The impact of vaccination with conjugated pneumococcal vaccine on pneumococcal carriage and disease caused by pneumococci in Icelandic children.

Samuel Sigurdsson

Thesis for the degree of Philosophiae Doctor

Supervisor:
Ásgeir Haraldsson

Advisor:
Karl G. Kristinsson

Doctoral committee:
Helga Erlendsdóttir (chair)
Ásgeir Haraldsson
Birgir Hrafnkelsson
Karl G. Kristinsson
Þórólfur Guðnason

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Áhrif bólusetningar með prótein-tengdu pneumókokkabóluefni á pneumókokka í nefkoki og sýkingar af völdum pneumókokka í íslenskum börnum

Samuel Sigurdsson

Ritgerð til doktorsgráðu

Umsjónarkennari:
Ásgeir Haraldsson

Leiðbeinandi:
Karl G. Kristinsson

Doktorsnefnd:
Helga Erlendsdóttir (formaður)
Ásgeir Haraldsson
Birgir Hrafnkelsson
Karl G. Kristinsson
Þórólfur Guðnason

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Ágrip

Pneumókokkar eru oft bornir einkennalaust í nefkoki, sérstaklega í börnum. Þeir eru einnig með algengustu bakteríum sem valda bæði alvarlegum og mildari algengum sýkingum í börnum og fullorðnum. Alvarlegustu sýkingarnar eru ífarandi pneumókokka sýkingar (IPS) sem eru á meðal þeirra sýkinga sem valda mestri örkumlun og dauða um allan heim. Þar að auki eru pneumókokkar með algengustu bakteríum sem valda lungnabólgum og bráðum miðeyrnabólgum (BMB), algengustu orsök sýklalyfjaútskrifta hjá börnum.

Tilgangur þessarar rannsóknar var að finna hugsanlegar breytingar í hlutfalli barna á leikskólum sem bera pneumókokka í nefkoki sínu og meta breytingar í faraldsfræði sýkinga barna vegna pneumókokka eftir upphaf bólusetninga með pneumókokka bóluefni tengdu við prótein D úr *Haemophilus Influenzae* (PHiD-CV) á Íslandi árið 2011.

Um 500 sýni voru tekin árlega frá 2009 til 2015 úr heilbrigðum börnum tveggja til sex ára í 15 leikskólum á höfuðborgarsvæðinu. Sýni voru ræktuð innan 4 – 6 klst frá töku með aðferðum sem auka greiningu pneumókokka sem mest úr sýnunum. Allir pneumókokkar voru hjúpgreindir með latex kekkjun og kjarnsýrumögnun. Næmi fimm mismunandi sýklalyfja var rannsakað og 2012 EUCAST aðferð og viðmiðin voru notuð. Bein áhrif bólusetningarinnar voru metinn með því að bera saman bólusett börn (fædd 2011 og síðar, VEC) við óbólusett börn (fædd 2010 og fyrr, VNEC). Hjarðónæmi var metið með því að bera saman sýni tekin úr eldri óbólusettum börnum fyrir og eftir upphaf bólusetningarinnar.

Áhrif bóluefnisins á heilsugæslukomur vegna BMB var metið með því að fylgja öllum börnum á Íslandi frá 2005 til 2015 eftir frá fæðingu til þriggja ára aldurs eða til enda rannsóknartímabilsins. Með því að nota kennitölur barnanna samtengdar við sjúkraskrár þeirra, var hægt að gera einstaklingsmiðaðar aðhvarfsgreiningar fyrir endurteknar komur og reikna út áhrif bóluefnisins (áhættuhlutfall [HR] – 1) x 100%.

Áhrif bóluefnisins á komur barna á bráðamóttöku barna vegna öndunarfærasýkinga var fundin með tvennu móti. Annars vegar með því að bera saman nýgengi sýkinganna fyrir og eftir upphaf bólusetningarinnar og hins vegar með þvi að bera saman nýgengi sýkinga barna í VNEC og VEC fæðingarárgöngum. Breytingar í innlögnum barna vegna

pneumókokkasýkinga var metið með því að kalla eftir öllum innlögnum sem báru greiningar tengdum öndunarfærasýkingu eða IPS frá 2005 til 2015 í börnum yngri en þriggja ára. Samanburður á milli VNEC og VEC var gerður til að reikna muninn í nýgengi og áhættu innlagna fyrir hvern greiningarflokk.

Heildarberahlutfall barna á pneumókokkum í nefkoki breyttist ekki eftir bólusetninguna. Berahlutfall bóluefnishjúpgerða lækkaði um 94% í bólusettum börnum og um 56% í sýnum teknum úr eldri, óbólusettum börnum eftir upphaf bólusetningarinnar. Berahlutfall hjúpgerða sem ekki eru í bóluefninu óx. Pneumókokkar sen óbólusett born báru voru næmari fyrir penisillíni og öðrum sýklalyfjum borið saman við óbólusett börn. Þar að auki fækkaði fjölónæmum pneumókokkum um 56% og pneumókokkum sem voru ónæmir fyrir öllum prófuðum sýklalyfjum fækkaði um 94%.

Komum á bráðamóttöku barna vegna bráðrar miðeyrnabólgu (BMB) fækkaði um 24% og vegna lungnabólgu um 23% í VEC samanborið við VNEC. Samhliða því fjölgaði komum vegna bráðrar berkjungabólgu um 53%. Komum vegna BMB á heilsugæslu fækkaði um 24%. Færri börn voru greind með BMB fyrir þriggja ára aldur og færri börn höfðu endurteknar BMB. Bólusetningin var verndandi gegn fyrstu tveim bráðum miðeyrnabólgum, en eftir að börnin höfðu fengið tvær BMB þá voru þau í jafnri áhættu og óbólusett börn að fá enn fleiri BMB.

Innlögnum vegna ífarandi pneumókokka sýkinga fækkaði um 93% í bólusettum börnum samanborið við óbólusett og engar bóluefnishjúpgerðir fundust í þeim sýkingum í bólusettum börnum. Innlögnum vegna lungnabólgu í bólusettum börnum fækkaði um 20% þrátt fyrir 32% aukningu í innlögnum vegna bráðrar berkjungabólgu. Fjölgunin í innlögnum vegna bráðrar berkjungabólgu var mest meðal barna yngri en sex mánaða en fækkunin í innlögnum vegna lungnabólgu var mest meðal 12 til 18 mánaða gamalla barna. Enginn munur var á innlögnum vegna BMB, en þær voru fáar á rannsóknartímabilinu.

Meginniðurstöður rannsóknarinnar eru því að bólusetning með próteintengdu pneumókokka bóluefni á Íslandi hefur valdið nánast upprætingu bóluefnishjúpgerða meðal barna á Íslandi og fækkun hefur orðið á flestum þeim mælikvörðum á pneumókokkasýkingum sem metnir voru í þessari rannsókn.

Lykilorð: Bólusetningar gegn pneumókokkum, faraldsfræði, bráð miðeyrnabólga, berahlutfall pneumókokka, lungnabólga

Abstract

Pneumococci are commonly carried asymptomatically in the nasopharynx, especially in children. Pneumococci are also one of the major bacterial pathogens causing both severe infections and non-severe infections in both children and adults. The most severe infections are invasive pneumococcal diseases (IPD) being one of the leading causes of mortality and morbidity worldwide. Additionally, pneumococci are among the most common bacterial pathogens causing pneumonia and acute otitis media (AOM) and the most common indication for antimicrobial prescriptions in young children.

The aims of the study were to investigate the possible changes in the carriage and serotypes of pneumococci in the nasopharynx of children attending day care centres and evaluate the change in the epidemiology of paediatric pneumococcal infections following the introduction of the pneumococcal nontypable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Iceland in 2011.

Around 500 samples were collected yearly from 2009 to 2015, from healthy children two to six years of age, attending 15 day care centres in the Reykjavík, capital area. Samples were inoculated and incubated within four to six hours of sampling using methods maximizing pneumococcal detection. All pneumococcal isolates were serotyped using latex agglutination and PCR. Antimicrobial susceptibility was determined for five common antimicrobials using the 2012 EUCAST methods and criteria. Direct impact was evaluated comparing children born prior to the initiation of the vaccination (vaccine not eligible cohort, VNEC) with children born after the vaccination (vaccine eligible cohort, VEC). The herd impact was evaluated by comparing samples collected from older non-vaccinated children before and after the start of the vaccination.

The impact on primary care visits due to AOM was evaluated by following every child born in Iceland from 2005 to 2015 until three years of age or end of the study period. Using unique social security numbers linked to patients' records allowed the use of individual based cox-regression model for repeated visits analysis to calculate the vaccine impact, (hazard ratio [HR] – 1) x 100%.

The vaccine impact on paediatric emergency department visits for respiratory tract infections was determined both by comparing the incidence of infections before and after the vaccination and by comparing VEC to VNEC. Changes in the admission rate because of pneumococcal infections were evaluated by extracting every admission due to specific study diagnoses

between 2005 and 2015 for children less than three years of age. Comparing the VNEC and VEC allowed calculating the changes in incidence rate, cumulative incidence rates and hazard of admissions for various pneumococcal infections.

The total pneumococcal nasopharyngeal carriage did not change following the PHiD-CV vaccination in Iceland. The carriage of vaccine serotype pneumococci was reduced by 94% in vaccinated children and the herd effect was 56% in older, non-vaccinated children sampled after the initiation of the vaccination. Pneumococcal isolates from vaccinated children had lower penicillin MICs and lower rates of non-susceptibility to other antimicrobials compared to non-vaccinated children. Additionally, multi-resistant pneumococci were reduced by 56% and pneumococci non-susceptible against all tested antimicrobials by 94%.

Paediatric emergency department visits due to AOM were reduced by 24% and due to pneumonia by 23% in VEC compared to VNEC. Conversely, visits due to acute bronchiolitis were increased by 53% for the same period. In addition, recurrent AOM was less frequent.

The vaccine impact on primary care visits due to AOM was 24%. Fewer children were diagnosed before the age of three, fewer children had recurrent infections and vaccinated children were diagnosed with fewer episodes of AOM. The vaccination was effective in preventing the first two episodes of AOM, with children already diagnosed two times having equal risk of contracting the third episodes, regardless of vaccine status.

Admissions due to culture confirmed IPD were reduced by 93%, with no vaccine type pneumococci isolated in the VEC. Admissions for pneumonia were reduced by 20% despite a 32% increase in admissions due to bronchiolitis. The increase in admissions due to bronchiolitis was mainly found in children less than six months of age while the decrease in admissions due to pneumonia was most profound in children 12 – 17 months of age. Conversely, no change was noted in admissions due to AOM, although few in number during the study period.

In conclusion, the initiation of PHiD-CV into the vaccination schedule has resulted in almost complete eradication of vaccine type pneumococcal disease among children in Iceland and reduction in most parameters of pneumococcal infections in Iceland measured in this study.

Keywords: Pneumococcal vaccination, acute otitis media, pneumococcal carriage, pneumonia, invasive pneumococcal disease

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List of abbreviations

ABS	Acute bacterial sinusitis
AOM	Acute otitis media
CAP	Community acquired pneumonia
CDC	Center for Disease Control and Prevention
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CPS	Capsular polysaccharide synthesis gene
DCC	Day care centre
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ENT	Ear, nose and throat
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
GAS	Group A streptococcus, *Streptococcus pyogenes*
HiB	*Haemophilus influenzae*, serotype B
HIC	High-income countries
HR	Hazard ratio
ICD-10	The 10th revision of the International Statistical Classification of Diseases and Related Health Problems
ICU	Intensive care unit
IgA	Immunoglobulin A
IPD	Invasive pneumococcal disease
IQ	Intelligence quotient
IQR	Interquartile range
IR	Incidence rate
IRR	Incidence rate ratio
ISPPD	International Symposium on Pneumococci and Pneumococcal Diseases
LMIC	Low- and medium-income countries

LRTI	Lower respiratory tract infection
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization Time of Flight
MEF	Middle ear fluid
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence type
NID	National identification numbers
NP	Nasopharynx
NPV	Negative predictive value
NSCMID	Nordic Society of Clinical Microbiology and Infectious Diseases
NT	Non-typeable
NTHi	Non-typeable *Haemophilus Influenzae*
NVT	Non-vaccine type
OECD	Organization for Economic Cooperation and Development
OM	Otitis media
OME	Otitis media with effusion
OR	Odds ratio
PCR	Polymerase Chain Reaction
PCV	Pneumococcal conjugate vaccine
PCV-13	13 valent pneumococcal conjugate vaccine
PCV-7	Seven valent pneumococcal conjugate vaccine
PHiD-CV	Pneumococcal nontypable Haemophilus influenzae protein D conjugate vaccine
PNSP	Penicillin non-susceptible pneumococci
PostVac	Post-vaccination period group
PPV	Positive predictive value
PPV	Polysaccharide pneumococcal vaccine
PreVac	Pre-vaccination period group
PSI	Pneumonia severity index
PspA	Pneumococcal surface binding protein A
rAOM	Recurrent acute otitis media

RCT	Randomized controlled trial
RSV	Respiratory syncytial virus
RT PCR	Real time Polymerase Chain Reaction
RTI	Respiratory tract infection
ST	Sequence type
URTI	Upper respiratory tract infection
US	United States of America
VAT	Vaccine associated serotypes, serotypes 6A and 19A
VE	Vaccine effectiveness
VEC	Vaccine eligible cohort
VIc	Vaccine impact against carriage
VNEC	Vaccine non-eligble cohort
VT	Vaccine serotypes, serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F
WHO	World Health Organization

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List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-IV):

I. Sigurdsson, S., Erlendsdóttir, H., Quirk, S. J., Kristjánsson, J., Hauksson, K., Andrésdóttir, B. D. I., Jónsson, A. J., Halldórsson, K. H., Sæmundsson, Á., Ólason, Ó. H., Hrafnkelsson, B., Kristinsson, K. G., Haraldsson, Á. (2017). Pneumococcal vaccination: Direct and herd effect on carriage of vaccine types and antibiotic resistance in Icelandic children. Vaccine, 35 (39).

II. Sigurdsson, S., Eythorsson, E., Hrafnkelsson, B., Erlendsdóttir, H., Kristinsson, K. G., Haraldsson, Á. (2018). Reduction in All-Cause Acute Otitis Media in Children less than 3 Years of Age in Primary Care Following Vaccination With 10-Valent Pneumococcal Haemophilus influenzae Protein-D Conjugate Vaccine: A Whole-Population Study. Clinical Infectious Diseases.

III. Sigurdsson, S., Kristinsson, K. G., Erlendsdóttir, H., Hrafnkelsson, B., Haraldsson, Á. (2015). Decreased Incidence of Respiratory Infections in Children After Vaccination with Ten-valent Pneumococcal Vaccine. Pediatric Infectious Disease Journal, 34 (12), 1385–1390.

IV. Sigurdsson, S., Eythorsson, E., Erlendsdóttir, H., Hrafnkelsson, B., Kristinsson, K. G., Haraldsson, Á. Impact of the 10-valent pneumococcal conjugate vaccine on hospital admissions in children under three years of age in Iceland (submitted)

In addition, the author contributed to the following original publications as co-first author, referred by their Roman numerals (V-VI)

V. Eythorsson E., Sigurdsson S., Hrafnkelsson B., Erlendsdóttir H., Haraldsson Á., Kristinsson K.G. (2018). Impact of the 10-valent pneumococcal conjugate vaccine on antimicrobial prescriptions in young children: A whole population study. BMC Infectious Disease

VI. Eythorsson E., Sigurdsson S., Erlendsdóttir H., Hrafnkelsson B., Kristinsson K.G., Haraldsson Á. Increase in tympanic tube placements despite pneumococcal vaccination: a whole population study (submitted)

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Declaration of contribution

- Study I. The doctoral student, Samuel Sigurdsson (SS), planned the research in co-operation with Ásgeir Haraldsson (ÁH), Helga Erlendsdóttir (HE) and Karl G. Kristinsson (KGK). He collected specimen from all children in 2015, while other students performed the collection in 2009 – 2014. In addition, SS collected samples in 2016 and 2018 (not included in this thesis). SS plated all isolates from 2015 and 2016 and was involved in performing anti-microbial susceptibility testing alongside HE. SS performed PCR on all isolates from 2009 – 2015 in which HE was unable to determine the exact serotype using latex agglutination. SS did the statistical analysis and ran the statistical analysis in cooperation with Birgir Hrafnkelsson (BH). SS drafted the paper and did critical revisions alongside his co-authors.
- Study II. SS planned the study with Elías Eyþórsson (EE), ÁH, HE and KGK. He analysed the data and was involved in the statistical analysis, which was performed by EE with help from BH. SS and EE drafted the paper and did critical revisions alongside his co-authors.
- Study III. SS planned the study alongside ÁH, HE and KGK. SS ran the statistical analysis in co-operation with BH. SS drafted the paper and did critical revisions in co-operation with his co-authors.
- Study IV, V & VI. SS planned the study with EE, ÁH, HE and KGK. He analysed the data and was involved in the statistical analysis, which was performed by EE. SS and EE drafted the paper and did critical revisions alongside his co-authors.
- SS wrote the thesis under guidance from his doctoral committee.

1 Introduction

Streptococcus pneumoniae (pneumococci) was first identified in 1880, independently in France by Pasteur and in the United States by Sternberg (Austrian, 1981; Catterall, 1999; Pasteur, 1881; Sternberg, 1881). They both demonstrated that by inoculating rabbits with human saliva containing lancet shaped diplococci, the rabbits developed deadly septicaemia. Post-mortem the same bacteria could then be detected in the rabbits' blood (Austrian, 1981; Catterall, 1999). They both drew the correct conclusion that the diplococci which they had seen were the rabbits' cause of death and published their findings in their respective countries (Pasteur, 1881; Sternberg, 1881). Although the lancet shaped diplococci had previously been described (Klebs, 1875; Koch, 1881), Pasteur and Sternberg were first to directly demonstrate the pathogenicity of the bacteria (Austrian, 1981; Catterall, 1999; Watson, 1993).

Pneumococci has been one of the most studied organisms in microbiology and studies on it have resulted in the discovery of many fundamental principles in biology and immunology including the discovery of DNA, the Gram stain and anti-sera treatment (Austrian, 1981; Watson, 1993).

The yearly worldwide under-five mortality rate from diseases caused by *Streptococcus pneumoniae* is enormous and is one of the leading cause of death in children in the low- and medium-income countries (LMIC) (Barber, 2017). Additionally, pneumococci is one of the leading bacterial causes of acute otitis media (AOM), which is the most common reason for health care visits and antimicrobial prescriptions in children (Ahmed, 2014).

Since the licensure of the first pneumococcal conjugate vaccine (PCV) in 2000-1, paediatric vaccination programs against pneumococci have become common, and have resulted in widespread reduction in both invasive and non-invasive pneumococcal disease (IPD) (Berglund, 2014; L. H. De Oliveira, 2016; Deceuninck, 2015; Fitzwater, 2012; Griffin, 2013; Nair, 2016; Tawfik, 2017; Taylor, 2012). By the end of 2016, 134 countries have introduced pneumococcal conjugate vaccines, with global coverage estimated at 42% (World Health Organization, 2018).

1.1 Characteristics of *Streptococcus pneumoniae*

Pneumococci are Gram-positive cocci, classically described as lancet shaped

cocci under the light microscope appearing in pairs or chains (Reller, 2008; Satzke, 2013). Pneumococci are facultative anaerobes and grow well in the presence of oxygen. They, like many other streptococci do however grow better under anaerobic conditions, where the colonies grow faster and become larger (Blaschke, 2011; Reller, 2008; Satzke, 2013).

Pneumococci are α-haemolytic, e.g. they partially haemolyse red blood cells, when cultured on blood agar, giving off a dark green zone around the colonies on blood agar (Blaschke, 2011; Reller, 2008; Satzke, 2013). This is a useful first step to identifying pneumococcus and to differentiate them from other Gram-positive bacteria, such as β or γ haemolytic streptococci (Pichichero, 1998). Several other streptococcal species are α-haemolytic, most relevant are the commensal streptococci of the naso- and oropharyngeal cavity (Blaschke, 2011; Reller, 2008; Satzke, 2013).

