PCV doses. Our findings are surprising and possible explanations for the differences in VT-pneumococci proportion may include lower vaccine impact, age at sampling, variations in the nasopharyngeal microbiome and/or secular trends. For instance, changes in HIV treatment regimens in South Africa and a community-wide azithromycin campaign in the Gambia are thought to have influenced the high vaccine effects in these countries (Hammitt et al., 2014; Roca et al., 2013; Roca et al., 2011).

The main strength of our study was the application of the WHO cluster sampling design which involved a systematic recruitment of all subjects from the same communities following the same procedures including sampling period/time and age range.

A major caveat in this study stems from the storage of NPS specimens (2013 collection) in a European port of entry not within the recommended laboratory conditions of −70 °C for over two months (Satzke et al., 2013; Kaijalainen and Palmu, 2015; Hill et al., 2016). This may have affected the viability of pneumococci in these samples (Satzke et al., 2013; Kaijalainen and Palmu, 2015; Hill et al., 2016). However, the overall pneumococcal carriage prevalences were nearly identical during both periods, yet variations in the prevalence for other bacteria, especially non-typeable *H. influenzae* (Figure 2) were observed. This suggests that our baseline carriage prevalence was probably under-estimated and therefore, no comparison between the vaccinated and non-vaccinated was performed. Moreover, our study used a different methodology in which PCV impact was assessed from a baseline of PCV13-unvaccinated 24–36 months old children, when routine vaccination had already been rolling for nearly two years. Therefore, the unvaccinated group was exposed to early indirect effects following routine infant vaccination (Roca et al., 2011). However, the indirect effects develop gradually, and the PCV13 uptake was low during the first 2 years of implementation (mean coverage for 2011 and 2012 was <55%) (World Health Organization, 2018).

In conclusion, the reported reductions in VT-pneumococci proportion are lower in our study compared to expected levels based on previous reports in similar settings. These suggest that either the vaccination programme with the 3 + 0-dose schedule is not eliciting sufficient protection against transmission of vaccine serotypes or there are programmatic challenges with the programme (Roca et al., 2015; Madhi and Nunes, 2016; Nzenze et al., 2015). More so, pneumococcal carriage is a precursor to invasive pneumococcal disease (IPD) (Sleeman et al., 2005) and as postulated by Boula et al., the prevalence of non-PCV13 serotypes among paediatric bacterial meningitis cases in Cameroon may play an increasing role in disease aetiology in the PCV13 era (Boula et al., 2019). Such findings as obtained in our study are important to identify which serotypes show increased propensity for IPD as well as in clinical practice and regulations on antibiotic prescription.

Authors' contributions

Conceived, planned and designed the study: JNL, HN, JPP and AAP; participated and supervised local data collection: JNL, PKN, MK and SKS; performed laboratory analyses: JV, KGH, MT, ON and JNL; contributed reagents and other logistics: AAP, MT and JV; drafted the manuscript: JNL and AAP; significantly contributed to the revised and final versions of the manuscript: JNL, KGH, MK, MT, PKN, ON, SKS, JV, HN, JPP and AAP

Conflicts of interest

AAP, JNL, TM, HN and ON are employed by the National Institute for Health and Welfare (THL), Finland which has received research funding from GlaxoSmithKline and Pfizer, Inc. "This does not alter our adherence to THE-IJID policies on sharing data and materials".

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PUBLICATION III

The prevalence of otitis media in 2–3 year old Cameroonian children estimated by tympanometry.

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The prevalence of otitis media in 2–3 year old Cameroonian children estimated by tympanometry

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ABSTRACT

Background: Acute otitis media is a common illness in children under-five years of age and associated with major health care resources in high-income countries. However, there is paucity of data on its epidemiology and clinical presentation in low-income countries. We estimated the prevalence of otitis media and assessed risk factors among children in Cameroon.

Methods: A community-based cross-sectional prevalence study of otitis media (OM) was performed on randomly selected children aged 2–3 years in Yaoundé, Cameroon from March to June 2013. OM was assessed by clinical inspection for chronic suppurative otitis media (CSOM) and tympanometry for otitis media with effusion (OME). CSOM was defined as draining of the middle ear with duration of more than two weeks and OME was defined as a flat 'type B' tympanogram.

Results: Out of 529 children enrolled in the study, 433 (56% males) subjects with available tympanograms were evaluated. Altogether, 9.7% (42/433) of children met the case definition of CSOM, OME or its complications. This consisted of 3 (0.7%) children identified with unilateral CSOM; 7 (1.6%) children with bilateral OME; 31 (7.2%) with unilateral OME and 1 (0.2%) subject with unilateral dry tympanic membrane perforation.

Logistic regression analyses showed statistically significant association between OM and parental reporting of "current symptoms of upper respiratory tract infections", Prevalence Odds Ratio (POR) = 3.71; 95% CI = 1.69–8.14).

Conclusion: As many as two out of a hundred children between the ages of 2–3 years were affected by significant middle ear disease i.e. CSOM or bilateral OME. These data could be useful as a baseline for estimating the impact of pneumococcal conjugate vaccines (PCV13) introduced in July 2011 for infants in Cameroon.

1. Introduction

Otitis media is reported as one of the most common respiratory illnesses affecting children under five years old worldwide [1–3]. The disease and its complications are diagnosed and treated more actively in developed countries than in resource-poor settings like Cameroon [3]. In most developing countries, acute otitis media goes usually undiagnosed and consequently, affected children are not timely identified to be treated. Otitis media may also occur as chronic otitis media with effusion or chronic suppurative otitis media, and it may remain persistent in early childhood [4]. Prolonged hearing loss and delayed development are potential long-term complications of otitis media. These long-term complications in children may amount to considerable socio-economic costs both to the children, parents and the public health system [5], especially in most communities in Cameroon where 24% of the population lived under the poverty line i.e. < $US2 daily [6].

Thus, access to care is not easy to everybody due to financial constraints. Although drugs are prescribed in the hospitals and available in pharmacies, it is a very common practice to buy antimicrobials over-the-counter at local markets. More so, the doctor-patient ratio is low

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and it was reported the country is experiencing a crisis in human health resources with an estimated ratio of 1 clinician and 8 nurses/midwives per 10000 people reported in 2010 [7]. However, there are many health institutions (both public and private) in the country staffed with nurses and general practitioners. A few specialists are available usually in major cities.

Epidemiologic studies on the burden of otitis media in Cameroonian children are lacking. However, the disease incidence is expected to be high considering data from other low-resource settings [8–11].

The 13-valent pneumococcal conjugate vaccine (PCV13) was introduced in Cameroon's Expanded Immunization Programme in July 2011. The primary aim of this study was to measure the prevalence of OM occurring in PCV-unvaccinated children 2–3 years old, as a baseline for estimating the PCV13 impact on OM disease burden and sequelae.

2. Methodology

2.1. Study description

PCV-unvaccinated children aged 2–3 years were enrolled from March to June 2013 in a community-based cross-sectional study to evaluate the prevalence of OM in children. Ethical approval was obtained from the Cameroon National Ethics Committee and from the Institutional Review Board of the Yaoundé Gynaeco-obstetrics and Paediatric Hospital (YGOPH). Further, informed consent was obtained from parents/caretakers in addition to permission from the local administrative authorities.

2.2. Sites selection and inclusion criteria

The study sites were situated within 80 km radius from Yaoundé, Cameroon's capital city. Yaoundé and the surroundings harbour a population of over 3.5 million out of which 18% are children aged from 2 to 3 years, based on 2010 National Population Census. The sites were chosen as they constitute a group of health institutions described as the pneumococcal disease sentinel surveillance sites. These include the Cite Verte Health District (urban) with the Mother & Child Reference Hospital (MCH) and four other health districts. Sites were partitioned into 40 blocks (clusters) using the health map with each cluster hosting at least one health centre/clinic either, public or private. Children were eligible if aged from 24 to 36 months, and residing in the area for at least six months. Enrolment was restricted to those who had not received any doses of pneumococcal conjugate vaccine, as was confirmed from child's vaccination card or registers. The starting household within the cluster was selected after spinning a pen, usually at a central location in the community. Selection of participants was done randomly after every 10th home within a cluster. One participant was selected per home even if two or more were eligible (in such an event, selection was with respect to birth order); and twenty-five children were enrolled per "cluster".

2.3. Study team and participants

Training of the study team members in the practical aspects of the study (recruitment of subjects, questionnaires administration, clinical examination and tympanometry) was done prior to the start of the study by the principal investigator. Two mobile study clinic teams, each with three trained study nurses and a study physician were established to enrol children. Families were informed about the study by community "social mobilisers" (in addition to radio announcements and fliers) a week prior to visiting a specific area and within the actual planned visit days. The study clinics were established at a central location (e.g. chief's or local leader's compound or at a health centre).

