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Impact of Pneumococcal Conjugate Vaccine on Pneumococcal Disease, Carriage and Serotype Distribution
Comparative studies in Sweden and Uganda

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By

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ABSTRACT

Background *Streptococcus pneumoniae* is a leading infectious cause of child deaths worldwide. Pneumococcal conjugate vaccine (PCV) was first introduced in the US in the year 2000, and included the major seven pneumococcal serotypes (PCV7) causing invasive pneumococcal disease (IPD) there. Current PCVs include 10 or 13 of the more than 97 known pneumococcal serotypes. In Stockholm County, Sweden, PCV7 was introduced for infants born from July 2007, at 3, 5, and 12 months of age and in 2010 it was changed to PCV13. Uganda started national PCV10 implementation in 2014.

Aims To study the effects of the introduction of PCV in the childhood vaccination program in Stockholm on incidence, serotypes and antibiotic resistance patterns of IPD, hospitalization due to severe sinusitis and pneumonia in children, and pneumococcal carriage. Also, to study pneumococcal carriage and serotype distribution in healthy children <5 years prior to PCV introduction in Uganda, and estimate the potential effectiveness of PCV.

Methods All cases of IPD in Stockholm registered in the national mandatory reporting system from 2005 to 2014 were included (n=2519). The pneumococcal isolates were characterized with serotyping (n=2336), including some with molecular typing and antibiotic resistance pattern. All hospitalizations from 2003 to 2012 in Stockholm, ICD-10 coded as sinusitis or pneumonia (N=678, 5051, respectively) in children, were collected from hospital registries. Nasopharyngeal pneumococcal isolates from children <5 years in Stockholm were collected at regular visits to Child Health Centers from 4 to 8 years after PCV introduction from 2011 to 2015 (N=916). Pneumococcal carriage was compared to carriage data in children attending day-care centers in 2004 (N=246), which was before vaccine introduction. OR for invasive disease potential of the pneumococcal isolates in carriage was calculated using data on IPD in all ages from 2011 to 2015. Nasopharyngeal carriage of pneumococci in children <5 in Uganda was assessed through collecting isolates at the Health and Demographic Surveillance Site in Iganga/Maygue districts (N=1761).

Results We show that PCV introduction in Stockholm has been successful in decreasing the incidence of IPD, from 28.4 to 10.3 cases /100,000 children <2 years (RR 0.36, 95% CI 0.2-0.6) when comparing the time periods 2005-2007 to 2009-2014, Serotypes included in the PCV7 decreased from 22.7 to 0 cases/ 100,000 in this age group (RR 0.0, 95% CI 0.0-0.1). The IPD incidence also decreased in older children and adults, excluding the elderly. However, PCV7 serotypes have decreased in all age groups. There was a decrease in hospitalizations due to severe sinusitis (RR 0.34, 95% CI 0.2-0.5) and pneumonia (RR 0.81, 95% CI 0.7-0.9) in children <2 years. A near elimination of most vaccine serotypes with a high invasiveness potential was seen in carriage. Emerging both in carriage among children and as cause of IPD (all ages) were instead non-vaccine types of lower invasive potential. Carriage data before PCV introduction in Uganda shows that vaccine serotypes were much less prevalent in children <5 years old (PCV10 for 42% and PCV13 for 54%) than what was observed in children <5 years old in Sweden before the PCV implementation (PCV10 63%, PCV13 82%), which may reduce potential vaccine effectiveness in Uganda.

Conclusions PCV introduction in Stockholm has had a positive overall impact on pneumococcal morbidity in young children, and serotypes included in the vaccine are decreasing in IPD and carriage. PCVs have the potential to save many children's lives in the coming years, both in Sweden and Uganda. The extent of the impact is still not known, as PCV effectiveness depends on factors such as pneumococcal serotype distribution in carriage before and after PCV implementation, the extent of serotype replacement in carriage as well as in IPD in different age groups following PCV, vaccination coverage, and the serotype content of future pneumococcal vaccines, which may cover more or all pathogenic serotypes.

LIST OF SCIENTIFIC PAPERS

I.   Galanis I *, **Lindstrand A***, Darenberg J *, Browall S, Nannapaneni P, Sjöström K, Morfeldt E, Wilson J, Naucler P, Blennow M, Örtqvist Å*, Henriques-Normark B*. Effect of PCV7 and PCV13 on Pneumococcal Invasive Disease and Carriage in Stockholm, Sweden. European Respiratory Journal. January 21, 2016 as doi: 10.1183/13993003.01451-2015*equal contribution

II. **Lindstrand A**, Bennet R, Galanis I, Blennow M, Schollin Ask L, Hultman Dennison S, Ryd Rinder M, Eriksson M, Henriques-Normark B, Örtqvist Å*, Alfvén T*. *equal contribution. Sinusitis and Pneumonia Hospitalization after Introduction of Pneumococcal Conjugate Vaccine. Pediatrics. 2014;134(6):1-9

III. **Lindstrand A,** Galanis I, Darenberg J, Morfeldt E, Alfvén T, Blennow M, Naucler P, Henriques Normark B*, Örtqvist Å*. *equal contribution. Unaltered Pneumococcal Carriage Prevalence Due to Expansion of Non-vaccine Serotypes of Low Invasive Potential Eight Years after Pneumococcal Conjugate Vaccine Introduction in Stockholm, Sweden. Submitted

IV. **Lindstrand A**, Kalyango J, Alfvén T, Darenberg J, Bwanga F, Kadobera D, Peterson S, Henriques Normark B, Källander K. Pneumococcal Carriage in Children Under Five Years in Uganda - Will Present Pneumococcal Conjugate Vaccines Be Appropriate? Submitted

CONTENT

LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices (US)
AOM	Acute Otitis Media
CI	Confidence Interval
DALY	Disability Adjusted Life Years
HDSS	Health and Demographic Surveillance Site
DTP 3	Three doses of vaccine against Diphtheria, Tetanus and Pertussis
DTwP-Hib-HepB	Diphtheria-Tetanus-whole cell Pertussis-*Haemophilus influenza* type b and Hepatitis B combination vaccine
DTaP-polio-Hib-HepB	Diphtheria-Tetanus-acellular Pertussis-polio-*Haemophilus influenza* type b and Hepatitis B combination vaccine
EPI	Expanded Program on Immunization
GAVI	Global Alliance for Vaccine Initiative
GVAP	Global Vaccine Action Plan
IRR	Incidence Rate Ratio
NT	Non-typeable (NT) is a serotype that is not possible to define as a certain serotype in the methods used.
NVT	Non-vaccine type (NVT) is a pneumococcal serotype which is not included in a specified pneumococcal vaccine.
MDG	Millennium Development Goals
IPD	Invasive Pneumococcal Disease
OR	Odds Ratio
PCV	Pneumococcal Conjugate Vaccine
PPV	Polysaccharide Pneumococcal Vaccine
RSV	Respiratory Syncytial Virus
SAGE	Strategic Advisory Group of Experts (WHO)
SDG	Sustainable Development Goals
UN	United Nations
VPDI	Vaccine Preventable Disease Incidence
VT	Vaccine-type (VT) is a pneumococcal serotype which is included in a specified pneumococcal vaccine
WHO	World Health Organization

DEFINITIONS

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Carriage prevalence	Proportion of a population with nasopharyngeal pneumococcal carriage.
Invasive pneumococcal disease (IPD)	IPD is defined as disease with growth of pneumococcal isolates from a sterile location; either blood or cerebrospinal fluid (CSF) or bone, or any other sterile location in the body.
Incidence rate (IR)	IR is the occurrence of a disease event, being a case or hospitalization, per 100,000 person-years of observation.
Incidence rate ratio (IRR)	IRR is the ratio of incidence rates.
Invasive disease potential	Invasive disease potential is measured as the odds ratio of the odds of a certain serotype causing invasive disease in a population, to the odds of the same serotype found in carriage during the same time and place.
Lower respiratory infection	Any infection in the respiratory tract below the larynx; for example pneumonia or bronchitis
Odds ratio (OR)	OR is the ratio between two odds, for example the odds of exposure among the cases to the odds of the exposure among the controls. Odds is the likelihood of an event occurring to the event of it not occurring.
Pneumonia	WHO definition: cough and/or difficult breathing, and fast breathing (with or without fever)
Pneumococcal carriage	Pneumococcal nasopharyngeal carriage is defined as growth of pneumococcal isolates from the nasopharyngeal cavity.
Pneumococcal disease	Pneumococcal disease is disease caused by pneumococci. It may be invasive or not. Main diseases are pneumonia, septicemia, meningitis, sinusitis, and acute otitis media.
Serotype distribution	Serotype distribution is the proportion of different serotypes, or the proportion of vaccine-types to non-vaccine types, in a given time period.
Vaccine effectiveness	Vaccine effectiveness is the ability of a vaccine to reduce the incidence of an outcome of interest in the "real world." Depends on host factors, vaccine characteristics and match to circulation strains etc.
Vaccine efficacy (VE)	VE is the % reduction of incidence of a disease in a vaccinated group compared to an un-vaccinated group, under optimal conditions (RCT).

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Child survival

Global child survival has been improved tremendously in the last decades. WHO estimates that 12.7 million children died before their 5th birthday in the world in 1990, while in 2013 it was estimated to be 5.9 million children (1). This is a decrease from 35,000 deaths per day to 16,000, however, still an unacceptably high number. Lower respiratory disease, diarrhea, neonatal disorders and malaria are the most common causes of death in children (figure 1). Every minute six children die from pneumonia and diarrhea alone. The most important pathogens causing these child deaths are pneumococci for pneumonia and rotavirus for diarrheal disease (2). Lower respiratory infection was the main cause of life years lost in children less than five years old in 1990 and still in 2013. In all ages, it was the second largest cause of life years lost, after ischemic heart disease, in 2013 (2, 3). Measured instead in DALYs lost (disability adjusted life years), summing the burden of both mortality and morbidity in one measure, lower respiratory infection was the major cause of DALYs lost in 1990 for all ages, but was in 2013 in third place after ischemic and cerebrovascular disease (4). In 2013, an estimated 2.7 million people (2.4-2.8) died from lower respiratory disease, and 900,000 of those were children less than 5 years of age (5, 6). Although viruses are the most common etiology of pneumonia among children, RSV 29% and influenza 17% of all episodes (7), a third of deaths due to pneumonia are caused by *Streptococcus pneumoniae* (7, 8).

Pneumococcal conjugate vaccines (PCVs), with a high potential to decrease global child mortality, are currently being rolled out in national vaccination programs in many low- and middle income countries. Pneumococcal disease is actually the vaccine preventable disease with the highest potential of decreasing child mortality, since measles mortality already has decreased by 75% in the last decades due to effective vaccines.

Figure 1. Causes of death globally in children under five years of age in 2013 (2)
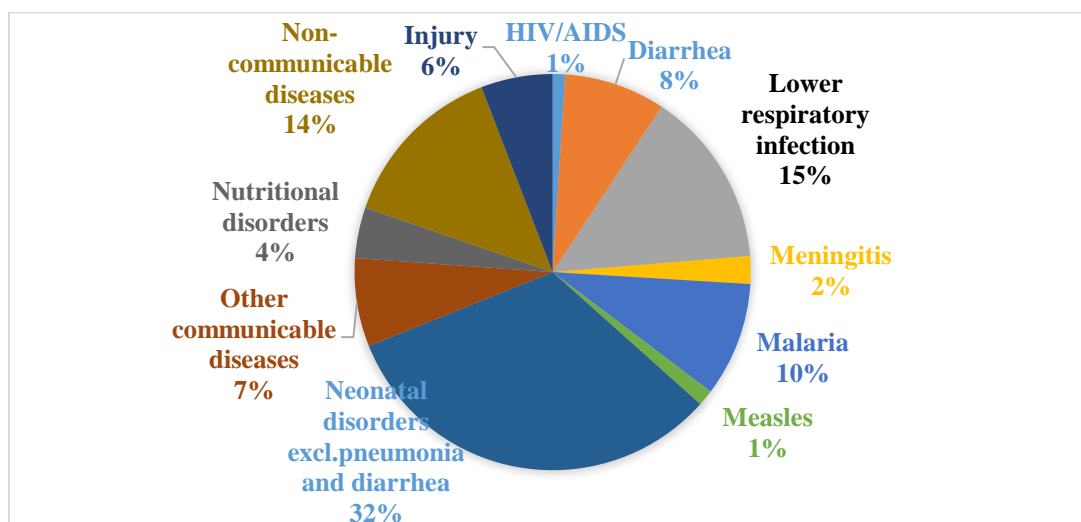
1.1.2 Pneumococcal disease burden

Pneumococcal disease causes a range of illnesses, from severe invasive pneumococcal disease (IPD) such as meningitis, septicemia and invasive pneumonia to less severe respiratory mucosal infections such as otitis media and sinusitis (9). Pneumonia may occur either with or without bacteremia (10)(table 1).

About 11.9 million episodes of severe pneumonia, and 3.0 million very severe pneumonia episodes occurred globally in children younger than 5 years in 2010 (11). An estimated 18.3% of severe pneumonia cases are associated with *Streptococcus pneumoniae* (8). WHO estimated that 541,000 (95% CI 376,000-594,000) deaths in children less than 5 years were due to pneumococcal disease in 2008 (12). Out of these deaths, more than 50% occurred in Africa: 247,000 (95% CI 167,000-274,000), and 6,800 (95% CI 5,000-7,800) in Europe. Incidence and mortality due to pneumococcal disease is higher in low-income countries, and most deaths occur within Africa and Asia (8, 11, 13, 14).

In Europe, the incidence of IPD for all ages varied between 20-174/100,000, with a mean annual incidence of 44/100,000 before the introduction of pneumococcal conjugate vaccine (15, 16). In the US and Australia, the IPD incidence pre-PCV was 28-214/100,000 (17). In Africa, IPD incidence varied between 60-797/100,000 (18-20) (table1).

Children under five years of age and the elderly above 65 years of age are the age groups at highest risk for severe pneumococcal disease (16, 21). A recent systematic review of IPD in neonates estimated the incidence to 16/100,000 in high income countries as compared to 370/100,000 in a study from a low income countries (22).

People who are HIV positive are at a higher risk of morbidity and mortality due to pneumonia and to invasive pneumococcal disease, except if well-treated with anti-retroviral treatment (2, 23). In HIV positive children under five, 64,900 (95% CI 44 500-72 800) deaths occurred, almost exclusively in Africa (12). Other risk groups are malnourished children and children with low birth weight (24).

Case fatality rate in infants in low-income countries may reach 20% for pneumococcal septicemia and 50% for pneumococcal meningitis (25). In a recent study from South Africa, case fatality rates in children >15 years and adults were as high as among infants: 23% for pneumococcal septicemia and 55% for meningitis (26). In high-income countries pre-PCV, the case fatality rate for all ages was 5% for pneumococcal pneumonia, 20% for septicemia and 30% for meningitis (27-29). In another study in Europe, the mean annual case-fatality rate for IPD in children <5 year old was 7.4% (range 0.7-34) (16).

Seasonal peaks of pneumococcal disease occur during winters. Pneumococcal co-infection with influenza increases the risk of mortality during influenza seasons (30). One explanation is that the co-infection of these respiratory pathogens damage the respiratory epithelium and decreases clearance of bacterial carriage and consequently increases the risk for invasive pneumococcal infection (31).

Table 1. Estimated pneumococcal burden in children in Europe and Africa (references in the table)

	Invasive Pneumococcal Disease			Non-invasive Pneumococcal Disease		
	IPD age <2years No of cases /100,000	Meningitis < 5 years No of cases /100,000	Bacteremia <5 years No of cases /100,000	Pneumonia <5 years with or without bacteremia No of cases /100,000	Sinusitis < 5 years all causes No of cases /100,000	Acute otitis media < 5years No of cases /100,000
Europe	44 (range 20-174)[15, 16, 32]	7.5 (range 0.7-22)[16]	31 (range 31-49)[33]	462 (uncertainty range 359-574)[33]	200[34]	1,475(range 1,470-1,498)[35, 36]
Africa	60-797[18-20]	10-48[33, 37, 38]	139 (range 104-174)[38]	3,397 (uncertainty range 2,643-4,227)[33]	-	5,702 (range 5,532-5,875)[35, 36]

Consequently, pneumococcal disease gives a high disease burden. This thesis explores the potential impact of PCVs on morbidity due to pneumococcal disease. However, firstly, a background explaining the larger policy framework, including UN development goals, the importance of a health system thinking while introducing a new vaccine in the childhood vaccination program and the need for an integrated approach, including to decrease pneumonia mortality.

1.1.3 From Millennium Development Goals to Sustainable Development Goals

The Millennium Development Goals (MDG) was an important UN framework for global health policy, and instrumental in the reduction of child mortality from 1990 to 2015. It was achieved by political commitment and by setting concrete goals and measuring progress with the help of monitored indicators by countries and regions (39). At the end of 2015, the MDG were exchanged for the Sustainable Development Goals (SDG 2015-2030). These new development goals have now been broadened to include many societal sectors, and are relevant for low-, middle- and high-income countries. The newly launched UN Sustainable Development Goal 3 (40) sets as a target the reduction of child mortality and "b*y 2030, end preventable deaths of newborn and children under five years of age, with all countries aiming to reduce neonatal mortality to at least as low as 12 per 1000 live births and under-five mortality to at least as low as 25 per 1000 live births*". There is an ongoing debate in the global health community whether the SDG will be able to strengthen the health pillar in development, since health was represented in three of the eight MDG, and now only in one of the seventeen SDG (41). WHO argues that health is an integral part of many of the other

SDGs and has proposed indicators to follow the progress of this issue (42). In the report "Health in 2015: from MDG to SDG", WHO points out the importance of a well-functioning health system for sustainable development (39). Of the thirteen SDG health goals, nine are measurable indicators, and the last four are means of implementing targets. One of these targets is related to vaccines and says: "*Support the research and development of vaccines and medicines for the communicable and non-communicable diseases that particularly affect developing countries, provide access to essential medicines and vaccines.*" WHO estimated that global vaccine coverage for three doses of diphtheria-tetanus and pertussis vaccine (DTP-3) was 86% in 2014, while only 31% for pneumococcal vaccine and 19% for rotavirus vaccines (43).