1.1.1 Identification

In cultures, pneumococci are differentiated from other α-haemolytic streptococci by their susceptibility to optochin, its bile solubility and its capsule, while other streptococci are generally; non-capsulated, resistant to optochin and bile insoluble (Blaschke, 2011; Reller, 2008; Satzke, 2013). This definition is not entirely specific, as some pneumococci are non-encapsulated or resistant to optochin, appearing more like viridans streptococci than pneumococci (Blaschke, 2011; Clark, 2015; Satzke, 2013; Song, 2013). Furthermore, *Streptococcus pseudopneumoniae*, an alpha haemolytic streptococci, can be either sensitive or resistant to optochin (Arbique, 2004; Keith, 2006; Satzke, 2013; Song, 2013), further complicating diagnosis. The specificity of culture of clinical samples is excellent, despite the above considerations which are more relevant in carriage studies, than in patient samples. The sensitivity is also high under ideal circumstances, with several factors which can limit it, causing false negative results. Recent antibiotic usage, suboptimal quantity or quality of samples and autolysis of the pneumococci are all common and reduce the sensitivity (Bjarnason, 2017; Blaschke, 2011; Clark, 2015). Consequently, other methods, such as antigen-based or molecular-based detection, where viable bacteria are not required, is often used, complementing the diagnosis (Blaschke, 2011; Clark, 2015).

Antigen assays, such as Binax NOW©, an assay for detection of C-polysaccharide can be useful in rapid identification of pneumococcal disease (Blaschke, 2011; Clark, 2015; Navarro, 2004; Reller, 2008; Song, 2013). It is most widely used as an urine antigen test, often positive, when an infection such as pneumonia is present (Blaschke, 2011; Navarro, 2004; Song, 2013).

When meningitis is suspected, it can also be very useful in rapid detection of pneumococci in cerebrospinal fluid, where the sensitivity has been determined to be 95 – 100% and the specificity 100% (Reller, 2008; Song, 2013). The sensitivity and specificity is also high when applied directly to other high quality patient samples, such as pleural effusions (Blaschke, 2011; Clark, 2015; Reller, 2008), bronchioalveolar lavage or blood (Blaschke, 2011; Reller, 2008).

The urine antigen test is useful when pneumonia is suspected in an adult patient (Blaschke, 2011; Clark, 2015; Navarro, 2004; Reller, 2008), and has high specificity (>90%) in adults, but lower, sensitivity, 70 – 80% (Reller, 2008). It is therefore, a suitable complementary test to other diagnostic tools, especially in patients in which no other samples can be obtained. However, its applicability in children is severely limited due to high rate of false positives, mainly due to pneumococcal carriage and recent vaccinations (Blaschke, 2011; Clark, 2015; Navarro, 2004; Reller, 2008; Song, 2013). Importantly, for all age groups, pneumococcal antigens are secreted into the urine, causing a positive test, even in absence of current infection, for weeks following infection, severely limiting its usability in re-infections (Blaschke, 2011; Reller, 2008; Song, 2013).

Detecting the presence of pneumococci straight from patient samples by Polymerase Chain Reaction (PCR), where specific nucleic acid strands are amplified, complementing culture-based detection with many different genes with varying specificity and sensitivity (Bjarnason, 2017; Blaschke, 2011; Carvalho, 2007; Clark, 2015; Lorente, 2000; Murdoch, 2003; Satzke, 2013; Song, 2013). Currently, simultaneous real time PCR (RT PCR) for both LytA and ply genes (Carvalho, 2007; Clark, 2015; Messmer, 2004; Satzke, 2013), gives the highest specificity and sensitivity of the nucleic acid amplification tests for detection of pneumococci (Satzke, 2013).

In addition to the above, antigen detection by latex agglutination and antibody detection by the Quelling method is available, although they are more useful in serotyping (Clark, 2015; Reller, 2008; Song, 2013). Other techniques such as MALDI-TOF in which cultured bacteria can be rapidly identified, can also be useful in the detection of pneumococci (Clark, 2015; Song, 2013). However, the interpretation of the results must take into account, that it cannot reliably discriminate between pneumococci and other streptococci (Clark, 2015; Song, 2013).

Although the above non-cultural based methods can be fast and relatively accurate, they have their limitation, with the lack of information on antimicrobial susceptibility and limited possibility of serotyping (Reller, 2008).

1.1.2 Serotyping and anti-serum treatment

Soon after the first discovery of pneumococcus, it was recognized that pneumococci were of different types. Consequently, serotyping methods were developed, many of which centred around the principle of capsular polysaccharides reaction to anti-serum antibodies (Austrian, 1982; Geno, 2015; Henrichsen, 1999). By 1932, 32 serotypes had been discovered (Geno, 2015) and unspecific pneumococcal immune serum was being used at some sites as treatment for pneumococcal infections, such as pneumonia (Austrian, 1982; Geno, 2015; Henrichsen, 1999).

In 1939 the Danish prince Valdemar, died of pneumococcal pneumonia by a previously unknown serotype from serogroup 9 despite receiving immune serum for both serotypes 9L and 9N. Later, the serotype was named after the prince, 9V. Additionally, the prince's death served as a catalyst for development of faster and more specific serotyping and treatment, with Denmark in the forefront (Geno, 2015). As a result, only a few years later, 75 serotypes had been discovered (Eddy, 1944), with the current numbers approaching a century (Bogaert, 2004; Geno, 2015). In the system developed by the Danes, each serotype was defined by their distinctive chemical and immunologic characteristics, with serotypes grouped as serogroups due to shared serologic features, such as cross-reactive antibodies (Geno, 2015). The discovery of sulfamethoxazole and later penicillin, practically eliminated anti-sera as treatment for pneumococcal infections (Austrian, 1982; Henrichsen, 1999)

1.1.3 Serotyping methods

The Quellung method, described by Neufeld in 1902 (Neufeld, 1904) is the current gold standard for serotyping of pneumococci. The method involves mixing anti-sera with pneumococcal isolate, with the pneumococcal capsule appearing swollen under the light-microscope in positive reactions (Jauneikaite, 2015; Reller, 2008; Satzke, 2013). Limiting factors for using the Quellung method include that expensive reagents are needed, have a short shelf life and extensive operator experience is needed for reliable detection (Jauneikaite, 2015; Reller, 2008; Satzke, 2013). Latex agglutination tests, however are faster, cheaper and easier to learn and are an acceptable alternative for serotyping. Type-specific antibodies from the serum bind to the pneumococcal antigens and clumping is visible by the naked eye in positive assays (Jauneikaite, 2015; Reller, 2008; Satzke, 2013; Slotved, 2004).

Monoplex, duplex or multiplex PCR are also recommended alternatives. These methods can be used either for primary detection via sequential multiplex reactions, or following conventional methods used to detect possible serogroups, which can be more accurately specified with multiplex PCR reactions (Jauneikaite, 2015; Jin, 2009; Morrison, 2000; Pai, 2006). PCR is a fast, sensitive and relatively easy method to master. Despite the above advantages of PCR it may be difficult to accurately discriminate between serotypes within some serogroups, requiring a diverse collection of DNA primers for detection of rare serotypes (Jauneikaite, 2015; Satzke, 2013).

Many other methods have been mentioned and are used in varying capacity in both research and clinical settings such as dot blot, microbead assays, ELISA, flow cytometry and microarray, to name a few (Jauneikaite, 2015; Satzke, 2013).

1.2 Carriage of pneumococci

The human nasopharynx (NP), especially in children seems to be the only natural reservoir of the pneumococcus (Bogaert, 2004; Gudnason, 2014; Simell, 2012; Tomasson, 2005). Although experimental animals are widely used in the study of pneumococcal disease, they are not thought to be natural colonisers of pneumococci without human contact (Chi, 2007; Gritzfeld, 2014; Kilian, 2008; Köndgen, 2017; van der Linden, 2009). However in a single study pneumococci were found in animals in which strict restriction in human contact had been adhered to (Chi, 2007). The authors reported that the same strain of pneumococci, was recovered from several dead chimpanzees over a few years period in two distinct chimpanzee communities with no shared borders. This led the authors to speculate that other animals in the sanctuary might serve as intermediates, perhaps indicating natural carriage in those animals (Chi, 2007). Further analysis of the clones, revealed serotype 3 of a novel sequence type (ST), previously not listed among the 6300 ST of serotype 3 found in the MLST data base (Denapaite, 2011).

1.2.1 Dynamics of carriage

The carriage of pneumococci is a dynamic process. Acquiring new strains and clearing previous ones is a continuous process. It is well documented that there are multiple independent risk factors for the carriage of pneumococci. The most often cited risk factors are recent viral infections, young age and number of siblings at home, crowding and being of, or living in a low socioeconomic census tract (Table 1).

Table 1. Risk factors for nasopharyngeal carriage of pneumococci and carriage of PNSP. PNSP: Penicillin non-susceptible pneumococci.

Factors affecting carriage	Effect on carriage of pneumococci	Effect on carriage of PNSP
Recent upper respiratory tract infection	**Increased risk:** (Bogaert, 2004; Desai, 2015; Gudnason, 2014; Huang, 2004; Peltola, 2004; Zuccotti, 2014)	**Increased risk:** (Gudnason, 2014; Huang, 2004; Kristinsson, 1997)
Young age	**Increased risk:** (Bogaert, 2004; Desai, 2015; Gudnason, 2014; Simell, 2012; Zuccotti, 2014)	**Increased risk:** (Gudnason, 2014; Kristinsson, 1995, 1997)
Recent antimicrobial usage	**Decreased risk:** (Gudnason, 2014; Huang, 2004; Zuccotti, 2014)	**Increased risk:** (Arason, 1996, 2006; Bogaert, 2004; Gudnason, 2014; Hjaltested, 2003; Kristinsson, 1995, 1997)
Smoking at home	**No impact**: (Desai, 2015; Gudnason, 2014) **Increased risk:** (Bogaert, 2004)	**No impact:** (Gudnason, 2014)
Breastfeeding, short	**No impact:** (Gudnason, 2014) **Increased risk:** (Huang, 2004)	
Young siblings at home	**No impact:** (Gudnason, 2014) **Increased risk:** (Bogaert, 2004; Desai, 2015; Huang, 2004; Mosser, 2014; Roca, 2013; Zuccotti, 2014)	**No impact:** (Gudnason, 2014)
Low socioeconomic status	**Increased risk:** (Bogaert, 2004; Huang, 2004) **No impact:** (Desai, 2015)	**Increased risk:** (Huang, 2004)
Crowding	**Increased risk:** (Bogaert, 2004; Huang, 2004)	**Increased risk:** (Huang, 2004)
Out of home day care	**Increased risk:** (Bogaert, 2004; Desai, 2015; Zuccotti, 2014) **Increased risk for high socioeconomic status:** (Huang, 2004)	**Increased risk:** (Kristinsson, 1997)

The prevalence of carriage differs greatly with reported carriage rate in young children in the high-income countries (HIC) (Bogaert, 2004; Desai, 2015; Dunais, 2015; Gounder, 2015; Hamaluba, 2015; Hussain, 2005; Shak, 2013; Sigurdsson, 2017; Simell, 2012; Tomasson, 2005; Zuccotti, 2014). Nasopharyngeal carriage prevalence likely peaks before the second or third birthday reaching around 50% (Bogaert, 2004; Shak, 2013; Sigurdsson, 2017;

Simell, 2012; Tomasson, 2005), thereafter slowly decreasing, until reaching a lower, adult carriage rate at adolescence (Bogaert, 2004; Hussain, 2005; Shak, 2013). Additionally, the cited studies vary greatly in setting and risk factors.

As corroboration, native populations of western countries, such as aboriginals of Australia, Alaskan Inuit and Native Americans, where, on average socioeconomic status and hygiene is low and crowding high, incidence of carriage is very high, with almost all children being colonized by pneumococci by few months of age (Bogaert, 2004; Collins, 2013; Leach, 1994; Millar, 2009). Similar results have been established in low resource settings in Africa (Hill, 2010).

1.2.2 Carriage of pneumococci non-susceptible to antimicrobials

Numerous antimicrobial classes are effective against pneumococci. The most commonly used in Iceland are beta lactams and macrolides (NHS, 2008; The European Committee on Antimicrobial Susceptibility Testing, 2018). Narrow spectrum beta lactams, such as benzylpenicillin and amoxicillin are the first line treatment for both pneumococci susceptible and intermediately non-susceptible to penicillin (Landlæknisembættið, 2017; NHS, 2008). Macrolides are first choice for many pneumococcal infections in patients allergic to beta lactam drugs (Landlæknisembættið, 2017; NHS, 2008).

Penicillin non-susceptible pneumococci (PNSP) are defined according to the EUCAST clinical breakpoints as pneumococci in which the minimum inhibitory concentration of benzylpenicillin is greater than 0.064 mg/L (The European Committee on Antimicrobial Susceptibility Testing, 2018).

The most important risk factor for carriage of PNSP are young age, recent antimicrobial prescriptions and crowding (Table 1). Antimicrobial use exerts selective pressure and facilitates spread of PNSP within communities by killing susceptible organisms in other hosts (Arason, 1996; Gounder, 2015). Consequently, the rate of PNSP is higher in communities with high antibiotic usage (Arason, 1996, 2006; Austin, 1999; Bogaert, 2004; Hjaltested, 2003; Kristinsson, 1995).

Pneumococci can acquire resistance genes either spontaneously by remodelling of the genes causing increased fitness of the strain (Arason, 1996; Hjálmarsdóttir, 2014; Kalin, 1999) or by horizontal gene transfer from other pneumococci or alpha-haemolytic streptococci in the nasopharynx (Arason, 1996; Hjálmarsdóttir, 2014; Kadioglu, 2008; Kalin, 1999; Kilian, 2008).

Before the introduction of PHiD-CV in Iceland, 44.9% of the capsulated pneumococcal patient isolates had reduced susceptibility to penicillin with vaccine type, 19F being cultured in 82% of the isolates (Hjálmarsdóttir, 2014). Similarly, 19F was the leading cause of PNSP and multi-resistance in pneumococci isolated from the nasopharynx in healthy carriers. After the introduction of PHiD-CV, rate of 19F carriage and with it rate of PNSP carriage in healthy carriers greatly diminished (Sigurdsson, 2017). In Europe, in general the rate of PNSP is higher in the south of the continent, where antibiotic use is higher compared to countries in the northern part of the continent where prescription is more restricted (Adriaenssens, 2011; Liñares, 2010). Iceland has struggled with higher prevalence of PNSP than the other Nordic countries, but lower than the countries in Southern Europe (Hjálmarsdóttir, 2014). This is partly due to high antimicrobial usage in Iceland compared to other Nordic countries (Gefenaite, 2014; Howitz, 2017; Kronman, 2014; Lau, 2015; Eythorsson, 2018).

Mainly two clones have been responsible for most of the antibiotic resistance in Iceland in the last two decades. In the 1990s, a multi-resistant clone of serotype 6B, originating from Spain was highly prevalent (Kristinsson, 1995, 1999) and after 2004 a multi-resistant clone of 19F expanded and became the most prevalent serotype in Iceland until the initiation of the vaccination (Hjálmarsdóttir, 2014).

Overuse of antimicrobials is a worldwide challenge that has contributed to an epidemic of antimicrobial resistance (O'brien, 1997; Spellberg, 2008). In a 2016 study on antimicrobial prescriptions in the United States, 30% of all antimicrobial prescriptions were deemed inappropriate (Fleming-Dutra, 2016).

Many factors contribute to the overuse of antimicrobials (Fleming-Dutra, 2016; Fletcher-Lartey, 2016; Lucas, 2015; Stille, 2008), which in turn results to increase in antimicrobial resistance (Arason, 1996; Costelloe, 2010). Contributing factors cited by physicians in causing over-prescriptions include uncertainty of diagnosis, fear of disease complications, lack of perception of harmful effects of antimicrobials, not perceiving their own prescription practices to be a problem, pressure by patients, limited time, fear of damaging doctor-patient relationship in addition to language, cultural and educational barriers (Cabral, 2016; Fleming-Dutra, 2016; Fletcher-Lartey, 2016; Lucas, 2015; Stille, 2008).

Educating the public on why antimicrobials are ineffective in most RTIs can reduce antimicrobial expectation. However, many parents, even well informed about inappropriate and overuse of antimicrobials in the community, still

pressure for prescriptions for their own children. Courses directly aimed at increasing physicians skills when communicating individualized rationale behind withholding antimicrobials is therefore as important as training the physicians in safe prescribing and to safely differentiate between which infections need treatment (Cabral, 2016; Fletcher-Lartey, 2016; C. R. Lee, 2013; Lucas, 2015; Stille, 2008). In addition to reducing human consumption of antimicrobials, reducing its use in livestock farming is even more important (Tang, 2017), although its discussion is beyond the topic of this thesis.

1.2.3 Competition within the nasopharyngeal microbiome

In addition to pneumococci, many other bacteria colonize the human nasopharynx, many of which are commensal and of low or no virulence living in symbiosis with its host (Bogaert, 2011; Devine, 2015; Mika, 2017; Pettigrew, 2012; Shak, 2013). Complex competition between the various species is well-known (Bogaert, 2004; Devine, 2015; Kadioglu, 2008; Pettigrew, 2012; Shak, 2013), with decreased density of commensal species often found in disease states, such as AOM (Devine, 2015; Pettigrew, 2012; Shak, 2013). The most well-known colonisers of the nasopharynx are, beside pneumococci, α-haemolytic streptococci, non-typeable *Haemophilus influenzae* (NTHi), *Moraxella catarrhalis, Staphylococcus aureus, Neisseria meningitidis,* and *Streptococcus pyogenes* (GAS) (Bogaert, 2004, 2011; Devine, 2015; Shak, 2013; Vuononvirta, 2011), in addition to many other genera (Bogaert, 2011; Mika, 2017).

1.2.4 Duration of carriage

Duration of nasopharyngeal carriage of pneumococci differs between individuals and serotypes, with younger children carrying pneumococci more often and for longer periods than older children (Hill, 2010; Högberg, 2007; Hussain, 2005; Melegaro, 2007; Mosser, 2014; Shak, 2013). The reason is probably their less developed immune system, as compared to older children and adults (Mosser, 2014). Additionally, older children and adults living with younger children are at increased risk of acquisition of pneumococcus in their nasopharynx (Hill, 2010; Mosser, 2014). The mean duration of carriage for children is around 20 – 60 days (Cauchemez, 2006; Högberg, 2007; Melegaro, 2007; Shak, 2013) in high-income settings, in contrast, a significantly longer duration, up to 12 – 28 weeks is found in very low-income settings (Hill, 2010)

There is strong evidence for serotype specific duration of carriage (Högberg, 2007; Melegaro, 2007; Sleeman, 2006). Several studies report 6B to be carried for longer duration than other serotypes (Högberg, 2007;

Melegaro, 2007; Sleeman, 2006), while other serogroups, such as 9, 14 and 15 are carried for a shorter period (Högberg, 2007; Melegaro, 2007; Sleeman, 2006). Additionally, there is some evidence that non-vaccine serotypes have a shorter duration of carriage (Melegaro, 2007), although other studies have not confirmed differences between vaccine and non-vaccine serotypes (Cauchemez, 2006). Some serotypes, such as 1, 4, 5 and 7F are rare in carriage studies and are possibly only transiently carried (Hill, 2010; Simell, 2012; Sleeman, 2006), despite having been an important cause of IPD prior to introduction of higher valent PCVs (Hill, 2010; Simell, 2012; Sleeman, 2006). The reason for this might be connected to their immunogenicity and invasiveness, discussed in detail in Chapter 1.3.

In animal models, knocking out certain receptors of the innate immune system causes delayed clearing of pneumococci (Sun, 2004). The likelihood of transformation from colonization to infection is negatively associated with a longer duration of colonization (Hill, 2010; Simell, 2012; Sleeman, 2006). The risk of infection is apparently the highest soon after acquisition and decreases as colonization endures (Simell, 2012; Smith T, 1993). This may indicate that serotypes with low virulence have lower immunogenicity and are more likely to cause asymptomatic carriage state than progressing to invasive disease. On the other hand, more invasive strains will either infiltrate the mucosal barrier causing clinical infections or evoke immune response and be cleared (Simell, 2012; Smith T, 1993).

1.2.5 Using nasopharyngeal samples to predict aetiology of disease

Predicting the aetiology of respiratory tract infections (RTIs) can be challenging without access to the site of infection, as discussed in Chapter 1.1.1. Considerable research has been done in evaluating the utility of using nasopharyngeal samples in predicting the aetiology of RTIs, mainly AOM. Several studies have reported the calculated positive predictive value (PPV) and negative predictive values (NPV), of a given pathogen in a NP sample also being found in the middle ear fluid (MEF). In this chapter, those studies, their implications and limitations will be discussed.