2.4. Data collection

In order to enhance compliance of the children, inspection to detect draining ears was done first, followed by tympanometry. Clinical and visual examination involved a thorough inspection of the external ear structure for signs of drainage or cerumen accumulation in the outer third of the ear canal as recommended [12]. Pneumatic otoscopy was performed, but since most subjects had considerable cerumen accumulation and we lacked appropriate equipment to clean-up the wax in the field conditions, the otoscopic data were sparse and not used for this analysis. Tympanometry was performed using the Middle Ear Analyser Grason Stadler tympanometer (GSI-38 Autotymp, Grason-Stadler Inc., Milford, NH, USA). Tympanograms were recorded with a 226 Hz probe tone with a pressure varying from +200 daPa (daPa) to −400 daPa in a time of 7 s. Tympanometry was not performed on draining ears. Tympanometry was followed by parental questionnaire. It consisted of questions on potential risk factors, i.e. demographic characteristics, family socio-economic status, number of children under 18 years living in household, the number of children sleeping in the same bedroom, parental smoking status, source of household cooking, duration of breastfeeding and antibiotic use. In addition, the parents were asked about current symptoms of any respiratory tract infections.

2.5. Interpretation and classification of tympanograms

Tympanograms were independently interpreted by two researchers in retrospect. In an event of discordance in the interpretation, a third researcher interpreted for a final decision. The tympanograms (Table 1) were classified based on a modified version of Liden/Jerger's classification [13]. In this categorisation, flat, 'type B' tympanograms indicated the presence of middle ear fluid (MEF). Tympanograms with curve types A, As, C, or Cs suggested absence of MEF. High external ear canal volume (ECV > 1.0 cm^3) and with a 'flat curve' was interpreted as perforation of the tympanic membrane (TMP) i.e. type P

Table 1
Classification criteria used for reporting tympanograms in this study [13].

Curve Type	Criteria	Clinical Presentation
A	TPP ≥ −100 daPa, SAA ≥ 0.2 cm^3	Normal middle ear pressure (MEP), normal static admittance and no MEF
B	Flat curve; ECV = 0.3 to 1.0; no values for TPP	Consistent with Middle ear pathology (MEF)
C	TPP < −100 daPa, SAA ≥ 0.2 cm^3	Significant negative MEP, normal static admittance, no MEF
As	TPP ≥ −100 daPa, SAA ≤ 0.2 cm^3	Reduced admittance, Normal MEP, no MEF
Cs	TPP < −100 daPa, SAA ≤ 0.2 cm^3	Reduced admittance, decreased MEP, no MEF
F	Erroneous peaks (no distinct curves) or ECV < 0.3 in the absence of a distinct curve	Failed tympanogram, child unstable in process or probe in contact with ear canal or ear wax
P	No Peak (or flat curve); ECV > 1.0	Tympanic Membrane perforated

SAA = Static acoustic admittance; TPP = Tympanometry peak pressure; daPa = deca-pascals; MEF = Middle ear fluid; **ECV** = Ear can volume. The difference between A and As (and C & Cs) at SAA = 0.2cm3 was dependent on the graphical display of the curve. When the curve exceeded the lower limit of the graphic normal box, it was described as A (or C, depending on the TPP); A, As, C, Cs = Healthy ears; B = Diseased ear, F = Failed tympanogram; P = Perforation. Curves type B, P and F all have undetermined acoustic reflexes but could not be distinguished from each other based on the measure of the ear canal volume.

tympanogram. Curves with erroneous peaks due to artefacts or movements of the child and curves with ECV below 0.3 cm^3 without any recording of a normal curve were interpreted as failed (type F tympanogram).

2.6. Case definitions

In this study, ears observed by clinical inspection with draining and parental reporting to have lasted more than two weeks were considered as CSOM based on the World Health Organization's criteria [14]. We defined OME as flat, 'type B' tympanogram and a dry TMP was distinguishable from a 'type B' flat curve when the ECV was above 1.0 cm^3. For each subject, one of the following mutually exclusive categories was assigned: CSOM, dry perforation, bilateral OME, unilateral OME and healthy ears.The first four categories were considered to have OM or its complications.

2.7. Statistical analyses

Estimations were made to sample at least 250 participants from each of the five health districts. In determining otitis media prevalence, we first included subjects with CSOM (n_1) in the analysis (Fig. 1). Of the remaining subjects, those with no tympanometry data (n_2) were excluded. Subjects with CSOM (n_1) and subjects with available tympanogram data (n_3, n_4, n_5, and n_6) were included in the analyses. Multivariable logistic regression analysis was performed to assess risk factors for OM. The presence of OM was the primary outcome. In the multivariate analyses, inclusion of covariates (see Table 2) was restricted using a statistically significance level of alpha < 0.05. Statistical analyses were conducted using the statistical software programme, SPSS 24.0 version.

3. Results

3.1. Enrolment and baseline characteristics of subjects

Of the 529 enrolled in the study, ninety six subjects with no tympanogram measurement were excluded and 433 (56% males) were evaluated. The main reason for missing tympanometry data was electrical power cuts. The baseline characteristics of the participants are shown in Table 2. Forty percent of subjects were sleeping alone and the remainder shared their bedrooms with at least one other sibling. Also, majority of subjects were living in same household with at least one sibling who was ≤18 years old. Day care amenities are uncommon in this setting for this age group so all children were enrolled during home visits.

3.2. Preliminary analysis

We noticed in otoscopy that many children had ear wax accumulation, which could block the ear canal and therefore, give a flat 'false type B' tympanogram curve. Thus, we first addressed this question by examining the distributions of the ECV measurements by tympanogram type. In total, there were seventy six flat tympanograms distributed across all the ECV categories. However, we observed that many flat tympanogram curves initially interpreted as 'type B' had low ECV values i.e. 0.3 cm^3 and 0.4 cm^3 in comparison to other tympanogram types (Fig. 2) and, there was a statistically significant difference in the mean ECV values of flat and 'Other type' tympanograms. We interpreted this difference to be probably due to ear wax accumulation resulting in flat tympanograms in many children. Based on the distribution of the ECV values in 'Other type' tympanograms with discernible curves (A, As, C and Cs), we noticed that 90% of these were in the ECV categories of between 0.5 cm^3 to 1.2 cm^3, and 10% were distributed between 0.3 cm^3 and 0.4 cm^3 ECV categories. With respect to this, we therefore adjusted the number of original 'type B' tympanograms with low ECV values of 0.3 cm^3 and 0.4 cm^3 (n = 42, including 12 bilateral i.e. from 6 subjects) to follow the same ECV distribution for the selected categories of 0.3 cm^3 and 0.4 cm^3. Thus, the estimated number of initial flat tympanograms with low ECVs dropped from 42 to 4 i.e. 38/42 flat tympanograms with low ECVs were considered false positive 'type B' i.e. failed tympanograms, and only 4/42 were considered true positives. Therefore, the final number of true 'type B' tympanograms fell from 76 to 38.

3.3. Point estimates for otitis media prevalence

Among the subjects included in the analysis, 42/433 (9.7%) were diagnosed with at least one form of otitis media or its complications. This consisted of 3 (0.7%) children identified with unilateral CSOM, 7 (1.6%) children with bilateral OME and 31 (7.2%) with unilateral OME and 1 (0.2) subject with unilateral dry tympanic membrane perforation (ECV > 1.0 cm^3). In the stratified logistic regression analyses, no statistically significant association was observed between OM and any of the predictors evaluated (Table 2). However, there was a statistically significant association observed between OM and parental reporting of "notice of any current symptoms of upper respiratory tract infections", Prevalence Odds Ratio (POR) = 3.71; 95% CI = 1.69–8.14; p = 0.001.

4. Discussion

In the present study, we enrolled 2 to 3 year-old PCV-unvaccinated children and, estimated the prevalence of otitis media and associated risk factors in a low-resource setting. The results indicate a 9.7% overall prevalence of otitis media or its complications in the study population. Presence of current symptoms of upper respiratory tract infections was statistically significantly associated with OM.