Achieving high vaccination coverage requires a well-functioning health system that also covers disadvantaged and hard-to-reach areas. In the evaluation of the progress of the MDGs it was concluded that not enough attention had been focused on strengthening health systems and that there was too much focus on reaching targets instead of achieving equity (39). This means that although an overall indicator in a country may have a positive development, the poorest and most disadvantaged people may experience no improvement at all. Therefore one ambitious indicator in the SDG health goal is: "*Achieving universal health coverage, including financial and risk protection, access to quality essential health care services and access to safe, effective quality and affordable medicines and vaccines for all.*"

1.1.4 Health systems

A health system can be defined as "*all organizations, people and actions whose primary interest and focus is to promote, restore and maintain health*" (44). Strong health systems are fundamental to achieve good health for populations in any society as described in the WHO/ Alliance for Health Policy and Systems Research report from 2009 (44). The various health system building blocks in the model (figure 2) cannot operate alone, but need coordinated and connected interaction with the other blocks – a system. The conditions for health systems in low- and high-income countries are vastly different with regard to for instance health financing, access to human resources and service delivery. Weak health systems do not manage to deliver the needed health care, particularly to the poorest and most rural populations. Any added burden on a weak health system, for example the introduction of a new vaccine, may exhaust not only financial, but also logistical and human resources, and a health systems approach is therefore needed in any such decision.

The introduction of new vaccines into child vaccination schedules has been proposed as a vehicle for strengthening health systems. However, this issue is complex and more research is needed in order to make appropriate adjustment necessary to get the most positive effects possible on immunization and health systems when introducing new vaccines as described in a recent SAGE report (45). The benefits for health systems of implementing new vaccines may include added resources for new refrigerators, roads built and strengthened logistical chains. The Global Alliance for Vaccine Initiative (GAVI) supports eligible countries in their efforts to introduce new vaccines by negotiating reduced vaccine prices and trying to identify

problems in the health system that need to be addressed before a vaccine is introduced, and also by providing support to countries by helping to make improvements in their health systems' performance.

Figure 2. The building blocks of the health system - a dynamic architecture and interconnectedness. WHO framework. (44)
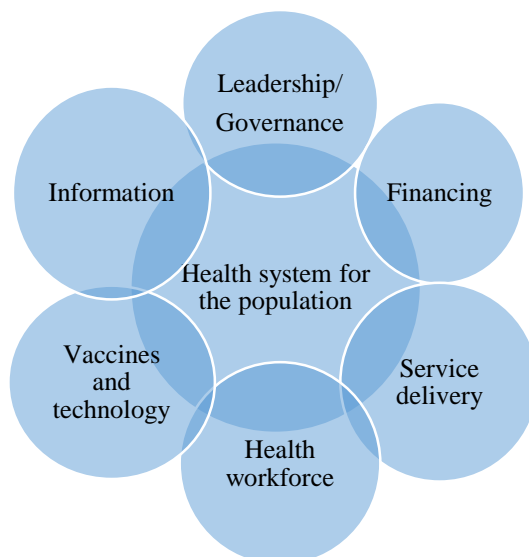
1.1.5 Introducing new vaccines

Introducing a new vaccine into a national childhood vaccination program is a multifaceted and complicated task that demands skilled people with multidisciplinary competences, from the fields of policy and science to logistics and communication. WHO published a report on principles and considerations for adding new vaccines to national immunization programs (figure 3) (46). It is a fourteen step process that resembles the health system framework by Don de Savigny et.al. described above (figure 2) but which also adds details to each step and points out the importance of knowing disease burden and its distribution in the population, as well as adverse events, monitoring and evaluation. The process is similar in low- and high income countries, regardless of differences in resources. However, evidence regarding vaccine efficacy and effectiveness is useful and important knowledge for both settings. The disease burden is often higher in low income countries which makes the potential impact of a vaccine easier to show. On the other hand, some population based surveillance systems in high income countries may make monitoring of effectiveness after implementation of a new vaccine more feasible, even though the vaccine preventable disease incidence is lower. Results from efficacy-, and even more so effectiveness evaluations, of vaccines do not always carry over between high and low income countries. Some vaccines, like rotavirus vaccines, have been shown to have a vaccine efficacy (VE) of 40-60% in low income countries, while it has a VE of 80-90% in high income countries. The reasons for this lower VE are not fully understood, but may have to do with differences in intestinal flora, malnutrition, differences in food intake etc. Pneumococcal conjugate vaccines seem to have a more equivalent efficacy for the serotypes included in the vaccines in different contexts (25, 47).

Figure 3. Framework for adding new vaccines to national immunization programs, adapted from WHO 2014 (46)
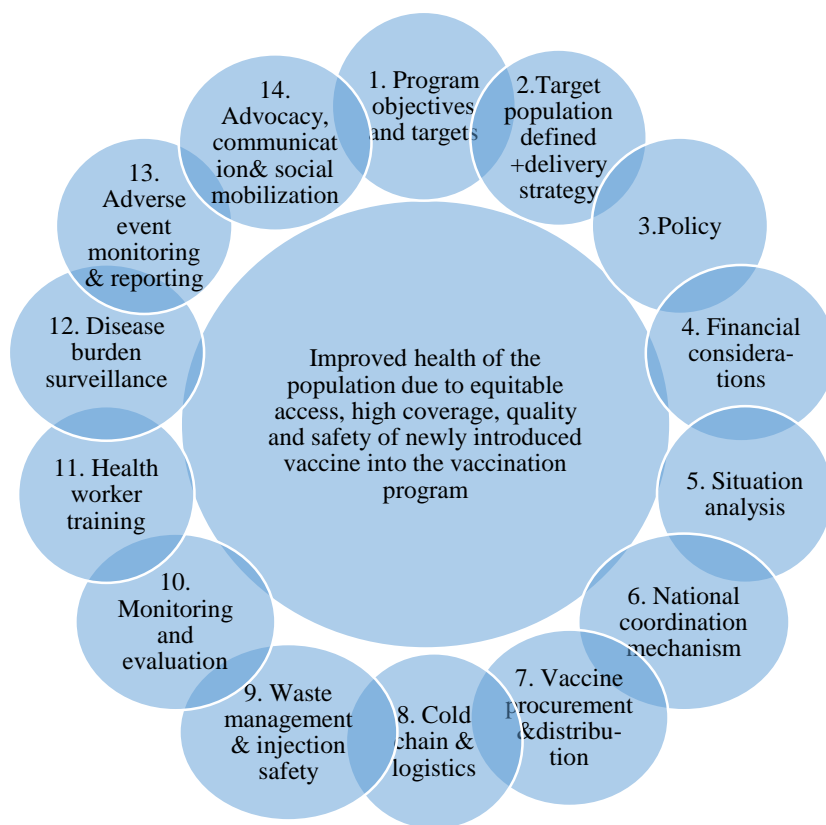
1.1.6 Global Vaccine Action Plan - GVAP

The Decade of Vaccines was endorsed by 194 member states at the World Health Assembly in May 2012. It contains an action plan called The Global Vaccine Action Plan (GVAP) and stretches from 2011 to 2020 (48). If the goals are reached it is estimated that between 24.6 and 25.8 million lives will be saved before the end of the Decade of Vaccines.

The goals of the GVAP by 2020 are to:

- ✓ Achieve a world free of poliomyelitis;
- ✓ Meet global and regional disease elimination targets (for measles, neonatal tetanus, rubella and congenital rubella syndrome);
- ✓ Meet vaccination coverage targets in every region, country and community;
- ✓ Develop and introduce new and improved vaccines and technologies;
- ✓ Exceed the Millennium Development Goal (MDG) 4 target for reducing child mortality.

The strategic objectives for the GVAP are.

- ✓ All countries commit to immunization as a priority.
- ✓ Individuals and communities understand the value of vaccines and demand immunization as both their right and responsibility.
- ✓ The benefits of immunization are equitably extended to all people.

- ✓ Strong immunization systems are an integral part of a well-functioning health system.
- ✓ Immunization programmes have sustainable access to predictable funding, quality supply and innovative technologies.
- ✓ Country, regional, and global research and development innovations maximize the benefits of immunization.

The Strategic Advisory Group of Experts (SAGE), WHO´s technical advisory group on immunizations, is monitoring the GVAP and in its last report from 2015 (49) Uganda was among the top ten countries with most children un-immunized against diphtheria-tetanus-pertussis (DTP-3). The other countries were India, Nigeria, Pakistan, Indonesia, Ethiopia, the Democratic Republic of Congo, the Philippines, Iraq and South Africa. Much effort is still needed to increase vaccination coverage of these older vaccines in these countries.

The Global Alliance for Vaccine Initiative (GAVI) provides crucial support to low-income countries to introduce immunization using newer vaccines that are still underutilized. These are vaccines that may improve child survival, for example the rotavirus vaccines and the pneumococcal conjugate vaccines (13). GAVI, in view of the high pneumococcal disease burden and high pneumonia mortality, created the pneumococcal advanced market commitment, in collaboration with donors and the pharmaceutical industry, in order to support the development and production of affordable pneumococcal vaccines. The ambition was to prevent 7 million childhood deaths by the year 2013 (50).

1.1.7 *Streptococcus pneumoniae* – the bacteria

Streptococcus pneumoniae is a gram-positive, extra-cellular, facultative anaerobic bacterium, often called pneumococcus. It is identified in laboratories by its colony morphology, often crater-like and alfa-hemolytic, which is shown as a light halo around bacterial colonies as they grow on blood agar plates, due to the production of hydrogen peroxide by the bacteria. (figure 4). Pneumococcus is optochin susceptible and bile/deoxy chocolate soluble; this forms the basis for routine detection tests (51).

Pneumococci are grouped according to immunological similarities into so-called serogroups. These serogroups are given a number, for example 19 or 23. The pneumococci are then further classified into serotypes based on their polysaccharide containing capsule, named with a letter, for example 19A or 23F. The serogroups and serotypes are identified based on reaction to specific antisera.

Pneumococcal bacteria have several virulence factors that contribute to their pathogenicity that are also central to their capacity to cause disease. These include polysaccharides surface antigen included in the bacterial capsule, surface proteins (pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), autolysin (LytA) and pneumococcal surface adhesion (PsaA), excreted proteins (IgA protease), and cytoplasmic proteins (pneumolysin) (30, 52). The mechanisms of these virulence factors are explained in the following list:

- PspA blocks the binding of complement component C3, thereby hindering the opsonisation and phagocytosis of the pneumococcal bacteria.
- PspC mediates adherence of the bacteria to the cell of the epithelium of the host. It also binds IgA and factor H, further inhibiting the immune defense of the host.
- Autolysin, LytA leads to cell wall auto-destruction so that intracellular cytoplasmic pneumolysin can be released.
- Pneumolysin is a cytoplasmic toxin that binds to cholesterols in the cell wall of the host cells, opening the cell wall and thereby leading to the lysis of the host cell.
- PsaA is an extracellular membrane protein that transports magnesium into the cell.
- IgA protease targets human immunoglobulin A, which is important in the respiratory immune defense of humans.
- Pili, filamentous surface structures, which are expressed on some, but not all pneumococcal isolates, seem to enhance bacterial adhesion and thereby help in colonization.

However, the most important virulence factor in the pneumococcal bacteria is its polysaccharide capsule, which forms the outer surface layer. Un-capsulated pneumococci are normally not virulent, except in immunocompromised patients. The polysaccharides are covalently attached to the cell wall, and most are highly charged and highly anti-phagocytic. The polysaccharide capsule defines which serogroup and serotype the pneumococcal bacteria belongs to.

Figure 4. Pictures of alfa-hemolytic pneumococcal colonies on agar gel, optochin susceptibility and the pneumococcal formation as a diplococci.*

*Pictures from freely available ppt presentations from the former PneumoADIP initiative (http://www.preventpneumo.org/about_us/).

1.1.8 Pneumococcal serotypes

To date, there are 46 known serogroups and 97 serotypes of pneumococcus identified (53). The serotype capsules are made up of repeated units of two or more monosaccharides, but may also be branched with side chains. The capsule thereby vary in thickness and ability to activate the complement pathways, and in ability to induce an antibody response, and therefore to resist phagocytosis (9). Consequently, the serotypes' association with invasive disease varies. Invasiveness does not always lead to high case-fatality. Some serotypes with high invasive disease potential cause low mortality and conversely, some serotypes with low

invasiveness may have a high case-fatality rate (54). Before vaccination with PCVs, 6-11 serotypes caused more than 70% of all invasive pneumococcal infections (IPD) as described in a systematic review with data from 70 countries (14). The most common serotypes in IPD in descending order were: 14, 6B, 1, 23F, 5, and 19F. The disease caused by specific serotypes differs depending on which age groups they cause disease in, and in the clinical outcome (disease syndrome and severity). Serotypes also vary naturally with time and geography (55, 56). Some of the characteristics of selected serotypes are listed in table 2.

Table 2. Characteristics of selected commonly isolated serotypes of pneumococci in invasive disease and carriage, and which vaccines (PCV) cover the different types.

Serotype	Vaccine-type or non-vaccine type*	Mainly isolated in IPD or carriage or both	Clinical importance and comments (references)
4	PCV7	IPD	Frequently causing septicemia (57) Found in both children and adults (58)
6B	PCV7	IPD	Commonly isolated in acute otitis media (9) More commonly isolated from CSF than blood (9, 16) Commonly found in the youngest children (58)
9V	PCV7	IPD	Found in both children and adults (58)
14	PCV7	IPD	Frequently causing septicemia and meningitis (38, 57) Commonly isolated in acute otitis media (9) Commonly found in the youngest children (58)
18C	PCV7	IPD	Cause high mortality (59) More common in children than adults (ref)
19F	PCV7	IPD+Carriage	Commonly isolated in acute otitis media (9) Commonly isolated in pediatric meningitis and bacteremia (16)
23F	PCV7	IPD+Carriage	Frequently causing meningitis (60) Commonly isolated in acute otitis media (9) Elderly but also found in both children and adults (58)
1	PCV10	IPD	Frequently causing septicemia and severe pneumonia (57) Older children 2-4 years more common than infants (14) Affecting younger adults. High invasiveness but low mortality (59) Causing empyema (55) More common in low than in high income countries (9)
5	PCV10	IPD	High invasiveness (9) More common in children than adults (9) More common in Africa than in Europe (14)
7F	PCV10	IPD	High invasiveness but low mortality (59) Found in both children and adults (58)
3	PCV13	IPD	Cause high mortality (59) Frequently causing middle ear infection and severe pneumonia (57) Causing empyema (55) Found in both children and adults (58)
6A	PCV13	IPD	Cause high mortality (59) Frequently causing meningitis (60)
19A	PCV13	IPD	Frequently causing middle ear infection (57) Causing empyema (55)
10A	NVT	Carriage	More commonly isolated from CSF than blood (9)
11A	NVT	Carriage	Cause high mortality in elderly and risk-groups (59)
22F	NVT	Carriage+IPD	Cause meningitis in adults (61)

* PCV7, 10 and 13 means that this serotype is included in respective pneumococcal conjugate vaccine (PCV), see table 3. NVT are not included in any PCV.

In general, serotype specific antibodies are protective, and immunity develops after an infection. There is a small degree of cross protection between certain similar serotypes, such as 6B and 6A (62). Between others, however, even within the same serogroup, little or no cross protection has been seen, for example 19F and 19A (63, 64). Certain serotypes, such as 6B, 9V, 14, 19A, 19F, and 23F, are more commonly associated with antimicrobial resistance than others (55, 65). These serotypes are more often isolated from middle ear infections as compared to other serotypes, probably because they are carried for a longer period, with probable high exposure to antibiotics. This is as an effect of the common treatment of otitis media with antibiotics. Pneumococci in the upper respiratory tract mucosa are easily accessible to antibiotics (9).

Capsular switch is a phenomenon whereby the pneumococcal bacteria change their capsular expression of serotype by a genetic shift of material. This has been suggested to be caused partly by selection under antibiotic pressure or pneumococcal vaccines, but is also part of the genius natural evolution of the pneumococcal bacterium (66, 67).

1.1.9 Pneumococci – an interplay between carriage and illness

Pneumococci are spread by aerosol, droplets or direct contact from person to person. Even though invasive pneumococcal disease is a severe disease, the pneumococcal bacteria may also merely cause asymptomatic colonization in the upper respiratory tract. Colonization is a prerequisite for invasive pneumococcal disease (figure 5). The pneumococcal bacteria attach to the epithelial cells of the nasopharyngeal mucosa and either stay as a harmless colonizer for days, weeks or months, or spread locally to the ears, sinuses, or via the respiratory tract to the lungs. The bacteria become more harmful if they penetrate the mucosal wall and enter the blood stream where they may cause septicemia and sometimes spread further via the blood stream to cause osteomyelitis or, if crossing the blood-brain barrier, cause meningitis (30, 68).

Carriage of pneumococci is both age and serotype dependent (9). Carriage is more prevalent under the age of five years, peaking in children from 1 to 3 years of age, ranging from 23-85% of the children being colonized (25), while it is less than 10% among adults (30). Carriage prevalence have been shown to be higher in low- and middle income countries than in high income countries (69, 70). IPD is higher in populations with the high carriage prevalence in low- and middle income countries (33). Also, IPD incidence is highest in children under 2 years of age. The carriage stage may be considered to be the stage where humans build up their immunity against different pneumococcal serotypes (9, 71). This protective immunity is shown with a markedly lowered incidence of IPD after five years of age, which lasts until about the age of 65.

Figure 5. Progression and spread of pneumococcal bacteria (72, 73)*
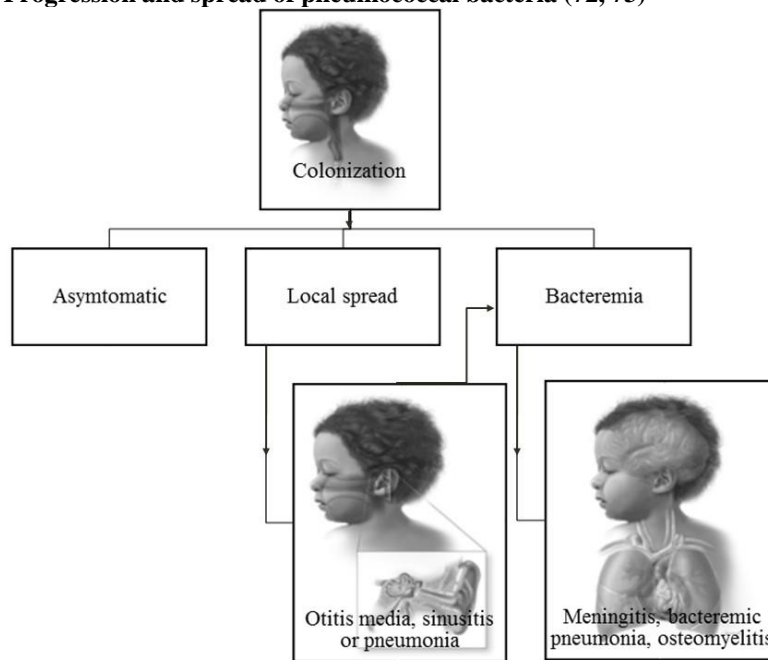
*Pictures from freely available ppt presentations from the former PneumoADIP initiative (http://www.preventpneumo.org/about_us/).