Three papers, reporting data from seven studies evaluated the PPV of finding the same pathogen in the middle ear as in the nasopharynx and the NPV of the absence of the pathogen in the nasopharynx being predictive of it also being absent in the middle ear (Kaur, 2014; Syrjanen, 2006; Van Dongen, 2013). Kaur et al. found a PPV and NPV of 49% and 93%, respectively for pneumococci and 67% and 94%, respectively for NTHi. Additionally, they

found a PPV of 95% for carriage of oxacillin resistant pneumococci in the NP also being in the MEF (Kaur, 2014). Syrjanen et al. conducted a study on children <2 years of age, where both the serotype and clonal type of pneumococci were compared in MEF and NP. In their study the PPV and NPV was 50% and >99% for pneumococci and 64% and 93% for NTHi (Syrjanen, 2006), results which supports using NP to rule out a certain pathogen in the middle ear. Van Dongen et al. compared six studies, including the one by Syrjanen et al., calculated PPV and NPV for pneumococci was found to be 50% and 70% and 58% and 80% for NTHi (Van Dongen, 2013).

Discrepant to those studies, Yatsyshina et al. showed a high PPV of 92%, with lower 79% NPV for pneumococci. For NTHi their result showed a PPV and NPV of 64% and 92%, respectively. Importantly, Yatsyshina et al. used PCR for detection, which increases sensitivity, especially in children with current antibiotic use and MEF samples were taken as part of routine tympanic tube procedure, perhaps promoting increased risk of selection bias (Yatsyshina, 2016).

In summary, the value of NP sampling in predicting pathogens in AOM seems to only be useful in detecting isolates with reduced susceptibility to antimicrobials, thus aiding the choice of treatment. The NPV may indicate absence of pneumococci and NTHi but probably with uncertain clinical value. However, it seems to be inadequate, at least with current culturing methods to predict with sufficient accuracy which pathogen is causing the infection.

In pneumonia and IPD, sampling of the nasopharynx is generally not helpful in distinguishing causative bacterial pathogens (Catterall, 1999; Clark, 2015; Simell, 2012), although quantitative RT PCR might play a part in distinguishing carriage from lower respiratory tract infections (Bjarnason, 2017).

1.3 Virulence

The pneumococcus is classified among the streptococcal pneumoniae-mitis-pseudopneumoniae cluster, with its closest relatives being the paradigm commensal bacteria (Kilian, 2008). Phylogenetic analysis showed close genetic relationship of pneumococci to other species of the pneumoniae-mitis-pseudopneumoniae cluster, even closer relation than the intraspecies variability (Kilian, 2008). They suggest that rather than commensal species acquiring virulence factors to become pathogens, as suggested by others (Raskin, 2006), the cluster has evolved from a common pathogenic ancestor. While pneumococci have retained their virulence factors, the other species have slowly lost part of their virulence factors, adapting for symbiosis with

humans (Kilian, 2008). They also suggest that the same is happening with pneumococci, although at a slower rate, using non-capsulated pneumococci as their example (Kilian, 2008).

Despite their substantial virulence, pneumococci are for the most part, commensals like their closest relatives, as discussed in Chapter 1.2. Therefore, one might speculate that colonization or commensalism as opposed to disease state is the most beneficial for bacteria, with the greatest fitness for strains able to spread without eliciting strong immune response or killing its host (Kadioglu, 2008; Tlaskalová-Hogenová, 2004). Commensal bacteria thread a delicate balance, they must be able to circumvent the innate immunity while not eliciting the adaptive immune response. As corroboration, highly invasive pneumococcal strains are rare in carriage (Hill, 2010; Simell, 2012; Sleeman, 2006), as they likely elicit strong immune response and are either cleared or invade the hosts' defences (Tlaskalová-Hogenová, 2004).

1.3.1 Virulence factors

Pneumococci have various virulence factors which aid in both their circumvention of the innate immunity in carriage, suppressing competing bacteria and facilitating and maintaining invasion to underlining tissue and blood (Bogaert, 2004; Simell, 2012). In this section, main emphasis will be on the pneumococcal capsule, an important virulence factor, necessary for the pneumococcus to cause disease in an immunocompetent host (Catterall, 1999; Epstein, 1995; Kalin, 1998).

1.3.1.1 Capsule

As the most important virulence factor of the pneumococcus, the capsule has been extensively studied (Austrian, 1981). It is made up by repeated units of different oligosaccharides, with different configurations for each serotype (Kadioglu, 2008; Kalin, 1998; Llull, 2001). The capsular polysaccharide synthesis genes A – D (Cps A, B, C and D) are highly preserved in all pneumococcal serotypes, except 3 and 37 (García, 1997; Kadioglu, 2008; Llull, 2001; Morona, 2004; M. L. S. Oliveira, 2003), with manipulation of gene expression genes reducing or eliminating virulence (Morona, 2004)

Pneumococcal colonization can occur without the capsule, but at a lower density. On acquisition, the negatively charged molecules of the capsule prevents mucus entrapment, thus increasing the chance of reaching epithelial cells (Kadioglu, 2008; Nelson, 2007; Paterson, 2010; Simell, 2012). On reaching the epithelial cells, capsular density is decreased, as a thick capsule acts as a hindrance to adherence (Kadioglu, 2008; Nelson, 2007). In

concordance, pneumococci sampled from nasopharynx are more likely to form transparent colonies, while those isolated from the middle ear are more likely to form thicker, opaque colonies (Arai, 2011).

It seems that one of the main virulent functions of the pneumococcal capsule, is its protective function against opsonisation and subsequent phagocytosis (Epstein, 1995; Hyams, 2010; Kadioglu, 2008; Simell, 2012). Although anti-phagocytosis might also play a part in acquisition, it does not seem to be the major determinant (Nelson, 2007). Phagocytosis is mediated by binding of Fcy receptors on phagocytes to soluble factors (opsonins), bound to bacterial surfaces (Kumar, 2012). The capsule protects the bacteria from opsonisation and decreases the activity of both the classical and alternative pathways (Hyams, 2010; Kadioglu, 2008; Paterson, 2010). Additionally, it protects the pneumococcus from trapping in neutrophil extracellular traps and offers some protection against antimicrobial lysis (Kadioglu, 2008)

1.3.1.2 Other virulence factors

As discussed above, the capsule is the major virulence factor of the pneumococcus (Catterall, 1999; Epstein, 1995; Kalin, 1998). However, it is not the only weapon in its arsenal. Many of the major virulent components, are expressed extracellularly, on the cell wall. The best described include autolysin, choline binding protein A, hyaluronate lysase, neuroaminidases, pili and pneumococcal surface protein A (PspA) (Catterall, 1999; Epstein, 1995; Hava, 2002; Kadioglu, 2008).

The cell wall is dynamic in structure, remodelling continuously with insertion and releasing of its glycopeptide cell wall components, varying by the bacteria's need. Its main role is to keep an equilibrium within the bacteria, even in harsh environment. Lysing of the cell wall invariably causes cell death. By bacterial regulation of capsular expression, cell wall antigens are exposed, aiding binding to host cells and activation of the host immune response. The components are highly chemotactic, stimulating leukocyte recruitment, thus increasing permeability of host´s tissue and blood flow. This in turn aids the pneumococcal infiltration from site of infection to the bloodstream (Catterall, 1999; Epstein, 1995; Hava, 2002; Kadioglu, 2008; Simell, 2012).

Additionally, both secretory and intracellular components contribute variably to pneumococcal virulence. Immunoglobulin A (IgA) protease is an enzyme excreted by some microbes that hydrolyses human IgA, increasing the pathogen's survival against the body's immune system, and exposing attachment sites (Catterall, 1999; Kadioglu, 2008). Pneumolysins are

intracytoplasmic toxins, produced by pneumococci which are released only on bacterial cell lysis, and destroys host cells (Catterall, 1999; Epstein, 1995; Kadioglu, 2008)

With the development of new laboratory methods, various other virulence factors have been described in recent years (Catterall, 1999; Epstein, 1995; Kadioglu, 2008).

1.3.2 Invasiveness index

As previously mentioned, the propensity of different pneumococcal serotypes to cause disease is not equal, with only a few serotypes causing the majority of invasive pneumococcal disease, prior to the advent of pneumococcal conjugate vaccine (Simell, 2012). Several studies, discussed in more detail in the following section, have reported a serotype specific invasiveness index or disease potential of serotypes. This invasiveness index has been defined as the relative prevalence of serotypes isolates in pneumococcal disease compared to the prevalence in healthy carriers. Although, mostly used for IPD, it can also be utilized for AOM and pneumonia, then more often referred to as disease potential. A high invasiveness index or disease potential indicates that the serotype is disproportionally often found in disease compared to carrier studies.

1.3.2.1 Invasiveness and IPD

In IPD, the invasiveness index varies greatly between serotypes (Brueggemann, 2003; Hanage, 2005; Kronenberg, 2006; Rivera-Olivero, 2011; Sá-Leão, 2011; Shouval, 2006; Zemlickova, 2010). However, due to small samples sizes, researchers often have difficulties estimating invasiveness index, especially for rare serotypes.

Six papers presented the comparison of serotype distribution between healthy carriers and IPD (Brueggemann, 2003; Hanage, 2005; Rivera-Olivero, 2011; Sá-Leão, 2011; Shouval, 2006; Zemlickova, 2010). One study presented the nasopharyngeal carriage in outpatient children presenting with AOM or pneumonia as controls for IPD (Kronenberg, 2006).

In a study from the UK (Brueggemann, 2003) data is reported on children <5 years, in Finland (Hanage, 2005) for children <2 years, while carriage data for children <6 years compared to IPD data from all ages is reported from Portugal (Sá-Leão, 2011). In the Czech Republic (Zemlickova, 2010) data was included for children <6 years, <3 years in Israel (Shouval, 2006), 3 – 36 months in Venezuela (Rivera-Olivero, 2011) and on children <7 years from

Swiss (Kronenberg, 2006), all prior to initiation of the conjugate vaccines (Table 2).

In those seven studies, a total of 8575 pneumococcal isolates were included, 3600 from invasive disease and 4975 from carriage. The studies varied in size and therefore the power to detect significant differences between individual serotypes varied. Invasiveness indexes for several serotypes were in concordance between the studies, serotypes 1, 4, 5, 7F, 8, 12, 14, 19A with

Table 2. *Serotypes invasiveness indexes significantly differing from 1.0 as reported from selected studies. More invasive serotypes, defined as having index >1.0 and less invasive serotypes with index <1.0.*

Country, study period	n of isolates IPD / carriage	More invasive serotypes (Invasiveness index)	Less invasive serotypes (Invasiveness index)
UK, 1994-2001, (Brueggemann,2003)	150 / 351	1[a] (10), 4[a] (12), 14[a] (9), 18C[a] (6)	23F[a] (0.4)
Finland, 1995-1999 (Hanage, 2005)	224 / 217	6B[a] (2), 14[a] (4), 18C[a] (3), 19A[b] (3)	6A[b] (0.5), 11A (0.05), 35F (0.2)
Portugal, 2001-2003 (Sá-Leão, 2011)	475 / 769	1[a] (78), 3[b] (3), 4[a] (14), 8 (46), 9L (2 – ∞)[c], 9N (10), 14[a] (2), 12B (4 – ∞)[c], 18C[a] (2), 20 (4 – ∞)[c]	11A (0.4), 6A[b] (0.3), 6B[a] (0.3), 15B/C (0.09), 16F (0.04), 19F[a] (0.1), 23F[a] (0.4), 34 (0.3), 35F (0.1), 37 (0.0)
Israel, 2000-2004 (Shouval, 2006)	189 / 1763	1[a] (25), 5[a] (9), 12F (9)	6A[b] (0.5), 6B[a] (0.6), 15A (0.4)
Swiss, 2002-2004 (Kronenberg, 2006)[d]	2388 / 1540	1[a] (3), 4[a] (7), 7F[a] (7), 8 (6), 9V[a] (3), 14[a] (2), 22 (2)	6[e] (0.5), 6A[b] (0.0), 6B[a] (0.6), 10 (0.5), 11 (0.4) 15 (0.3), 18C[a] (0.6), 19F[a] (0.2), 23[f] (0.5)
Czech Republic, 2004-2005 (Zemlickova, 2010)	138 / 153	9V[a] (11), 14[a] (5)	6A[b] (0.3), 23F[a] (0.4)
Venezuela, 2006-2008 (Rivera-Olivero, 2011)	36 / 182	7F[a] (16), 18[a] (16)	

[a]Serotypes included in PHiD-CV10 vaccine. [b]Additional serotypes included in the PCV-13 vaccine. [c]INF: Infinity, due to serotype not being found in carriage. Using the rule of threes (Hanley, 1983)*, upper bound of 95% confidence intervals, were found to be between 3000 – 8000. [d]Invasiveness indexes calculated from data presented in paper. [e]Not 6 A/B. [f]Not 23F.*

high indexes (all >1.0) and serotypes 6A, 11A, 15, 19F, 23F, 35F with low indexes (all <1.0). Conversely, 6B was reported to have a high index in one

study (Hanage, 2005), but low in three other studies. 18C was reported with high index in five studies, but low index by one (Kronenberg, 2006) (Table 2). This discordance could represent different distribution of serotype clones between countries, as invasiveness differs between serotype clones (Brueggemann, 2003). It is assuring that five of the eight serotypes with significant indexes >1.0 are included in the PHiD-CV10, while the serotypes most commonly cultured from healthy children in Iceland after initiation of PHiD-CV10, 15, 11, 23B, 6C and 6A (Sigurdsson, 2017) have indexes <1.0 (Table 2).

1.3.2.2 Invasiveness and Pneumonia

Establishing serotype specific disease potential for pneumonia is challenging due to hindrance in acquiring representative samples from site of infection (Catterall, 1999; Clark, 2015; Simell, 2012; Song, 2013), discussed in length in other chapters. Therefore, fewer studies have attempted estimating the disease potential in non-bacteraemic pneumonia.

In a study conducted in Israel prior to PCV-7 licensure (Greenberg, 2011), nasopharyngeal samples taken at time of radiographically confirmed alveolar pneumonia were compared to serotype prevalence in an ongoing surveillance in healthy children in the area (Greenberg, 2011). In that study, serotypes with disease potential significantly higher than 1.0 were serotypes 1, 22F, 5, 7F, 14, 9V, 19A with indexes ranging from 1.7 to 16.8 (all $p<.05$). Serotypes with indexes significantly lower than 1.0, were: 6B, 6A, 15A, 23A and 35B with indexes ranging from 0.2 – 0.5 (all $p<.05$) (Table 3, Figure 1). For several other serotypes, the null hypothesis could not be rejected, thus those serotypes may have a disease potential close to 1, although for some serotypes a larger study would be needed (Table 3).

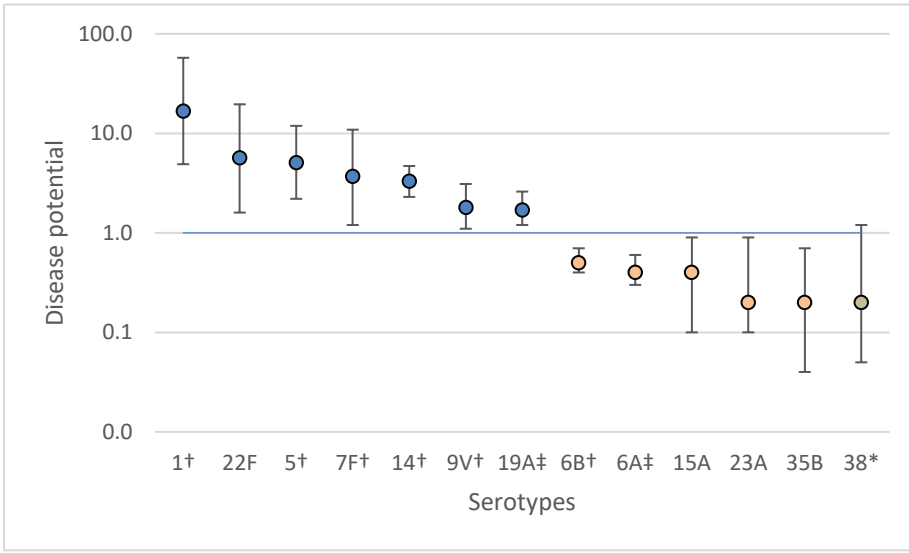

Figure 1. The pneumonia disease potentials for selected serotypes. Adapted from (Greenberg, 2011). *Not significantly different from 1.0. †Serotypes included in PHiD-CV10 vaccine ‡Additional serotypes included in the PCV-13 vaccine

1.3.2.3 Invasiveness and AOM

In contrast to IPD, serotype distribution in mucosal infections such as OM is closer to what is found in carriage studies of healthy children (Hjálmarsdóttir, 2017; Simell, 2012). However, there are differences in the disease potentials. In a large study from Israel including 3200 MEF isolates compared to healthy carriers, serotypes 3, 5, 1, 12F, 19A and 19F all had disease potential significantly higher than 1.0. Additionally, 6B, 6A and 15A had indexes significantly lower than 1.0 (Shouval, 2006). In another study serotypes 19F and 23F were found more likely to cause AOM, while 33F was less likely (Hanage, 2004). This contrast in serotype distribution, might indicate that the risk of invasive disease is more strongly connected to the ability to cross the first barrier and survive there, while local spread might be more dependent on other factors such as density of growth (Simell, 2012).

Table 3. The pneumonia disease potentials for selected serotypes. Odds ratios (OR) were calculated using the odds of the serotype being found in disease compared to in carriage. An OR of 1 indicates equal odds of finding serotype in carriage and in disease. Adapted from (Greenberg, 2011)

Serotype	% disease	% carriage	Odds ratio	95% CI
1 †	4.5	0.2	16.8	4.9 – 57.5
22F	1.0	0.3	5.7	1.6 – 19.6
5†	4.1	0.6	5.1	2.2 – 11.9
7F†	1.8	0.5	3.7	1.2 – 10.9
14†	15.3	5.5	3.3	2.3 - 4.7
9V†	4.5	2.9	1.8	1.1 – 3.1
19A‡	8.5	5.3	1.7	1.2 - 2.6
33F*	2.2	1.1	1.9	0.8 – 4.5
19F*†	9.3	9.4	1.2	0.8 – 1.7
18C*†	1.2	2.1	1.1	0.4 – 2.9
34*	1.0	0.9	0.9	0.3 – 2.7
15B/C*	2.7	2.2	0.9	0.5 – 1.6
23F*†	8.5	9.2	0.9	0.6 – 1.3
11A*	1.2	1.7	0.8	0.4 – 2.0
21*	1.5	2.9	0.7	0.3 – 1.5
16F*	0.8	1.8	0.4	0.1 – 1.2
6B†	7.8	13.0	0.5	0.4 – 0.7
6A‡	6.6	12.8	0.4	0.3 – 0.6
15A	1.2	2.1	0.4	0.1 – 0.9
23A	0.5	2.1	0.2	0.1 – 0.9
35B	0.3	2.9	0.2	0.04 – 0.7
38*	0.3	1.3	0.2	0.05 – 1.2

*†Serotypes included in PHiD-CV10 vaccine ‡Additional serotypes included in the PCV-13 vaccine *Not significantly different from 1.0*

1.4 Otitis media

1.4.1 Acute otitis media

Acute otitis media (AOM) is the most commonly diagnosed infectious disease in children (Ahmed, 2014; Bergenfelz, 2017; Daly, 2000; Monasta, 2012; Ramakrishnan, 2007). In children between the age of one and four, the worldwide incidence rate (IR) has been estimated to be 0.61 episodes per person-year (Monasta, 2012). It has been estimated that more than 700 million episodes of AOM occur annually, with half occurring in under five year old children, resulting in more than 20 thousand worldwide deaths annually due to OM and its complications (Acuin, 2004). The IR varies between countries, lowest in European countries (Liese, 2014; Monasta, 2012; Usonis, 2016), higher in Iceland (Gudnason, 2013), the United States (Ahmed, 2014; Marom, 2014), and in low resource countries (Daly, 2007; Monasta, 2012; O'Brien, 2003, 2008).