The 9.7% prevalence of OM (including 2.3% of significant middle ear disease) obtained in our study is within the range of findings reported in previous studies. OM prevalence ranging between 2.5% and 41% has been reported from studies in the USA, Turkey, Italy, Nigeria, Kenya, India and Saudi Arabia using varying methodologies [1,15–18]. Earlier studies, which depended on hospital-data rather than community–based data have either focused on OM prevalence and associated risk factors in the first two-years of life or early infancy to the early teens [19–21]. Although studies on otitis media in low-resource settings are limited, age discrepancies may also limit comparisons with the present results. The current study targeted children aged from 2 to 3 years; whereas, previous studies have covered children aged from 0 to 20 years [1,11,15–17,22]. As such, few studies like the present one have focused on screening of otitis media in the general population. Fifteen percent prevalence for otitis media was reported in one Nigerian study on the epidemiology of otitis media in a community of children aged 0–12 years old. In that study, those between the ages of 1–4 years showed the highest prevalence (22.5%) of the disease [11]. Another study in Nigeria reported a prevalence of 37.7% and 43.7% among day-care and non-day-care attendees aged between 6 and 24 months, respectively [23]. Moreover, Minja & Machemba carried out a study among rural and urban school children aged from 5 to 20 years in Dar es Salaam, Tanzania and reported a prevalence of 9.4% and 1.3%, respectively [22]. Further, a Turkish study to determine the prevalence of OME in primary school children reported 10% prevalence [24]. A similar study conducted in the Qassim Region of Saudi Arabia reported 7.5% prevalence [25]. Additionally, an Australian study reported a 15% prevalence of CSOM in 709 Aboriginal children aged 6–30 months in remote communities [26]. Therefore, it appears that the OM prevalence is high in resource-low settings, especially in early childhood.

The relationship between OM and the lone factor observed in this study, i.e. current symptoms of upper respiratory tract infections (URTI) was expected. Children with prevailing symptoms of URTI are known to have a higher risk of developing otitis media than others [27,28]. In addition, the clinical symptoms of acute otitis media are generally

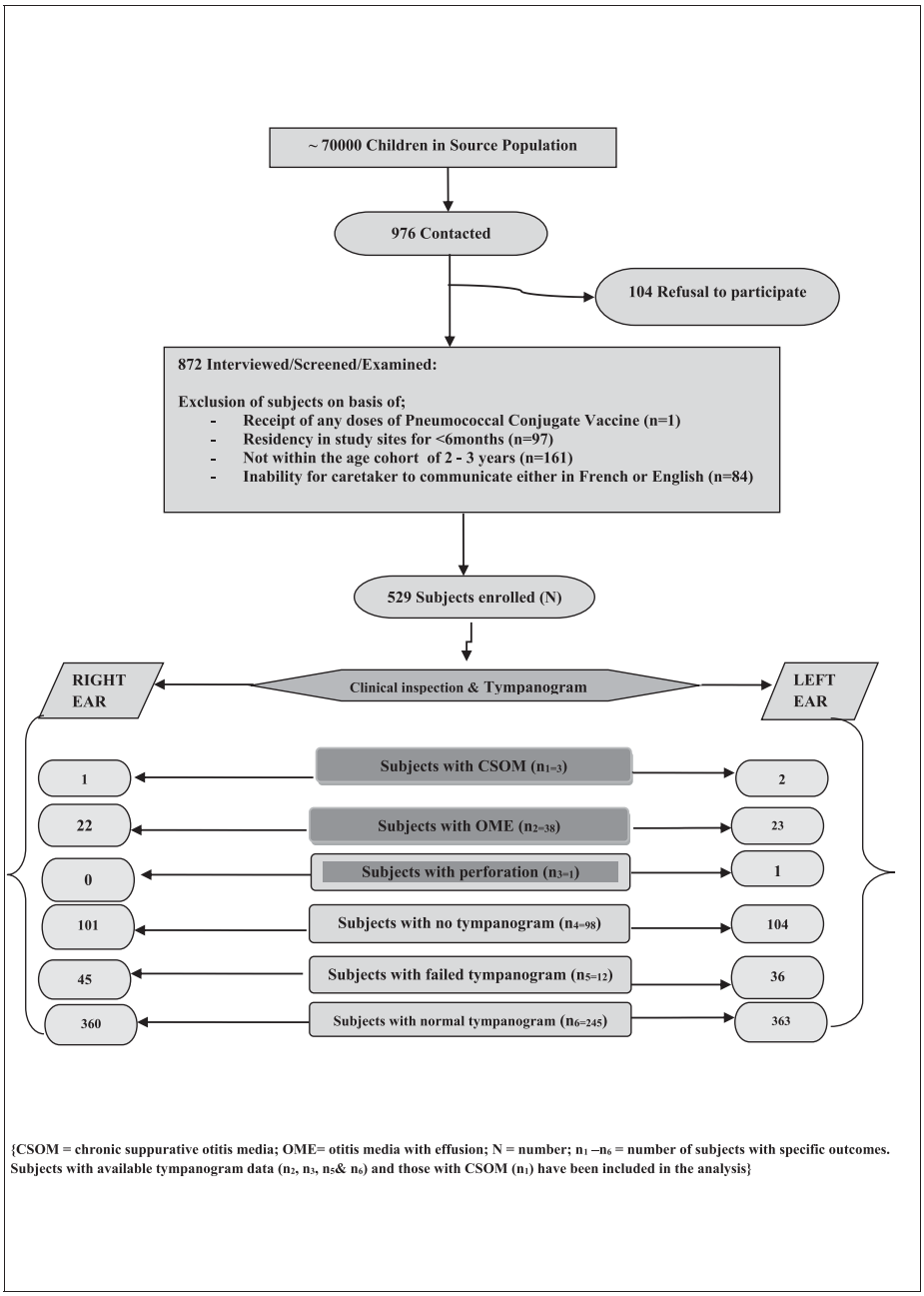

Fig. 1. Flow chart of data collection process and results by ears. {CSOM = chronic suppurative otitis media; OME = otitis media with effusion; N = number; n_1 –n_6 = number of subjects with specific outcomes. Subjects with available tympanogram data (n_2, n_3, n_5& n_6) and those with CSOM (n_1) have been included in the analysis}.

similar to those of URTI [29,30,33]. Although we did not find any risk factor associated with OM in our study apart from the URTI, several other factors have been reported in earlier studies [1,30,31]. No statistically significant association was observed between number of siblings and OM in the present study. This was surprising because families were largely extended with about 60% of the children having at least 3 other siblings living in the same home. Thus, the result is not in line with the finding that increase in the number of persons in homes or

Table 2
Baseline characteristics and their association with Otitis Media (OM) in 2–3 years old children in Cameroon, *N = 429*.

Characteristics	N (%)	Prevalence of OM (%)	Multivariable logistic regression (Modelling for OM)		
			POR	95% CI	p-value
Gender of child					
Male	240 (55.9)	21/240 (8.8)	1.0		
Female	189 (44.1)	17/189 (9.0)	1.03	0.53–2.01	0.929
Age (group) of child in months					
24 to 29	154 (35.9)	12/154 (7.8)	1.0		
30 to 35	109 (25.4)	9/109 (8.3)	1.07	0.43–2.62	0.891
36	166 (38.7)	17/166 (10.2)	1.35	0.55–2.53	0.447
No. of siblings sleeping in same bedroom ≤18years					
Alone	170 (39.6)	14/170 (8.2)	1.0		
One	141 (32.9)	11/141 (7.8)	0.94	0.41–2.15	0.889
Two	84 (19.6)	9/84 (9.6)	1.34	0.55–3.23	0.518
≥ Three	34 (07.9)	4/34 (10.7)	1.49	0.46–4.83	0.510
No. of siblings living in same home ≤18years					
One	76 (17.7)	10/76 (13.2)	1.0		
Two	98 (22.8)	9/98 (9.2)	0.67	0.25–1.74	0.407
≥ Three	255 (59.4)	19/255 (7.5)	0.53	0.24–1.20	0.127
History of previous otitis media					
No	344 (80.2)	27/344 (7.8)	1.0		
Yes	85 (19.8)	11/85 (12.9)	1.75	0.83–3.68	0.143
Breastfeeding period					
≤ 6months or not breastfed	37 (8.6)	2/37(5.4)	1.0		
≤12 months	182 (42.4)	15/182 (8.2)	1.57	0.34–7.19	0.560
> 12 months	210 (49.2)	21/210 (10)	1.94	0.44–8.67	0.383
Antibiotic use when child is sick					
No	147 (34.3)	10/149 (8.1)	1.0		
Yes: with/ without medical report	282 (65.7)	28/280 (9.2)	1.51	0.71–3.20	0.282
Noticed any current URT symptoms					
No	342 (79.7)	26/342 (7.6)	1.0		
Yes	47 (11.0)	13/47 (23)	3.71	1.69–8.14	0.001
Unknown	40 (9.3)	1/40 (2.5)	0.31	0.41–2.36	0.259
Parental educational level (SES1)					
≤ Primary school	146 (34.0)	12/146 (8.2)	1.0		
≥ Secondary school	212 (49.4)	23/212 (10.8)	1.36	0.65–2.83	0.412
≥ University Education	71 (16.6)	3/71 (4.2)	0.49	0.13–1.80	0.285
Parental occupational/income level (SES2)					
No Education	210 (49.0)	15/210 (7.1)	1.0		
No Education, some income	92 (21.4)	7/92 (7.6)	1.07	0.42–2.72	0.886
Some Education	11 (2.6)	1/11 (9.1)	1.30	0.16–10.85	0.808
Higher Education	57(13.3)	5/57 (8.8)	1.25	0.43–3.60	0.679
Student and others	59 (13.8)	10/59 (16.9)	2.65	1.12–6.27	0.026
Parental smoking status					
Non-smokers	370 (86.2)	32/370 (8.6)	1.0		
Smokers	59 (13.8)	6/59 (10.2)	0.95	0.48–2.99	0.703
Using wood/cool as household cooking fuel					
No	127 (29.6)	9/127 (7.1)	1.0		
Yes	302 (70.4)	29/302 (9.6)	1.39	0.64–3.03	0.404