1.1.10 Risk factors for pneumococcal disease

One of the main risk factors for pneumococcal disease is age, both low and high. Children under the age of 2 years are at the greatest risk for severe disease, followed by age groups above 65 years, as mentioned earlier. Young children who spend time in crowded settings such as day-care centers are at higher risk of IPD. Boys, particularly those under 2 years, are at higher risk of invasive pneumococcal disease than girls (52, 74).

Immunocompromised persons are at high risk of morbidity and mortality due to pneumococcal infection. This includes those infected with HIV, malignancies, or with lack of an anatomical or functional spleen. Individuals with sickle cell disease, diabetes or chronic heart, lung or liver disease are at higher risk of severe disease and complications due to pneumococcal diseases. There may also be recurrent pneumococcal diseases in patients with skull defects after fracture, patients with cerebrospinal leaks, patients with cochlear implants or with immunodeficiency (75, 76).

In a recent systematic review of risk factors for mortality due to lower respiratory infection in low- and middle income countries, the following were associated with increased mortality: having very severe pneumonia, age below two months, underlying chronic diseases, HIV positivity, young maternal age, low maternal education, low socio-economic status, second-hand smoke exposure, and indoor air pollution (77). Protective factors were immunization, and good antenatal practices (77).

1.2 MANAGEMENT OF PNEUMOCOCCAL DISEASE

Protect, Prevent and Treat is a WHO/UNICEF slogan employed in the effort to increase the use of life-saving interventions against pneumonia and diarrhea in a program called Global Action Plan for the Prevention and Control of Pneumonia and Diarrhea (GAPPD) (78). This plan includes targets with precise indicators that are followed in all countries with a high burden of these two diseases. In 2009 the Global action plan for the prevention of pneumonia (GAPP) was launched, and from the year 2013 diarrhea was added to the plan. Preventive measures in focus in this plan are high coverage of vaccination, prevention and management of HIV infection, improvement of nutrition and breastfeeding, reduction of children born with low birth weight, and reduction of indoor air pollution (79). The plan also focuses on quality case management. Even though effective antibiotics exists, pneumococcal pneumonia is still estimated to cause nearly a fifth of all deaths in children under five years of age, and further preventive measures are clearly needed to limit the disease burden (80, 81). In 2013, Lancet published a series of articles on the epidemiology of pneumonia and diarrhea and what public health actions possible and needed (13, 47). This thesis focuses of the impact and potential impact of pneumococcal conjugate vaccines (PCV) on pneumococcal disease. The framework by Bhutta et. al. in figure 6, however, put the use of PCVs in a perspective, as one important piece in a larger plan of public health actions needed to lower mortality and morbidity due to pneumonia.

Figure 6. Protection, prevention and management of pneumonia. Framework by Bhutta et al. adapted by Lindstrand. Interventions to address deaths from childhood pneumonia and diarrhea equitably: what works and at what costs? Lancet 2013 (47)
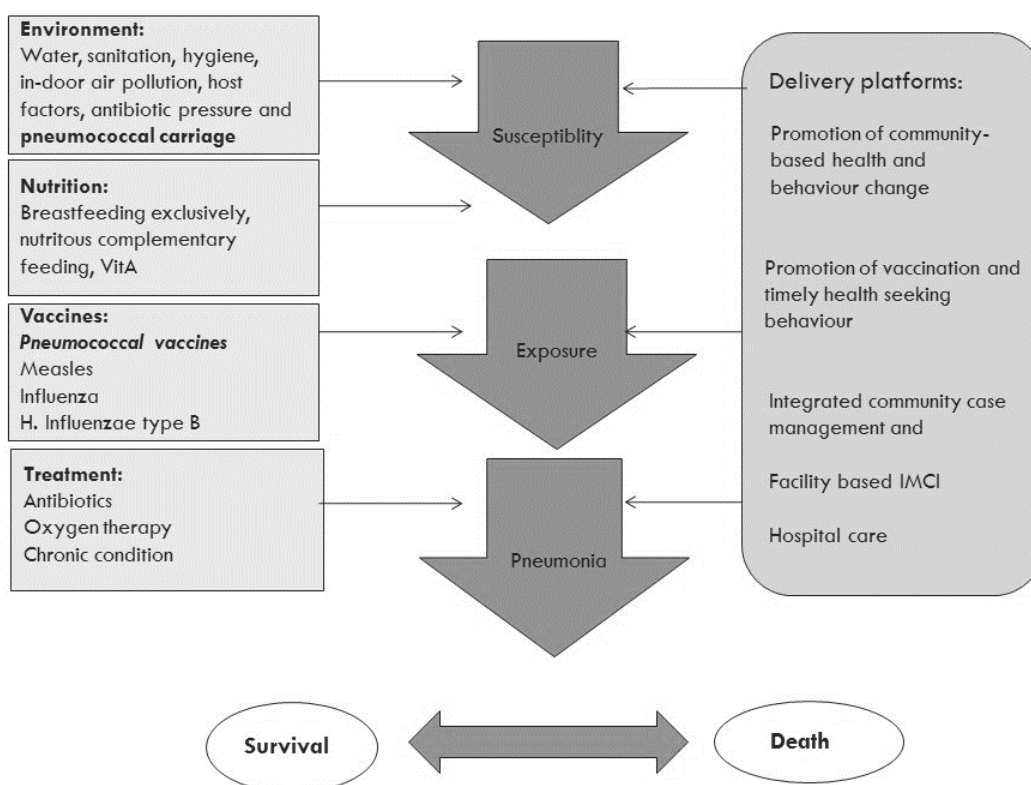
1.2.1 Prevention of pneumococcal disease

Preventive measures, other than pneumococcal vaccination, include exclusive breastfeeding for six months. To avoid malnutrition, which is a major risk factor for pneumonia, it is crucial to give nutritious complementary feeding alongside continued breastfeeding as the infants adapt to eating solid foods. Vitamin A supplementation is important as it has an immunomodulatory effect and has been shown to decrease both incidence and mortality from pneumonia (47, 82).

As pneumococcal disease may evolve as a secondary infection after influenza and measles infection, vaccination against these diseases also helps to reduce the burden of pneumococcal disease (83). According to the framework above (figure 6) on the management of pneumonia, it is also important to include vaccination against *H.influenzae* type b in national vaccination schedules to prevent pneumonia, as it causes 16% of all pneumonia deaths (8, 47).

Other known risk factors for pneumococcal disease such as tobacco smoke exposure and indoor air pollution, are two factors which are preventable risks for pneumococcal disease (84).

1.2.2 Treatment of pneumococcal disease and antibiotic resistance

Penicillin, discovered in 1928, has been used as the treatment of choice for pneumococcal disease since the 1940s (52). It was not until the 1970s that pneumococcal resistance towards penicillin started to appear, and since then, resistance has become widespread. Penicillin, a β-lactam antibiotic, as treatment against meningeal pneumococcal disease with intermediate or fully resistant strains, needs to be replaced with another type of antibiotic. Non-meningeal disease may, however, still be treated with higher doses of penicillin. Another strategy is to combine different types of antibiotics. Antibiotic resistance against macrolides, trimethoprim, fluoroquinolones, and vancomycin is however increasing (27). In pneumococcal isolates from IPD cases in children <18 years of age in a European review from 2010, the proportion with penicillin G non-susceptible was 31% (range 0% in Sweden and Finland, to 48% in France) (16). IPD isolates in children <5 years old were resistant in 35% (range 7-53%) of cases for erythromycin and in 9% (range 0-36%) for cefotaxime or ceftriaxone, in the same review.

In Sweden and the Nordic countries, antibiotic resistance is still at low levels (85). Penicillin is therefore recommended in treatment guidelines as first-line antibiotic of acute otitis media, sinusitis and community acquired pneumonia in Sweden (86). For community acquired pneumonia in children < 5 years old, the recommendation is penicillinV 20 mg/kg three times a day for seven days, or alternatively amoxicillin 15 mg/kg three times a day for five days. In a study in Uganda in 2008, 99% of pneumococcal strains carried by young children showed resistance towards trimethoprim sulphamethoxazole (co-trimoxazole), while 80% showed intermediate resistance towards penicillin (87). Co-trimoxazole was the first-line treatment for pneumonia at that time in Uganda, but recommendations subsequently changed to amoxicillin in 2010. WHO now recommends oral amoxicillin 40 mg/kg twice daily for 5 days (3 days in low HIV endemic areas) for treatment of uncomplicated pneumonia, and for

severe pneumonia the recommendation is ampicillin 50 mg/kg or benzylpenicillin 50000 units/kg IM/IV six times daily for at least five days, and gentamycin 7,5 mg/kg once a day for at least five days (88).

Reducing antibiotic resistance is a global priority. Interventions to combat the spread of resistance include the development of better diagnostic tools to distinguish between viral and bacterial disease, decrease irrational antibiotic use, strengthening of hygienic routines, and the use of pneumococcal vaccines (89). The causal relationship between pneumococcal vaccines and decreased antibiotic resistance may be through a diminished burden of infections requiring antibiotics (otitis media and sinusitis) or by increasing pneumococcal vaccination coverage for serotypes carrying antibiotic resistance (90, 91). However, the opposite may happen if pneumococcal vaccines were to instead target less antibiotic resistant serotypes, which could lead to an increase in antibiotic resistant serotypes or clones (90).

1.2.3 Pneumococcal vaccines

There are two different principles behind the two available types of vaccines against pneumococcal disease: a vaccine containing only pneumococcal polysaccharides (PPV) and different protein-polysaccharide conjugate vaccines (PCV). Both types of vaccine have based their antigen content on the serotype specific polysaccharide capsule antigens and include different numbers of serotypes.

Pneumococcal polysaccharide vaccine (PPV)

The first PPV was introduced in the US in 1977 and included 14 serotype antigens, a so called 14-valent vaccine. In 1983 it was changed to a 23-valent vaccine. PPV is given as a single dose.

The immune response to a polysaccharide vaccine is T-cell independent and therefore does not induce a memory T-cell function. Instead, immunity is antibody mediated and transient, and the activity of serotype specific antibodies and opsonisation decreases after about five years (92-94). PPV is effective in reducing invasive pneumococcal disease in immunocompetent patients (50% 95%CI 21-69% in age groups >65(95)), less so in immunocompromised patients, and a weak or no association has been shown for pneumonia in age groups older than 65 (93, 95). Response has been shown to a PPV23 booster dose 5 years after the first PPV23 dose (96), and revaccination after 5 years is recommended in some countries, however there is a lack of data on revaccination effectiveness against IPD for healthy elderly persons. Revaccination has been tried, however, in immunocompromised patients (25). Furthermore, the vaccine does not induce a mucosal immunity, and therefore has no effect on carriage at all (94). Thus it does not contribute to herd immunity. Children under the age of two respond poorly or not at all to polysaccharide vaccines due to their immature immune system (92, 97). The advantage of the PPV is that it covers more serotypes than the PCVs. It is recommended in most high income countries to medical risk groups and persons older than 65, and there is increasing evidence that a combination of PCV and PPV is

advantageous for high risk groups (92). PPV23 includes all PCV13 serotypes, except 6A, and 11 additional serotypes (table 3).

Pneumococcal conjugate vaccine (PCV)

PCV7, which includes seven serotypes, was first introduced in the US in the year 2000. In 2009, two new PCVs were marketed, the PCV10 and PCV13, with their respective broader coverage of serotype protection. Serotypes included in the pneumococcal vaccines are called vaccine types (VT) and the other serotypes are called non-vaccine types (NVT). Here, the polysaccharide antigen of each included serotype is chemically bound to a protein carrier. This conjugated antigen-protein complex changes the immune response to a T-cell dependent response. The vaccine therefore induces an immune memory with a longer duration than the PPV and produces a booster response upon subsequent doses of the vaccine (97). The duration of protective immunity that is induced by the PCV is, however, not known. PCV10 includes capsular antigens of 10 serotypes, within which 1, 4, 5, 6B, 7F, 9V, 14 and 23F are conjugated to a protein carrier Protein D, serotype 19F to diphtheria toxoid, and 18C to tetanus toxoid. Protein D is composed of the outer membrane protein for non-typeable *H. influenzae*, and a significant 33.6% decrease in overall incidence of otitis media, possibly due to Protein D, was shown in one earlier study of PCV10 impact (98). This added effect on otitis media incidence has also been shown in Australia (99). PCV13 includes capsular antigens of 13 serotypes all individually conjugated to a non-toxic diphtheria protein carrier CRM 197. Both PCV10 and 13 use aluminum phosphate as adjuvant. PCV13 is licensed in Europe for use in all ages, and PCV10 up to 5 years of age. Both vaccines are recommended to be given with three primary doses with at least four weeks between doses and a booster dose at least six months after the last primary dose (3+1 schedule). Vaccination scheduled with two primary doses, two months apart, and one booster dose six months later, may also be used (2+1 schedule). Both schedules show a good vaccine efficacy against IPD (100, 101). Children in older age groups are recommended fewer PCV doses depending on age and type of vaccine.

In a systematic review from 2009, Johnson et al. showed that Africa and Asia (49 and 51% respectively) had the lowest PCV7 serotype coverage rates (14), meaning covering the IPD cases due to the seven serotypes included in the first PCV vaccine developed. Europe and North America had much higher PCV7 coverage rates, of 72% and 82% respectively, prior to PCV vaccination. In another review, serotype coverage for IPD in children <5 years old in Europe pre-PCV, ranged from 67-93% for PCV10 and 56-95% for PCV13 (16).

Pneumococcal vaccination recommendations

Since the year 2007, WHO recommends PCV vaccination for all children in the regular childhood vaccination program. The recommendations were changed from PCV7 to either PCV10 or PCV13 in 2012 (25). WHO states that countries with an under-five mortality of more than 50/1000 live births should make the introduction of the PCVs a "high priority".

The US Advisory Committee on Immunization Practices (ACIP) recommends PCV13 for all adults >65 years old, followed after one year by a PPV23 (102, 103). It also recommends that all medical risk groups below 65 years of age who have had a PPV23 should take a dose of PCV13 followed within a year of a second PPV23 (102, 104). In Sweden, current pneumococcal vaccine recommendations are being updated, but the suggestion from the Public Health Agency includes that medical risk groups of all ages at higher risk of IPD and or complications due to IPD should obtain one PCV13 vaccine, followed by a dose of PPV to protect against a broader range of serotypes (105).

Table 3. Vaccines against pneumococcal disease (9)

Named	Serotypes included in the vaccine	Protein carrier	Adjuvants and antigen content	Brand name and company	Licensed for ages
PCV7	4, 6B, 9V, 14, 18C, 19F, 23F	CRM197	Aluminium phosphate	Prevenar7, Pfizer	< 5years
PCV10	4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F	*H influenza* protein D and tetanus and diphtheria toxoid	Aluminium phosphate	Synflorix, GSK	≤ 5 years
PCV13	4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, 6A, 19A	CRM197	Aluminium phosphate	Prevenar13, Pfizer	All ages
PPV23	4, 6B, 9V, 14, 18C, 19F, 23F, 1, 5, 7F, 3, -, 19A	-	-	Pneumovax23, Merck	All ages > 2years
	2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, 33F				

Future pneumococcal vaccines

Concerns arise, due to serotype replacement after PCV implementation, to the long-term effects of vaccines that only cover a limited number of serotypes. There are new pneumococcal conjugate vaccines at various pre-licensure stages, including higher valent PCVs. One 15-valent that has passed the phase I and II trials, includes, apart from the 13 in PCV13, also 22F and 33F serotypes (106).

Finally, and what many pneumococcal experts call for, is a protein based vaccine. Since they are directed towards antigens on the pneumococcal cell wall below the polysaccharide capsule, they are serotype independent (97). In children from 10 month of age there is evidence of a broad increase in immunity against pneumococcal disease, simultaneously covering many serotypes, suggesting a serotype independent immunity developing (97). Phase I and II studies in Europe and Africa, show initial safety and immunogenicity against a

pneumococcal histidine triad D (PhtD) and pneumolysin toxoid (pPly) protein based vaccine, also in combination with antigens of 10 serotypes (107, 108). PhtD is expressed on the pneumococcal surface and inhibit complement deposition and is important for host colonization and invasion. Pneumolysin is an exotoxin involved in bacterial autolysis and facilities intrapulmonary bacterial growth and invasiveness. Other interesting pneumococcal proteins in focus for vaccine development are pneumococcal choline-binding protein A (PcpA) and pneumococcal surface adhesion A (PsaA) (97). A protein sub-unit approach with combinations of common proteins is suggested a solution to minimize immune escape, which pneumococci is so capable of (109). Another possible approach is using whole cell vaccine technology and phase ½ trials are ongoing (110). Any of these candidate vaccines needs to be efficient not only on IPD but also on pneumococcal carriage. Close monitoring of vaccine escape is then needed to assess if it emerges against pressure conserved protein antigens (97).

1.2.4 Experiences of conjugate pneumococcal vaccines worldwide

Evidence from the clinical trials

The Cochrane review of the efficacy of pneumococcal conjugate vaccines studied in six randomized controlled trials in Africa, US, Philippines and Finland with more than 57,000 children receiving PCV7 and 56,000 placebo, concludes a good efficacy of PCV7 against IPD and x-ray confirmed pneumonia (111). The pooled vaccine efficacy (VE) in children <2 years against VT-IPD was 80% (95% CI 58-90%), all-type IPD 58% (95% CI 29%-75%), x-ray defined pneumonia 27% (95% CI 15-36), and clinical pneumonia 6% (95% CI 2%-9%). A non-significant decrease in overall mortality by 11% (95% CI -1%-21%) was shown (p=0.08) (111). A clinical trial in Gambia was stopped due to the shown efficacy of PCV on overall mortality decreasing by 16% (95% CI 3-28%) (20).

A large double blinded RCT of PCV10 in Latin America in children (n=24000) showed a wide effect against pneumococcal disease from an infant 3+1 schedule after 23 months of follow-up. Vaccine efficacy (VE) was 22% (95% CI 7.7-34%) for bacterial community acquired pneumonia, 67.1% (95% CI 17-87%) for serotype confirmed acute otitis media, 100% (95% CI 74-100%) for vaccine type IPD, and 65% (95% CI 11-86%) against any IPD (112).