Recent prospective study, conducted after the introduction of PCV in the US estimated that 23% were diagnosed with AOM by their first birthday, 23% by their second and 60% by their third (Kaur, 2017). This is lower than the 80% at three years of age, estimated by Teele et al. in 1989 (Teele, 1989). In Denmark, the cumulative incidence is 38.8% and 60.6% at 18 months and 7 years of age, respectively (Todberg, 2014)

1.4.2 Pathogenesis

The eustachian tube in young children is shorter, with a narrower isthmus and less acute angle than in older children and adults (Daly, 2000; Harmes, 2013; Ramakrishnan, 2007; Rosenfeld, 2013). Often the spark for otitis is a mild upper respiratory viral infection, acquisition of pneumococci or allergic rhinitis (Daly, 2000; Ramakrishnan, 2007; Zola, 2008), which can cause congestion and subsequent swelling of mucus membranes in the nose, eustachian tube and middle ear. This in turn causes delayed clearing and development of effusion in the middle ear due to development of negative pressure in the middle ear. Viruses or bacteria or both can secondary infect the effusion causing otitis media (Ramakrishnan, 2007).

The most common bacterial causes of AOM are pneumococci, NTHi and *Moraxella catarrhalis*, while GAS can be the causative agent in severe cases, often in older children (Bluestone, 1992; Casey, 2004; Dagan, 2016; Kaur, 2017; Leibovitz, 2004; Ngo, 2016; Van Eldere, 2014). Pneumococci are more common in young children, and in a child's first infection. Middle ear infections, especially caused by pneumococci can disrupt the mucosa, increasing the risk

of subsequent infections by less virulent bacteria. Thus in recurrent and complex disease, other pathogens, especially NTHi are more common (Dagan, 2016; Van Eldere, 2014). In preventing or delaying first presentation of pneumococcal AOM, the risk of subsequent non-pneumococcal AOM is decreased (Dagan, 2016).

1.4.3 Diagnosis

The clinical symptoms of AOM are often nonspecific such as fever, pulling at ears, irritability, lack of sleep, headache, loss of appetite, anorexia, vomiting, diarrhoea, rhinitis and otalgia, although otalgia is less common in infants and young children (Bergenfelz, 2017; Ramakrishnan, 2007; Rothman, 2003). As the presentation of AOM varies, differentiation between AOM, otitis media with effusion (OME) and other upper respiratory tract infections (URTI) is often difficult using only the clinical symptoms (Ramakrishnan, 2007).

The most common signs of AOM found on otoscopic examination are inflammation in the middle ear, seen as bulging, erythematous tympanic membrane and absent or displaced light reflex, representing presence of purulent or non-purulent effusion behind the membrane. In some cases perforation of the membrane and spontaneous draining of otorrhea is present (Bergenfelz, 2017; Rothman, 2003). Severe headache, focal neurological signs or confusion warrant further testing to rule out intracranial spread of infection, a rare but potentially devastating complication (Ramakrishnan, 2007)

Additionally, pneumatic otoscopy, tympanometry, acoustic reflectometry and otomicroscope all improve the accuracy of diagnosis, when used by experienced providers (Ramakrishnan, 2007; Rothman, 2003)

1.4.4 Risk factors for acute otitis media

The main risk factors for AOM is young age and crowding, while several others are also well established (Table 4). Additionally, the risk is higher in children of indigenous populations, possibly due to high carriage prevalence, overcrowding, lack of hygiene and low socioeconomic status, although genetic dispositions cannot be ruled out (Macintyre, 2010; Ramakrishnan, 2007).

1.4.5 Chronic otitis media

1.4.5.1 Otitis media with effusion

Otitis media with effusion (OME), often called secretory otitis media or ´glue ear´, is the more chronic type of otitis media, often more prevalent in older children, in late preschool and beginning of primary school (Bergenfelz, 2017).

OME can occur spontaneously due to eustachian tube dysfunction or more commonly following or in reaction to an infection (Ramakrishnan, 2007). Longstanding effusion is more prevalent in children with previous history of recurrent or severe AOM, especially at a young age (Acuin, 2004; Dagan, 2016). Its main clinical symptoms are discomfort connected to the presence of non-purulent fluid in the middle ear cavity, such as hearing impairment (Acuin, 2004; Mortensen, 2013; Roberts, 2004) in contrast to symptoms of active inflammation as in AOM (Bergenfelz, 2017),

Table 4. The risk factors for developing initial AOM in children

Factors effecting	Initial AOM
Young age	**Increases risk:** (Baraibar, 1997; Daly, 2000; Macintyre, 2010; Marom, 2014; Ramakrishnan, 2007; Todberg, 2014)
Breastfeeding <3 months	**Increases risk:** (Baraibar, 1997; Ramakrishnan, 2007)
DCC attendance	**Increases risk:** (Baraibar, 1997; Daly, 2000; Ramakrishnan, 2007)
Male sex	**Increases risk:** (Baraibar, 1997; Daly, 2000; Macintyre, 2010; Ramakrishnan, 2007)
Exposure to cigarette smoke	**Increases risk:** (Baraibar, 1997; Daly, 2000; Macintyre, 2010; Ramakrishnan, 2007)
Pacifier use	**Increases risk:** (Baraibar, 1997; Ramakrishnan, 2007)
Season: fall winter	**Increases risk:** (Baraibar, 1997; Ramakrishnan, 2007)
Comorbidities; allergic rhinitis, cleft palate, Down syndrome	**Increases risk:** (Baraibar, 1997; Ramakrishnan, 2007)
Siblings / crowding	**Increases risk:** (Baraibar, 1997; Daly, 2000; Macintyre, 2010; Ramakrishnan, 2007)
Maternal age, low	**Increases risk:** (Macintyre, 2010)
Birth weight	**Decreased risk with high:** (Macintyre, 2010), **Increased risk for low**: (Daly, 2000),
Gestational age	**No effect:** (Macintyre, 2010), **Increased risk in prematurity**: (Baraibar, 1997; Daly, 2000)
Previous antibiotic use	**Increases risk of antimicrobial failure:** (Ramakrishnan, 2007)
Previous otitis media	**Increases risk of antimicrobial failure:** (Ramakrishnan, 2007)

1.4.5.2 Recurrent acute otitis media

Recurrent AOM (rAOM), defined as three well-documented separate AOM episodes within six months or four within the span of 12 months, is fairly common and can like SOM have negative impact on quality of life for children

and their parents during active episodes (Crawford, 2017; Greenberg, 2003; Holl, 2015).

As discussed in Chapter 1.4.2, it has been hypothesized that a child's first OM, especially in young children, is more often caused by the more virulent serotypes of pneumococci (Dagan, 2016; Leibovitz, 2004), inflicting damage to the immature middle ear, facilitating recurrence of otitis with less virulent organisms.

Observational studies have shown a correlation between early diagnosis of AOM to rAOM (Dagan, 2016; de Hoog, 2016; Teele, 1989; Todberg, 2014). In a recent study, a linear connection was established between age at first infection and risk of recurrent OM. The study showed that with every month younger the children were at first diagnosis of AOM, the risk of developing rAOM increased by 6 – 9% (de Hoog, 2016). Similarly, in another study, children diagnosed with AOM before the age of one were seven times more likely to develop rAOM than those diagnosed after first birthday (Macintyre, 2010).

1.4.6 Treatment of otitis media

Although up to 80% of cases of acute otitis media resolve spontaneously (Ramakrishnan, 2007), the majority of episodes are treated with antimicrobials (Landlæknisembættið, 2017; Ramakrishnan, 2007).

It has become more and more apparent that antimicrobial treatments do not improve outcomes in general, except for certain patient age groups. Consequently, in recent years the indications for antimicrobial treatment has been more focused, with the most recent guidelines in Iceland on AOM from 2009 and antimicrobial usage from 2017 (Landlæknisembættið, 2009, 2017), adopted from National Institute of Health and Clinical Excellence (NICE) guidelines (NHS, 2008). The recommendations are that children less than 12 months of age with suspected AOM and children between the ages of 12 and 24 months with confirmed bilateral or severe AOM should be treated with antimicrobials (Landlæknisembættið, 2009, 2017; Ramakrishnan, 2007), (Figure 2). Additionally, antimicrobial treatment is warranted in children older than 12 and in adults. For others, watchful waiting, and delaying of prescription is recommended if tight follow-up is possible (Landlæknisembættið, 2009, 2017).

If antimicrobial treatment is warranted, an empiric regimen of amoxicillin 45 – 60 mg/kg/day is recommended. For children with clinical suspicion of antimicrobial resistant pathogens, such as in repeated infections or following

treatment failure, higher dose or broader spectrum antimicrobials are warranted (Figure 2) (Landlæknisembættið, 2009, 2017).

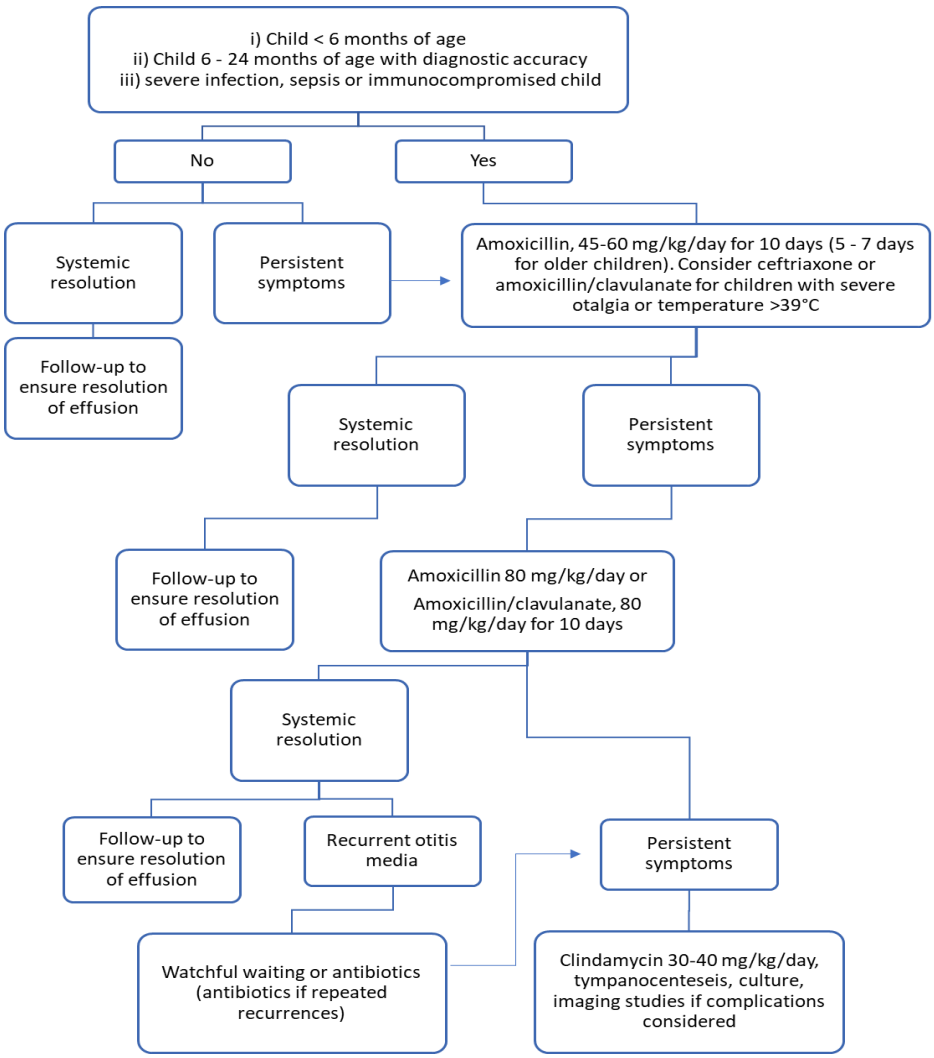

Figure 2. Algorithm for AOM treatment, adapted from references (Landlæknisembættið, 2017; Ramakrishnan, 2007)

In addition to antimicrobial treatment and for children, where antimicrobial therapy is not warranted, analgesia and anti-pyretic treatment is recommended (Bergenfelz, 2017; Landlæknisembættið, 2009, 2017). Anti-histamines and other congestion medications are not indicated as they can prolong effusion (Landlæknisembættið, 2009; NHS, 2008; Ramakrishnan, 2007).

For children with middle ear effusion without the suspicion of acute inflammation, watchful waiting is warranted. In children with long-standing effusion, insertion of tympanic tube is often considered (Browning, 2010; Paradise, 2005; Rosenfeld, 2013).

1.4.7 Cost of otitis media

In addition to hardship endured by children and parents alike due to otitis media, the societies cost of OM is high (Ahmed, 2014; Greenberg, 2003; Niemelä, 1999).

In 2009, the annual direct health care expenses due to AOM in the US was estimated to be $2.88 billion (Ahmed, 2014), with each child diagnosed contributing to direct cost of $314 (Ahmed, 2014). In Finland, the lifetime cumulative cost per child due to OM by the age of entering primary school was estimated to be $2549 (Niemelä, 1999).

Interestingly, direct cost contributes only to a minority of the total cost (Ahmed, 2014; Greenberg, 2003). Indirect cost includes parental absence from work and leisure time, increased cost of transportation and baby-sitting expenses (Ahmed, 2014; Greenberg, 2003; Niemelä, 1999). The mean yearly parental loss of work days due to AOM was 1.3 days per child aged seven and younger in Finland (Niemelä, 1999) and 2.2 days per instance of AOM in children younger than three in Israel (Greenberg, 2003).

1.4.8 Long term sequalae of otitis media effusion

The impact of longstanding or recurrent otitis media on development is controversial (Hall, 2014; Mortensen, 2013; Roberts, 2004). A 2004 meta-analysis on prospective studies concluded that a very small to no association on speech and language development in otherwise healthy children existed (Roberts, 2004).

However in a 2014 prospective study (Hall, 2014), which controlled for various factors including maternal age and education level, socioeconomic status, smoking during pregnancy, parity, birth weight, gestational age and sex it was found that long standing effusion and hearing problems during childhood increased the risk of both reduced verbal and performance intelligence quotient (IQ) (Hall, 2014). Consequently, the children most severely affected had mean seven points lower verbal IQ and five points lower performance IQ at age four than those unaffected. Conversely, at age eight, the effect was milder, 4 points in verbal IQ and non-significant reduction in performance IQ. The HOME questionnaire measures, among other things, how much cognitive

stimulation the child receives at home. When stratifying the children based on the HOME score, the difference was more pronounced in those that had lower HOME score, but mild or non-existing in children with high at-home stimulation.

This underlines the importance of cognitive stimulation at home for children and having an open eye for early intervention in children at-risk due to other reasons, such as unrelated development problems (Hall, 2014; Mortensen, 2013; Roberts, 2004).

In a large population study on male army recruits in Denmark, 18 – 20 years of age, those that had been hospitalized for OM at any point before the age of 8, were 15% more likely to be in the bottom quartile cognitive function than those that hadn't. However, no significant difference was found in proportion with secondary education. Additionally, they were more likely to suffer from hearing impairment (Mortensen, 2013).

1.5 Respiratory tract infections

Respiratory tract infections are classically divided in to upper and lower respiratory tract infections, with the larynx considered part of the upper respiratory tract and everything below it lower respiratory tract (Kumar, 2012).

1.5.1 Upper respiratory tract infections

Upper respiratory tract infections are extremely common, most often with unspecific symptoms from the nose, throat, ears and the conjunctiva. They are most often caused by viruses and generally resolve without consultations with health-care professionals (Kumar, 2012).

1.5.1.1 Common cold and sinusitis

The common cold is likely the most common infectious disease of humans, young children can expect to have 6 – 8 episodes every year, while adults can expect between two and four infections annually (Heikkinen, 2003; Johnson, 1996). Its hallmark symptoms are rhinorrhoea, nasal congestion, sneezing, cough and sore throat. Additionally, low-grade fever, headache, and malaise are often present.

It often occurs concurrently with viral sinusitis and are often collectively called rhinosinusitis. Rhinoviruses are the most common agent, causing up to 80% of episodes, depending on the season (Heikkinen, 2003; Kumar, 2012). The incubation time of rhinoviruses is short, and the mean duration of the common cold is little over a week. As of yet, treatment is purely symptomatic

as research on antiviral therapy has not been convincing (Heikkinen, 2003; Kumar, 2012).

Although a benign condition, it is a common catalyst for other disease such as AOM and acute bacterial sinusitis (ABS). Consequently, due to its extreme commonness its economic impact is considerable, contributing yearly to loss of 20 million work-days and 22 million school-days in the US (Heikkinen, 2003).

ABS can develop as a complication of rhinosinusitis, and has been estimated to develop in around 0.5 – 2% of episodes (Heikkinen, 2003; Johnson, 1996). Additionally they can occur secondary to other conditions such as allergy or with nasal obstruction (Johnson, 1996; Kumar, 2012). ABS is characterized by similar, but more severe and longstanding symptoms as rhinosinusitis. Additionally, early purulent discharge, sinus pain or pressure can signify bacterial infection (Kumar, 2012).

The sinuses become secondarily infected by the nasopharyngeal bacterial microbiome, with pneumococci, NTHi and *M. catarrhalis* accounting for majority of cases (Johnson, 1996; Kumar, 2012). As a self-limiting disease, symptomatic treatment and watchful waiting is often warranted, in uncomplicated disease. However, in patients, where follow-up is doubtful, or worsening symptoms, in some cases, antibiotic treatment should be considered. In Iceland, amoxicillin is the first line treatment (Landlæknisembættið, 2017). Due to high over-diagnosis of acute bacterial sinusitis, impact of the pneumococcal vaccination on ABS is unlikely.

1.5.1.2 Tonsillopharyngitis

Pharyngitis is a common respiratory tract infection, accounting annually for 12.5 million primary care visits in the US (Schappert, 2006) and is caused by viruses in majority of cases (Barnett, 2014; Kumar, 2012). Even though bacteria, such as group A streptococci (GAS) are the causative organism in only around 10% of cases, one study estimated that around 60% (95%CI:57-63%) of tonsillopharyngitis episodes were treated empirically with antibiotics (Barnett, 2014). Even though, bacterial tonsillopharyngitis is usually a self-limiting infection which should only be treated if symptoms warrant it, the treatment of GAS tonsillopharyngitis (strep throat) is almost universal (Kumar, 2012).

Generally, sore throat is the principal symptom, while fever, lymphadenopathy, headache and malaise are more common with a bacterial

cause, while concurrent upper respiratory symptoms are more typical in viral infections. These symptoms are however not pathognomic (Kumar, 2012).

1.5.1.3 Other upper respiratory tract infections

Infections below the throat, such as laryngopharyngitis are generally benign, often caused by viruses and presents with hoarseness in adults. Children often develop stridor, tachypnoea and dyspnoea, which although generally benign, can be highly distressing to both for the patient and parents (Sharland, 2011).

Epiglottitis, a bacterial infection of the glottis, is a devastating infection, most often caused by *Haemophilus influenzae* serotype B (HiB) (Swingler, 2003), although it can also be caused by other bacteria, including pneumococci. Epiglottitis is very rare to due effective conjugate vaccine against HiB (Berndsen, 2012; Swingler, 2003).

1.5.2 Lower respiratory tract infections

1.5.2.1 Acute bronchitis

Acute bronchitis is a common, mostly benign disease. It is most often due to viruses, although smokers and patients with chronic obstructive pulmonary disease (COPD) can be secondarily infected by pneumococci or *H. influenzae* (Kumar, 2012). Uncommon in children, but very common in older adults, especially current smokers. The main symptoms include chest tightness, dry cough and shortness of breath. Wheezing is dominant on auscultation, although occasional crackles can be heard (Kumar, 2012). Acute bronchitis should not be treated with antibiotics except in special cases, as antibiotic treatment has not been associated with better outcomes or earlier recovery (Kumar, 2012; NHS, 2008).

1.5.2.2 Acute bronchiolitis

Acute bronchiolitis is very common, especially in children <2 years of age (Bordley, 2004; Fitzgerald, 2004; Ralston, 2014) and characterized by inflammation and oedema in the epithelial cells of the terminal bronchioles due to respiratory viruses (Bordley, 2004; Fitzgerald, 2004; Ralston, 2014). Following a prodrome of coryza, the children often presents with fever, cough with variable levels of respiratory distress, with fast breathing, chest retractions and wheezing (Bordley, 2004; Fitzgerald, 2004; Ralston, 2014; Sharland, 2011).

Respiratory syncytial virus (RSV) is the most common cause of acute bronchiolitis followed by rhinovirus, parainfluenza, human metapneumovirus

and others being less common (Mansbach, 2008; Midulla, 2010; Ralston, 2014).