POR = Prevalence Odds Ratio; 95%CI = Ninety-five percent confidence interval; N = Number; % = percentage; OME = Otitis Media with Effusion; URT = upper respiratory tract.

groups facilitates the transmission of URTI and thus development of OM [11,32]. Hence, we expected a positive association as Amusa et al. reported in a Nigerian study [11]. In the current study we did not observe any statistically significant association between exposure to passive smoke and OM, but the number of parents who were smokers was small. A strong correlation between household cooking fuel and OM has been reported in previous studies [1,11,31]. Considering that most of the households depended on wood/coal as cooking fuel, we observed that children were generally more exposed to pollutant smoke from firesides. Similarly, a study conducted in Egypt did not either find any significant association between OM and passive smoke through parental smoking [34]. Some investigators have reported an association between seasonal changes and OM disease prevalence [35], but our study was not designed to measure variations in prevalence in different seasons.

With challenges of data paucity on otitis media in developing countries, this is to our knowledge the first community-based study of otitis media in Cameroon. Moreover, the methodology we have used is not biased by diseases leading to health care attendance or health-care seeking habits. Thus, the findings could be useful as a baseline for evaluating the effectiveness of the pneumococcal conjugate vaccines introduced in 2011 against otitis media disease and sequalae.

Otitis media can be diagnosed using different methods. Although pneumatic otoscopy is the standard tool [36], interpretation of tympanic membrane findings is dependent on a straight visual access, prone to errors and subject to inter-observer variation [37]. Tympanometry, an application of impedance audiometry is a more objective measurement suitable for the diagnosis of middle ear effusion, assessment of tympanic membrane perforations and for the estimation of middle ear pressure [13,38].

In interpreting this result, caution is needed since 96 of the 529 children did not have tympanograms available because of power cuts, and the outcomes for these children remained unknown. Further, the possibility of selection bias should also be carefully considered since 447 of the 976 children contacted could not be enrolled in the study as most of their parents declined [39] (Fig. 1). Additionally, data on otoscopy would have provided more detailed information e.g. of the presence of acute otitis media were not available. This was because we lacked sufficient equipment at field conditions to clean occluded ears.

Tympanometry needs an airtight seal between the probe and the external auditory canal which may pose problems in uncooperative children [13]. Hence, this may result in misclassification as another potential source of bias in this study, i.e. the challenges of distinguishing 'type B' tympanograms from 'type F' or 'type P' tympanograms as they all generate graphically 'flat curves'. The observed differences in the ECV values suggest that we initially misinterpreted many occluded ears as 'type B'. We corrected this by adjusting the majority of these to 'type F' curves based on the ECV distribution in normal tympanograms. Despite of the challenges, our findings provide accurate estimates obtained by tympanometry as it is objective and reliable to detect middle ear effusion in this age group [13,26,37].

The results of OM prevalence from our study population concords with those previously reported elsewhere. The presence of current symptoms of URTI was strongly associated with OM in this population. Lack of adequate materials at field conditions for ear wax removal was one of the main obstacles encountered in the study. However, with limited data on OM in Cameroon and most of Sub-Saharan Africa, our results add to the knowledge on OM from remote settings and could serve as a useful baseline for future vaccine impact studies in Cameroon.

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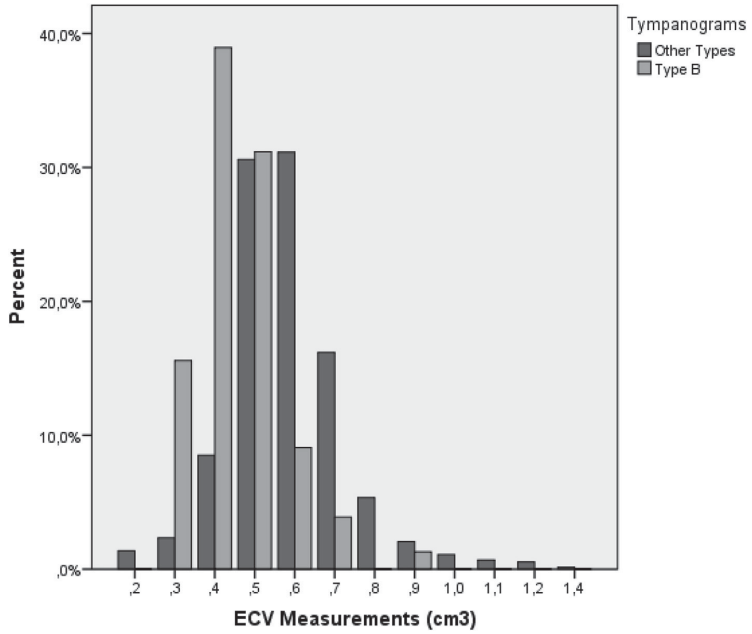

Fig. 2. Distribution of ear canal volume (ECV) measurements on tympanograms types.

interpretation of the data, the writing of the report, and the decision to submit the article for publication.

Declaration of interest

Conflict of interest: none.

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PUBLICATION IV

Impact of 13-valent pneumococcal conjugate vaccines on otitis media in 2 to 3 years old Cameroonian children.

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Impact of 13-valent pneumococcal conjugate vaccines on the prevalence of otitis media in 2 to 3 years old Cameroonian children

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Abstract

Objectives: We assessed the 13-valent pneumococcal conjugate vaccine (PCV13) impact on otitis media (OM) in Cameroon where infant vaccination was implemented in July 2011 using a 3+0-dose schedule at 6, 10 and 14 weeks of age.

Methods: We used a retrospective cohort design in which OM prevalence among PCV13-vaccinated children aged 24 to 36 months in 2015 was compared with an age-matched PCV13-unvaccinated cohort of 2013. OM was diagnosed by clinical inspection for chronic suppurative otitis media (CSOM) and tympanometry for otitis media with effusion (OME). CSOM was defined as draining of the middle ear lasting over two weeks and OME was defined as a flat 'type B' tympanogram.

Results: Altogether, 111 OM cases were identified; 42/433 (9.7%) in the PCV13-unvaccinated in 2013 and 69/413 (16.7%) in the PCV13-vaccinated cohort in 2015. In 2013, 3/433 (0.7%) children were diagnosed with unilateral CSOM and 7/433 (1.6%) with bilateral OME, compared to 12/413 (2.9%) and 9/413 (2.2%), respectively, in 2015.

Conclusion: OM prevalence in 2015 was higher among PCV13-vaccinated cohort compared with PCV13-unvaccinated in 2013, although this was not statistically significant. Confounding due to the predominance of other bacteria as causative pathogens in otitis or waning immunity in PCV13-vaccinated children or increase in non-vaccine replacement disease.

Key words: PCV13, Vaccinated children, Otitis media, Unvaccinated children, Vaccine effectiveness

1. Introduction

Middle ear infections are a predominant cause for healthcare visits in the early years of life [1]. Otitis media (OM) is a very common middle ear infection in children globally, contributing to excessive antibiotic consumption in most countries and to a substantial burden of deafness and suppurative complications in developing countries [2]. Peak incidence period of the disease is reported to occur between the ages of 6 and 11 months based on data mostly from high-income nations. Moreover, an estimated 70% of children aged under-five years worldwide do encounter at least one episode of OM before their fifth birthdays [3]. The most frequent bacterial pathogen associated with OM is *Streptococcus pneumoniae,* followed by non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* [4,5]. These three pathogens are reported to account for more than 95% of all OM cases with a bacterial aetiology, although viral infections may play a role in the pathophysiology [4,5]. The type of OM disease witnessed in developed countries differs widely to what pertains in developing countries [2]. The major differences seem to be the frequency of complications and sequelae such as hearing loss due to chronic suppurative otitis media (CSOM) or otitis media with effusion (OME) reported in developing countries, rather than the incidence of acute otitis media (AOM) frequently reported in developed countries [2].