One randomized double blind, placebo-controlled trial has evaluated the effect of PCV13 on adults older than 65 years (n=84 495) on pneumococcal pneumonia (113). This so-called CAPITA study in the Netherlands showed a vaccine efficacy of 75% (95% CI 41-91%) against IPD due to vaccine-type strains, 44% (95% CI 22-62%) against vaccine-type community acquired pneumonia, and 45% (95% CI 14-65%) against vaccine-type non-invasive, non-bacteremic community acquired pneumonia. No effect of the PCV13 on mortality was shown compared to the placebo group (113).

Effectiveness studies

Experiences of PCVs in national childhood immunization schedules show a similar pattern of a rapid decrease of IPD incidence in vaccinated age groups, followed by a more slow decrease in unvaccinated age groups, a so-called herd effect. Feikin showed in a meta-analysis of 21 surveillance site datasets from 16 high-income countries, that incidence of IPD in children <5 years old decreased the first year of the program to RR 0.55 (95% CI 0.46-0.65) and remained stable for seven years. This overall decrease in IPD in children <5 years was mediated by VT-IPD decreasing annually to RR 0.03 (95% CI 0.01-0.10), but non-vaccine type (NVT)-IPD increased to the 7th year RR 2.81 (95% CI 2.12-3.71). This, however did not counterbalance the positive VT effect. The meta-analysis also showed a decrease in VT-IPD in all other age groups within seven years after introduction, but a barely significant decrease in overall IPD due to increase in NVT-IPD (114). Fitzwater et al. reviewed the effect of PCV7 in nine high–income countries and found that incidence of VT-IPD in young children decreased with 79-97%, and the decrease in IPD of all-serotype was between 38-80%. They also saw a decrease in hospitalization due to all-cause pneumonia by 13-65% and an impact of all-cause otitis media by between 13-42% (115).

In a cluster randomized trial in Finland, PCV10 effectiveness was evaluated in children 3-18 months using the schedules 3+1 or 2+1 (116). Of the 47,366 participating children, 30,527 were assessed for effectiveness against IPD. Vaccine effectiveness (VT) for children <1 year after about 2 years of follow-up was 100% for children with 3+1 schedule and 92% for children with 2+1 schedule (116). Palmu et al. continued to study the vaccine effectiveness on clinically suspected IPD cases through national registry data excluding the laboratory confirmed cases. They observed a 71% (95% CI 52-83%) vaccine effectiveness after the diagnosis of the patients with suspected IPD were verified in medical records. They concluded that the incidence rate of laboratory confirmed IPD was markedly lower than the clinically suspected IPD incidence and that the true vaccine effectiveness is underestimated using only laboratory confirmed cases (117).

PCV effect on carriage

The overall pneumococcal carriage prevalence has, in most countries, not decreased significantly after PCV introduction. However, there is switch from VT to more dominance of NVT in the countries where this has been studied (115, 118-122). Carriage of VT pneumococcal serotypes was shown to be reduced in a recent systematic review by Fleming-Dutra et al., for the PCV schedules of 2+0, 2+1, 3+0, and 3+1 (123). Another review including 16 RCTs by Nicholls, showed no change in VT carriage after the first one or two doses, but that the changes in decrease of VT and increase of NVT, appears only after the second dose at and after about 7 months of age (124).

Antibiotic resistance

Following the introduction of pneumococcal conjugate vaccines, there has been a decrease in antibiotic resistance in serotypes causing IPD and in carriage (65). However, before the switch from PCV7 to higher valent conjugate vaccines, the antimicrobial resistant prone serotype 19A increased in, for example, the US and Israel (125, 126).

Serotype replacement=replacement in disease?

S.aureus and *S.pneumoniae* are both common colonizers in the nasopharynx, and both give rise to serious disease. *S. aureus* causes skin infections, endocarditis and toxic shock syndrome (127). Since PCV decreases the VT pneumococci in carriage, there is rising concern that *S.aureus* will not only replace *S. pneumoniae* in the nasopharynx, but that it will rise as a cause of disease. This fear of replacement leading to replacement disease has mainly proved unsubstantiated (127). Studies from the randomized control PCV trials in the Netherlands show a shift in the microbiota of the nasopharynx, but the rise in *S. aureus* following PCV was transient and had disappeared by the age of 24 months (128). In post-licensure studies there has been a reported shift in etiology of rhinosinusitis (129, 130) and a rise in incidence of empyema as a complication after pneumonia (131). This needs close monitoring as *S aureus* emerging may cause more complications and carry antibiotic resistance problems in treatment.

Serotype replacement after PCV implementation resulting in increase in NVT pneumococcal serotype, may however, result in replacement disease if the emerging NVT carry a high invasiveness potential (132). It may also be that IPD disease will change in its clinical character or age of onset if the emerging serotypes expand in ages and in immunocompromised groups of individuals who are more vulnerable to disease by those NVT (132).

Viral co-infection, for example between influenza and *S. pneumoniae* is associated with pneumococcal pneumonia (133). PCV may therefore, as an additional benefit, decrease complicating pneumonia after influenza and other viral infections (83).
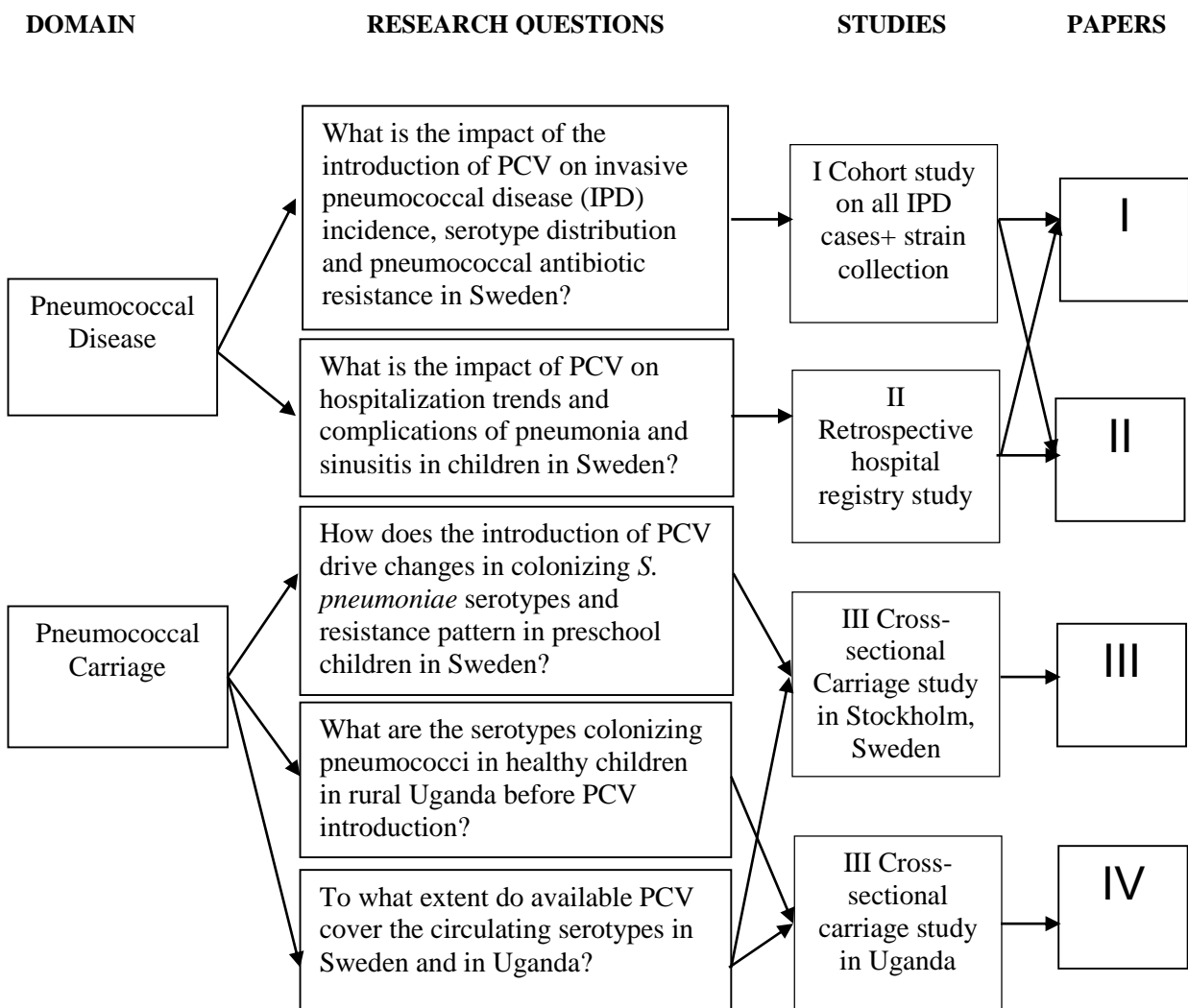
1.3 CONCEPTUAL FRAMEWORK

Pneumococcal disease needs to be managed at many different levels in society and in the health care system, as explained in the background of this thesis. Pneumococcal vaccines is one intervention needed in an integrated approach.

The current thesis focuses on pneumococcal disease and carriage, and explores the impact or the potential impact of pneumococcal conjugate vaccine in two very different contexts: Sweden and Uganda.

The conceptual framework below outlines the domains, research questions, studies and papers included in the thesis:

Figure 7. Conceptual framework of the thesis.

DOMAIN	RESEARCH QUESTIONS	STUDIES	PAPERS
Pneumococcal Disease	What is the impact of the introduction of PCV on invasive pneumococcal disease (IPD) incidence, serotype distribution and pneumococcal antibiotic resistance in Sweden?	I Cohort study on all IPD cases+ strain collection	I
	What is the impact of PCV on hospitalization trends and complications of pneumonia and sinusitis in children in Sweden?	II Retrospective hospital registry study	II
Pneumococcal Carriage	How does the introduction of PCV drive changes in colonizing *S. pneumoniae* serotypes and resistance pattern in preschool children in Sweden?	III Cross-sectional Carriage study in Stockholm, Sweden	III
	What are the serotypes colonizing pneumococci in healthy children in rural Uganda before PCV introduction?		
	To what extent do available PCV cover the circulating serotypes in Sweden and in Uganda?	III Cross-sectional carriage study in Uganda	IV

1.4 STUDY RATIONALE

Pneumonia is the major cause of death in children worldwide. If the Sustainable Development Goals of an under-five mortality rate of less than 25 per 1000 live births is going to be achieved before 2030, a considerable renewed and combined effort is needed in all areas of protection, prevention and treatment from community to tertiary level health care in low-income countries. Vaccines are one of the most cost-effective interventions in public health. WHO and UNICEF have estimated that 2-3 million children's lives are saved yearly due to vaccination against measles, diphtheria, tetanus, and pertussis (43). Higher coverage of equitably and safely distributed vaccines is therefore a priority. (47).

Similarly to the new rotavirus and human papillomavirus vaccines currently being implemented at a global level, the pneumococcal vaccines do not cover all sero- and genotypes affecting humans. There are 97 different serotypes that may cause disease, varying in severity by age and risk groups. The ecological niche, or microbiota, in the nasopharynges of young children harbor and transmit disease. Since the PCVs only reduce 10 or 13 serotypes in carriage and invasive pneumococcal disease, with a small degree of cross protection, inevitably there will be a new equilibrium of pneumococcal strains evolving after PCV immunization in children. With newer generations of PCVs covering more serotypes, we will see even greater effect on the nasopharyngeal microbiota and consequently effects on IPD and herd protection, and possibly on other infectious diseases. This so-called "ecological experiment" is important to monitor in order to give information for future vaccine choices and policies, as well as information concerning future need of treatments for the diseases that develop.

Few places have the opportunity to do quality surveillance in population based studies on both carriage and IPD in the same population and at the same time. In Stockholm County, Sweden, this was possible, both pre-and post-PCV implementation. In the Health and Demographic Surveillance Site in Iganga/Mayuge districts in Uganda, we could do population based carriage studies pre-vaccination, and use this data to calculate the potential vaccine efficacy.

Knowledge is still lacking from large, longitudinal population based studies on the effectiveness of PCVs in low-, middle-, and high-income countries, and on how to further the understanding on what is the long-term impact of an altered pneumococcal epidemiology, due to a change in the ecological niche. This is needed in order to further adapt vaccine policies and decrease mortality in children due to pneumococcal disease. This thesis addresses this lack of knowledge.

2 AIMS AND OBJECTIVES

General Aim:

To increase knowledge of the possible impact of pneumococcal conjugate vaccination on invasive pneumococcal disease, pneumonia, and sinusitis, as well as impact on carriage in healthy children

Specific Objectives:

To study the impact of the pneumococcal conjugate vaccine on pneumococcal carriage, on hospitalization trends and complications of pneumonia and sinusitis in children, as well as incidence, serotypes and resistance patterns of invasive pneumococcal disease (IPD), after introduction into the general vaccination program in Stockholm, Sweden

To study pneumococcal carriage and serotype distribution in healthy children <5 years prior to PCV introduction in Uganda, and estimate the potential effectiveness of the PCV

Research Questions:

- What is the impact of the introduction of PCV on invasive pneumococcal disease (IPD) incidence, serotype distribution, and pneumococcal antibiotic resistance in Sweden?
- What is the impact of PCV on hospitalisation trends and complications of pneumonia and sinusitis in children in Sweden?
- How does the introduction of PCV drive changes in colonizing *Streptococcus pneumoniae* serotypes and resistance patterns in preschool children in Sweden?
- Which pneumococcal serotypes colonized healthy children in rural Uganda before PCV introduction?
- To what extent do available PCV cover the circulating serotypes in Sweden and in Uganda?

3 MATERIALS AND METHODS

3.1 STUDY AREAS AND POPULATIONS

Studies I to III were carried out in Stockholm County in Sweden and study IV was carried out in a Demographic and Health Surveillance site (HDSS) in Iganga/Mayuge districts in the southeastern part of Uganda.

Stockholm County

Stockholm County had a population of 2.2 million in 2015. Of these, 22% were under than 18 years of age, and 7 % under five years (Statistics Sweden, www.scb.se). Stockholm, being the capital of Sweden, is mainly urban, but about half of its population lives in suburb municipalities. The total area of the Stockholm County is 6519 km^2. Data for the three studies were collected from 2003 to 2015 in this region. PCV7 was introduced, free of charge, for all infants born from the 1st of July, 2007 at age 3, 5 and 12 months, concomitantly with the hexavalent vaccine (DTaP-polio-Hib-HepB) or pentavalent (DTaP-polio-Hib) offered at Child Health Centers. This was started 15 months before the inclusion of the PCV in the national child vaccination schedule. In January 2010, PCV7 was exchanged for PCV13. No catch-up vaccination of older children had been offered. If a child had started the vaccination series with PCV7 it was changed to PCV13 in the final doses.

Health and Demographic Surveillance Site in Iganga/Mayuge districts

The Health and Demographic Surveillance Site (HDSS) is located 112 kilometers from the Ugandan capital, Kampala. Its total area is 155 km^2 out of which 90% is considered rural. There are 65 villages in the HDSS, with a population of 70,000 living in 13,000 households. 11,000 children are under the age of five years, which is about 16% of the total population. As in most low-income country settings, the major cause of death in the HDSS, apart from death due to neonatal causes, are pneumonia, malaria and diarrhea (134, 135). There are 13 health facilities and 121 pharmacies and private clinics serving the area. Our study IV was set in the pilot (2008), the baseline (2009) and the follow-up (2011) of a cluster randomized controlled trial cRCT that evaluated the impact of integrated community case management (iCCM) of malaria and pneumonia in children using antimalarials and antibiotics. In this trial, half of the villages were randomized to continue the national program with Community Health Workers (CHW) treating malaria in the villages and half of the villages to treat malaria, and additionally, pneumonia with amoxicillin. In April 2013, with the financial support of GAVI, PCV vaccination was launched in Iganga as the first district in Uganda. It was planned to be launched in other districts in a phased manner, but implementation has been delayed due to necessary preparations, such as cold chain systems, training of personnel, and storage capacity. PCV vaccination was therefore relaunched in January 2014 in 94 of the 112 districts. It is given simultaneously with other vaccines (DTwP-Hib-HepB and oral polio vaccine (OPV)) at 6, 10 and 14 weeks, together with inactivated polio vaccine (IPV), also

administered at 14 weeks. Uganda uses the 10-valent PCV. The PCV10 vaccination coverage rate of three doses was estimated at 50% nationally in 2015 (136).

The two study sites and populations represent a high- and a low-income country setting, very different in their health and demographic indicators (table 4).

Table 4. Health and demographic comparison between Sweden and Uganda, estimated in 2014-2015

Health or demographic indicator	Sweden*	Stockholm** County	Uganda*	Iganga*** Mayuge
Total Population (thousands)	9,800	2,200	37,700	93
Under five mortality rate (deaths/1000 live births)	3	-	66	114
Total fertility rate (children/woman)	1,9	1.8	5,9	4.8
Life expectancy at birth (years)	82	82	57	65
Maternal mortality ratio (maternal deaths/ 100 000 live births)	4	-	360	253
Gross national income per capita (PPP# in USD)	44,760	-	1370	-

*WHO accessed January 2015: http://apps.who.int/gho/data/node.country

** Center for Epidemiologic Statistics, Stockholm County

*** HDSS data from 2014 collected at the surveillance site. Due to differences in data collection it may show inconsistencies in relation to nationally reported data to WHO.

PPP is the purchasing power parity

Sampling

In study I, all reported IPD cases in Stockholm County from 2005-2014 were included. In study II all hospitalized children with ICD-10 coded exit diagnoses of interest in Stockholm County from 2003-2012 were included (specified in paper II). In study III all consenting children (2011-2015) and parents (2014-2015) visiting one of the 23 Health Care Centers chosen to be geographically representative of the Stockholm County were included.

Simple random sampling using probability proportionate to population size was only used in the HDSS in Uganda (IV). For the 2011 study, the sample size calculations of the cluster randomised trial were presented in the paper by Kalyango et al. (137).

Study I was a cohort study of all reported IPD cases in Stockholm County from 2005 to 2014 that had cultured pneumococcal isolates. Data on carriage in study I and III was collected prospectively from August 2011 to May 2015 in 23 of the largest Health Care Centers, chosen to geographically represent different areas of Stockholm County. For convenience and

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out of respect for the work load of the centers, we collected samples one week at a time at the different centers during a week chosen by the center. Each center was visited once per semester, and there had to be a month's interval between samples for a child to be sampled twice. In study III, IPD cases from Stockholm County in study I from August 2011 to December 2014 and an additional five months during January-May 2015, was used to calculate the invasive disease potential of colonizing pneumococcal strains.

Study II was a retrospective hospital register study of all children hospitalized in Stockholm County due to pneumonia, sinusitis and empyema.