Treatment for bronchiolitis is mainly supportive, with no pharmacological treatment recommended in most cases in non-asthmatic children (Fitzgerald, 2004; Ralston, 2014; Sharland, 2011). Studies on inhaled or oral bronchodilators, nebulized adrenaline, nebulized hypertonic saline, oral, intravenous or inhaled glucocorticoids have not demonstrated any significant improvement in clinical parameters, admission rates and lengths of stay (Fitzgerald, 2004; Gadomski, 2014; Hartling, 2011; Heikkilä, 2018; Ralston, 2014), although the most severely sick children were often excluded from the cited studies. The benefit of anti-virals is reserved to immunocompromised children with severe disease and immunoglobulins have not been shown to be effective (Fitzgerald, 2004; Rodriguez, 1997; Ventre, 2004). Antibiotics should only be used when secondary bacterial infections are suspected (Fitzgerald, 2004; Peltola, 2004; Ralston, 2014). It is recommended to administer supplemental oxygen as needed and to keep oxygen saturation above 92% (Fitzgerald, 2004; Ralston, 2014). In more advanced cases high-flow warm humidified oxygen is given by nasal cannula, continuous positive airway pressure (CPAP) used and in the most severe cases endotracheal intubation is required (Javouhey, 2008; Pierce, 2015). Additionally, most children likely benefit from mechanical nasal suction, to relieve nasal obstruction (Fitzgerald, 2004; Gadomski, 2014; Hartling, 2011; Heikkilä, 2018; Ralston, 2014; Sharland, 2011).

1.5.2.3 Pneumonia

Pneumonia is an inflammation of the parenchyma of the lungs, the alveoli, alveolar ducts and respiratory bronchioles. Most often this classification is limited to inflammation caused by an infectious agent, with inflammation due to other causes rather referred to as pneumonitis (Kumar, 2012; Remington, 2014; Sharland, 2011). Pneumonias are commonly classified as either community-acquired (CAP) or hospital-acquired (Kumar, 2012), with this introduction focussing mainly on CAP in an immunocompetent person.

Annual worldwide under-five mortality from pneumonia has been estimated to be 1.3 million, most of which occur in LMIC (Fischer Walker, 2013). In HIC, hospitalizations and deaths due to pneumonia are much lower, (Fischer Walker, 2013) with a recent study estimated that 0,2% of children less than five years of age in the US are hospitalized annually for pneumonia (G. E. Lee, 2010). Admissions due to CAP in young adults are also uncommon, increasing with age. In Iceland prior to introduction of PCVs, the IR of admitted CAP was

20.6 cases per 10,000 person-year of follow-up in adults older than 18 years. The IR varied greatly between age groups, ranging from 8.3 in patients 18 to 49-year old to 127.3 per 10,000 in patients 80 years and older, with pneumococci detected in 20% of cases (Bjarnason, 2018).

The most common symptoms of pneumonia regardless of aetiology are cough, dyspnoea, tachypnoea, fever, chest pain and sputum production. In addition confusion, weakness and falls are common in the elderly and retraction and other indicators of increased breathing effort in children (Kumar, 2012; McCracken, 2000; Metlay, 1997; Remington, 2014). In addition to the above, physical examination often reveals coarse crackles, sometimes absent in dehydrated children (McCracken, 2000; Metlay, 1997).

Pneumonia severity scoring systems, used to predict the risk of mortality should be used when evaluating all adult patients presenting with pneumonia (Chalmers, 2010). The Pneumonia severity index (PSI), CURB-65, CRB-65 scores are the most commonly used, and have been determined to be roughly equal when applied correctly (Chalmers, 2010).

Severity assessment in children rely more on physical examination and vital signs with high fever, high respiratory rate and signs of laboured breathing indicative of severe lower respiratory tract infection, requiring admission to hospital (Sharland, 2011) (Table 5).

Table 5. Severity of lower respiratory tract infections in infants and children. Adapted from (Sharland, 2011)

	Infants		**Older children**	
	Mild	**Severe**	**Mild**	**Severe**
Temperature	< 38.5°C	> 38.5°C	< 38.5°C	> 38.5°C
Respiratory rate, per min	< 60	> 60	< 50	> 50
Feeding	Full feeding	Not feeding		
Physical examination	Mild retractions	More severe retractions with nasal flaring, grunting and intermittent apnoea	Breath-lessness	Severe difficulty in breathing, nasal flaring, grunting
Cyanosis	No	Yes	No	Yes

In diagnosis of pneumonia, chest radiographs are still the imaging study of choice. Pneumococcal or other bacterial pneumonia are often characterized by lobar consolidation, while interstitial or bilateral consolidation are more indicative of viral pneumonia (McCracken, 2000; Metlay, 1997). These findings are however not pathognomonic, with high level of cross-over between etiologic agents (McCracken, 2000; Metlay, 1997; Sharland, 2011). The presence of a large pleural effusion in suspected pneumonia is however highly specific for a bacterial agent (McCracken, 2000; Metlay, 1997). Blood test showing leucocytosis with predominant left shift and increase in serum values of acute phase reactants, can be helpful in diagnosis, despite lack of specificity (Sharland, 2011; Williams, 2015).

Pneumococci are the most common bacterial cause of CAP, being the causative agent in up to 50% of cases of bacterial CAP in adults and 30% in children (Berg, 2016; A. Bjarnason, 2017; Catterall, 1999; Fischer Walker, 2013; Heiskanen-Kosma, 2003; Howie, 2014; Jonnalagadda, 2017; Kumar, 2012; Remington, 2014; Sharland, 2011). Other agents include *Mycoplasma pneumoniae, Haemophilus influenzae, Staphylococcus aureus*, respiratory viruses and many more (Kumar, 2012; Remington, 2014; Sharland, 2011). Determining the microbiological agent is extremely important, and sputum collection should be performed for all admitted adult patients. For adult patients with a CURB-65 score of more than two, urine should be collected for pneumococcal and Legionella antigen testing and blood-cultures should be drawn in highly-pyretic patients regardless of age (Kumar, 2012; Remington, 2014). The utility, challenges and upcoming techniques of determining the microbial cause of pneumonia are discussed in detail in Chapter 1.1.

Empiric treatment of CAP depends on severity on presentation. Narrow spectrum beta lactams can safely be used on an outpatient basis in milder cases, while admission and broader spectrum antimicrobial treatment may be warranted in more severe cases (Kumar, 2012; Remington, 2014). In addition, supportive care is vital in treatment of CAP, with principles described in Chapter 1.5.2.2. also applying to patients with CAP.

1.6 Invasive pneumococcal diseases

Pneumococcal infections are defined as invasive when pneumococci are detected in specimens from normally sterile sites of the body. Blood, cerebrospinal fluids, joints and pleural fluid are the most common, while the middle ear, although normally sterile is not included in the definition (Epstein, 1995). IPD are among the deadliest infections, causing annually an upwards

of 800,000 deaths worldwide in children less than five, most of them living in low income countries (O'Brien, 2009).

The main risk factors for IPD in children are similar as those for carriage of pneumococci, mainly low age, crowding, respiratory tract viral co-infections, immunocompromise, parental smoking, winter months and being colonized with a new serotype (Mehr, 2012).

Microbial detection in suspected IPD is summarized in Chapter 1.1.

1.7 Pneumococcal vaccines

With the principles learned from over a century of vaccinations (Baxby, 1999) and the more contemporary pneumococcal anti-sera treatments (Austrian, 1981), many early 20th century researchers were highly interested in preventing pneumococcal diseases. As early as in 1911 Wright and colleagues attempted to vaccinate against pneumonia. They concluded that the vaccine was inefficacious, although had the study been structured differently, it might have been able to demonstrate protection (Austrian, 1981). Several studies in the 1930s and 1940s on low valent vaccines showed promise (Butler, 1999; W., 1982) and a 1945 study by Hodges and Bernhard which used polysaccharides from five serotypes showed it to be efficacious in preventing pneumococcal pneumonia caused by those serotypes (MacLeod, 1945). After the introduction of antimicrobials in the 1950s, the interest in pneumococcal vaccines dwindled (Butler, 1999).

1.7.1 Polysaccharide vaccines

In 1964 in a classical paper by Austrian et al. the death rate of bacteraemic pneumonia was determined to be very high despite optimal antimicrobial treatment. In the paper, the researchers showed that the 5-day survival rate was comparable between patients receiving penicillin, serum treatment and in untreated patients. After five days the survival lines diverged and the untreated patients had much worse prognosis (Austrian, 1964). The study showed the need for prevention and caused renewed interest in pneumococcal vaccines. Building on the research by Hodges and Bernhard, Robert Austrians' team developed a 14-valent polysaccharide pneumococcal vaccine (PPV) that was licensed in 1978 (Butler, 1999; Rytel, 1982) and the current polysaccharide vaccine, PPV-23, a 23-valent polysaccharide vaccine in 1983 (Butler, 1999). Although efficacious in preventing serotype specific adult disease (Shapiro, 1991) it was largely ineffective in preventing disease in infants and young children, due to limited immune memory for most polysaccharides, due

because of immature T-cell independent response (Ahmad, 1999; Jakobsen, 1999; Stein, 1992).

The 23 serotypes included in PPV-23 were the cause of close to 90% of all bacteraemic pneumococcal disease in the US and the UK before the initiation of the protein conjugate vaccines (Ahmad, 1999; Catterall, 1999; Epstein, 1995; Mehr, 2012).

1.7.2 Protein conjugate vaccines

In the late 20th century, both meningococcal and HiB conjugate vaccines had been introduced with dramatic success in prevention of serious paediatric infectious diseases (Robbins, 1996). Unsurprisingly, due to high mortality and morbidity caused by pneumococci in addition to increasing antimicrobial resistance, the interest in developing pneumococcal conjugate vaccines soared. Few different vaccines were in development in the 1990s (Ahmad, 1999; Pichichero, 1997), with only one, Wyeth's (later acquired by Pfizer) 7-valent Prevnar being licensed in 2000.

1.7.2.1 PCV-7

Prevnar (PCV-7) was a protein conjugate vaccine, in which Diphtheria CRM197 protein was conjugated to capsular polysaccharides from serotypes, 4, 6B, 9V, 14, 18C, 19F and 23F (FDA, n.d.). RCTs showed that PCV-7 was safe, immunogenic and efficacious in children against both invasive and non-invasive pneumococcal disease (Black, 2000; Eskola, 2001; Fireman, 2003; O'Brien, 2008).

In a 2012 review, the post-marketing studies on PCV-7 were summarized (Fitzwater, 2012). The vaccine impact on VT IPD ranged from 79 – 100%, while the impact on all cause IPD, ranged from 37 – 80%. The variance between effect sizes, was likely due to difference in both the vaccine uptake and pre-vaccine serotype prevalence between studies (Fitzwater, 2012). In a 2013 meta-analysis, four post-marketing impact studies on IPD were summarized (Schönberger, 2013). The authors calculated the incidence rate ratio (IRR) for each study and used a random effect model to calculate the summary estimate. The overall result was that the incidence of IPD had been reduced by 90% (95%CI:70 – 96%) (Schönberger, 2013).

The impact on hospitalized pneumonia was also high, albeit lower than IPD ranging from 13 – 65% between studies reviewed, with the same reasons quoted for variance as for IPD (Fitzwater, 2012). In contrast, researchers were

unable to consistently show an impact on outpatient visits due to pneumonia (Fitzwater, 2012).

Studies have also consistently shown decreased out-patient visits due to AOM with effect sizes ranging from 13 – 43% (Fitzwater, 2012; Magnus, 2012; Taylor, 2012). Hospitalizations due to AOM were reported to have reduced by an impressive 36% in an single study from Italy (Durando, 2009), with a lack of other studies agreeing with the result.

Carriage of vaccine serotypes was universally lowered, with impact ranging from 48 – 92%, with most surveillance programs seeing no change in the total carriage prevalence a few years after initiation due to an increase of NVT (Fitzwater, 2012).

1.7.2.2 Serotype replacement

Offsetting the large decreases in VT pneumococcal carriage and disease (discussed in 1.8.2.1), was the increasing carriage and disease caused by non-vaccine serotypes due to serotype replacement (Rose, 2009). As the replacing serotypes in carriage had lower disease potentials, as discussed in Chapter 1.3.2 the net-effect was still a large-scale reduction for most infections. The serotype replacement showed that extended coverage pneumococcal conjugate vaccines were needed (Jefferies, 2011; Weil-Olivier, 2012).

1.7.2.3 PCV-10 (PhiD-CV10)

GlaxoSmithKline's protein D, *Haemophilus influenzae* pneumococcal conjugate vaccine, (PHiD-CV10, Synflorix) was first licensed in Canada in December 2008 and in Europe in 2009 (GlaxoSmithKline, 2009). In addition to the serotypes included in the Prevnar-7 it contained serotypes 1, 5 and 7F, most of which are conjugated to Protein D from *Haemophilus* (Croxtall, 2009; GlaxoSmithKline, 2009; Plosker, 2014; Prymula, 2009). Serotype 3, which had previously been included in an earlier vaccine formulation (11Pn-PD) was not part of the licensed product, due to lack of lasting immune response and possible interactions with other serotypes in the vaccine (Croxtall, 2009).

The safety and side effect of PHiD-CV10 was comparable to PCV-7 (Chevallier, 2009; Plosker, 2014) and immunogenic non-inferiority to PCV-7 was established (Croxtall, 2009; Plosker, 2014; Prymula, 2009). Additionally, functional antibodies against all the included serotypes was found via an opsonophagocytic activity assay (Croxtall, 2009).

A RCT evaluating the vaccine effectiveness (VE) of 11Pn-PD on AOM showed a VE of 57.6% against VT AOM and 33.6% against all-cause AOM

(Prymula, 2006). Additionally, a barely significant 35.6% reduction was noted in the intention to treat analysis for AOM caused by NTHi (p=0.032) (Prymula, 2006). Similarly, in RCT evaluating the PHiD-CV10, the VE was 16.1% for all-cause AOM, and 67.1% against AOM caused by vaccine serotypes (Tregnaghi, 2014). For pneumonia, the VE ranged from -1 to 17% for clinical diagnosed pneumonia (Black, 2002; Cutts, 2005; Klugman, 2003; Tregnaghi, 2014) in RCT on PHiD-CV10 and its predecessors. Additionally, RCTs showed high VE against IPD, 65% and 100% against all-cause IPD and vaccine serotype IPD, respectively in the per-protocol analysis, consistent with the intention-to-treat analysis (Tregnaghi, 2014).

Observational studies have been in accordance with most studies showing favourable results. In countries without previous PCV, IPD was reduced by up to three quarters, with even higher effect on vaccine type disease (Jokinen, 2015; Knol, 2015; Verani, 2015). Impact on pneumonia hospitalization varied by studies, in Finland hospitalizations were reduced by 23% (Palmu, 2017), while it ranged from 7 – 49% in 8 Latin America studies reviewed in a systemic-review (L. H. De Oliveira, 2016). Additionally the impact on AOM has been favourable, with reductions up to 25% (Sartori, 2017; Sigurdsson, 2018).

1.7.2.4 PCV-13

Like in its predecessor, PCV-7, Prevnar-13 (Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein), PCV-13), contains capsular polysaccharides conjugated to Diphtheria CRM197 protein. It is a 13-valent PCV, first licensed in Chile in 2009 and in the US in 2010. It includes serotypes 3, 6A and 19A in addition to the serotypes included in the PHiD-CV10.

PCV-13 established immunologic non-inferiority for PCV-7 serotypes and showed significant functional antibodies against the additional six serotypes, with comparable safety and side effect profile (Bryant, 2010; Esposito, 2010; Kieninger, 2010; Snape, 2010).

Post marketing studies have shown that conversion from PCV-7 to PCV-13 conveys additional protection against pneumococcal carriage and disease (Andrews, 2014; Ben-Shimol, 2016; Flasche, 2011; van Hoek, 2014; Weinberger, 2016). Following the vaccination, carriage of the additional six serotypes in PCV-13 serotypes was rare (Flasche, 2011; van Hoek, 2014). Similar studies on otitis media are in accordance, showing further reduction in incidence (Ben-Shimol, 2016; Marom, 2014; Taylor, 2012).

In Latin America countries without prior PCV-7 vaccination, IPD was reduced by up to 65% and up to 85% against vaccine type disease (L. H. De

Oliveira, 2016). The hospital out-patient visits were also reduced by up to 30% (L. H. De Oliveira, 2016). In European countries that had prior PCV-7 the vaccine effectiveness against IPD caused by the extra six serotypes was 88% in Germany (Weinberger, 2016) and 76% in the UK (Andrews, 2014).

1.7.2.5 Direct comparison of the higher-valent vaccines

In the Canadian province of Quebec, the three vaccines were taken up subsequently, first PCV-7 in December of 2004 with catch-up schedule for children <5 years of age, then PHiD-CV in June 2009 and PCV-13 in January 2011, both with-out catch-up (Deceuninck, 2015). In a 2015 study the impact of PHiD-CV and PCV-13 was compared. The researchers found that for all-cause IPD, 19A IPD and IPD caused by PCV-13 serotypes, that the two vaccines were comparable in all three categories (Deceuninck, 2015).

In Taiwan a case control study evaluating the different effectiveness between different kinds of mixed-schedules was conducted (Su, 2016). It was found that using PCVs of different valences were protective against IPD. The PCV7/PHiD-CV mixed schedule was inferior to PCV13 schedule or mixed PCV7/PHiD-CV + PCV13 booster schedule in preventing any serotype IPD, 48% (95%CI:32 – 60%), 76% (95%CI:61 – 85%) and 78% (95%CI:56 – 89%), respectively. Likewise the PCV7/PHiD-CV schedule had less impact against 19A IPD (VE:31% (95%CI:4 – 51%)) than the other two schedules (VE:82% (95%CI:63 – 91%) and 87% (95%CI:61 – 96%), respectively) (Su, 2016).

In Sweden around half of 21 counties used each of PHiD-CV and PCV-13. Comparing counties based on which vaccine was used, no significant difference was found in the impact on IPD (Naucler, 2017). In another Swedish study, otitis media and related diagnosis were decreased in both groups, often more profoundly in the counties that used PHiD-CV. They however did note that the incidence was in general higher prior to the vaccination in the PHiD-CV counties (Gisselsson-Solen, 2017).

1.7.2.6 Future vaccine strategies

After almost ten years use of extended valence PCVs, the serotype diversity of nasopharyngeal carriage and disease is higher than before the PCV era (Myint, 2015), meaning that it is not as easy to decide which serotypes should be included in the next generation of conjugate vaccines. Formerly very rare serotypes have now become more common (Myint, 2015; Sigurdsson, 2017).

Currently many different options for future vaccine are being explored. First several extended spectrum PCVs are in development, including a 15 valent

and 23 valent PCVs from Merck (Rupp, 2018; Skinner, 2018) and patent from Pfizer for a 20 valent PCV has been filed (US patent: 9,492,559 B2). Additionally protein vaccines for various pneumococcal proteins are in pre-clinical phase through phase 2 (Entwisle, 2018; Hammitt, 2018; Leite, 2018; Mann, 2018). The protein vaccines are either designed to be co-administered with current vaccines or to replace them (Entwisle, 2018; Hammitt, 2018; Leite, 2018; Mann, 2018). Other strategies include vaccinations with a pneumococcal pep 27 mutant (Ghosh, 2018).

1.8 Introduction to this study

In late 2000s there was an increased interest in Iceland on the possibility of introducing a pneumococcal conjugate vaccine (Fréttablaðið, 2009; Morgunblaðið, 2009). In early 2008, an parliamentary advisory group recommended that preparation for uptake of a pneumococcal conjugate vaccine should be undertaken in Iceland (Friðleifsdóttir, 2009). The vaccination wasn't introduced into the paediatric vaccination schedule until 2011 (Sóttvarnarlæknir, 2010) due to the 2008 economic crisis (Morgunblaðið, 2009). The vaccine was introduced with a 2+1 schedule, given at 3, 5 and 12 months of age with no catch-up schedule.

2 Aims

The aims of this thesis were to measure the impact of adding the ten-valent protein conjugated pneumococcal vaccine (PHiD-CV) into the national immunisation program in Iceland on:

I. The nasopharyngeal carriage of all serotypes of *Streptococcus pneumoniae* (antimicrobial susceptible and non-susceptible) in healthy children attending day care centres (Paper I).

II. The incidence of acute otitis media in primary care among children (Paper II).

III. The incidence of emergency department visits due to respiratory tract infections in children (Paper III).

IV. The incidence of hospital admissions due to respiratory tract infections and invasive pneumococcal disease among children (Paper IV).

3 Materials and methods

This thesis is based on four studies, published in the four papers mentioned in the start of the thesis.