The frequent occurrence of OM in children under-five years of age and the associated direct and indirect cost is a heavy toll to both parents and the healthcare providers [6]. Even when parents do not seek medical care for a child, they may still need to be absent from work to care for them [1]. In some countries, costs related to parental loss of productivity have been reported to represent a 50% average relative to the total costs of OM, depending on country, age, and number of previous episodes [6]. As such, the use of currently available and future vaccine formulations to prevent OM is considered the most promising approach to affect disease burden and consequences, both in developed and developing countries [2].

Prior to the availability of the PCV13 in 2010, studies using previous PCV formulations (PCV7 or the investigational prototype PCV11) involving over 54000 children under-five years old in randomized control trials (RCTs) conducted in the USA and Europe demonstrated modest to insignificant protection against AOM [7]. In 2001, a 6% vaccine efficacy was reported in the Finnish Otitis media (FinOM) trial using the PCV7 with endpoint on reducing the number of vaccine-type AOM episodes [8]. Another efficacy trial in northern California, USA (with the endpoints on preventing IPD and clinical episodes of AOM) had a 7.8% efficacy against AOM [9]. Both the Finnish and California studies demonstrated the PCV's potential role in reducing the bacterial causes of OM in immunized subjects, although the vaccine impact has been suggested to be overshadowed by an overall increase in non-PCV7 serotypes [8,10]. These findings were corroborated by reports from three other RCTs

(PCV7-OMPC, Native American and POET), which also estimated endpoints on AOM incidences [4,7,11]. However, AOM is described as a transit state of ear disease and its assessment is suggested not to sufficiently represent the actual OM burden in a developing country-perspective: defined by assessing OM sequelae including OME, recurrent, non-responsive, and chronic OM [7,12].

Further, a prospective-based active surveillance study in Israel reported dramatic reductions in endpoints on rates of pneumococcal and overall OM in children <2years old, resulting in near-elimination of PCV13 serotypes following sequential introduction of PCV7 and PCV13 [13]. Another observational study in the United Kingdom including an endpoint on overall OM incidence reported first a 22% significant reductions in OM in children aged <10 year-olds using the PCV7; and an additional 19% reduction after PCV13 introduction [14].

In Sub-Saharan Africa, where most of the OM disease burden and sequelae are reported [2], PCV effectiveness or impact studies on any OM endpoints are yet to be documented; as most studies have focused on the impact of the vaccines on pneumonia, carriage and IPD [15–19]. PCV's impact on nasopharyngeal colonization plays an important role against bacteria causing IPD and mucosal infections such as OM [20,21].

In July 2011, Cameroon introduced the 13-valent pneumococcal conjugate vaccine administered following the Expanded Programme on Immunization (EPI) 3-dose schedule to infants at 6, 10 and 14 weeks of age. Two studies on children aged from 2 to 3 years in Cameroon from the same population conducted in 2013 and 2015 respectively, on nasopharyngeal carriage prevalence demonstrated a slight increase in non-PCV13 type carriage and a remarkable increase in *Moraxella catarrhalis* and *Haemophilus influenzae* [22]. Additionally, the overall proportion of PCV13-serotypes in carriage was 18.0% for the PCV13-vaccinated group in 2015 and 21.2% for the PCV13-unvaccinated group in 2013 [22]. Also, earlier we conducted a study of baseline prevalence of OM in 2013 sampling 24 to 36 months old PCV13-unvaccinated children in which an OM disease prevalence of 9.7% was reported [23]. In this study, we investigated the impact of the PCV13 on OM as case-defined (CSOM and OME), by comparing the prevalence of OM in PCV13-vaccinated and PCV13-unvaccinated children.

2. Subjects and Methods

2.1 Study design

Two rounds of community-based surveillance surveys were conducted. First in the year 2013 to assess the prevalence of OM in PCV13-unvaccinated subjects and two years later, in 2015 PCV13-vaccinated subjects were sampled, using the same sampling methods [23].

2.2 Study population and study areas

We targeted children born between June 2010 and June 2011 (baseline data) and between June 2012 and June 2013 (comparison data). Cameroon has an annual birth cohort of approximately 856000 according to the 2016 report of The Vaccine Alliance – GAVI (www.gavi.org/country/cameroon). Selection of subjects was done systematically following the WHO cluster sampling method and guided by the inclusion and exclusion criteria. The study areas have been earlier described [23]. Briefly, they included localities situated within an 80 km radius from Yaoundé, Cameroon's capital city. Yaoundé and its surroundings harbour a population of over 3.5 million, out of which 18% are children aged under- five years, based on 2010 National Population Census. The sites were chosen as they constitute a group of health institutions involved with invasive disease sentinel surveillance. Sites are partitioned into 40 communities (clusters) using the health map and with each cluster hosting at least one health center/clinic, either public or private [23]. The starting household within the cluster was selected after spinning a pen, usually at a central location in the community. As previously reported during the 2013 baseline study, selection of participants was done randomly after every 10^{th} home within a cluster. One participant was selected per home even if two or more were eligible (in such an event, selection was with respect to birth order); and twenty-five children were enrolled per "cluster" [23]. Using the sample size calculator (http://www.raosoft.com/samplesize.html), we estimated to sample at least 384 participants during each study period.

2.4 Inclusion and exclusion criteria

Those included in the study were children aged from 24 to 36 months, residing in the study area for at least six months, availability of parental signed consent and subjects who were PCV-unvaccinated (for the baseline group in 2013). In 2013, enrolment was restricted to those who had not received any doses of the PCV as was confirmed from child's vaccination card or registers. Children had to have at least two documented doses of PCV13 in order to be eligible for inclusion in the comparison group in 2015.

2.5 Data Collection

The data collection process during the baseline study had earlier been reported [23] and in the 2015 study the procedure was similar. Briefly, inspection to detect draining ears in subjects was done first, followed by tympanometry. Clinical and visual examination involved a thorough inspection of the external ear structure for signs of drainage or cerumen accumulation in the outer third of the ear canal as previously recommended [23]. Using a Welch Allyn with Siegel's speculum, pneumatic otoscopy was done and many of the subjects had cerumen stacked in the middle ear, but we lacked sufficient material to clean earwax at field conditions during the baseline study [23]. Therefore, AOM evaluation could not be done. Tympanometry was performed using the middle ear analyser Grason Stadler

tympanometer (GSI-38 Autotymp, Grason-Stadler Inc., Milford, NH, USA). Tympanograms were recorded with a 226Hz probe tone with a pressure varying from +200 deca Pascals (daPa) to -400 daPa in a time of 7 seconds. We did not perform tympanometry on draining ears. As earlier reported, study-specific case report forms were used for parental interviews to obtain socio-demographic and clinical history of study subjects [23].

2.6 OM case definitions

In this study and using the same criteria as previously defined in the 2013 baseline cohort [23], ears observed by clinical inspection with draining and from parental reporting to have lasted more than two weeks were considered as CSOM based on the WHO's criteria [24]. We defined OME as 'type B' tympanogram with no peak as observed in tympanometry. Dry tympanic membrane perforation was denoted when the ear canal volume (ECV) was > 1.0 cm^3, in an event of a flat curve. For each subject, one of the following mutually exclusive categories was assigned: CSOM, dry perforation, bilateral OME, unilateral OME and healthy ears. We considered the first four categories to have OM or its complications [23].

2.7 Interpretation and Classification of tympanograms

The tympanograms were independently interpreted by two researchers in retrospect, as was previously reported [23]. In an event of discordance in the interpretation, a third researcher interpreted for a final decision. The tympanograms (Table 1) were categorized following a modified version of Liden & Jerger's classification [25], in which flat 'type B' tympanograms indicated the presence of middle ear fluid (MEF). Tympanograms with curve types A, As, C, or Cs suggested absence of MEF. High external ear canal volume (ECV>1.0cm^3) with a 'flat curve' was interpreted as perforation of the tympanic membrane (TMP) i.e., type P tympanogram. Curves with erroneous peaks due to artefacts or movements of the child and curves with ECV below 0.3cm^3 without any recording of a normal curve were interpreted as failed or 'type F' tympanogram [23].