Study IV consisted of cross-sectional surveys, with repeated collection of nasopharyngeal samples during a three year period, in healthy children under 5 years of age in Iganga/Mayuge HDSS.

3.2   DATA SOURCES AND COLLECTION

3.2.1   Mandatory reporting of IPD cases and strain collection (I)

It has been mandatory to report all cases of IPD since 2004 in Sweden. Cases have to be reported both by clinicians and microbiological laboratories. The patient is reported with his or her national personal identification number, which makes it possible to merge the laboratory and clinical reports. It is entered into software called SmiNet, accessed only after a personal identification control of authorized health professionals. The County Council medical officer and the Public Health Agency have full access for surveillance at County and national level, respectively.

Microbiological laboratories that performed the initial isolation of the pneumococcal strain sent the strains to the Public Health Agency of Sweden, Department of Microbiology, where serotyping and molecular typing were performed.

3.2.2   Register data (II, IV)

In study II, hospital registry data on discharge diagnosis was collected for all children 0-17 years of age hospitalized for pneumonia, sinusitis and empyema. Consequently we identified all children whose discharge diagnoses (first or secondary diagnosis) were coded as bacterial pneumonia, sinusitis or empyema by a senior pediatrician.

Pyelonephritis was used as the control diagnosis for general hospitalization trends. The diagnoses asthma, obstructive bronchitis, RSV infection and viral pneumonia were used to control that any change in bacterial pneumonia incidence was not due to a change in tradition of how to code a respiratory disease diagnosis. ICD-10 codes for all these diseases were identified. Data on age, gender and month of admission was recorded.

In study IV, data on socio-demographic indicators was collected from the database of the HDSS in Uganda. All families in the HDSS area were visited every three months for data collection in order to update the database on socio-demographic indicators. All children in the

HDSS database had an individual identification number that was connected to the household. Data on age, gender of the sampled child, and wealth quintile (87) of the household was collected from the database.

3.2.3   Questionnaires (I, III, IV)

Questionnaires were used in study I, III and IV. In study I, questionnaires were sent to reporting clinicians for all IPD cases. The questionnaires for patients older than 17 years of age were included (N=790) for 2007-2013. To increase the completeness of the clinical information on children, medical records were also studied for all children with IPD below 18 years of age. In study III all parents to participating children were asked questions on medical history and treatment, on-going or in the past. The questionnaire also included questions on risk factors for carriage such as having siblings, smokers in the family, lack of breastfeeding, having travelled abroad recently, as well as chronic illnesses. In study IV, questions focused on medical history and treatment. The answers were recorded, on paper during the 2008 and 2011 study and in personal digital computers during the 2009 study, by the research assistants during an interview. The questionnaire in 2008 and 2009 were nearly identical and limited to less than ten item, but the 2011 study questionnaire was a longer more comprehensive questionnaire since it also included in the evaluation of community health workers practice and performances and knowledge on malaria and pneumonia disease and intervention (137).

3.2.4   Medical records (I, II)

Clinical information and the diagnosis was verified in their medical records for all reported cases of IPD in children 0-17 years in Stockholm, between 2005 and 2014 (N=161) (study I). Information collected included chronic underlying disease, clinical development, microbiological results, diagnosis, treatment, and outcome in the form of mortality, recovery, or complications lasting more than three months.

In study II we verified all cases of sinusitis through studying and checking the medical records of all cases for a correct clinical diagnosis (N=678). This led to an exclusion of 76 cases due to incorrect diagnosis (skin infection, conjunctivitis or insect bite) or no clinical signs of sinusitis. We also validated 50 cases of pneumonia before and after PCV introduction. Concerning frequency of chest x-ray, chronic conditions, and severity, we found no major difference pre- and post PCV.

3.2.5   Nasopharyngeal samples (I, III, IV)

Nasopharyngeal samples were collected in Sweden and Uganda for study I, III and IV using two different methods.

In Sweden, an electric suction machine was used to collect the nasopharyngeal aspirates. A thin catheter was inserted vertically in the nasopharyngeal cavity until it was stopped at the posterior wall of the nasopharynx, and a sound of mucus being sucked was heard. We used this because our target population was children 0-<5 years, and with 18% of the children

being below 3 months of age, the suction tube was considered less painful. We also wanted to collect a large mucus sample to be able to divide the sample for future research. The mucus was divided into three tubes after being placed in three ml of NaCl, and the tubes were kept in a cool box at 5-8°C for a maximum of eight hours before arriving at the Microbiology Laboratory at Karolinska Institutet, Solna.

In Uganda, due to covering a large area with unsure electricity supply as well as sampling children mainly from 6 months of age, nasopharyngeal swabs were used for specimen collection. The swab was introduced vertically in the nasopharyngeal cavity until it reached the posterior wall of the nasopharynx, then slightly turned and then removed and placed in a sterile Amies medium. They were kept at ambient temperature until arriving, within 12 hours, to the Microbiology laboratory of Makerere University Hospital.

3.3 LABORATORY ANALYSIS

Strain isolation

To identify pneumococcal strains both in Sweden and in Uganda, colony morphology, α-haemolysis, optochin susceptibility, and deoxy chocolate/bile solubility were used. In Sweden, horse blood agar and chocolate agar were used when samples arrived in the Microbiologic Laboratory of the Karolinska University Hospital. In Uganda, the Department of Microbiology at Kampala University Hospital used sheep blood agar and chocolate agar for the initial identification of colonies. The next day colonies were identified and isolated on a blood agar plate with tests for antibiotic resistance.

Antibiotic resistance

MIC-values, the Minimal Inhibitory Concentration, for penicillin G using discs and Etests were used in studies I, III, and IV. In study III we also tested for antibiotic susceptibility using discs for erythromycin and clindamycin. In study IV antibiotic susceptibility testing was done, and the results were presented for the survey in 2008 (87). These will be presented elsewhere for the years 2009 and 2011. The EUCAST system of species-related breakpoints was used to classify isolates as sensitive, intermediate or resistant, S, I and R respectively. For penicillin G, the MIC breakpoint in mg/L was S≤0.06 and R>2. For erythromycin it was S≤0.25 and R>0.05. For clindamycin it was S≤0.5 and R>0.5.

Serotyping

Serotyping for all strains (both IPD (I) and carriage (III, IV)) was done by the Public Health Agency of Sweden. Serotyping was performed with a gel diffusion method, including 46 serogroup antisera. Isolated pneumococcal strains were cultured overnight on blood agar plates, harvested and diluted in 1% dextrose broth supplemented with 10% horse serum (DS solution), and again cultured overnight. The samples were then centrifuged, and the resulting pellet was dissolved in MQ water. Droplets of sample and antisera were put on gel in separated wells and incubated until the next day. The gel plates were controlled for precipitation to decide the serogroup. Isolates that were of a serogroup with several serotypes

were further analyzed with relevant serotype antisera and factor sera. The Quellung reaction was used for serotyping when serotype had to be confirmed after the gel diffusion method.

The antisera used came from SSI, the Danish Serum Institute, in Copenhagen, Denmark.

Molecular typing methods

Molecular typing methods were only used on selected isolates in study I to understand the genetic content of the isolates in addition to the identification of the outer polysaccharide serotype structures. The methods used were pulse-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

3.4 STATISTICAL METHODS

Descriptive statistics

Descriptive statistics of the study population, for example to show frequencies of pneumococcal carriage, antibiotic resistance, and pneumococcal serotypes, were conducted, and characteristics of the study participants were compared using chi-squared (χ^2) or Fischer's exact test statistics in all four studies. Fisher's exact test was used to compare variables between different groups (in study III: carriers/non-carriers and VT/NVT). Bivariate analysis of individual characteristics (age, sex, vaccination status, history of illness and drug intake) and socio-economic factors (wealth quintiles) in relation to carriage of a resistant strain were also calculated using χ^2 statistics (categorical values and proportions) or ANOVA (multiple observations, tests any significant difference between for example years (study IV)). Two-sided p-values <0.05 were considered statistically significant.

Incidence Rate and Incidence Rate Ratio

Incidence rate ratios (IRR) and their respective 95% confidence intervals (CI) were also calculated to compare the pre- and post-vaccine periods in both study I and II. Incidence rate was first calculated using number of cases (IPD or hospitalizations) divided by the 100,000 mid-year population per study year. Incidence rates from after PCV implementation were then divided with incidence rates before implementation to get the IRR.

Trend analysis

Trend analysis was used in study II to depict and analyze trends in hospitalization due to sinusitis, pneumonia and empyema before and after PCV implementation, excluding the years of introduction. Segmented regression analysis was applied to evaluate the monthly hospital admission rates (138, 139). Generalized linear models (GLM), assuming a Poisson distribution for the monthly admission rates, were fitted, while the Negative binomial distribution was preferred at the presence of over dispersion. Generalized additive models where used instead of GLM when necessary, in order to adjust for a seasonal effect. All models contained three basic parameters accounting for the pre-intervention trend, the change in level from the last pre-intervention point to the first post-intervention one and the

difference in trend between the two periods. The post-intervention trend and its standard error were derived from the combination of the first and third parameter. Correlograms were used to check for autocorrelation in the residuals, and the models were adjusted for first-order autocorrelation, when necessary.

Univariate logistic regression was used in order to study trends in antibiotic resistance and changes in proportion of individual serotypes over time in study III.

Simpson's diversity index

Simpson´s diversity index was used in study I and III to depict the diversity of serotype distribution. The Simpson's diversity index D is interpreted as the probability of two randomly chosen cases being infected with different serotypes. So when the diversity D increases, the likelihood of two cases being infected with the same serotype decreases, taking into account the number of cases and the number of different serotypes identified in one year. An increased diversity of strains may indicate serotype replacement if new and previously non-vaccine types emerge as a cause of IPD (140).

Multivariate analysis

Multivariate logistic regression was performed to evaluate risk factors for pneumococcal carriage and to estimate its association with the variables in study III and IV, after adjusting for confounding. In study IV, variables with p-value<0.2, and in study III with p-values<0.5 in the bivariate analysis, were tested in the multivariable model. Odds ratios (OR), p-values and 95% confidence intervals were calculated, and p-values of less than 0.05 were considered significant.

Invasive disease potential

Invasive disease potential was calculated for different serotypes in study III. The following formula was used for the serotype specific odds ratio: OR = [ad]/[bc], where *a* was the number of invasive X isolates, *b* was the number of carriage X isolates, *c* was the number of invasive non-X isolates, and *d* the number of carriage non-X isolates. An OR of >1, with its 95% confidence interval not including 1, indicated increased invasiveness while Holm's method was used to adjust for the multiple comparisons (141).

3.5   SUMMARY OF METHODS

Study focus	Design and methods	Study population and study sample	Statistical analysis	Years
I. Invasive pneumococcal disease and carriage in Stockholm, Sweden before and after PCV introduction	Population based cohort IPD cases by mandatory reporting system and collection of clinical data from medical records of children 0-<18 years Serotypes and clones of pneumococcal strains Comparing 3 years before and 7 years after PCV	All patients (n=2519) with IPD, all ages, in Stockholm County. Carriages strains N=260 before PCV N=647 after PCV of children <5 years.	Incidence rates, Serotype distribution and trends, Simpsons diversity index Chi square of clinical data on the children	IPD 2005-2014 Carriage 2004 and 2011-2014
II. Pneumonia and sinusitis and empyema hospitalization in children in Stockholm, Sweden before and after PCV introduction	Population based retrospective hospital registry study of discharge diagnosis Comparison of 4 years before and after PCV introduction Validation of the diagnosis in all cases of sinusitis and in a sub-set of 100 cases of pneumonia	All children 0-17 years hospitalized due to sinusitis (n=678) or pneumonia (n=5018) or empyema (n=60)	Incidence Incidence rate ratios Trend analysis Chi square	2003-2012
III. Carriage in Stockholm, Sweden after PCV implementation	Cross sectional study Nasopharyngeal aspirates and questionnaire data. All strains isolated from invasive disease, all ages in Stockholm.	Children <5 years visiting Child Health Centers (n=3024) and their parents (N=787).	Carriage prevalence Serotype distribution and PCV serotype coverage rates Chi square and logistic regression of risk factors for carriage Invasive disease potential	2011-2015
IV. Carriage in Uganda before PCV implementation	Cross sectional studies in a demographic and health surveillance site in south eastern Uganda NPH swabs and questionnaire HDSS socioeconomic data	Children <5 years (N=1761) 2008 n=150 2009 n=587 2011 n=1024	Carriage prevalence Serotype distribution and PCV serotype coverage rates Chi square and logistic regression of risk factors for carriage	2008, 2009, 2011

3.6 ETHICAL ISSUES

Ethical approval for all the studies was given by the Central Ethics Committee (national)(I) and Regional Ethics Committee (Stockholm)(I, II, III, IV). Study IV was first approved by the Uganda National Council for Science and Technology following review by the Institutional Review Board of Makerere University School of Public Health. Written consent was obtained from all participants where nasopharyngeal samples (Study III and IV) and questionnaire data (study III and IV) were collected. In the study in Uganda, interviewers were recruited locally and the questionnaires as well as the interview and consent forms were written in Lusoga, the local language. In Sweden, consent forms and questionnaires were available in Swedish and English.

It could be discussed whether to inform parents about the laboratory results of the nasopharyngeal carriage or not. Being a carrier of pneumococci in the nasopharynx may be part of the natural asymptomatic carriage and therefore not necessary or beneficial for parents to know about. However, being colonized with resistant pneumococci could guide future choice of antibiotics if the child turns ill shortly after being sampled. In Sweden, the families of the few children who carried pneumococci resistant to penicillin were contacted and reported according to the mandatory procedures to the public health authorities. In Uganda informing families in this way was not logistically possible. Another ethical consideration in Uganda was the set-up of the Health and Demographic Surveillance Site in itself. Residents of this area were subjected to all kinds of surveys in addition to the regular surveillance every three months. Community meetings with heads of villages and community health workers were conducted in order to explain the purpose of the study and promote awareness of the signs and symptoms of pneumonia and the importance of treatment. Verbal consent was also obtained from village and district leaders. The local leaders were instrumental in explaining the purpose of the study in order for the study participants to be able to make an informed decision on whether or not to participate.

4 RESULTS

4.1 INVASIVE PNEUMOCOCCAL DISEASE

The main aim of study I was to evaluate the impact of the pneumococcal conjugate vaccine on incidence, serotypes and resistance patterns of invasive pneumococcal disease (IPD) after introduction in the general vaccination program in Stockholm County, Sweden

In total there were 2529 cases of IPD in all ages notified in the mandatory reporting system, and over 90% of the isolates were serotyped (N=2336) from 2005-2014. We showed a good PCV effect on IPD for children <2 years (N=91). Incidence of IPD decreased from 28.4 to 10.3 cases /100 000 children <2 years of age (RR 0.36, 95% CI 0.2-0.6) overall and for the vaccine-types included in the PCV7 from 22.7 to 0 cases/100,000 children (RR 0.0 95% CI 0.0-0.1) comparing the time periods 2005-2007 to 2009-2014. However, in children <2 years old, the vaccine types not included in the 13-valent vaccine increased (RR 7.1, 95% CI 1.7-30).

All medical records of children <18 years old were studied in order to verify the clinical diagnoses (N=161). There was a significant decrease in meningitis (RR 0.43, 95% CI 0.2-0.9), septicemia (RR 0.32 95% CI 0.1-0.8) and rhinosinusitis (RR 0.11 95% CI 0.02-0.5), but not in pneumonia with bacteremia in children <2 years (RR 0.76, 95% CI 0.3-2.1). In children 5-<18 years there was a significant decrease only in pneumonia with bacteremia (RR 0.30, 95% CI 0.1-0.8).

One vaccine failure occurred in a one year old boy who had received two doses of PCV (6B). In post-PCV period, 95% of children aged 1-<2 years and 47% of children between 2-<5 years old were fully vaccinated with three doses of PCV. Data on vaccination status was available for 97% (36/37) of children 0-<2 years and 93% (14/15) of children 2-<5 years. In children 5-<18 years, no one of those with available vaccination data (33%, 5/15) had been vaccinated with PCV.

A decreased incidence of IPD was also shown in older children 2-<18 years (RR 0.39, 95% CI 0.2-0.7, p=0.002) and adults 18-<65 years (RR=0.62, 95% CI 0.5-0.7). However, in adults 65 years and older the overall rate of IPD remained unchanged (RR 0.90, 95% CI 0.8-1.0), due to an expansion of NVT. For the age group ≥80 years the overall IPD incidence was also comparable to the pre-PCV period (RR 1.04, 95% CI 0.8-1.3).

There was a significant decrease in PCV7 serotypes in all ages. All specific PCV7 serotypes except 4 and 18C, decreased after PCV7 vaccination in IPD compared to pre-vaccination. However, serotypes 4 and 18C decreased significantly during the PCV13 period, compared to pre-PCV7. Serotype 3 increased significantly among adults, especially older persons, despite being included in the PCV13, which was due to a clonal expansion of two preexisting clones. Of the 6 extra serotypes in PCV13 only 7F decreased significantly after PCV13 introduction in IPD in all ages. Among non-vaccine serotypes, type 8 decreased significantly post-PCV13 compared to pre-PCV, while the majority of the largest NVT increased significantly,

particularly after the PCV13 implementation. Two other preexisting clones expanded as well, in 22F in IPD and 11A mainly in carriage, but also in invasive isolates. Of the NVT, 23A, 10A, 22F and 33 F increased significantly post-PCV13 compared to pre-PCV in IPD.

The diversity of serotype strains in IPD as measured by the Simpson index of diversity increased significantly (p<0.001).

Overall the susceptibility to penicillin was unaltered after vaccine introduction among IPD isolates (4.4% before as compared to 6.0% after had a reduced susceptibility to penicillin G, p=0.84). There was an increase in the proportion of strains with decreased susceptibility to penicillin G in PCV7 serotypes, from 5.5% pre-PCV to 26% post-PCV13 (p<0.001).

Incidence rates of IPD were used as the standard measure for best comparability between studies. To understand the magnitude of the IPD decrease in the different age groups in a population of 2 million inhabitants, the mean number of annual cases/age group is used (table 5).

Table 5. Mean annual number of reported IPD cases per age group in Stockholm County before (2005-2007) and after (2012-2014) PCV introduction (population 2.2 million).

Age groups (years)	Mean annual number of cases 2005-2007	Mean annual number of cases 2012-2014	Total mid-year population in each age group in 2014*
0-<2	15	6	50 090
2-<18	9	3	416780
18-<65	144	92	1377070
65+	103	117	345100

*Population data from Statistics Sweden: www.scb.se

4.2 SINUSITIS AND PNEUMONIA

The aim in study II was to estimate the effect of the pneumococcal conjugate vaccine on hospitalization trends and complications of sinusitis, pneumonia and empyema in children after introduction in the general vaccination program in Stockholm County, Sweden.