- Study I was a study on the impact of the vaccination on pneumococcal nasopharyngeal carriage in healthy Icelandic children. Nasopharyngeal samples were collected annually in the years 2009 – 2015.
- Study II was a study on the impact of the vaccination on AOM in children, evaluated by visits to primary care physician due to AOM including every child born in Iceland from 2005 – 2015 followed from birth to third birthday or the end of study period.
- Study III was a study on the vaccine impact on paediatric emergency department visits due to RTI. All visits for children less than 18 year for RTI were recorded from 2008 – 2013.
- Study IV was a study on the impact of the vaccination on hospital admission for RTI or IPD for children less than three years of age. Every child born in Iceland between 2005 and 2015 was followed to third birthday or end of study period. All admissions to the Children's Hospital due to study diagnosis were recorded.

3.1 Nasopharyngeal carriage of healthy children attending Day-care Centres (Study I)

3.1.1 Design

Study I was a repeated cross-sectional study conducted annually from 2009 to 2015. In the study, nasopharyngeal (NP) samples were collected annually in March at 15 day-care centres (DCC). Five DCCs were selected from each of the three largest capital municipals (Reykjavík, Hafnarfjörður and Kópavogur). The included DCCs were selected so they would be representative both geographically and socially for the Reykjavík capital area. Additionally, when inviting DCCs to participate, DCCs with prior experience with other carriage studies were preferred if available. The same 15 DCCs participated during the whole study period with only two exceptions when they were exchanged for other DCCs within the same municipal, due to scheduling difficulties.

3.1.2 Recruitment of children:

In general, children gain admission to a DCC in Iceland in the autumn of their second year, although it differs between individual day care centres. Children usually attend DCCs until they start primary school, in the autumn of the year when they turn six years old.

Each year all children attending the selected DCCs were invited to participate in the study. Signed informed consent forms were obtained and guardians were asked to fill in questionnaires on current and previous 30-day usage of antimicrobials, repeated antimicrobial prescriptions in the last six months and previous diagnoses of AOM, sinusitis or pneumonia by physician in the previous six months.

NP samples were taken from all children present at the day of sampling when i) they consented to the sampling and ii) guardian signed informed consent had been obtained. In general, parents are asked not to send their children to DCCs when ill, so the children that participated were therefore considered healthy at the time of sampling. Children were eligible to participate more than once in subsequent years, were they still enrolled to the DCC at the next sampling occasion, with each sampling, considered independent of the previous sampling.

3.1.3 Isolation and identification of pneumococci

Using COPAN transport medium swabs, (Copan®, Italy) a single sample was taken from the nasopharynx of the child. The swabs were inoculated on to appropriate media within six hours of sampling and selectively cultured for pneumococci. They were plated on blood agar containing 5mg/L gentamicin (to prevent growth of Gram-negative bacteria normally found in the nasopharynx) and then an optochin disc was placed on the inoculum. The agar was incubated under anaerobic conditions at 35°C for 18-20 hours (using GasPak envelopes, Thermo Scientific™).

Pneumococci were identified by morphology and susceptibility to optochin. The next day two to three colonies were picked for antimicrobial susceptibility testing and plated on regular blood agar plates and incubated overnight in 5% CO^2 at 35°C. If different colony morphologies were macroscopically identified, two to three colonies of each morphology were picked and plated. Subsequently all pneumococcal isolates were stored at -80°C in glycerol broth.

3.1.4 Antimicrobial susceptibility of isolates

Disk susceptibility testing was done on all the pneumococcal isolates for

oxacillin, erythromycin, clindamycin, tetracycline and trimethoprim/sulfa-methoxazole. The 2012 EUCAST methods and criteria were used (www.eucast.org.eu).

The minimum inhibitory concentration (MIC) for penicillin was measured for all oxacillin resistant isolates (< 20 mm) using the E-test (AB biodisk, Sweden). Isolates defined as PNSP (MIC ≥ 0.094), were further divided as low MIC and high MIC, due to the post-hoc observation of high variability in sensitivity of isolates, that were classified as intermediately resistant according to EUCAST, with only few isolates being classified as resistant (MIC > 2). Isolates that were non-susceptible to three or more antimicrobial classes were defined as multi-resistant isolates. Other definitions were according to the 2012 criteria and definitions and can be seen in Table 6.

Table 6. Antimicrobials tested in this study and the definition for non-susceptibility. Adapted from the 2012 EUCAST criteria and definitions. PNSP isolates were further divided in two brackets low and high PNSP.

Antimicrobial	**Zone diameter breakpoint (mm)**		
	Sensitive		
Oxacillin[a]	≥ 20		
Erythromycin	≥ 22		
Clindamycin	≥ 19		
Tetracycline	≥ 25		
Trimethoprim-sulfamethoxazole	≥ 18		
	Sensitive	**Low MIC PNSP**	**High MIC PNSP**
Penicillin E-test, MIC	≤ 0.064	0.094 – 0.5	> 0.5

[a]*For oxacillin resistant isolates, an MIC test was used to determine if resistant against penicillin.*

3.1.5 Serotyping of isolates

Serotyping was done using the pneumococcal Latex antisera, Pneumotest (Statens Serum Institute, Copenhagen, Denmark). The same senior biomedical scientist conducted all the serotyping for the whole study period. When isolates were serotyped into the serogroups in Table 7, PCR was used.

PCR was used only on isolates previously serotyped using latex agglutination. PCR was done by the following steps:

1. After culturing the pneumococci as described above, three pneumococcal colonies of identical morphology were picked and dissolved in 500 µL of 5% Chelex 100® (Bio-Rad laboratories, Hercules, CA, USA). The DNA was extracted by heating the solution to 100°C for 10 minutes, then centrifuging it at 14000 rpm for 10 minutes, thus to separate the heavier parts, such as cellular debris and Chelex from the DNA. 250 µL of the float was then collected and stored.
2. For duplex or multiplex PCR, one to three different specific primers (TAG Copenhagen, A/S, Copenhagen, Denmark) were chosen depending on which serotypes were likely to be detected. As an example, if the latex agglutination showed serogroup 23, primers specific for 23F, 23A and 23B were used (Table 7). In addition, a primer specific for capsular gene *cpsA* was used as a positive internal control for all duplex and multiplex reactions. The four primers were mixed with isolate DNA, sterile water and Taq 2x Master Mix (New England Biolabs, Inc., Ipswich, MA, USA).
When doing PCR on isolates from serogroup 6, three different monoplex reactions were done for each isolate (Table 7). When doing the monoplex reactions, only one primer and no internal control could be used. Amplitaq Gold® PCR Master Mix (Applied Biosystems, Foster City, CA, USA) with hotstart polymerase was added to the single primer, isolate DNA and sterile water.
3. The reactions were done in 2720 Thermal Cycler (Applied Biosystems) where first denaturising was done at 94 – 95°C for 4 – 5 minutes followed by 30 – 35 PCR circles. Each circle started with denaturation for 30 – 45 sec at 94°C, followed by annealing at 54°C – 62°C for 0.75 – 1 minute and elongation at 65 – 72°C for 1 – 2.5 minutes, with specific temperatures and time differing based on the reaction.
4. Next, gel electrophoresis was done on the PCR product. From each PCR tube, 6 µL of product was pipetted to a cup in the gel. DNA ladder was pipetted in one of every 16 cups. The products from the serogroup 6 reactions were mixed with 6x DNA Loading Dye (Fermentas International Inc., Burlington, Canada) before pipetting to cups. The gels were electrophoresed at 120V for 10 – 40 minutes, depending on size of the gels.
5. Next, the gels were coloured with Ethidium bromide (0.57 µg/mL) for 20 minutes and washed with distilled water and finally, the gels were photographed under UV light and analysed.

Table 7. Overview of polymerase chain reaction run in instances when latex agglutination was inconclusive. Possible serotypes indicate serotypes which are possible according to the result of the latex agglutination. PCR primers available to our laboratory for reactions are listed for each category. When the internal positive control (IPC) was positive and none of the PCR primers in the reaction were positive, the isolate was labeled in its serogroup or pool without further sub-group.

Result from agglutination	Possible serotypes	Reactions
6, 6A or 6B	6A, 6B, 6C, 6D	3 monoplex PCRs: 1. 6A/C, 2. 6B/D, 3.6C/D
23, not 23F	23A, 23B, 23F[a]	Multiplex PCR: cpsA, 23A, 23B, 23F
9, not 9V	9A/L or 9N	Duplex PCR: cpsA, 9V/A
Pool D	16F, 16A, 36, 37	Multiplex PCR: cpsA, 9V/A, 11A/D, **16F**
Pool E	21, 39	Multiplex PCR: cpsA, 10A, **21**
Pool G	29, 34, 35F, 35A, 35B, 35C, 42 47F, 47A	Two multiplex reactions: 1. cpsA, 29, 34, **35F/47F** 2. cpsA, **35B**, 42 (35A/C), 47A,
Pool I	25F, 25A, 38, 43, 44, 45, 46, 48	Multiplex PCR: cpsA, **38** (25F)

[a]*Isolates that had positive agglutination reactions for 23F were not tested further with PCR. All reactions positive for 23 but not 23F, were however all tested for 23F with PCR.* ***Bold*** *indicate most common serotype in reaction.*

3.1.6 Statistical analysis

Study I was divided into two substudies, both published in Paper I, referred to as Study IA and IB. Study IA was designed to examine the total impact of the vaccination, Study IB was designed to examine the indirect impact of the vaccination.

Study IA was designed to estimate the total impact of the vaccination on children eligible for the vaccination. Children born 2011 and later (Vaccine Eligible Cohorts, VEC) were compared to children born in 2010 and earlier (Vaccine Non-Eligible Cohort, VNEC). Only children less than four years of age were included, to prevent bias caused by longer follow up of the VNEC children. Additionally, to prevent bias caused by herd effect induced by

vaccinated children entering the DCCs in 2013, children in the VNEC that were sampled in 2013 - 2015 were excluded.

Study IB was designed to estimate the herd effect on older children that were not eligible for the vaccination. Only children that were born in 2010 and earlier were included and compared before and after the initiation of the vaccination.
Children sampled in 2009 to 2011 were considered in the PreVac period group and children sampled in 2013 to 2015 were considered in the PostVac period group. 2012 was classified as a transition year, and thus isolates from that year were excluded. In study B, only children >3.5 years of age were included for equal age distribution between the two groups.

For both study IA and study IB non-typeable pneumococci were excluded in analysis of antimicrobial non-susceptibility, as they do not generally cause disease in immunocompetent hosts.

When testing for differences between clinical characteristics of the children, the answers to their questionnaires and both their overall carriage prevalence of pneumococci and of antimicrobial resistant pneumococci two-sided Fisher's exact test and *z*-test were used for categorical outcomes and *t*-test for continuous outcomes. Alpha =0.05 was used as a significance level.

Odds ratios and 95% confidence intervals were calculated for the risk of carrying individual and pooled serotypes in the vaccine and non-vaccine groups. The vaccine impact against carriage (VIc) was calculated as (1 – OR) x 100%. Large sample theory was used to construct approximate confidence intervals for VIc and a hypothesis test for the difference between two VIc coefficients.

When testing the null hypothesis that the VIc differences were equal then the distribution of the test statistic under the null hypothesis, was approximated with a standard normal distribution. Conservative one-sided 95% confidence interval for vaccine impact was found by replacing the observed zero with one when there were zero observation for any of the serotypes in either of the groups. Due to dual carriage in some children, the denominator was the number of children in that group, with children with dual carriage being counted twice. Children were eligible to participate in the study more than once, provided that they were still attending the day care centre the following year, on the next sampling occasion. The events were considered independent and not analysed in the main analysis. In a descriptive post hoc sub-analysis

children that carried 19F in 2012 or later were analysed for repeated carriage of 19F.

Statistics were done using the statistical software R, Version 3.3.2 for all studies.

3.2 Vaccine impact on infections (Study II – IV)

3.2.1 The Icelandic health system

Primary care for children in Iceland is handled by 69 primary health care centres around the country. In addition to regular visits, each primary health care handles walk-ins during office hours for acutely ill patients. Primary care physicians run out of hours clinics that are operated at evenings and weekends in the Capital-area and in Akureyri, but at all hours in other areas. All visits are collected to the primary health care database, operated by the Directorate of Health, which is used in study II.

Private paediatricians operate various clinics accepting both appointments made by parents and referrals from primary care for most non-urgent matters requiring specialist services. The largest paediatrician clinic operates after-hours urgent walk-in clinic during evenings and weekends in Reykjavík. Visits to private paediatricians were not included in this study due to coding differences. Although it may have been interesting to include this clinic we maintain that these visits did not have a substantial effect on the results. Both do they not represent a huge proportion of the cohort but more importantly this inclusion system for the study remained unchanged for the whole study period.

Finally, a paediatric emergency department is operated at the Children's Hospital - Landspitali, University Hospital in Reykjavík, visits from which are included in study III and admissions to hospital in study IV. An Urgent care department is operated at the Akureyri Hospital in Akureyri, the capital of Northern Iceland region (visits not included in this study). In other towns, urgent care is handled by local primary care physicians (included in study II). The paediatric emergency department handles walk-ins during all hours triaged by nurses in addition to referrals by primary care physicians and private paediatricians. Therefore, only emergency visits missing in this study are those handled by the urgent care at Akureyri Hospital. These are also the only two hospitals with specialized paediatric inpatients ward, although other smaller hospitals observe children with mild diseases. The Children’s Hospital – Landspitali University Hospital handles primary care for majority of children in

Iceland and tertiary care for all children in the country. This was unchanged during the study period.

3.2.2 Design, participants and samples

Children born in 2010 and earlier were defined as vaccine non-eligible cohorts (VNEC) and children born in 2011 and later were defined as vaccine eligible cohorts (VEC) for all studies.

3.2.2.1 Impact on primary care visits due to AOM (Study II)

Study II was a whole-population, individual level, observational cohort study of primary health care visits due to AOM in Iceland. All children in Iceland born in 2005 through 2015 were included and followed from birth until three years of age, or until the end of the study period in December 2015.

3.2.2.2 Impact on emergency department visits and hospital admissions (Study III and IV)

Study III and IV were single centre, individual level, observational cohort studies conducted at the Children's Hospital Iceland, Landspitali University Hospital.

In Study III all children less than 18 years of age that visited the paediatric emergency department due to respiratory tract infections from January 1st, 2008 to December 31st, 2013 were included. The Children's Hospital serves as a primary hospital for the capital-area. Approximately 50,000 children (<18 years old; www.statice.is), were living in the area and were used as an estimate for the uptake population in Study III. The hospital also serves as tertiary hospital for all children in Iceland. Therefore, children from outside the primary uptake area but seeking medical assistance at the Children's Hospital could count in the numerator in Study III, but not the denominator.

For Study IV, all children in Iceland born in 2005 through 2016 were included and followed from birth until three years of age, emigration or the end of the study period in December 2016. All children that immigrated to the country after birth were excluded. Individual level immigration and emigration data was collected available from Statistic Iceland (statice.is).

3.2.3 Outcome measures/variables

3.2.3.1 Study II

All children in Iceland born in 2005 through 2015 were followed from birth to

third birthday or end of the study period 31st of December 2015.

An AOM-visit was defined if an ICD-10 diagnostic code of suppurative otitis media (H66) was recorded anywhere on the record. All 69 primary health care centres in Iceland were included and visits to health-care providers, other than physicians were excluded. Information on visits was extracted from the primary health care database of the Icelandic Directorate of Health, which includes all visits to all primary care health clinics in Iceland and corresponding ICD coding. Visits to paediatricians or otolaryngologist in private clinics were not included due to coding differences, as discussed above. Data extracted, included ICD-10 codes linked to the visit and demographic information.

Children were identified using their individual national identification numbers (NID), allowing chronological tracking regardless of the primary care centre visited. All citizens and permanent residents of Iceland have NIDs. Visits by individuals without NIDs, namely temporary residents and travellers were excluded, to remove possible bias due to increased tourism in Iceland, during the study period. Repeated visits within 30 days from the initial visit were considered to be the same episode and excluded from the analysis. Consequently, children were removed from the risk-set for 30 days after each AOM visit.

3.2.3.2 Study III and IV

Study III included all visits to the paediatric emergency department at the Children's Hospital, Landspitali, University Hospital coded with one of the study's ICD-10 codes for infections (bold in Table 8). Study IV included all admissions due to the study's diagnosis (all except those marked with asterisk in Table 8). The included visits and admissions were extracted from the Hospital patient's registry. Additionally, in Study IV, all admissions linked to an invasive pneumococcal infection, defined as a positive pneumococcal culture sampled from normally sterile site were included, regardless of diagnosis. In the study, the Center for Disease Control and Prevention's (CDC) definition of IPD and normally sterile sites was used (Center for Disease Control and Prevention, 2017).

All children less than 18 years of age were included in Study III and children less than three years of age in Study IV. Only patients with permanent residence or citizenship were included (those with Icelandic social security number). Therefore, travellers in Iceland were not included in the study. In Study III every new visit was counted. In Study IV readmissions within 30 days from discharge for the same diagnosis was regarded as recurrence of the

same episode. This approach detected all emergency ward visits and hospital admissions.

When a patient's chart contained more than one ICD-10 diagnosis, an algorithm was created to identify the most relevant diagnoses for the study. For Study III the order was: i) pneumonia or AOM, ii) complications of pneumonia or AOM and iii) other diagnoses. For Study IV the algorithm was i) meningitis, ii) sepsis, iii) pneumonia, iv) AOM, v) acute upper respiratory tract infection (URTI) and vi) lower respiratory tract infection (LRTI). When the record contained more than one diagnosis in the same level, the diagnosis that was coded earlier on record was used. Only one diagnosis was used for each visit.

3.2.4 Statistical analysis

Statistics were done using the statistical software R, Version 3.3.2 for all studies.

3.2.4.1 Study II

Analyses were either done on individual birth-cohorts or on grouped cohorts, as described above. In this study the VNEC was defined as birth cohorts 2005 – 2010 and the VEC 2011 – 2015. Analysis were done in Version 3.3.2. of R using the survival package (Therneau, 2000).

For each birth-cohort, divided into 4-month age-groups, crude incidence rates (IR) were calculated per 100 person-years at-risk. Additionally, for each age-bracket crude incidence rate ratios (IRR) were calculated between VNEC and VEC, and assuming the Poisson distribution and confidence intervals were estimated.

The cumulative incidence of AOM episodes diagnosed for each child before three years of age was calculated and compared using the Chi-squared test of homogeneity. Additionally, the IRR of experiencing 0, 1 – 4 or >5 episodes of AOM before three years of age was calculated between groups, and confidence intervals adjusted for multiple testing using the Bonferroni correction. Birth-cohorts 2013 – 2015 were excluded from the analyses on cumulative incidence due to incomplete follow-up time at time of writing.

Using the Andersen-Gill extension of the Cox regression model for repeated events (Cook, 2007), the data was modelled on individual level, accounting for censoring of follow-up time. The correlation between successive visits by the same individual was corrected using the Sandwich variance estimates. Careful attention was given to the risk-set and children were excluded from the risk-set

for 30 days following an AOM diagnosis, excluding re-visits within 30 days. Hazard ratios (HR) of AOM visits of each birth-cohort compared to the reference birth cohort (the 2010 cohort, the last vaccine non-eligible) were derived from the model, correcting for both the number of previous AOM episodes and gender. The vaccine impact of PHiD-CV on AOM was defined as 1 – (HR) x 100%, where HR denotes the hazard ratio between the last vaccine eligible birth-cohort (2015 cohort) and the reference cohort (2010 cohort).

Characterizing the mechanism by which the vaccine reduces the risk of AOM, the generalized Nelson-Aalen estimator (Cook, 2007) was used to calculate the hazard ratio between VNEC and VEC, stratified by the number of previous AOM episodes. Finally, using the same model, mean number of AOM episodes by age for both VNEC and VEC was calculated. Absolute number of prevented episodes of AOM prevented by the vaccination were estimated by multiplying each child's follow-up time by the corresponding mean number of episodes. The absolute incidence rate reduction was calculated by dividing the number of prevented episodes with the total person-time of the VEC.

3.2.4.2 Study III

The patients were stratified according to age group, sex and diagnosis. The age stratifications were 0, 1, 2, 3 – 6 and 7 – 17 years.

The mean incidence rate (IR) in a 3-year period before the immunization (2008–2010, PreVac) was compared with the mean IR in a 3-year period after the initiation of the vaccination (2011–2013, Post Vac). IR was calculated as number of cases per 10,000 children each year in the area, using population data from Statistics Iceland (www.statice.is). Each diagnostic and age groups were studied separately.