2.8 Methodological approach

As previously reported [23], many children had occluded ear canals due to earwax and therefore, produced a false flat type B tympanogram curve with low ear canal volume (ECV). We resolved this by examining the distributions of the ECV measurements by tympanogram type, and observed that higher proportion of the initial 'type B' tympanograms had low ECV values i.e., 0.3cm^3 and 0.4cm^3 in

comparison to other tympanogram types (Figure 2b). We interpreted this difference to be probably due to earwax accumulation resulting in flat tympanograms. Based on the distribution of the ECV values in 'other type' tympanograms with discernible curves (A, As, C and Cs), it was evident that 90% of these were in the ECV categories of between 0.5cm^3 to 1.2cm^3, and 10% were distributed between the ECV categories <0.5cm^3. Therefore, we adjusted the number of original 'type B' tympanograms with ECV values below 0.5cm^3 to follow the same ECV distribution for the aforementioned categories [23]. It was in this respect that the adjustment was done i.e., of all flat curves, only 10% with lower ECV values were true positive "type B". The same approach was used for the 2015 (PCV13-vaccinated) group (Figure 2a).

2.9 Statistical analyses

The prevalence of OM in PCV13-vaccinated children in 2015 who had received at least two doses of PCV13 was compared with that of children with no PCV13 vaccination in 2013. Additionally, we explored associations between OM prevalence and potential risk factors. The Chi-square test was used to compare differences in OM prevalence of baseline characteristics between PCV13-vaccinated and PCV13-unvaccinated groups (Table 2). In the multivariate logistic regression analyses (Table 3), variables with p-values ≤0.05 were entered to obtain the adjusted prevalence odds ratios (PORs). We computed PORs and prevalence difference (PD) with their 95% CI at a statistical significance level of 5%. PD was estimated by subtracting the prevalence in the PCV13-unvaccinated group from that in the PCV13-vaccinated group. Statistical analyses were performed using the International Business Machines (IBM) corporation's Statistical Package for Social Sciences (SPSS) version 25.0.

3. Results

3.1 Study population

A total of 846 children aged from 24 to 36 months were included in the analysis, consisting of 433 children in 2013 baseline (PCV13-unvaccinated) and 413 children in 2015 (PCV13-vaccinated) groups. Apart from those who refused to participate, the remainder were excluded either for not having a tympanogram or outside the age range of 24 to 36 months or due to lack of a signed parental consent (Figure 1). All children in PCV13-vaccinated group had received at least two doses of the vaccine and vaccine coverage was >88% in the year 2015 for all PCV13 doses received [26]. There were 111 cases of OM in total (Figure 1).

3.2 Point estimates for OM prevalence in PCV13-unvaccinated and PCV13-vaccinated groups

A diagnosis for OM or its complications (excluding AOM) was obtained for 42/433 (9.7%) of PCV13-unvaccinated children compared with 69/413 (16.7%) in PCV13-vaccinated children (PD = 7% [95%CI: 2.5 to 11.6], p=0.003. This included 3/433 (0.7%) children identified with unilateral CSOM in the baseline survey in 2013, compared with 9/413 (2.2%) in subjects with CSOM in the PCV13-vaccinated group in 2015 (PD 1.5% [95%CI: -0.2 to 3.5], p= 0.067). Bilateral OME was diagnosed in 7/433 (1.6%) PCV13-unvaccinated children and 12/413 (2.9%) of PCV13-vaccinated children (PD =1.3% [95%CI: -0.8 to 3.6%], p=0.2013). Proportions of children with unilateral OME were 31/433 (7.2%) amongst the PCV13-unvaccinated group compared with 48/413 (11.6%) in the PCV13-vaccinated group (PD = 4.4% [95%CI: 0.5 to 8.4], p=0.028). There was no significant difference in the proportions of subjects with unilateral dry tympanic membrane perforation (ECV >1.0 cm^3) between the two groups, 0.2% and 0%, respectively (PD = 0.2% [95%CI: -0.7 to 1.2], p=0.365).

3.3 Multivariate analyses

The crude estimates from logistic regression analyses showed that PCV13-vaccinated children were significantly associated with more OM (POR = 1.76[95%CI: 1.12 to 2.68], p = 0.013) compared to PCV13-unvaccinated children (Table 3). However, in the multivariate analyses adjusting for significant baseline risk factors in Table 2, we observed that compared with the PCV13-unvaccinated cohort, PCV13 vaccination was associated with more OM, but this was not statistically significant (adjusted POR = 1.50 [95%CI: 0.84 to 2.67], p = 0.171). Additionally, non-significant results were found for the other risk factors assessed (Table 3).

4.0 Discussion

Our surveillance found the prevalence of OM was higher in children who received PCV13 vaccination in infancy compared with those who did not receive PCV13 vaccination, but this was not statistically significant. In the PCV13-vaccinated group, there were seven more cases of OM infections per 100 children than in the PCV13-unvaccinated group within the study period.

Our findings are compatible with those from an Australian community-based cross-sectional study of indigenous population of children under the age of 36 months, which used a similar categorisation of middle ear statuses as we did (including healthy ears, OM without perforation, OM with perforation, dry perforation and CSOM). Subjects vaccinated with the 10-valent pneumococcal *Haemophilus influenzae* Protein-D conjugate vaccine (PHiD-CV10) had less CSOM than those vaccinated with

PCV7. However, there was a simultaneous increase in asymptomatic OME in these subjects, so the overall risk of OM was similar between the two groups [27].

Differences in baseline epidemiology and disease incidence, study design, case definitions, cases ascertainment, and local practices make it difficult to compare the results of clinical trials/observational studies of PCV efficacy/effectiveness or impact analyses [7–10,28–32]. Others have primarily evaluated vaccine effectiveness on the endpoint of AOM incidence, whereas we assessed PCV13 impact on the prevalence of CSOM, OME and dry perforation. As a result, such discrepancies may limit our ability to extensively compare our findings to previous research.

Our findings could be explained by a variety of factors. First, differences in the patho-physiology of AOM, compared to that of IPD, such as bacteraemia and meningitis, may be a contributing factor [33]. The polymicrobial nature of AOM disease may play a role, as it is believed that even if high efficacy could be obtained, protection against pneumococcal AOM alone would have only a limited impact on the overall burden of OM disease [10]. This signifies that PCV may offer only limited protection against OME/CSOM. It is unclear whether the infants were exposed to early AOM episodes prior to vaccination, giving that children in low-resource settings are more exposed than those in more affluent communities to experience bacterial nasopharyngeal colonization shortly after birth [33]. In addition, nasopharyngeal carriage not only provides an ecological niche for *S. pneumoniae,* but also for other pathogens that can cause middle ear infection [34]. PCV vaccination prior to this chain of patho-physiological events may play a significant role in preventing recurrent and complicated infections in otitis-prone children [31,33]. But, once this chain of events has commenced, PCV vaccination is thought to have little or no effect [31,33]. Other studies, however, have found no correlation between early vaccine-type AOM and an increased risk of subsequent AOM when compared to AOM caused by other confirmed bacterial aetiologies [35]. This supports the hypothesis that the higher OME prevalence among PCV13-vaccinated subjects in our study may have resulted from other bacterial aetiology or the replacement of PCV13 serotypes by non-PCV13-type pneumococci, when compared to the PCV13-unvaccinated. Hence, it is likely that the predominance of non-vaccine type pneumococci and/or other pathogens are primarily responsible for the unexpected findings in our study.

Furthermore, the lack of indirect effects or vaccine waning effects in children as they get older may be consistent with our findings, especially since the PCV13 is being rolled-out using the 3+0 EPI dose-schedule with no booster [36]. The possible vaccine waning effects after the first year of life [36], and an increasing OM prevalence among the PCV13-vaccinated supports recommendation that a booster dose be introduced to counter observations of waning immunological effects over time [37]. This could be achieved by shifting to a 2 +1 schedule in which the third dose is given at 9- to 12-months of age may be more effective [37]. However, a number of published studies from other countries have shown

that protection against OM has been lower compared to IPD and pneumonia; and PCVs effectiveness against severe pneumococcal diseases and hospitalizations remained remarkable [15,38–40]. Real-life studies have demonstrated that vaccinations with PCVs have shown more than 30% effectiveness in preventing AOM episodes in children [13,14], which surpasses the 6 -7% obtained from clinical trials [7,8,11]. The implications of these differences in effectiveness suggest that Cameroon should consider introducing a booster shot, as its PCV13 programme implementation follows that of an accelerated primary vaccination schedule being administered at early infancy [41], with no booster.