During the study period 2003-2012, there were 678 children with severe sinusitis, 5018 children with pneumonia coded as bacterial, and 60 children with empyema, hospitalized and registered at any of the four hospitals treating children in the Stockholm County. The year of introduction of PCV (2007-2008) was excluded from analysis. The years were counted from July to June, to include the winter season in the study year.

Hospitalizations due to sinusitis decreased significantly in children aged 0-<2 years, from 70 to 24 cases/100 000 (RR=0.34, p<0.001), when comparing incidence rates from before the implementation, 2003-2007, to after PCV implementation, 2008-2012 (figure 8). The time trend analysis for sinusitis showed a significantly decreasing month to month trend in the post-intervention period for children 0-<2 years and 2-<5 years (p=0.018 and 0.004, respectively). There was also a drop in incidence from the last month pre-PCV to the first month post-PCV, however not significant (p=0.055).

Figure 8. Incidence of hospitalization due to sinusitis by age group in children in Stockholm County 2003-2012*
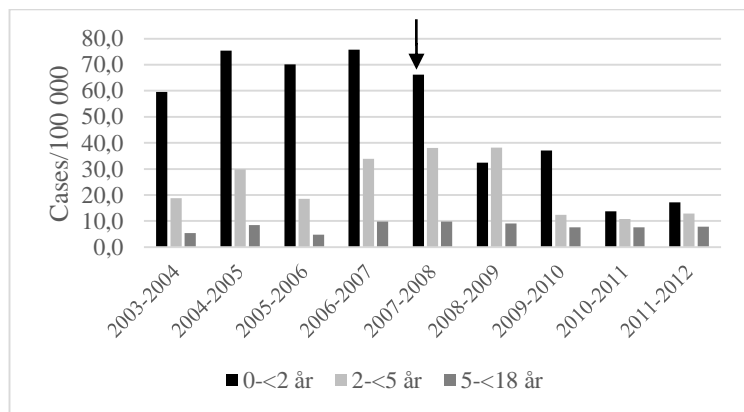
*The arrow marks the year of PCV7 introduction

Hospitalizations for pneumonia decreased significantly in children aged 0-<2 years, from 450 to 366/100,000 (RR=0.81, p<0.001) and in those aged 2-<5 years, from 250 to 212/100,000 (RR=0.85, p=0.002) comparing incidence rates before, with those after PCV implementation (figure 9). Trend analyses showed increasing month to month hospitalization rates for pneumonia in children 0-<2 years old pre-PCV. There was however a significant drop in hospitalization rates from the last month pre-PCV to the first month post-PCV in children 0-<2 years (p=0.002) and a decrease in month to month hospitalization rates due to pneumonia coded as bacterial in children aged 2-<5 years old post-intervention (p=0.02).

Figure 9. Incidence of hospitalization due to pneumonia coded as bacterial by agegroup in children in Stockholm County 2003-2012*
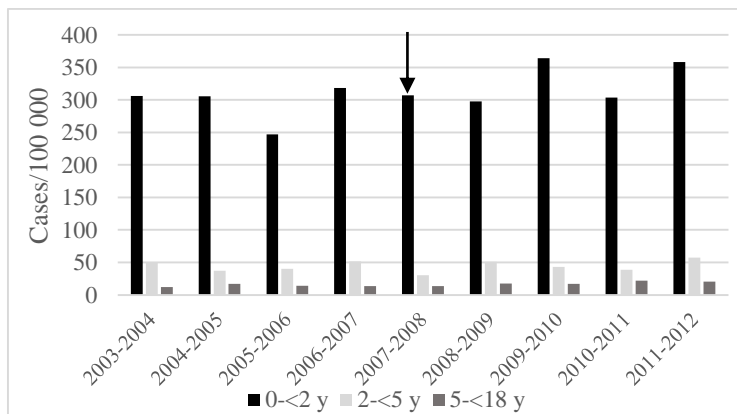

*The arrow marks the year of PCV introduction

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Hospitalization for empyema did not increase in any of the three age groups. The total number of hospitalized children due to empyema was low (N=60).

As a control for changes in hospitalization trends we collected data on a control disease not related to pneumonia (pyelonephritis). There was a significant increase in hospitalizations due to pyelonephritis in children <2 years (p=0.03) and 5-<18 (p=0.002) (figure 10). In the time trend analysis the month to month trend remained stable for these age groups both in the pre- and post-vaccine time.

Figure 10. Incidence of hospitalization due to the control diagnosis pyelonephritis in children in Stockholm County 2003-2012*

*The arrow marks the year of PCV introduction

To study if our results were due to changes in habits of physicians to mark diagnosis that were related to pneumonia coded as bacterial over the study period, we also collected data on hospitalization trends for viral pneumonia, asthma/obstructive bronchitis and respiratory syncytial viral infection. An increasing trend was found in the number of hospitalizations due to viral pneumonia in children<2 years (p=0.01) and respiratory syncytial virus (RSV) in all age groups (p=0.001) (figure 11). For viral pneumonia, however, the increase (from N=70 to 115 cases/year) was not considered in the same magnitude as the decrease of the number of pneumonias coded as bacterial (N=914 to 836 cases/year).

Figure 11. Incidence of hospitalization due to the control diagnosis respiratory syncytial virus (RSV) in children in Stockholm County 2003-2012*
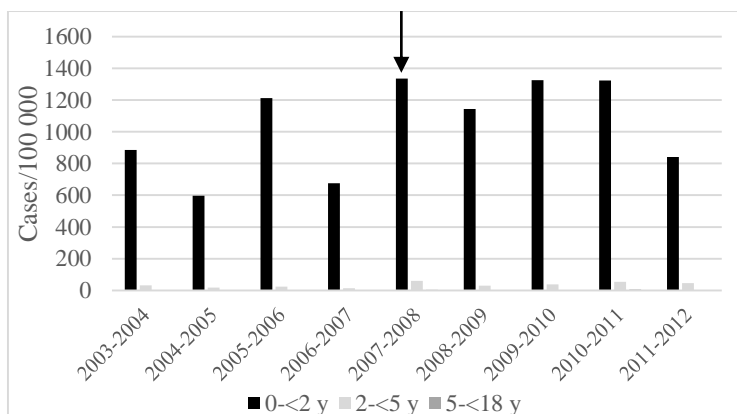
4.3 PNEUMOCOCCAL CARRIAGE IN SWEDEN

The aim of study III, and partly of study I, was to determine the impact of the pneumococcal conjugate vaccine on pneumococcal carriage, its serotype distribution and resistance patterns after introduction in the general vaccination program in Stockholm County, Sweden. Study III was done between 4 and 8 years after the introduction of PCV7. PCV7 was exchanged for PCV13 in 2010.

In total, 3024 children and 787 parents were included and sampled in this cross-sectional study, on regular visits at 23 Child Health Centers in Stockholm County between August 2011 and May 2015. Among these, 907 children and 27 of the parents carried pneumococci. Study I included the first three years of this carriage study, from August 2011 to July 2014 (N=647 isolates).

Carriage prevalence of pneumococci in children <5 years between 2011 and 2015 was 30% in total. Carriage prevalence were remained stable over the four years. However, there was a continued serotype replacement, with a shift from VT to NVT serotypes taking place during the study period (P=0.04). The percentage of NVT increased from 85% at the start to 94% at the end of the study period.

In study I we showed that there was a change in serotype distribution from VT to NVT comparing data from day-care centers in 2004 with data from August 2011 to July 2014 in carriage. Hence, most of the serotype replacement had already taken place before the start of carriage study III.

The top ten serotypes in proportion of all serotypes in the pre- and post PCV period is presented in table 6 for both IPD cases and carriage. In the pre-vaccine period most serotypes in both IPD and carriage were PCV13-VT, except serotypes 8 and 18F in IPD, and 16F in carriage. In the post PCV period, 5 of 10 top ten serotypes were PCV13-VT, but only one of 10 in carriage.

Table 6. The top ten serotypes in invasive disease in all ages compared to carriage in children in descending order. (PCV13 serotypes in bold), Stockholm, Sweden, 2005-2015

	Before PCV	After PCV
IPD all ages 2005-2007 and 2011-2015	**14, 9V, 7F, 4, 6B, 23F, 3**, 8, 18F, **19F, 19A**	**3**, 22F, **7F, 19A, 4**, 9N, 23A, **14**,11A, 8
Carriage in children 2004 and 2011-2015	**19F, 6B, 6A, 23F, 14, 18C, 3**,16F, NT, **9V**	11A, 35F, 23A, 21, 15B, 23B, 6C, **19A**, 33F, 35F

The diversity of serotype strains in carriage as measured by the Simpson index of diversity increased, however, non-significantly (p=0.06).

The major serotypes represented in colonizing pneumococcal isolates were all NVT: 11A, 23B, 35F, 21, 15B and 15C. The most prevalent VT was 19A, but that serotype decreased during the study period from 2 to 0% (p=0.02). In 2015, there were no 19F nor 19A neither in carriage nor in IPD in Stockholm, showing the effectiveness of PCV13 on these serotypes. Serotype 3 was the most common serotype in IPD of all ages, 2011-15, 14% (117/824). All but three of these IPD cases occurred in adults and serotype 3 carriage in children was also rare 0.3% (3/916).

No significant change in antibiotic resistance against penicillin G, erythromycin nor clindamycin was found. Antibiotic resistance levels were low relative to the situation internationally.

Risk factors for pneumococcal carriage were age 3 months-<3 years, day-care and open day-care attendance, having travelled abroad last year, and having siblings (all P<0,001).

Invasive disease potential was significantly increased (OR>1) for four NVT; 22F, 9N, 8 and 12F, however they represented only 3.6% (33/916) of the total number of carriage isolates in children. The majority of the NVT, however, 66% (525/799), had a significantly lower invasive disease potential (OR<1).

4.4 PNEUMOCOCCAL CARRIAGE IN UGANDA

The main aim in study IV was to show the pneumococcal carriage and the serotype distribution in healthy children <5 years of age prior to PCV introduction in Uganda. The secondary aim was to evaluate to what extent the available PCVs cover the circulating serotypes.

In total 1761 children under five years of age were included, 150 in 2008, 587 in 2009 and 1024 in 2011. 1723 children had a valid nasopharyngeal sample taken, and out of those 957 were identified as carrying pneumococci. The total nasopharyngeal pneumococcal carriage prevalence was therefore 56%. Carriage prevalences were 59%, 56% and 55% in 2008, 2009 and 2011, respectively. The highest carriage prevalence were found in the age group 6-11 months.

Out of the 957 pneumococcal isolates 852 (89%) were serotyped. The most common serotypes were 19F, 23F, and 6A. 75 children had 2 serotypes isolated. The most common non-vaccine serotypes were 29, 13, and 34.

The proportion of serotypes found among healthy children less than 5 years that are covered by PCV10, the vaccine included in the national vaccination program, was 42%. The serotype coverage for PCV13 was 54%. The proportions of non-vaccine PCV13 serotypes were 47, 45 and 46% in 2008, 2009 and 2011, respectively.

5 DISCUSSION

5.1 MAIN FINDINGS

The main finding was that PCV in Stockholm County has been successful in reducing both the incidence of IPD and hospitalization due to severe sinusitis and pneumonia in vaccinated groups. IPD incidence also decreased in unvaccinated age groups, except in the elderly (65 years and older). This effect seems to be mediated by the near elimination of most vaccine serotypes with high invasiveness in carriage, in children less than five years of age. Emerging in both IPD and carriage were instead non-vaccine types of pneumococci, mainly with lower invasiveness: a so called serotype replacement. Serotype diversity increased in IPD and carriage due to new emerging clones, likely affecting future vaccine strategies.

Carriage data before PCV introduction in Uganda showed that the PCV vaccine serotypes were much less prevalent in young children than they were in children before the PCV implementation in Sweden, which may potentially affect vaccine effectiveness in Uganda.

5.1.1 Pneumococcal morbidity before and after PCV introduction

Our results show a reduced incidence of IPD in vaccinated children (0-<2 years), in older unvaccinated children (2<18 years) and adults (18-<65 years), but an unaltered incidence in the elderly (>65 years) after vaccine introduction (Study I). Severity of clinical IPD was unchanged in children. Non-vaccine pneumococcal types in IPD increased. IPD caused by PCV13 types 3 and 19A increased post PCV7 vaccination, and new expanding serotypes/clones were identified in IPD after PCV13 was introduced.

The vaccine effectiveness, measured as reduction in IPD incidence, for all serotypes for children <2 years old was 64% (RR 0.36, 95% CI 0.2-0.6)(-18 cases/100 000) in Stockholm, Sweden. This effectiveness was rapid in this age group and as already observed after the PCV7 period (2009-2010), the risk of IPD was reduced by 60% (RR 0.40 95% CI 0.2-0.7). The PCV effect in Stockholm is similar to most European countries such as Norway, Denmark, England, and Germany (142-146). The PCV7 effectiveness in North America and Australia was even better, with incidence reductions in vaccinated younger children ranging from 73-85% (115).

In young children, meningitis, rhinosinusitis and bacteremia decreased significantly as causes of IPD, but bacteremic pneumonia did not. Other countries have reported significant decrease in pneumococcal meningitis after PCV (61, 147), but a recent study from the US did not observe any decrease in pneumococcal meningitis after the change from PCV7 to PCV13 (148). Our study is to our knowledge the only study that has specifically shown a significant decrease in sinusitis as underlying focus of IPD in children after PCV introduction. Most studies report only on the IPD outcomes: meningitis, bacteremia and bacteremic pneumonia. The verification of all diagnoses of children with IPD in Stockholm from 2005-2014 (N=161) strengthens the validity of our results. Additionally, hospitalization due to sinusitis, and pneumonia coded as bacterial, was shown in study II to be reduced by 19% and 66%

respectively. Based on a number of clinical parameters it was concluded that the children had the same clinical severity (days of treatment, proportion in intensive care, fever, CRP, etc) in the pre- and post PCV period. These clinical parameters were statistically compared individually between the pre- and post PCV time periods.

Initially, data was analyzed with the PCV7 and PCV13 periods together, but later we conducted separate analyses of two years of PCV7 use and four years of PCV13 use. This gave us useful insights into how individual serotypes are responding to PCV. There were no cases of the additional six PCV13 serotypes in the final years of the study in children <2 years old, showing that PCV13 gives protection against all included serotypes.

In children 2-<18 years of age the decreased risk of IPD after the PCV7 period was non-significant, possibly due to a lack of power. After the PCV13 period, a significant decrease in RR for IPD in ages 2-<18 years was observed which was almost as high (RR 0.39 95% CI 0.2-0.7) as that for the children <2 years (RR 0.36 95% CI 0.2-0.6). It seems therefore that herd effects may be expected, but only after a few years' time, as seen in the UK (149). Also, nearly half of the children 2-<5 years were fully vaccinated in the post-PCV period 2008-2014.

The absence of herd protection for the population >65 years of age of our study in Sweden, resulting in no impact on overall invasive disease, could be explained by an increase in non-vaccine serotypes, but also by the six additional serotypes in PCV13 still causing IPD cases in this age group. This was a surprising result because in many other countries, there has been a considerable decrease in IPD also in the elderly, for example in the US, UK and in Norway (149-151). In Quebec however, there was a non-significant increase in IPD incidence in people >60 years of age (152). The herd effect of PCV in older age groups therefore seem context dependent (132). One plausible explanation may be that the non-PCV13 serotypes spreading in the Swedish context cause IPD more easily in the elderly. Another explanation might be that the time of observation was not long enough to show a herd effect in Sweden in the elderly, compared to other countries. In the meta-analysis by Feikin et.al. from 16 countries it was reported that it took seven years before a barely significant decreased RR for IPD in adults 18-49 years of age and those >65 years of age was shown. Even if VT IPD decreased significantly already after five years of PCV use in children, this was counterbalanced by an increase in NVT (114). In Stockholm, Sweden, the RR was not compared yearly but for time periods, before (2005-2007), after PCV7 (2009-2010), and after PCV13 (2011-2014) respectively. The study in Stockholm continued seven years after the PCV introduction (1 year of introduction (2008), 2 years of PCV7 and 4 years of PCV13), but still the overall IPD incidence did not change.

Another reason for delayed or low PCV herd effect in the elderly may be fewer intergenerational social interactions in Sweden compared to other countries studied. However, results show that the PCV7 serotypes decreased in IPD for ages >65, by 55% (RR0.45 (95% CI 0.3-0.6) after the PCV7 period and by 85% (RR0.15 95% CI 0.1-0.2) after the PCV13 period. NVT increase in IPD increased however, and this indicated a herd effect

of the PCV rather than absence of contacts with vaccinated children or due to a natural serotype fluctuation.

One could argue that vaccinating children should be done for their benefit only, without necessarily focusing on the effect of vaccinating children on other age groups. PCV effectiveness is naturally most often measured in vaccinated children, but in a high income country, with low child mortality due to pneumonia or other pneumococcal diseases, the number of cases and incidence of IPD in the ages >65 years far outnumbers that of young children (table 5). Any measurement of the cost-effectiveness of PCV vaccination in children thus needs to include the herd effect on other age groups as this will influence the results (153, 154). An recent example of this is a cost-effectiveness study in Australia, post-implementation 2005-2010, that concluded that the PCV childhood vaccination program had averted about 5,900 hospitalizations and 160 deaths from IPD in all ages (155). The Australian vaccination program was however not cost-effective unless the herd effect of non-invasive pneumonia deaths in the elderly was included in the analysis.

Preliminary Swedish national IPD data from 2015 show a continued decrease in cases <5 years with 23 cases in 2015 (n=35 in 2014), but an increase to 1314 cases in all ages (1160 cases in 2014) (data from the Public Health Agency of Sweden). This trend demands further close surveillance. The gain in IPD incidence among children may be outnumbered by the emergence of IPD cases due to NVT in the elderly.

Hospitalization due to sinusitis, pneumonia or empyema

PCV7/PCV13 vaccination led to a 66% decreased risk of hospitalization due to sinusitis and a 19% decreased risk of hospitalization for pneumonia in children aged 0-<2 years, when comparing four years before with four years after vaccine introduction. A decreased risk of pneumonia hospitalizations in children has been shown in other countries, but the decreased risk of hospitalization due to sinusitis had to our knowledge not been documented in a population-based study previously.