To better evaluate the impact of the vaccination, a sub-analysis including only children between 3 months of age and <2 years of age was done. Children born in 2011 (vaccine eligible cohort, VEC) were compared to children born 2008–2010 (non-vaccine eligible cohort, NVEC).

To evaluate the possible impact of misdiagnosis of pneumonia as acute bronchiolitis, the yearly incidence of both diagnoses were calculated separately and the trends analysed. In each analysis, the algorithm was used to prioritise coding of pneumonia or bronchiolitis, respectively.

The possible impact of annual influenza was evaluated by calculating the incidence of RTI with and without influenza cases as primary diagnosis. The

diagnostic and admission practices at the hospital did not change during the study period.

Difference between IRs of study groups were tested using a large sample *z* test. Asymptotic confidence intervals (CIs) for IR and incidence rate ratio were constructed using large sample theory.

3.2.4.3 Study IV

Birth-cohorts 2005 – 2010 were grouped as the vaccine non-eligible cohorts (VNEC) and birth-cohorts 2011 – 2016 as the vaccine eligible cohorts (VEC).

The Welch two sample *t*-test was used to calculate the mean age at admission and compared between groups of diagnosis when appropriate. The difference between the individual birth-cohorts was tested by using Analysis of Variance followed by Tukey's Honest Significant Difference. The Wilcoxon Rank Sum test was used to calculated and compare the median length of stay between diagnostic groups. For each birth-cohort crude incidence rates of admissions were calculated by diagnostic group and age group. Assuming Poisson variance, IRR were calculated between the VNEC and VEC. Additionally, the proportion of admissions converted to ICU admission was calculated for each birth-cohort and diagnostic group.

Both event-free survival and the event-free survival difference of VNEC compared to VEC for each of the diagnostic groups was calculated using the Kaplan-Meier product limit estimator. Re-admissions were defined as an admission with the same discharge diagnoses within 30 days of previous discharge and were excluded from analysis. Consequently, the children were excluded from the risk-set for 30 days after discharge. The hazard ratio of admission between the VNEC and VEC was calculated using Cox regression.

Table 8. The study diagnosis ICD-10 codes which were collected from hospital registry in studies in Paper I and IV. Bolded ICD-10 codes were included in Paper II while Paper IV included all codes, except those marked with an asterix (*).The ICD codes and their corresponding subcodes were included, unless otherwise specified.

ICD-10 Diagnosis Name	ICD-10 Code
Acute sinusitis	**J01**
Other upper respiratory tract infections	J02, J03, J04, J05, **J06**
Influenza	**J09, J10, J11**
Pneumonia and complications	**J12-J14, J15, J18, J85, J86, J90**
Other lower respiratory tract infections	**J20, J21, J22**
AOM and complications	**H66, H70, H72**
Sepsis	**A40, A41**
Other diseases caused by pneumococcus or *Haemophilus influenzae*	**A49.1*, A49.2*, B95.3*, B96.3***
Meningitis	G00

3.3 Ethics

The studies were approved by: The National Bioethics Committee (VSNb2013010015/03.07) with later adaptations, The National Data Protection Authority (2013010100VEL/–), The University Hospital medical director, the Directorate of Health (1301266/5.6.1/gkg) and the appropriate directorates of the DCC's.

4 Results

4.1 Impact on nasopharyngeal colonization (Paper I)

4.1.1 Impact on pneumococcal carriage (Paper I)

Two studies were presented in Paper I. Study IA, the total impact on pneumococcal nasopharyngeal carriage in vaccine eligible children and Study IB, the indirect impact on older, vaccine non-eligible children (Figure 3).

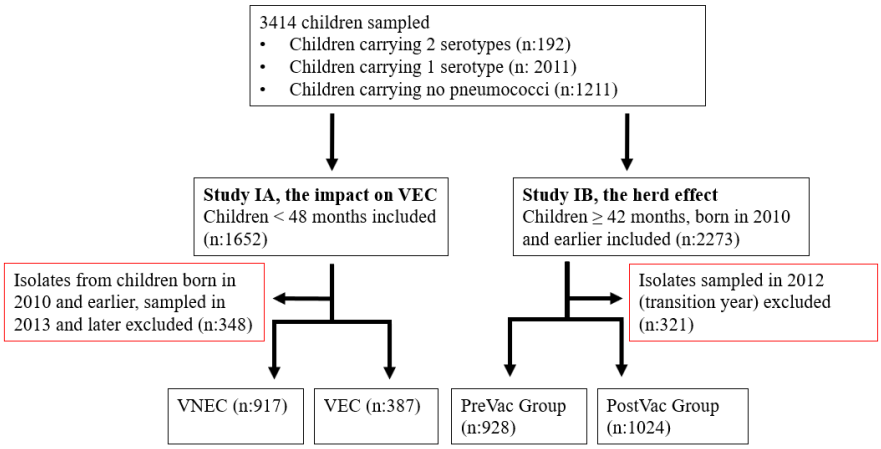

Figure 3. The design of Study I. Two independent studies were conducted. In **Study IA** (vaccine impact, left) only children less than 48 months of age were included, and children born in 2010 and earlier that were sampled in 2013-2015 were excluded. Children born before the vaccine initiation (born 2010 and earlier, Vaccine Non Eligible Cohort, VNEC) were compared to children born after the vaccine initiation (2011 and later, Vaccine Eligible Cohort, VEC). In **Study IB** (herd effect, right) only children born before the vaccination (born 2010 and earlier) were compared, according to years of sampling, children sampled in 2009-2012 compared to children sampled in 2013-2015. In Study IB only children older than 42 months of age were included.

4.1.1.1 Direct impact on vaccinated children

In this study, 1,224 children were included, 371 vaccine-eligible (VEC) and 853 vaccine non-eligible (VNEC). A total of 917 and 387 isolates were included in the study. Of those isolates 656 and 276 yielded pneumococci for the VNEC and VEC, respectively (Figure 3). No difference was found in overall carriage rate of pneumococci between the groups, 69.5% vs 70.1% (p=0.84). Likewise, no significant difference was found in the rate of dual carriage between the groups, VNEC rate of dual carriage was, 7.5%, while 4.3% of children in the VEC were dual carriers (p=0.051).

The mean ages of the children were slightly lower in the VEC. The mean ages were 2.80 in VEC and 2.89 in VNEC ($p<0.05$) and median ages were 2.8 and 2.9. There was no significant difference in the gender distribution, with 51.4% and 53.7% of the children being males for VNEC and VEC, respectively ($p=0.45$).

Questionnaire answers regarding recent RTIs were similar between the groups, with no significant differences in reported physician diagnosed RTIs (Table 9). Likewise, no significant difference was found between the parent-reported current antimicrobial use (5.9% vs 5.7%, $p>0.99$). Fewer parents of children in VEC reported any antimicrobial use in the last three months (excluding current) or more than three doses in the last six months. Additionally, aggregating the answers on antimicrobial use, significantly fewer parents in the VEC reported any antimicrobial use, 21.0% vs 28.0% (OR:0.68 (95%CI:0.50 – 0.92)) in the VEC and VNEC, respectively (Table 9).

The impact of the vaccination on carriage of vaccine type (VT) pneumococci was 96% (95%CI:91 – 96%). The impact on individual vaccine serotype carriage ranged from 84% for 19F to 100% for 9V, 14 and 18C (Table 10). The impact on vaccine associated serotypes (VAT) 6A and 19A was 33% (95%CI:1 – 55%). Serotype replacement was considerable, with non-vaccine serotypes replacing VT in the VEC. Serotype 15 was the most prevalent serotype in the VEC, while serotype 23B increased the most in prevalence (Table 10).

No significant difference was found in the rate of Penicillin Non-Susceptible Pneumococci (PNSP) between the two groups, 11.2% vs 10.4% (p:0.76)., When stratifying to susceptible, low minimum inhibitory concentration (MIC) PNSP and high MIC PNSP, most children from the VEC were stratified in the low MIC group, while most of the children in the VNEC were classified in the high MIC group (Table 11).

Table 9. Results from parent answered questionairres regarding physician diagnosed respirtatory tract infections (RTIs) and the children's antimicrobial usage. for Study IA: VNEC: Vaccine non-eligible cohort. VEC: Vaccine eligible cohort. For Study IB: PreVac: The pre-vaccine era. PostVac: The post-vaccine era.

	Study IA, total impact on vaccine eligible children			**Study IB, indirect effect on non-vaccine eligible children**		
	VNEC %	**VEC %**	**OR (95%CI)**	**PreVac**	**PostVac**	**OR (95%CI)**
RTIs, last 6 months:	43.8	41.8	0.9 (0.7 – 1.2)	27.6	21.4	0.7 (0.6 – 0.9)[b]
Otitis media	36.0	32.9	0.9 (0.7 – 1.1)	17.9	14.4	0.8 (0.6 – 1.0)[a]
Sinusitis	9.3	6.5	0.7 (0.4 – 1.1)	7.7	5.0	0.6 (0.4 – 0.9)[a]
Pneumonia	12.4	12.7	1.0 (0.7 – 1.5)	8.3	6.8	0.8 (0.6 – 1.2)
Antimicrobial use:	28.0	21.0	0.7 (0.5 –0.9)[a]	13.8	11.1	0.8 (0.6 – 1.1)
Current use	5.9	5.7	1.0 (0.5 – 1.7)	4.3	2.5	0.6 (0.3 – 1.0)[a]
Last 30 days	19.5	13.7	0.7 (0.5 –0.9)[a]	9.3	6.6	0.7 (0.5 – 1.0)[a]
≥3 times last 6 months	12.9	8.1	0.6 (0.4 –0.9)[a]	3.0	4.0	1.3 (0.8 – 2.3)

[a] *p<0.05* [b] *p<0.001*

A higher proportion of isolates cultured from children in VNEC were co-trimoxazole and erythromycin resistant than those from the VEC, 22.1% vs 12.1% (OR:0.48, 95%CI:0.34 – 0.69) and 13.1% vs 9.0% (OR:0.66, 95%CI:0.44 – 1.00), respectively. The prevalence of strains with reduced susceptibility to clindamycin, tetracycline or both erythromycin and penicillin did not differ between the two cohorts. Isolates with non-susceptibility to three or more antimicrobial classes were less common in the VEC than the VNEC 1.6% vs 9.4% respectively (OR:0.44, 95%CI:0.25 – 0.77). Likewise, isolates with non-susceptibility to all antibiotics tested were less commonly cultured from children in the VEC, 0.3% vs 4.4% in the VNEC (OR:0.06, 95%CI:0.003 – 0.37) (Table 17). 19F was the most common serotype found to be non-susceptible to three or more or all tested antimicrobials (Table 12).

Table 10. PHiD-CV vaccine impact on pneumococcal carriage, by serotype, comparing the VEC to the VNEC.

Serotype		VNEC, n (%)	VEC, n (%)	VIc (95% CI)	P
Vaccine type	**Total**	322 (35.1)	12 (3.1)	0.94 (0.91 – 0.96)	<.001
	6B	77 (8.4)	2 (0.5)	0.94 (0.84 – 0.98)	<.001
	9V	3 (0.3)	0 (0)	1.00 (-6.57 – 1.00)λ	.26
	14	57 (6.2)	0 (0)	1.00 (0.85 – 1.00)[a]	<.001
	18C	6 (0.7)	0 (0)	1.00 (-2.04 – 1.00)[a]	.11
	19F	84 (9.2)	6 (1.6)	0.84 (0.67 – 0.93)	<.001
	23F	95 (10.4)	4 (1.0)	0.91 (0.80 – 0.96)	<.001
Vaccine associate	**Total**	122 (13.3)	36 (9.3)	0.33 (0.01 – 0.55)	.04
	6A	76 (8.3)	22 (5.7)	0.33 (-0.09 – 0.59)	.1
	19A	46 (5)	14 (3.6)	0.29 (-0.31 – 0.61)	.27
Non- Vaccine Types	**Total**	169 (18.4)	207 (53.5)	-4.09 (-5.53 – -2.97)	<.001
	3	14 (1.5)	11 (2.8)	-0.89 (-3.14 – 0.14)	.11
	6C	8 (0.9)	25 (6.5)	-6.85 (-14.6 – -2.94)	<.001
	10	0 (0)	8 (2.1)	Not calculated	
	11	32 (3.5)	32 (8.3)	-1.49 (-3.07 – -0.53)	<.001
	15	36 (3.9)	37 (9.6)	-1.59 (-3.10 – -0.63)	<.001
	16F	9 (1)	2 (0.5)	0.48 (-1.37 – 0.88)	.4
	21	4 (0.4)	10 (2.6)	-5.05 (-15.9 – -1.17)	<.001
	22	2 (0.2)	5 (1.3)	-4.99 (-24.5 – -0.41)	.02
	23A	19 (2.1)	18 (4.7)	-1.31 (-3.37 – -0.22)	.01
	23B	7 (0.8)	30 (7.8)	-9.92 (-20.5 – -4.56)	<.001
	29/35B	5 (0.6)	9 (2.3)	-3.34 (-10.9 – -0.58)	.004
	33	13 (1.4)	2 (0.5)	0.64 (-0.51 – 0.91)	.16
	35F	0 (0)	10 (2.6)	Not calculated	
	38	8 (0.9)	1 (0.3)	0.71 (-1.09 – 0.96)	.22
	Other	7 (0.8)	6 (1.6)	-1.05 (-5.00 – 0.30)	.19
NT		47 (5.1)	22 (5.7)	-0.12 (-0.88 – 0.34)	.68
NONE		261 (28.5)	111 (28.7)	-0.01 (-0.32 – 0.22)	.94
Total		917 (100)	387 (100)		

VNEC: Vaccine non-eligible cohort, VEC: Vaccine eligible cohort, NT: non-typeable Children in the VNEC sampled 2013 and later were excluded to prevent possible bias caused by herd immunity. [a]Conservative one-sided 95% confidence interval for vaccine impact. The total number is higher than the number of children because some children carried more than one serotype.

Table 11. Antibiotic non-susceptibility for each of the antimicrobials and combinations of antimicrobials tested in the study for both study groups.

Non-susceptibility	VNEC, n (%)	VEC, n (%)	OR (95% CI)	PreVac, n (%)	PostVac, n (%)	OR (95% CI)
PNSP (all isolates)	97 (11.2%)	38 (10.4%)	0.92 (0.62-1.38)	42 (5.0%)	48 (5.0%)	1.02 (0.65-1.60)
Penicillin (Low MIC)	9 (1.0%)	33 (9.0%)	9.48 (4.54-20.3)	13 (1.5%)	22 (2.3%)	1.51 (0.72-3.30)
Penicillin (High MIC)	88 (10.1%)	5 (1.4%)	0.12 (0.05-0.31)	29 (3.5%)	26 (2.7%)	0.79 (0.44-1.40)
Erythromycin	114 (13.1%)	33 (9.0%)	0.66 (0.44-1.00)	52 (6.2%)	42 (4.4%)	0.70 (0.45-1.09)
Tetracycline	95 (10.9%)	31 (8.5%)	0.76 (0.48-1.16)	44 (5.2%)	48 (5.1%)	0.97 (0.62-1.51)
Clindamycin	54 (6.2%)	26 (7.1%)	1.15 (0.70-1.16)	30 (3.6%)	24 (2.5%)	0.70 (0.39-1.26)
Co-trimoxazole	192 (22.1%)	44 (12.1%)	0.48 (0.34-0.69)	113 (13.4%)	79 (8.3%)	0.59 (0.43-0.80)
Penicillin & Erythromycin	82 (9.4%)	30 (8.2%)	0.86 (0.55-1.33)	35 (4.2%)	35 (3.7%)	0.88 (0.53-1.47)
≥3 antibiotic classes	82 (9.4%)	16 (4.4%)	0.44 (0.25-0.77)	44 (5.2%)	31 (3.3%)	0.61 (0.37-1.004)
All 5 tested antibiotics	38 (4.4%)	1 (0.27%)	0.06 (0.003-0.37)	9 (1.1%)	6 (0.6%)	0.59 (0.17-1.86)
Total number of isolates	869	365		841	947	

For isolates with penicillin non-susceptibility non-typeable pneumococci were excluded. PNSP penicillin non-susceptible pneumococci. The denominator signifies the number of isolates (number of children sampled + number of children with dual carriage). VNEC: Vaccine non-eligible cohort. VEC: Vaccine Eligible Cohort

Table 12. Serotypes/groups on-susceptible to three or more antimicrobial classes and all antimicrobials tested for the study groups.

	Serotype (n of isolates)			
	VNEC	**VEC**	**PreVac**	**PostVac**
PNSP (0.064 – 0.5)	23F (3), 19A (3), 14 (1), 21 (1), 6C (1)	6C (14), 15 (9), 23B (5), 35B (3), 19A (2)	19F (3), 6B (4), 6C (2) 19A (2), 23F (1), 9V (1)	15 (6), 23B (5), 35B (4) 23F (3) 19A (3), 14 (1)
PNSP (> 0.5)	19F (75), 6B (7), 14 (6)	19F (5)	19F (25), 14 (2), 38 (1), 6B (1)	19F (22), 35B (2), 6B (2)
≥ 3 antimicrobial classes	19F (73), 6B (6), 14 (2), 6C (1)	15 (8), 19F (5), 6C (3)	19F (27), 6A (6), 6B (5), 6C (2), 38 (1), 15 (1)	19F (22), 15 (6), 6B (2), 14 (1)
All antimicrobials tested	19F (30), 6B (6), 14 (2)	19F (1)	19F (7), 6B (2)	19F (4), 6B (1)

4.1.1.2 Indirect impact on older children

In this study, 1,764 children were included, 831 children sampled in the pre-vaccination era (PreVac) and 933 children sampled in the post-vaccination era (PostVac). The carriage rate of pneumococci did not differ between the PreVac and PostVac groups, 62.6% vs 64.4% (p=0.42). Likewise, no significant difference was found in rate of dual carriage, with 5.8% and 5.0% children in the PreVac and PostVac groups carrying two serotypes (p=0.56). Consequently, 879 and 980 isolates were included in the study, of which 567 and 649 yielded pneumococci for the PreVac and PostVac groups, respectively. The mean and median ages of the children was similar for the groups, means 4.97 vs 4.98 (p=0.83) and medians 4.96 vs 5.00 for the children in the PreVac and PostVac, respectively. There was no significant difference in the gender distribution, with 49.4% and 51.2% of the children being males in the PreVac and PostVac groups, respectively (p=0.45).

Significantly fewer parents reported that their children had been diagnosed with AOM (14.4%) or sinusitis (4.9%) within last six months in the PostVac, compared to PreVac where 17.9% reported AOM diagnosis (OR:0.77 (95%CI:0.59 – 1.00)) and 7.7% sinusitis diagnosis (OR:0.62 (95%CI:0.41 – 0.93)) within the last 6 months (Table 9). No significant difference was found

in parent-reported diagnosis of pneumonia. Additionally, when aggregating the answers, significantly fewer parents reported their children having had any of the above RTIs within the last six months, 21.4% vs 27.6% for the PostVac and PreVac, respectively (OR:0.72 (95%CI:0.57 – 0.90)) (Table 9).

A significantly higher proportion of children in the PreVac were currently using antimicrobials, 4.3% vs 2.5% (OR:0.56 (95%CI:0.31 – 0.98)). Similarly, more parents reported any antimicrobial (excluding current) usage in the last 30 days in the PreVac, than the PostVac 9.3% vs 6.6%% (OR:0.70 (95%CI:0.48 – 1.00)). No significant difference was found in the proportion of parents reporting more than three antimicrobial prescriptions in the last six months or when aggregating the answers to the above questions on antimicrobial use (Table 9).

The impact of the vaccination on carriage of VT pneumococci was 56% (95%CI:44 – 65%). There was a significant impact on VTs 6B, 9V, 14, 18C and 23F ranging from 53% for 14 to 100% for 9V (Table 13). VTs 1, 5 and 7F were not detected in either group. Interestingly, no impact was found on serotype 19F (-3% 95%CI:-65 – -36%). The prevalence of 19F remained stable in the PreVac and PostVac groups with 4.2%, 2.7% and 4.7% for PreVac years (2009 – 2011) and 4.2%, 4.5%, and 3.1% for PostVac years (2012 – 2015). The impact on vaccine associated serotypes (VAT) was 33% (95%CI:7 – 51%), 44% against 6A and non-significant reduction for 19A (Table 13). Similarly, to the direct impact analysis the replacement of vaccine serotypes with non-vaccine serotypes was considerable, rising from 24.5% of PreVac isolates to 42.6% of PostVac isolates. Serotypes 3, 15, 23B and 11 were the most prevalent serotypes in the PostVac group, while serotype 23B increased the most in prevalence (Table 13).