To the best of our knowledge, this is Africa's first study to assess the impact of PCV13 on the prevalence of OM. However, we are aware of some constraints. As previously stated, AOM findings obtained using pneumatic otoscopy were not reported here because they were not evaluated in the same way in both groups. AOM is a serious problem worldwide [42], but in a low-resource setting like Cameroon, it often goes unnoticed in affected children [2]. Hence, we needed to define endpoints in our assessment that were realistic and reflective of local practices. More so, concerns from misclassification bias could not be ruled out i.e., the possibility of misinterpreting a 'type F' or 'type P' tympanogram for a true OME (type B), as they all produced graphically a "flat curve" as previously indicated [23]. In addition, because we did not examine the microbiology of OM disease causing serotypes, direct evidence of disease replacement of vaccine types by non-vaccine type pneumococci or other bacteria could not be determined. But, a study on nasopharyngeal carriage prevalence in same population found a slight increase in non-PCV13 type carriage as well as a significant increase in *Moraxella catarrhalis* and *Haemophilus influenzae* [22].

In developing countries, studies evaluating the impact of PCV13 infant vaccination on invasive pneumococcal diseases, pneumonia and nasopharyngeal carriage endpoints yielded vaccine impact estimates comparable to those obtained from high-income countries [17,18,43–45]. This study estimated the impact of the PCV13 programme on CSOM, OME and dry perforation which had not previously been measured in Africa. Despite being unexpected, our impact estimates showed that subjects who received PCV13 vaccination had 7 additional cases of OM infection per 100 children, compared to those who did not receive PCV13 vaccination. These findings should contribute to the possibility that PCV programmes may have limited ability to sustain appropriate immunogenic response against otitis in children who were vaccinated in infancy and/or the development of indirect effects was slow to interrupt the transmission of vaccine-type carriage [35].

In conclusion, the prevalence of OM in 2015 was higher in PCV13-vaccinated children compared with PCV13-unvaccinated children in 2013, although this was not statistically significant. Confounding due to unknown factors or the replacement with non-PCV13 serotypes or other bacteria may rapidly outweigh a positive effect. Another possibility could be waning immunity in PCV13-vaccinated

children with a 3+0 schedule, despite a reported vaccine uptake of >85% over time. More research is needed to assess these findings and potential causes, as well as to monitor the long-term impact of PCV13 programme implementation on the epidemiology of pneumococcal diseases, including otitis media in African settings.

5. Authors' contributions

Conceived, planned, and designed the study: JNL and AAP; participated and supervised local data collection: JNL, TNA, AVN, EE, YB, PKN, LEE, MK, and SKS; contributed logistics: AAP, MK, SKS and PKN; drafted the manuscript: JNL; significantly contributed to the revised and final versions of the manuscript: JNL, MK, RKS, TNA, AVN, EE, YB, LEE, PKN, SKS, HN, HH, JPN and AAP.

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7. Ethical considerations

The Institutional Review Boards (IRBs) of the Cameroon National Ethics Committee and the Yaoundé Gynaecology, Obstetric and Paediatric Hospital, approved the study. Signed informed consent forms were obtained from all parents. Additional permission for the study was obtained from local and administrative leaders.

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9. Conflict of interest statement

AAP, JNL, RKS and HN are employed by the National Institute for Health and Welfare (THL), Finland which has received research funding from GlaxoSmithKline and Pfizer, Inc.

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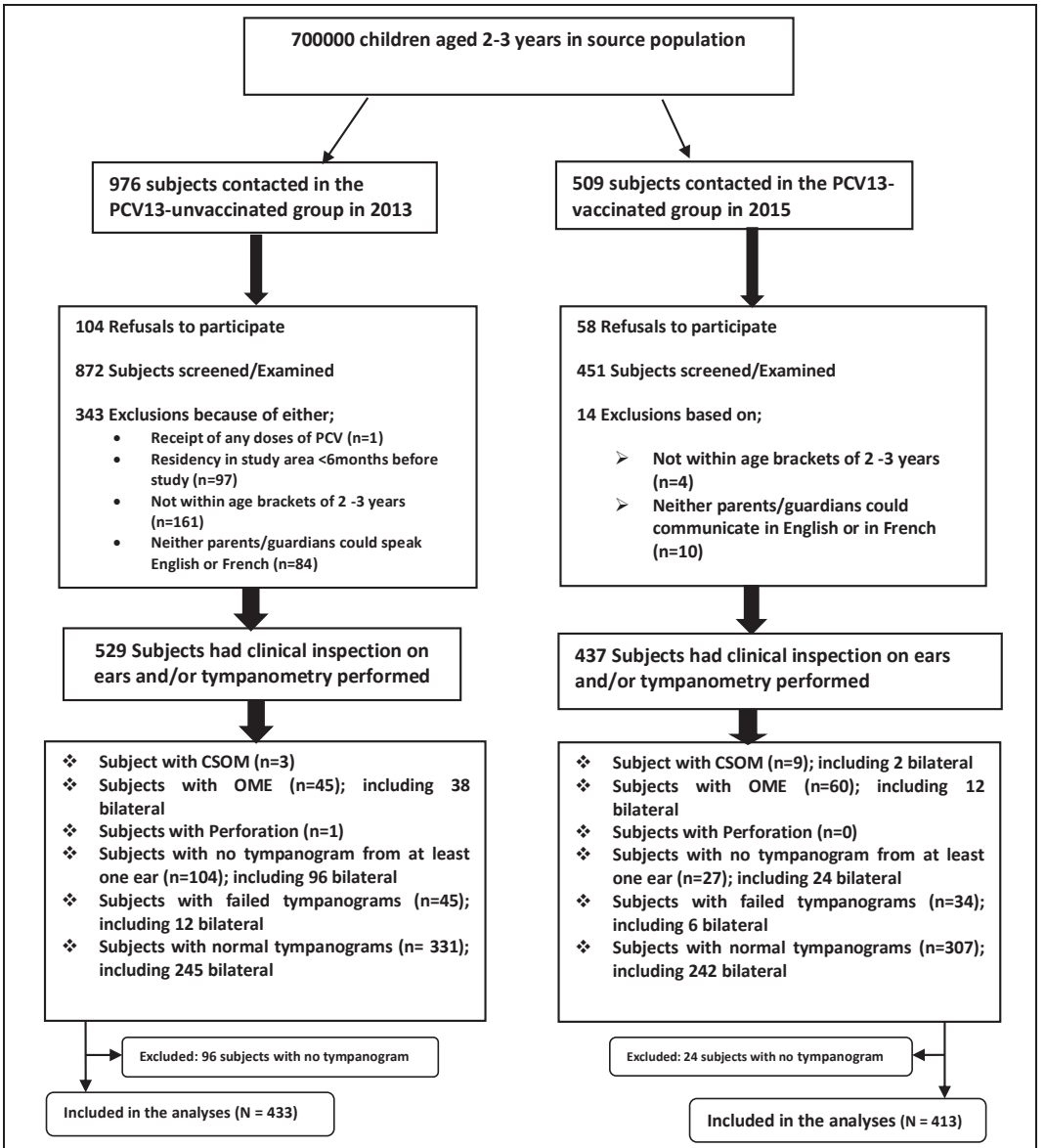


Figure 1. Study profile of subjects on the assessment of PCV13 effect on otitis media

CSOM = Chronic suppurative otitis media; OME = Otitis media with effusion; n= number of subjects with specific outcomes; PCV13 = 13-valent pneumococcal conjugate vaccine.

Figure 2a. Distribution of ear canal volume (ECV) measurements on tympanogram types among PCV13-vaccinated (2015 cohort) 2 to 3 years old children in Yaounde, Cameroon

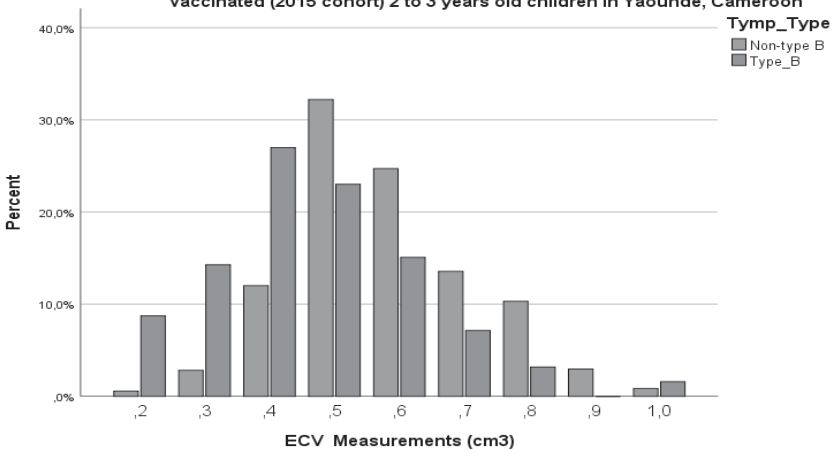
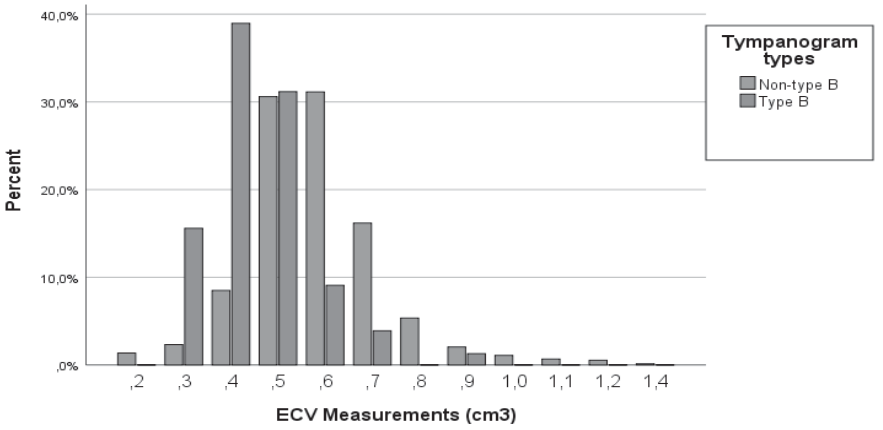


Figure 2b. Distribution of ear canal volume (ECV) measurements on tympanogram types among PCV13-unvaccinated (baseline in 2013) 2 to 3 years old children in Yaounde, Cameroon.