Other studies have indicated that PCV will likely have an impact on sinusitis, since between 20-40% of cases are estimated to be caused by pneumococci (156, 157). A study from the US showed no change in rates of outpatient visits from 1998-2007 in the US (158). In 2015, however, another study from the US also showed a decreased hospitalization rate due to rhinosinusitis. It also reported a slightly increased complication rate requiring surgical procedures in children, and the average age at hospitalization due to rhinosinusitis increased from 5 to 6 years after PCV7 (159). Both in the US and in Sweden the decreased hospitalization rate for rhinosinusitis occurred soon after PCV introduction. This effect is most probably due to a decrease in the VT included in the PCV7/13 in carriage, which then spreads locally to the sinuses. Unfortunately, we had no laboratory data available to confirm this hypothesis in our study. In study I it was however confirmed that invasive pneumococcal disease due to rhinosinusitis decreased significantly in children <2 years of age (160). The impact on the rates of complications needs to be further explored if, as suggested by other

studies (132), there is a replacement with other bacteria such as *S. aureus* as a cause for sinusitis. It might be that *S. aureus* is responsible for some of the increased complication rates seen in the US, but that still needs to be confirmed.

In our low-mortality context, we estimated that 715 hospitalizations for pneumonia in children <2 years and 644 children 2-<5 years were prevented during the four years of observation post-PCV. It was estimated that 393 hospitalizations due to sinusitis in ages 0-<2 years and 121 cases in ages 2-<5 years were prevented during the period of observation. This constitutes a considerable public health impact. Even if the decrease in incidence of hospitalization due to bacterial pneumonia after PCV was lower compared to the effect on hospitalization due to sinusitis, this effect may have a more significant public health impact, particularly if the results are transferable to low-income countries.

The results of the trend analysis on pneumonia hospitalization indicate that the decrease is due to the PCV introduction, as explained by the rapid decrease in pneumonia hospitalization after PCV introduction in children <2 years and a significant month to month decrease in children 2-<5 years old in the post-intervention years. It seems plausible that PCV first protects the vaccinated age groups and then provides a herd effect, combined with the fact that the vaccinated younger cohort grows older and becomes included in the older age groups.

An increase of empyema, as suggested in some post-PCV7 implementation studies (29, 131), could not be shown in our study. While there was an increasing trend of hospitalization with empyema, it was not significant, and a beta-error due to lack of power can not be ruled out. In the UK, empyema increased before PCV implementation, but remained stable after its introduction (161). A possible explanation for an increase in empyema complications is that other bacteria or serotypes, more prone to cause empyema, may emerge following pneumococcal VT decrease in the population. Serotypes 1, 3 and 19A, often seen in empyema, were not part of the first conjugate vaccine PCV7 (55).

This study adds to the evidence that PCV vaccine (PCV7/PCV13) prevents severe rhinosinusitis and pneumonia hospitalization in children, with implications for global child survival.

5.1.2 Pneumococcal carriage before and after PCV, comparing Sweden with Uganda

Carriage after PCV in Sweden

The shift from vaccine types (VT) to non-vaccine types (NVT) was nearly completed four years after introduction of the PCV vaccination and this serotype replacement continued to evolve from 4 to 8 years after PCV7 introduction in Stockholm County. At the end of the study only 6% of the serotypes in carriage in young children were of VT.

There is agreement in the scientific community that PCV gives rise to serotype replacement in carriage (132, 162, 163) as also shown in study III. It is less common to compare carriage

prevalence with population-based IPD incidence in the same time period. These data were available in Sweden, enabling an estimation of invasive disease potential of emerging NVT. Invasive disease potential is defined by the proportion of a certain serotype in IPD as compared to the proportion present in carriage. It was reassuring that the majority (66%) of the emerging NVT in carriage in Stockholm had lower invasive disease potential and only 3.6% had a significantly increased invasiveness potential. The emerging NVT serotypes in carriage in the Swedish context were mainly 22F and 9N. While 12F and 8 had even higher OR for invasiveness potential, it was rare to find them in carriage (0.1%). 24F has emerged post-PCV in several European countries, but was not identified in Stockholm (164-166). Invasiveness disease potential was also studied in France, and results similar to the Swedish ones were found, i.e. low OR for invasiveness for most NVT (164). 24F and 12F were the only NVTs with high invasiveness potential in France. In Alaska, invasive ratios of pneumococcal isolates in carriage did not change pre- and post PCV, but 66% of serotypes had high invasiveness (167). Other studies have used case:carrier ratio or attack rates to measure invasiveness of serotypes (168, 169). A study from Finland built a model to predict the optimal serotype composition in a given context, based on serotype distribution and case:carrier ratios (170). The study suggested that a new PCV vaccine should contain the serotypes 22 and 9N. This could also be concluded from the results in study III, since these serotypes were the ones most prevalent NVT in IPD and both had a high invasive potential in Sweden 2011-2015.

It must be remembered that not all serotypes causing IPD appear in carriage. In 2005-2006 in Stockholm, IPD in the age group 0-<2 had a PCV10 and PCV13 serotype coverage of 81% and 93% respectively. However, the PCV10 and PCV13 coverage in carriage in children <5 years was 63%, and 82% respectively in 2004.

There is a concern that there will be a decreased benefit of PCV with time due to a nearly complete decrease in VT in carriage and, to a lower extent, in invasive pneumococcal disease, and also an expansion of NVT in carriage and disease that will out-number the decrease in VTs. However, both modeling studies and effectiveness studies show continued overall benefits of PCV in Europe and the US (163, 171). There is a discrepancy with more serotype replacement in both carriage and disease in Europe compared to in the US (16, 118, 150). The reasons for this difference in magnitude of replacement is not known. In indigenous populations in Alaska and Australia there is also a more pronounced serotype replacement, in both disease and carriage, than in the general population in those countries (172, 173). Replacement in disease after PCV introduction, with increasing incidence of pneumococcal meningitis in children 5-15 years, was shown in France during a period of low vaccination coverage of PCV7 (174). Following high coverage of PCV13, this worrying trend in France disappeared. This shows the importance of how well the PCV program is implemented. In contrast to France, the US reached a high coverage of PCV7 within a few years. This program included a catch-up vaccination for children <5 years, which resulted in a fast and beneficial herd effect in other age groups (150, 175). The extent of the serotype replacement in carriage and disease has yet to be determined for the African continent after PCV rollout.

Carriage before PCV in Uganda

Nearly half of the serotypes colonizing healthy children (46%) in Uganda were serotypes not covered by any of the current PCVs. The serotype coverage rate was 42% for the 10 serotypes in PCV10, which is the vaccine currently being implemented in Uganda. Carriage data before PCV introduction in Uganda shows that PCV vaccine-types were much less prevalent in young children (PCV10 42%, PCV13 54%) than it was in children before the PCV implementation in Sweden (PCV10 63%, PCV13 82%), which may potentially affect vaccine effectiveness in Uganda.

Serotype distribution pre- and post-PCV is studied as a proxy for the expected effect of PCV on IPD and mucosal infection and it may provide information on the expected PCV prevention due to herd effects (176). Laboratory based active surveillance for IPD or hospital based electronic registries are generally lacking in low-income countries, and as a result cross sectional carriage data for the evaluation of PCV program effectiveness is more feasible to accomplish (177).

In a systematic review of low- and lower-middle income countries, carriage prevalence of pneumococci and other bacteria (*H. influenza, M. catarrhalis, S. aureus, N. meningitides*) were studied (69). Of the included studies, 38 were from African countries and 21 from Asian countries. The carriage prevalence of pneumococci varied between 20% and 93%. In our study in Uganda, the mean pneumococcal carriage prevalence was 56%, over the three years studied. The most common serotypes carried in the systematic review were 6A, 6B, 19A, 19F and 23F, but also 14 and 11A. In the African studies, between 36% and 56% of the carried serotypes in children less than 5 years were of PCV7-types, between 37-56% for PCV10 and 50-64% for PCV13. Our study in Uganda shows similar results for PCV10 serotype coverage, 42%, and 54% for PCV13 respectively. The most frequently isolated pneumococci were 19F (16%), 23F (9%), 6A (8%), 29 (7%) and 6B (7%), similar to the systematic review except NVT 29 being more prevalent, and 19A was only seen in the last year of the study at a prevalence of 2.7%.

The potential of saving childrens' lives with PCVs is higher in African countries, where the disease burden is higher, compared to Europe, even if VT serotypes are not as prevalent in Africa compared to pre-PCV Europe. Data on the impact of PCV in African countries has however up to now been scarce. In six West African countries a recent review on IPD serotypes showed a PCV10 serotype coverage of 68% overall, varying from 51-80%, except in Burkina Faso which had 39% (178). In East Africa an IPD surveillance network in 2009 published a study showing a IPD serotype coverage rate for PCV7 of 56% in children 6-29 months (179) while in Uganda, IPD serotype coverage was 56% for PCV7, 58% for PCV10 and 79% for PCV13. This difference in PCV serotype coverage in carriage, which was lower in our study, can partly be explained by serotype 1 and 5 which are rarely seen in carriage but accounted for 30% of the cases of bacteremia and 18% of the cases of meningitis (179). In Sweden there was also a discrepancy in serotype coverage when comparing IPD with

carriage, consisting of a pre-vaccination difference of 18% for PCV10 and 11% for PCV13 respectively (study I).

In a randomized controlled trial in children aged 6-51 weeks in the Gambia, the effect of the PCV9 candidate vaccine showed an all-cause mortality decrease by 11% (95% CI 3-28%), and for each case of IPD prevented, 15 cases of radiologically confirmed pneumonia were prevented (20). This trial also showed a 50% (95% CI 21-69%) overall VE for IPD incidence, from 380/100 000 in the placebo group to 190/100000 in the PCV9 group. In rural Gambia, pre-PCV coverage for IPD of PCV10 and PCV13 serotypes was 26.6% and 46.8% respectively in carriage in children < 5 years (180). In a RCT in South Africa of the PCV9 candidate vaccine, vaccine efficacy was 85% for first episode of vaccine-type IPD for HIV negative children, but only 63% for HIV positive children (181). These were however results from an optimal trial situation, and in real life, poorer vaccination coverage, lack of timeliness and completeness of PCV schedules, will likely reduce the effectiveness.

A few effectiveness studies post-PCV implementation are beginning to show promising reductions in IPD incidence in low- and middle income countries (176, 182-184). South Africa implemented PCV7 in 2009, in a 2+1 schedule at 6 weeks, 14 weeks, and 9 months respectively, and they have a nationally active laboratory based surveillance system that allows post-PCV impact surveillance (176). The vaccination coverage for three doses of PCV was 90% in 2011 and 99% in 2012 (185). In an ecological study comparing reported IPD cases in pre-PCV with post–PCV periods, rates of IPD in children <2 years decreased 69% (95% CI 65-72), from 55 to 17 /100 000, including a PCV7 serotype decline of 89% (95% CI 86-92)(184). This reduction in IPD reported is similar to what we showed in our high income setting (study I) in the same age group.

A population based surveillance study in the Gambia, five years after PCV13 introduction, showed a 55% decreased incidence of IPD in children 2-23 months old, from 253 to 113 per 100 000 population. The decrease was due to an 82% (95% CI 64-91) reduction in PCV13 serotypes (182). In Brazil, where PCV10 vaccination was implemented in 2010, IPD incidence in children 2-23 months d decreased by 44% (95% CI 16-72%); however the extra three serotypes in PCV13 increased in all ages, while IPD incidence increased in adults aged 18-<65, and as much as 79% (95% CI 62-97) in cases >65 years of age (183).

Effects of herd immunity protection by PCVs in adults in low- and middle- income countries is not well known due to scarce data on adult pneumococcal disease burden for different age groups and countries (19). However, in South Africa, the rates of PCV7 serotypes declined by 57% (95% CI 50-63%) in adults aged 25-44 in the national laboratory based active surveillance study, comparing pre-vaccine years with post-PCV year 2011-12 (184).

Vaccine effectiveness studies in African countries must take into account the HIV epidemic, both when it comes to the elevated pneumococcal disease burden and potential vaccine efficacy, in treated or un-treated people (176). In South Africa, a pre-PCV study has shown that the rate of acquisition of new serotypes in carriage was no different for mothers that were

either HIV positive or negative, 18.9% and 19.5% respectively, but that PCV7 serotypes were acquired more often by HIV-infected mothers (10% versus 6.4% p=0.03), and PCV7/13 serotype acquisition by mothers was associated with carriage of those serotypes in children. Therefore the authors suggested that there is a reservoir of PCV serotypes in HIV positive mothers which could delay the vaccine effectiveness in high HIV settings (186). The VE for IPD in HIV-infected children shows some conflicting results (176, 184, 187). However, most likely the benefit of PCV in HIV-infected populations will be greater that in HIV un-infected ones due to the higher disease burden in this group, and even more so if the HIV-infected people are undergoing ART treatment (132). Also, PCV13 decreased VT carriage in both HIV infected (OR 0.32) and un-infected (OR 0.37) children in Soweto (188).

Cost-effectiveness studies before and after the introduction of a new vaccine are important due to competing costs when resources are scarce (46). To introduce the PCV in Gambia, at 7 USD/dose, was estimated to raise the implementation cost of a fully vaccinated child by 45% (25 USD)(189). Uganda has a health budget of about 59 USD per capita per year (190). With the GAVI negotiated prices, a cost-effectiveness study in Uganda estimated that PCV could save 10 796 lives, and prevent 94 071 IPD cases of *S. pneumoniae*, without counting the non-invasive pneumococcal burden, and could be cost saving with a gain of 0.6 million USD in direct medical costs (190). The results remained highly cost-effective even at the non-GAVI subsidized price of 3.5 USD. A 42% reduction in the number of cases and deaths due to invasive pneumococcal disease was estimated, which seems reasonable in comparison to the effectiveness studies mentioned from the Gambia, South Africa and Kenya.

Our results of a moderate PCV serotype coverage in Uganda should definitely not discourage its use, however it may help in choosing between different PCVs.

5.2 METHODOLOGICAL CONSIDERATIONS

How to show the real public health impact of PCV?

The randomized placebo controlled trial, preferably double blinded, is the gold standard for evaluating vaccine efficacy in pre-licensure trials. It needs to be used in phase II to III trials in order to show the safety and efficacy of a new vaccine before licensing (191). The randomized controlled trials are labor-intensive and costly and focus on a few and pertinent outcomes for a vaccine, comparing vaccinated and un-vaccinated groups (192). Post-marketing studies following the large scale implementation of a new vaccine are important for determining the effectiveness of the vaccine in a natural population and to monitor rare side-effects that could not be found during the licensing process. The real public health impact of a vaccine may also be demonstrated through these kinds of studies.

Comparing some of the newer vaccines, like PCVs, with the older vaccines in the Expanded Program on Immunization (EPI) schedule, the well-defined end-point of a laboratory confirmed case is more seldom, or sometimes not at all, obtainable. PCVs were developed to protect against IPD, but this is only the tip of the iceberg of the disease burden of pneumococcal bacteria. Meningitis, septicemia and bacteremic pneumonia may be

underdiagnosed due to difficulties in pneumococcal bacterial growth. Furthermore, pneumococcal bacteria are isolated from only a small percentage of pneumonia cases and from an even smaller one in otitis media. There is a large potential to also protect other un-vaccinated older age groups from pneumococcal disease through PCV vaccination of children. Therefore, in order to measure the real public health impact of PCV we need to use proxy measures of the real effectiveness (193). Gessner et al. argues that the vaccine preventable disease incidence (VPDI) per 1000 person-years should be used (194). They argue that PCV has a higher vaccine efficacy than rotavirus vaccine (particularly in low-income countries), but that the VPDI is higher for rotavirus vaccines against severe diarrhea, as compared to PCV against meningitis or severe pneumococcal pneumonia. They also discuss the use of vaccine probes to evaluate unknown disease burdens (for example influenzae) following implementation of another vaccine (example PCV) (195).

Another example of the wider public health impact of PCV is its effect on hospitalization due to influenza as seen in for example the US (196), and decrease in respiratory syncytial viral (RSV) disease during a PCV trial in South Africa (83). PCV appears to have an impact on other infections, indicating that co-infection of pneumococci and viral diseases lead to more severe diseases (83, 197).

This thesis does not intend to demonstrate the full public health impact of the PCV implementation in Sweden, but attempts to go beyond looking solely at the impact on IPD in the vaccinated groups by including the impact of PCV on IPD for all age groups. A decrease in sinusitis and pneumonia hospitalization in vaccinated and un-vaccinated children is also demonstrated and the dynamics of carriage is studied to further explain the herd effect of the vaccine.

Study design

A retrospective (or historical) cohort study design was used in study I. The cohort was the whole population residing in Stockholm County from 2005 to 2014. In this population all reported cases of IPD were included. A case was defined as a positive pneumococcal bacterial culture from a sterile compartment, such as the blood, cerebrospinal fluid (CSF) or bone. In a cohort study the exposure (vaccination in this study) is usually measured at the start of the study and then the cohort is followed for the outcome (disease). This may be very costly, particularly if the studied disease is rare. We collected data on risk factors and clinical outcomes retrospectively for the IPD cases by submitting a questionnaire to reporting clinicians and validating the medical records of all children with IPD from 2005 to 2014. Incidence rates were calculated using mid-years population size per age group obtained from Statistics Sweden. It could be argued that Stockholm is not a closed cohort and that people move in and out during the year, and might fall ill away from Stockholm, thereby not contributing to the statistics. One limitation in this study was the low response rate on the clinical questionnaire including data concerning PPV or PCV exposure for the adults (54% in 2007 and 79% in 2009-13). By contrast, our data for children was complete since all medical records were accessible. Additionally, the County Medical Officer´s Office contacted all

families by phone to complete the vaccination data for each child. Another limitation is that the detection and thereby frequency of IPD cases recorded depends on the habit of taking samples of blood and CSF for bacterial culture by the clinicians. Clinicians confirmed that no major shift in sampling habits had taken place over the study period, but no denominator data on the total number of samples taken was available. Generally the disease severity of invasive pneumococcal disease calls for the taking of blood cultures, but the true number of cases might be underestimated through the exclusion of the less severe cases. It is believed that blood sampling is more frequently done in for example the US (150), which could be one explanation as to why the incidence of IPD pre-PCV was much higher than in for example Sweden or Norway (142, 198). Of importance to our study, though, is that blood sampling habits seem not to have changed pre- and post PCV. The decrease in incidence of IPD is therefore probably not due to less samples collected, which, although it cannot be excluded, no strong evidence exists for.