8.0 TABLES

Table 1: Classification criteria used for reporting tympanograms in this study as adapted from Liden/Jerger[25]

Curve Type	Criteria	Clinical Presentation
A	TPP ≥ -100daPa, SAA ≥ 0.2 cm^3	Normal middle ear pressure (MEP), normal static admittance and no MEF
B	Flat curve; ECV = 0.3 to 1.0; no values for TPP	Consistent with Middle ear pathology (MEF)
C	TPP < -100daPa, SAA ≥ 0.2 cm^3	Significant negative MEP, normal static admittance, no MEF
As	TPP≥ -100daPa, SAA ≤ 0.2 cm^3	Reduced admittance, Normal MEP, no MEF
Cs	TPP< -100daPa, SAA ≤ 0.2 cm^3	Reduced admittance, decreased MEP, no MEF
F	Erroneous peaks (no distinct curves) or ECV < 0.3 in the absence of a distinct curve	Failed tympanogram, child unstable in process or probe in contact with ear canal or ear wax
P	No Peak (or flat curve); ECV>1.0	Tympanic Membrane perforated

SAA= Static acoustic admittance; TPP= Tympanometry peak pressure; daPa= deca-pascals; MEF=Middle ear fluid**; ECV =** Ear can volume. The difference between A and As (and C & Cs) at SAA=0.2cm3 was dependent on the graphical display of the curve. When the curve exceeded the lower limit of the graphic normal box, it was described as A (or C, depending on the TPP); A, As, C, Cs = Healthy ears; B= Diseased ear, F=Failed tympanogram; P=Perforation. Curves type B, P and F all generate flat curves but could be distinguished from each other based on the measure of the ear canal volume.

Table 2. Baseline characteristics and clinical outcome in PCV13-vaccinated (N = 413) and PCV13-unvaccinated (N=433) 2 to 3 years old children screened for otitis media (OM) in Yaoundé, Cameroon

Characteristics/ Clinical outcome	PCV13-vaccinated		PCV13-unvaccinated		♫P-value
	N	%	N	%	
Gender of child					0.207
Male	212	51.3	241	55.7	
Female	201	48.7	192	44.3	
Age (group) of child in months					**< 0.001**
24 to 29	154	37.3	157	36.3	
30 to 35	44	10.7	110	25.4	
36	215	52.0	166	38.3	
No. of siblings living in same home ≤ 18 years					0.016
One	45	10.9	77	17.8	
Two	99	24.0	100	23.1	
≥ Three	269	65.1	256	59.1	
No. of siblings sleeping in same bedroom ≤ 18 years					**< 0.001**
Alone	37	9.1	172	39.7	
One	65	15.7	143	33.0	
Two	140	33.9	84	19.4	
≥ Three	171	41.3	34	7.9	
Otitis Media Status					**0.003**
Healthy	344	83.3	391	90.3	
Otitis positive	69	16.7	42	9.7	
History of previous otitis media					**< 0.001**
No	392	90.6	392	90.5	
Yes	21	9.4	41	9.5	
Breastfeeding period					**0.012**
≤ 6months or not breastfed	21	5.2	38	8.9	
≤ 12 months	145	36.1	179	41.7	
> 12 months	236	58.7	212	49.4	
Antibiotic use when child is sick					**0.001**
No	185	46.0	149	34.7	
Yes: with /without medical report	217	54.0	280	65.3	
Noticed any current upper respiratory tract symptoms					**< 0.001**
No	365	90.8	341	79.5	
Yes	32	8.0	48	11.2	
Unknown	05	1.2	40	9.3	
Parental educational level (SES1)	183	45.5	147	34.3	**0.001**
≤ Primary school	179	44.5	211	49.2	
≥ Secondary school	40	10.0	71	16.6	
≥ University Education					
Parental smoking status					0.588
Non-smokers	374	86.4	358	86.7	
Smokers	59	13.6	55	13.3	
Using wood/cool as household cooking fuel					**< 0.001**
No	98	24.7	128	29.6	
Yes	311	75.3	305	70.4	

PCV13 = 13-valent pneumococcal conjugate vaccines; N=number; ♫ = In bold denotes p-values less than 0.05; SES = Socio-economic status; % = percent

Table 3. **Risk factors for otitis media (OM) in PCV13-vaccinated (N = 402*) and PCV13-unvaccinated (N=429*) in children aged 2 to 3 years in Yaoundé, Cameroon**

Characteristics/Clinical outcome	OM Prevalence N (%)	Univariate analyses			Multivariate analyses♣		
		POR	95%CI	P-value	aPOR	95%CI	P-value
Vaccine cohort							
PCV13-Unvaccinated (2013)	38/429 (8.7)	1.0			1.0		
PCV13-Vaccinated (2015)	56/402 (14.4)	1.76	1.124 - 2.677	0.013	1.50	0.84 - 2.67	0.171
Age (group) of child in months							
24 to 29	39/307 (12.7)	1.0	0.543 - 1.295	0.427			
30 to 36	57/524 (10.9)	0.43					
No. of persons living in same household ≤18 years							
One	16/126 (12.7)	1.0		0.945			
Two	24/193 (12.4)	0.98	0.496 - 1.921	0.576			
≥ Three	56/512 (10.9)	0.84	0.466 - 1.528				
No. of siblings sleeping in same bedroom							
Alone	19/206 (9.2)	1.0					
One	23/205 (11.2)	1.24	0.655 - 2.361	0.505			
Two	27/218 (12.4)	1.39	0.748 - 2.588	0.297			
≥ Three	27/202 (13.4)	1.52	0.815 - 2.829	0.188			
Previous otitis media history							
No	74/710 (10.4)	1.0			1.0		
Yes	22/121 (18.2)	1.91	1.134 - 3.216	0.015	1.43	0.66 - 3.09	0.368
Breastfeeding period							
≤ 6months or not breastfed	5/56 (8.9)	1.0					
≤12 months	39/327 (11.9)	1.38	0.520 - 3.671	0.517			
>12 months	52/448 (11.6)	1.34	0.511 - 3.508	0.552			
Antibiotic use when child is sick							
No	13/172 (7.6)	1.0					
Yes: with / without medical report	83/659 (12.6)	1.76	0.957 - 3.245	0.069			
Noticed any current symptoms of URTI							
No	76/706 (10.8)	1.0			1.0		
Yes	19/79 (24.1)	2.63	1.487 - 4.633	0.001	2.15	0.95 - 4.90	0.067
Unknown	1/45 (2.2)	0.19	0.026 - 1.387	0.101	0.22	0.03 - 1.66	0.143
Parental educational level (SES)							
≤ Primary education	39/327 (11.9)	1.0					
≥ Secondary education	47/393 (12.0)	1.01	0.638 - 1.577	0.989			
≥ Tertiary Education	10/111 (9.0)	0.73	0.352 - 1.518	0.401			

URTI = Upper respiratory tract infection; PCV13= 13-valent pneumococcal conjugate vaccine; N= number; %= percentage; CI= confidence interval; ♣= OR was adjusted for symptoms of URTI and previous OM history; POR= Prevalence odds ratio; aPOR= Adjusted POR; *=subjects with ECV values < 0.5 cm3 with tympanograms type B initially adjusted for were excluded in logistic regression analyses (The observed differences in the ECV values suggested that many occluded ears were initially misinterpreted as 'type B'. We corrected this by converting the majority of these 'type B' to 'type F' tympanograms based on the ECV distribution in normal tympanograms)