An ecological study design was used in study II. In ecological studies, groups of people are compared in relation to an outcome. These studies are useful for generating a hypothesis based on a correlation between a phenomenon and an outcome. In our study it was the introduction of the PCV and the hospitalization of children due to pneumonia or sinusitis. In ecological studies, causal links cannot be made and it may be difficult to examine potential explanations for the findings (199). A difference in the selection of cases between study II and study I was that all IPD cases were laboratory confirmed cases (I), but no firm case definitions for bacterial pneumonia, empyema or sinusitis existed in the discharge diagnosis (II). To avoid over-interpretation of our correlations we also searched for alternative explanations to various hospitalization patterns, looking at unrelated diagnoses (pyelonephritis) and related respiratory diseases (asthma, obstructive bronchitis and viral pneumonia). We also validated all cases of sinusitis in children in the medical records and a sub-sample of 50 medical journals of children hospitalized due to pneumonia before and after PCV introduction. The validation of the pneumonia cases showed similar rates of x-ray and clinical parameters indicating that the diagnosis of pneumonia coded as bacterial was similarly determined before and after PCV. Despite this there was an increase in the hospitalization of viral pneumonias and RSV after PCV implementation as compared to before. One explanation for this may be the increased use of viral diagnostic tests at the pediatric hospitals. There may have been more children in the pre-vaccine era classified as having bacterial pneumonia rather than viral pneumonia which may have caused us to overestimate the true effect of PCV on bacterial pneumonia hospitalizations. The ecological study design does not exclude this possible misclassification between pneumonia coded as bacterial or viral or RSV, which is why the results must be interpreted with caution.

The pneumococcal carriage studies III and IV were of cross-sectional design. Cross-sectional studies may measure the prevalence of a disease and always measure exposure and effect at the same point in time. In cross-sectional studies it is however not possible to evaluate which came first: exposure or outcome. Natural fluctuations and clonal expansion in pneumococcal carriage and IPD (study I, III and IV) may of course be the results of other factors such as

antibiotic treatment practices, immunization coverage rates of influenza and PPV vaccine in the elderly and PCV in children and risk groups. However, questionnaire data in studies III and IV was used to evaluate factors affecting carriage, such as vaccination status (III), antibiotic use and socio-demographic data.

In studies III and IV, we performed repeated cross-sectional studies in the same population using simple random sampling in Uganda and a geographically representative sample in Sweden, which allowed us to study the longitudinal trend of carriage in these two populations. In both these studies we also collected information through questionnaires and also via the HDSS data base in Uganda on risk factors and sociodemographic indicators. A limitation of the study in Uganda was that although we used a random sampling and a similar questionnaire over the three years of the study, there were many differences in the study population over the three years (age, wealth quintiles, ill in the last 2 weeks, and symptoms during those weeks) (IV). This may be due to the fact that we did not use exactly identical questionnaires or survey methods during the period of study. Seasonal effects may have influenced the variance in the sampled children's data, e.g. disease burden. There was a school holiday during the last cross sectional year which might explain the lower percentage of included children that year. The studied population size varied from 150 to 587 to 1024 children between 2008, 2009 and 2011 respectively. To meet the requirements of the larger group of children studied, more field workers were recruited and may have asked the questions in the questionnaire differently. The population in the HDSS area is used to field workers asking questions, and there may also be a desirability bias of wanting to answer affirmatively to questions. This may for instance have led to an overestimation of the disease burden, if more respondents said that their children had been ill in the last two weeks than what was really the case. When it comes to the main outcome the pneumococcal carriage, the rates remained stable over the three years. So even if sampling and methods differed slightly across the study years, the serotype distribution probably reflects a representative sample in the child population in the area.

The study of carriage (study III) at Child Health Centers in Stockholm during four years, starting four years after PCV introduction, utilized standardized methods, and all data collection was done by the same five nurses, who had all received the same training and level of supervision.

In this thesis three out of four studies were population-based (study I, II and IV). This means that cases are sampled from the whole population at risk, allowing us to calculate incidence rates of disease. In study I and II we collected cases from mandatory reporting registries for IPD, and discharge diagnosis registries for sinusitis and pneumonia, from all available hospitals caring for children in Stockholm County. Children not residents of the Stockholm region were excluded (III). In study IV it was population based since all sampled children were registered in the HDSS data base and had an equal chance of being sampled. However, not everyone sampled was at home at the time of the survey. This limitation could have made the study population less representative, if for example certain maybe less ill travelled less.

Study III was not population based because the included Child Health Centers were not chosen randomly but rather chosen in order to be geographically representative of Stockholm, and in addition only large centers were included. Thus, the consenting parents may not be representative of the whole study population.

Selection bias:

Selection bias is a systematic error in epidemiology (199, 200). This is different from the random errors due to chance in the sampled subjects. Random errors can usually be corrected with a larger sampled base using a calculation of sample size with accurate assumptions. Selection bias, however, arises from procedures for selecting cases or factors that influence participation in a study so that the characteristics of the study subjects included are not the same as for those not included.

The risk of selection bias was most obvious in the carriage study in Sweden (study III). Families were offered to participate in the study following their regular visits at the Child Health Center. Before consenting they had received information about the study through posters posted weeks in advance at the Child Health Center and had received flyers informing about the study either at an earlier visit or in the mail. Then we asked the pediatric nurse to explain the purpose of the study, or at least to inform parents that they could get more information from the study research nurses. Unfortunately, due to the time constraints of both parents and nurses at the centers, we were not able to estimate the drop-out rate. We do not know how many actually read the information given. Parents who do not vaccinate their children may also be more hesitant to participate in a study concerning vaccines. However, the vaccination coverage for PCV is as high as 97% and very few actually do refuse. Our vaccination coverage rates in the study matched the population coverage. One risk we anticipated was that parents from higher educational levels would be more willing to participate. Indeed, the results show a selection of participants with higher level of education than the general population in Stockholm (study III). There is a pattern of higher antibiotic use in populations with lower socioeconomic status which may affect the pneumococcal carriage. However, travelling abroad was a risk factor for carriage in our study and this may be more common in wealthier population groups. Consequently, it is unclear how this selection bias may have affected the serotype distribution results.

Another potential risk of selection bias is missing data in the HDSS database in Uganda (study IV). Populations in the area that are squatters without a legal homestead may not be registered and therefore not sampled. There may also have been data missing on the age of some of the children registered in the database of the HDSS. However, experience of using the HDSS database shows that this is probably a minor problem and should not have affected the overall results. In study IV there is also the potential selection bias that some pneumococcal strains were more sensitive and selectively died during the transport from Uganda to Sweden.

Information bias

Information bias is caused by the collection of incorrect information (200). This can lead to misclassification of for example categorical variables. In a study when two groups are compared, such as study IV, with both ill and healthy study subjects, information on exposure or disease may have been systematically collected differently in the two groups. This may affect the interpretation of the results.

A classic example of an information bias is the recall bias. This is when two groups being studied, for example cases and controls, recall exposure differently. For example, a case might be keener to remember a certain exposure. In the study IV we used two a week recall for self-reported symptoms of illnesses and treatment. In study III we used longer recall – up to a year of illnesses, antibiotic treatments, travels, and hospital care. However, in both these studies, the participants did not know if they had the outcome (carriage), so the answers and therefore the results were probably not affected.

In study II there was a problem with a misclassification of the cases already described in the section of the study design (ecological) relating to the possible increased use of PCR, thereby leading us to believe that PCV decreased pneumonia hospitalization when in reality it could be misclassified disease. We think this misclassification may have affected the results, but we do not know not to what extent. Even if all 45 viral pneumonia cases per year had been classified as bacterial pneumonia, instead of viral, the incidence of pneumonia coded as bacteria would have increased from 366 to 385, but there would still have been a significantly decreased risk of hospitalization (RR 0.86, 95% CI 0.78-0.94). Our results pointing to a 19% decrease in hospitalizations due to pneumonia in vaccinated young age groups are also similar to other studies in different contexts (115, 201). ICD coding of discharge diagnoses, as used in study II, may lead to a risk of misclassification in any context. The classification of pneumonia becomes more accurate if it is confirmed with through x-ray. In study II this was controlled in the subsample where close to 100% of the hospitalized children were x-rayed. Therefore, we still believe that the classification was as good as it could be despite this chosen study design. Generally, the clinical definition of viral and bacterial pneumonia is difficult. High priority is put into research, to be able to diagnose and distinguish between viral and bacterial pneumonia and consequently prescribe antibiotics only to the cases who really need them, both in high, middle and low income countries (47, 81).

In study II we did not collect data on sinusitis treatment in out-patient clinics, only in in-patient clinics. If sinusitis patients were treated with oral antibiotics as outpatients to a larger extent post-PCV, there would be an information bias. This bias would be due to a change in treatment habits rather than a true decrease because of the exposure to PCV. No information from the active clinical colleagues on the research team however indicated that this was the case. It is also highly unlikely that such a change happens without an official change in treatment policy and concomitantly with the introduction of a vaccine.

Another information bias in study II was the lack of individual vaccination status for cases. This is rarely noticed in medical records and there was no national register available at the time of the study.

In study II we also excluded all H1N1 cases from the viral pneumonia group due to the mandatory reporting of all cases of pandemic flu since 2009. This lead to a disproportional increase in the reported number of cases. Including them would have given an overestimation of the incidence of influenza, or at least made it impossible to compare the viral pneumonia incidence over the study years.

Confounders

Confounder means that the effect of the exposure is mixed with the effect of another variable (200). This may in the extreme case be that the exposure has nothing to do with the outcome but rather that the outcome just happens to vary with another variable that has the actual effect in the outcome. A confounder must therefore be associated with the disease and also with the exposure, but it must not be an effect of the exposure. Logistical regression used to control for variations in risk factors in carriage was used to control for confounding in study III and IV.

A confounder we were not able to control for in study I was the use of PPV, since we had no individual data in the Stockholm region on the vaccine coverage of PPV in recommended risks groups; adults over 65 years, and persons of all ages with chronic conditions with increased risk of IPD or complications due to IPD. From 2009 to 2015, between 7,000 – 15,000 vaccinations with PPV were performed yearly in these risk groups, with no trend for either increase or decrease (County Medical Officer Åke Örtqvist, personal communication). Before the influenza pandemic of 2005-2008, the vaccination coverage with PPV was approximately two-four times as high, i.e. around 30 000 doses per year, but we lack the exact numbers. Although there were fewer vaccinated post than pre-PCV, the duration of protection of PPV makes it difficult to know if this would have had any impact on our results.

Generalizability

Generalizability has to do with the results being representative of the whole group of the population studied. Despite the methodological considerations listed above, this thesis contains three out of four population based studies and all four have a large sample size. All studies in Sweden were carried out on a defined population over a number of years, and include IPD and other morbidity data as well as carriage data – all pointing to the vaccine having an impact. A strength of study IV in Uganda was the use of random sampling to draw a representative sample from the population.

The use of risk difference vs risk rate ratio

Finally, the work on this thesis has been consistently filled with the question of how to present before-after PCV data. In study I incidence rate ratios was used. In study II we chose to present the data in two different ways, using trend analysis and incidence rate ratios. Trend

analysis has the advantage of it being visually easy to actually see a change in incidence at the time at and after exposure of the PCV. In table 7 some of the observed measures of impact of PCV implementation is presented for Sweden and the potential impact is estimated for Uganda.

Table 7. Different measures of vaccine effectiveness using data from Stockholm, Sweden (Study 1) and estimated impact in Uganda in children < 2 years of age.

Measure of effect on invasive pneumococcal disease	Stockholm, Sweden, results from study I	Uganda (estimated potential impact)	Assumptions in Uganda
Incidence rate ratio	0.36 (95% CI 0.2-0.6)	0.58	42% serotype PCV coverage (190)
Incidence rate difference	18.1 cases/ 100,000	105 cases/100,000	Pre-IPD incidence estimated at 250/100,000

6 CONCLUSIONS

Pneumococcus is currently the most important specific cause of child mortality. Pneumococcal conjugate vaccine (PCV) has a potential to alleviate at least a part of the pneumococcal disease burden. However, as this thesis points out, the current level of serotype coverage of PCVs limits their impact.

- After PCV introduction in Stockholm County, there has been a decline in IPD incidence due to meningitis, septicemia and rhinosinusitis in vaccinated age groups <2 years, as well as for bacteremic pneumonia in older children and septicemia in adults <65 years (I)
- Overall IPD incidence in the elderly did not decline due to an emergence of non-PCV13 vaccine-types (I)
- Antibiotic resistance levels in carrige and IPD remained low after PCV introduction in Stockholm county (I, III)
- After PCV introduction in Stockholm County there was a decline in hospitalizations due to sinusitis and pneumonia in vaccinated age groups 0-<2 years old, as well as in older un-vaccinated children 2-<5 years old (II)
- The shift from vaccine types (VT) to non-vaccine types (NVT) was nearly completed four years after introduction of the PCV vaccination and this serotype replacement continued to evolve from 4 to 8 years after PCV7 introduction in Stockholm County.
- Nearly half of the serotypes colonizing healthy children (46%) in Uganda were serotypes not covered by any of the current PCVs. The serotype coverage rate was 42% for the 10 serotypes in PCV10, which is the vaccine currently being implemented in Uganda.
- PCV serotype coverage in children under 5 was much higher in Sweden than in Uganda prior to PCV introduction (I, III, IV). Therefore, vaccine effectiveness in Uganda may not become as high as in Sweden (IV)

7 IMPLICATION FOR POLICY, PRACTICE AND RESEARCH

This thesis has attempted to answer a few research questions on the PCV impact on pneumococcal morbidity and carriage in Sweden and the potential impact in Uganda. The results may have implication for some of the policy, practice and research issues listed below:

Policy and practice:

- In Uganda, and in Sweden, the serotype distribution may be used to inform choices in PCV policies and future vaccine development.
    - In Sweden, future PCV is recommended to include NVT of high invasiveness potential: 22F, 9N, 8 and 12F, but also the NVT 33F, 23A and 11A due to their presence in both carriage and IPD.
    - In Uganda, PCV13 will offer a larger serotype coverage than the chosen PCV10.
- It is important to also carry out longitudinal surveillance studies on the impact of PCV in Uganda and other low-income countries where the disease burden and mortality is higher and PCV effectiveness data is scarce.
- Pneumcooccal conjugate vaccines could be included in policies to decrease antibiotic resistance.

Research:

- What is the perfect equilibirum in pneumococcal carriage and IPD, will it really be of advantage to eliminate all-type pneumococci in the nasopharyngeal niche? What kind of viral or bacterial pathogens will then develop and thrive?
- For how long will present antibiotics and vaccines be effective against pneumococcci? Will serotype replacment over time out-number the PCV gains in vaccine-type disease for vaccinated and/or un-vaccinated populations?
- More research is needed to understand mechanisms and how to positively impact the microbiota of the nasopharynx in ways to diminish disease.
- What other measure than PCV can be put in place to decrease IPD? Is it possible to administer asymptomatic bacteria on the mucosa of the nasopharynx to outnumber the invasive pathogens?
- Are contact patterns/social context between generations important for pneumococcal disease transmission and the impact of PCV?

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Barnadödligheten har minskat från 12,7 miljoner barn under fem till 5,9 miljoner per år sedan 1990. En ökad vaccinationstäckning mot mässling, stelkramp, difteri, kikhosta, och polio gör att mellan 2-3 miljoner barns liv räddas varje år. Den vanligaste enskilda sjukdomen som dödar flest barn under fem år, runt 900 000 per år, är lunginflammation. Många av dessa dödsfall beror på en bakterie som heter pneumokocker. Pneumokocker orsakar även andra allvarliga infektioner som hjärnhinneinflammation och blodförgiftning och mindre allvarliga som öroninflammation och bihåleinflammation. Sedan år 2000 finns ett vaccin mot pneumokocker som är effektivt även för barn under två år. Denna typ av vaccin, som kallas konjugerat pneumokockvaccin (PCV), rekommenderas av Världshälsoorganisationen för alla länder sedan år 2007. På senare år har PCV implementerats snabbt även i låg och medelinkomstländer. Men PCV vaccinationstäckningen är globalt på ungefär 31% och kunskap saknas hur effektiva PCV blir och vilka sekundära effekter de kan ge.

Pneumokocker delas in i över 97 serotyper beroende på ytstrukturer på bakteriekapseln. Bara 10 av dessa serotyper stod för 70% av alla fall av allvarlig pneumokocksjukdom innan PCV fanns. Man har utvecklat PCV så att de innehåller 10 eller 13 av de serotyper som gav mest och allvarligast sjuklighet. Vaccinerna är nästan bara effektiva mot serotyperna som ingår. Förutom att skydda barnet mot sjukdom mot dessa serotyper så minskar även bärarskapet. Detta betyder att vaccin serotyperna försvinner från nichen där de normalt finns och sprids, nämligen bakom näsan på yngre barn. Detta bidrar till att minska smittspridningen i omgivningen, men istället kan andra serotyper ta plats bakom näsan på barn och sprida andra sorters pneumokocker, sk serotyps-byte. PCV ges inom barnvaccinationsprogrammet i Stockholms län sedan 2007 och det implementeras i Uganda sedan år 2014. För att ta reda på effekten av PCV i Stockholms län, och den potentiella effekten av PCV i Uganda, gjorde vi uppföljning av alla fall av allvarlig pneumokocksjukdom, samt alla sjukhusbehandlade fall av barn med lunginflammation och bihåleinflammation i Stockholm och dessutom studier på pneumokockbärarskap på barn under fem år i både Stockholm och Uganda.

I Stockholm visades en 64%-ig minskad risk för allvarlig pneumokocksjukdom för barn under 2 år, men även en minskad risk för ovaccinerade äldre barn och vuxna, s.k. flockskydd, när man jämförde perioder innan och efter PCV. Flockskyddet av ovaccinerade sträckte sig dock inte till äldre över 65 år eftersom serotyper som inte ingår i vaccinet ökade så mycket att sjukligheten totalt inte påverkades. Dessutom visades en 66% minskad risk för sjukhusinläggning pga allvarlig bihåleinflammation och 19% minskad risk för lunginflammation. Den goda effekten kan delvis förklaras av ändrad bakterieflora i bärarskap hos barn, där de serotyper som ingår i vaccinet har utbytts mot mindre allvarliga serotyper som inte ingår i vaccinet. I Uganda såg vi en mycket lägre PCV serotypstäckning bland bärarskap hos barn under fem år, 42% jämfört med 63% innan PCV i Sverige. Men eftersom pneumokocksjukligheten och dödligheten är högre i Uganda uppskattas ändå värdet av PCV i Uganda bli betydande.

Avhandlingen bidrar med kunskap om PCVs effekt i Sverige och dess potentiella effekt i Uganda och kan bidra till val av vaccin och vaccinationspolicy i framtiden.

9 RELATED RESEARCH NOT INCLUDED IN THIS THESIS

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10 ACKNOWLEDGEMENTS

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