The rate of non-susceptibility to co-trimoxazole was significantly lower in the PostVac than in the PreVac, 8.3% vs 13.4% (OR:0.59, 95%CI:0.43 – 0.80), likewise resistance against three or more antimicrobial classes was just barely significantly lower in PostVac than PreVac, 5.2% vs 3.3%, OR:0.61 (95%CI:0.37 – 1.004, p:0.04). No change was noted in resistance against other antimicrobials (Table 11).

Serotype 19F was the most common serotype found to be PNSP, multi-resistant and resistant against all tested antimicrobials for both PreVac and PostVac (Table 12).

Table 13. Indirect PHiD-CV vaccine impact estimates by serotype for PreVac compared to PostVac.

Serotype		PreVac period	PostVac	VIc (95% CI)	p value
Vaccine type	**VT total**	219 (24.9)	125 (12.8)	0.56 (0.44 - 0.65)	<.001
	4	2 (0.2)	0 (0)	1.00 (-3.65 – 1.00)[a]	.14
	6B	51 (5.8)	27 (2.8)	0.54 (0.27 – 0.71)	.001
	9V	13 (1.5)	0 (0)	1.00 (0.68 – 1.00)[a]	<.001
	14	26 (3.0)	14 (1.4)	0.53 (0.10 – 0.75)	.02
	18C	28 (3.2)	10 (1.0)	0.69 (0.38 – 0.84)	<.001
	19F	34 (3.9)	39 (4.0)	-0.03 (-0.65 – 0.36)	.90
	23F	65 (7.4)	35 (3.6)	0.54 (0.30 – 0.69)	<.001
Vaccine assoc.	**VaT total**	95 (10.8)	74 (7.6)	0.33 (0.07– 0.51)	.01
	6A	50 (5.7)	32 (3.3)	0.44 (0.12 –0.64)	.01
	19A	45 (5.1)	42 (4.3)	0.17 (-0.28 – 0.46)	.40
Non-vaccine type	**Total**	215 (24.5)	417 (42.6)	-1.29 (-1.79 – -0.88)	<.001
	3	58 (6.6)	46 (4.7)	0.30 (-0.04 – 0.53)	.07
	6C	4 (0.5)	18 (1.8)	-3.09 (-10.17 – -0.50)	.006
	9A/N/L	9 (1.0)	13 (1.3)	-0.30 (-2.05 – 0.45)	.55
	10	4 (0.5)	13 (1.3)	-1.94 (-7.60 – -0.01)	.05
	11	42 (4.8)	42 (4.3)	0.11 (-0.38 – 0.42)	.61
	15	27 (3.1)	45 (4.6)	-0.52 (-1.46 – 0.06)	.09
	16F	14 (1.6)	8 (0.8)	0.49 (-0.20 – 0.78)	.12
	21	5 (0.6)	32 (3.3)	-4.90 (-12.6 – -1.56)	<.001
	22	5 (0.6)	39 (4.0)	-6.25 (-15.2 – -2.25)	<.001
	23A	16 (1.8)	33 (3.4)	-0.88 (-2.41 – -0.04)	.04
	23B	0 (0)	44 (4.5)	Not calculated	
	33	5 (0.6)	10 (1.0)	-0.80 (-4.21 – 0.38)	.28
	35B	3 (0.3)	26 (2.7)	-6.96 (-20.1 – -1.89)	<.001
	35F	0 (0)	31 (3.2)	Not calculated	
	38	12 (1.4)	5 (0.5)	0.63 (-0.01 – 0.87)	.05
	Other	11 (1.3)	12 (1.2)	0.02 (-1.23 – 0.57)	.96
	NT	38 (4.3)	33 (3.4)	0.23 (-0.24 – 0.52)	.28
	NONE	312 (35.5)	331 (33.8)	0.07 (-0.12 – 0.24)	.44
Total		879 (100)	980 (100)		

NT: non-typeable. Vaccine assoc.: Vaccine associated serotypes. Only isolates from children born 2010 and earlier and ≥3.5 years of age were included. [a]Conservative one-sided 95% confidence interval for vaccine impact. The total number is higher than the number of children due to some children carrying more than one serotype.

4.2 Vaccine impact on infections (Study II, III and IV)

The impact of the vaccination on infections were described in three studies, Studies II, III and IV. In Study II primary care visits due to AOM were described. In Study III the impact on paediatric emergency department visits due to RTIs is discussed. In Study IV admissions to the Children's Hospital due to RTIs and IPD are analysed.

4.2.1 Impact on primary health care visits due to AOM (Study II).

In Study II, 53,150 children, born in 2005 to 2015 were followed for a total of 140,912 person-years. During the study, 74,802 primary health care AOM visits were registered, 16,008 were repeated visits within 30 days and were excluded, leaving 58,794 visits included in the analysis of this study.

4.2.1.1 Crude incidence rate analysis

The crude incidence rate of visits due to AOM was 43.6 per 100 person-years in the VNEC and 38.0 in the VEC with the overall IR during the whole study period being 41.7. The highest absolute IR reduction was in children less than one year of age from 48.3 to 34.2 per 100 person-years, while the age group with the highest IR, children one year of age, were reduced from 57.2 to 51.9 per 100 person-years. Children two years of age also had significantly lower IR, from 29.1 to 25.5 per 100 person-years in VNEC and VEC, respectively (Table 14).

Table 14. The incidence rate and total number of visits for children <1, 1 and 2 years of age.

Age groups	VNEC		VEC	
	IR (n)	Person-years	IR (n)	Person-years
< 1	48.3 (13,474)	30,436	34.2 (7,232)	21,150
1	57.2 (17,419)	30,436	51.9 (8,692)	16,750
2	29.1 (8,872)	30,436	25.5 (3,105)	12,199

VNEC: Vaccine non-eligible cohort. VEC: Vaccine eligible cohort. The number of person-years of follow-up for each of the age groups within the VNEC is identical, as the same 30,436 children were followed from birth to third birthday, with no loss to follow-up.

Looking at each birth-cohort divided in 4-month age brackets, the incidence rate was highest in children 8 – 11 months and 12 – 15 months of age, but lowest in children <4 months of age with significant reduction noted in all age groups after the vaccination (Figure 4).

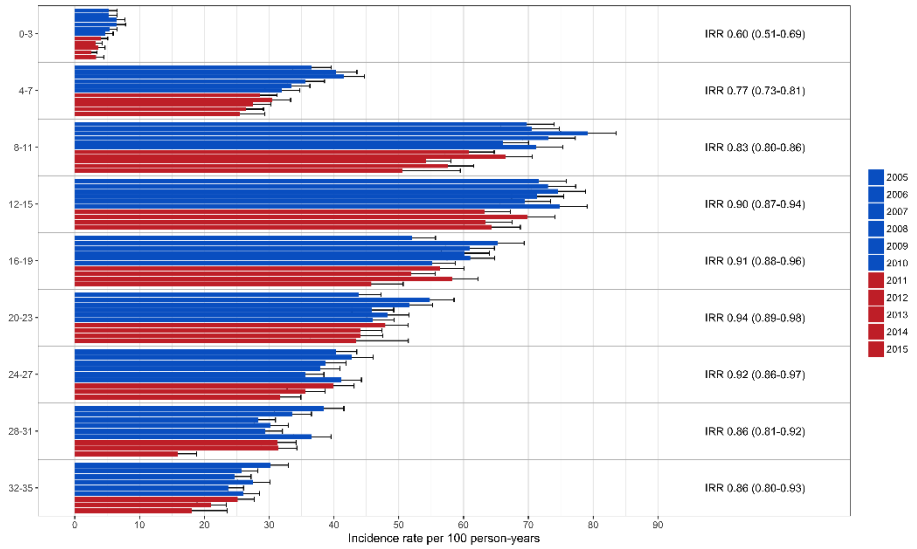

Figure 4. The incidence rate per 100 person-years with 95% confidence intervals are shown for each study birth-cohort by four-month age-brackets. The vaccine non-eligible cohorts are colored blue and the vaccine-eligible cohorts red.

The proportion of children that with zero visits due to AOM increased by 8% in the VEC compared to the VNEC, 43.6% vs 40.3%, respectively (IRR:1.08, 95% CI:1.05 – 1.12). Concurrently fewer children were diagnosed with AOM one to four times or five or more times in the VEC compared to the VNEC (Table 15).

Table 15. The proportion of children that were diagnosed with 0, 1-4 or ≥ 5 episodes for each of the study's groups and the IRR between them.

No episodes	Incidence proportion		IRR (98.3% C)
	VNEC	VEC	
0	40.3% (12,267)	43.6% (4,329)	1.08 (1.05-1.12)
1-4	55.5% (16,886)	52.9% (5,143)	0.95 (0.93-0.98)
> 5	4.2% (1,283)	3.6% (346)	0.84 (0.73-0.97)

4.2.1.2 Regression analysis

For each new episode of AOM, the hazard for contracting additional episodes increased. Thus, children that had one episode, were more likely to have a second episode compared to children without a prior episode.

Of the vaccine non-eligible cohorts, only the 2007 birth-cohort had significantly different hazard than the reference birth cohort, 2010 (HR 1.07, 95% CI:1.02 –

1.12). Conversely, each vaccine eligible cohort had HRs significantly lower than 1.0, ranging from 0.78 – 0.89 (Figure 5). Using the last VEC cohort, the estimated PHiD-CV10 vaccine impact on AOM visits was 22% (95% CI:12 – 31%)

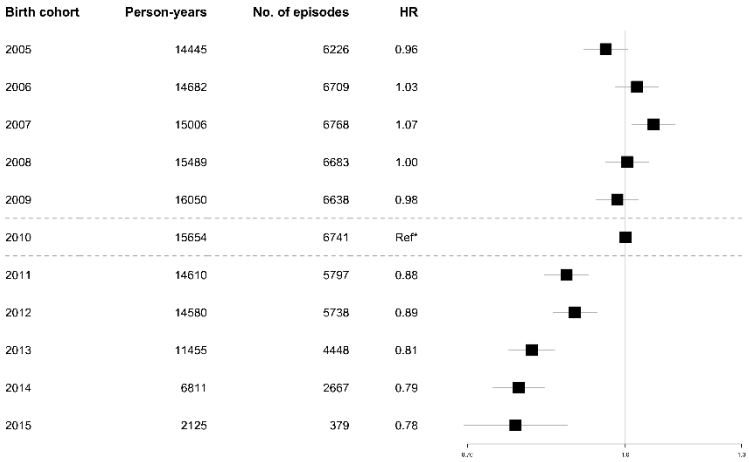

Figure 5. The hazard ratios (HR) of being diagnosed with acute otitis media for each of the birth-cohorts compared to the reference birth cohort (2010). Boxes indicate point estimates and bars the 95% confidence intervals. 2005 – 2010 belong to the Vaccine non-eligible cohorts (VNEC) and 2011 – 2015 to the Vaccine eligible cohorts (VEC).

When stratified by number of previous episodes, the hazard of the first two episodes of AOM were reduced. Children with no previous visits were 16% less likely to be diagnosed with their first AOM in the VEC than in the VNEC. Similarly, children in the VEC with one previous visit for AOM, were 5% less likely to be diagnosed with a second episode (HRs:0.84 (95%CI:0.82 – 0.86) and 0.95 (95%CI:0.93 – 0.98), respectively). For children with two or more episodes, no difference in hazard of subsequent visits was found between the groups.

In Figure 6 the mean number of visits for both the VNEC and VEC from birth to third birthday is displayed. Children belonging to the VEC had significantly lower mean number of visits per child, with the difference being significant from birth and increasing as the children aged. At three years of age the mean number of visits per child in the VEC was 1.37, compared to 1.61 per child born in the VNEC. Consequently, at the end of 2016, 4 years and 9 months after the initiation of the vaccination, the absolute number of prevented AOM episodes in vaccine eligible children less than 3 years of age were 4,187

(95%CI:3363 – 5011), with an absolute IR reduction per 100 person-years of 8.4 (95%CI:6.8 – 10.1).

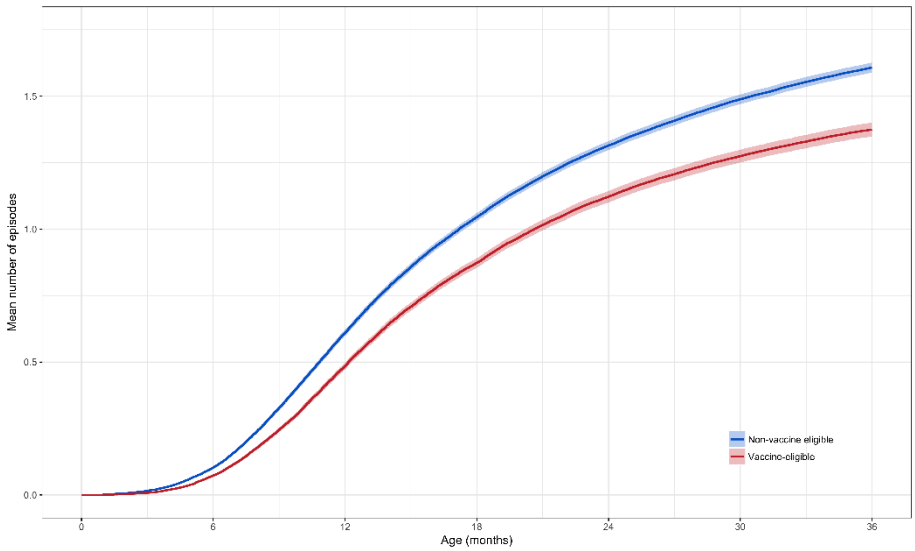

Figure 6. The mean number of acute otitis media episodes for children from 0-36 months of age. The blue line indicates the mean number of visits for the Non-vaccine eligible cohort (VNEC) and the red line for the vaccine eligible cohort (VEC). The 95% confidence intervals are shown with the shaded areas around the lines.

4.2.2 Impact on paediatric emergency department visits due to RTI (Study III)

4.2.2.1 Descriptive analysis

Included in the study were 11,752 paediatric emergency department visits for RTI by 7,158 patients less than 18 years of age. Most patients had only one visit (67.5%), while 18.0% had two, 7.7% three and 6.8% four or more visits due to study diagnosis during the six-year study period.

The median age of visiting children was 1.5 years and males were 56% of the patients. The incidence rate of RTI was highest during the winter months and 49.4% of visits occurring in December, January, February and March, the four coldest months. The overall quarterly incidence rate per 10,000 child-years for each diagnostic cluster during the whole study period, can be seen in Figure 7. The total paediatric population less than 18 years of age in the area ranged from 49,107 (January 1st, 2008) to 50,716 (January 1st, 2014) and the yearly number of visits to the paediatric emergency department increased from 12,229 in 2008 to 13,525 in 2013.

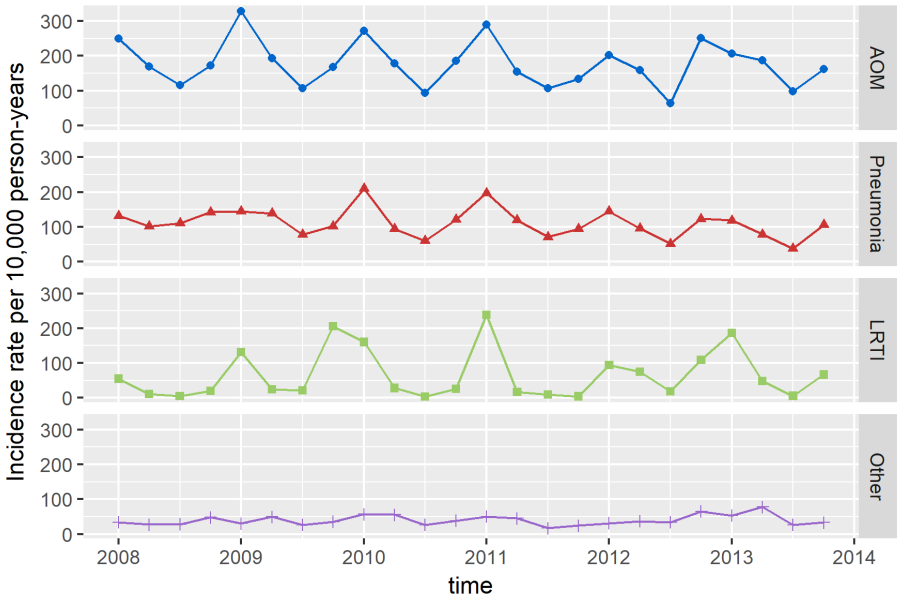

Figure 7. Quarterly incidence rate per 10,000 person-years to the paediatric emergency department for children less than 18 years of age due to study diagnosis from 2008 – 2013. AOM: Acute otitis media, LRTI: Lower respiratory tract infections (most often acute bronchiolitis). Other: other study diagnosis, not included in other diagnostic clusters.

4.2.2.2 Incidence rate analysis

The all cause RTI incidence rate was the highest for children 1 year of age and declined by 15% (95%CI:8 – 22%) from 2,322 before the vaccination to 1,967 per 10,000 children in this age group after the vaccination. Similarly, significant 15% (95% CI:0 – 28%) decrease was noted in children 7 – 17 years of age, while a 13% (95% CI:2 – 24%) increase was noted in children less than one year of age. IR was unchanged in other age-groups, (2 years and 3 – 6 years of age, Table 16). Visits due to OM decreased by 26% (95% CI:17 – 34%) in children 1 year of age from 1,426 to 1,058 per 10,000 children, with no significant changes noted in other age groups (Table 16).

Visits due to pneumonia were decreased in children in the age groups less than 1 and in children 1 year of age. The reduction was 30% (95% CI:11 – 45%), from 245 to 172 per 10,000 person-years in children less than 1 year of age and 23% (95%CI:10 – 34%) from 596 to 460 per 10,000 person-years in children 1 year of age. The IR was unchanged in other age groups (Table 16).

Conversely, increase in visits due to acute bronchiolitis was found in the same period in the age groups of children less than 1, 1 and 2 years of age.

The increase was 51% (95%CI:33 – 85%) from 423 to 637, 96% (95%CI:63 – 172%) from 149 to 292 and 94% (95%CI:46 – 240%) from 54 to 105 per 10,000 person-years for children less than 1, 1 and 2 years of age, respectively (Table 16).

Table 16. The incidence rate and incidence rate ratio between the Pre-vaccine and Post-vaccine study groups for all cause study visits, acute otitis media, pneumonia and acute bronchiolitis.

Groups (years)		Pre IRR (n)	Post IRR (n)	At risk pre/post	IRR (95% CI)
All cause study visits	**<1**	1724 (1642)	1948 (1779)	3174/3044	1.13 (1.02-1.24)[a]
	1	2322 (2101)	1967 (1844)	3016/3125	0.85 (0.78-0.92)[c]
	2	907 (788)	919 (876)	2896/3176	1.01 (0.90-1.15)
	3 – 6	229 (744)	204 (715)	10826/11660	0.89 (0.75-1.06)
	7 – 17	78 (689)	66 (574)	29559/29051	0.85 (0.72-1.00)[a]
Acute Otitis Media	**<1**	910 (867)	980 (895)	3174/3044	1.08 (0.94-1.23)
	1	1426 (1290)	1058 (992)	3016/3125	0.74 (0.66-0.83)[c]
	2	371 (322)	315 (300)	2896/3176	0.85 (0.70-1.03)
	3 – 6	60 (194)	63 (221)	10826/11660	1.06 (0.83-1.34)
	7 – 17	8 (74)	10 (87)	29559/29051	1.19 (0.86-1.67)
Pneumonia	**<1**	245 (233)	172 (157)	3174/3044	0.70 (0.55-0.89)[b]
	1	596 (539)	460 (431)	3016/3125	0.77 (0.66-0.90)[b]
	2	358 (311)	388 (370)	2896/3176	1.09 (0.89-1.32)
	3 – 6	93 (346)	91 (319)	10826/11660	0.98 (0.79-1.23)
	7 – 17	39 (348)	32 (275)	29559/29051	0.80 (0.64-1.01)
Acute Bronchiolitis	**<1**	423 (403)	637 (582)	3174/3044	1.51 (1.28-1.77)[c]
	1	149 (135)	292 (274)	3016/3125	1.96 (1.56-2.47)[c]
	2	54 (47)	105 (100)	2896/3176	1.94 (1.60-2.90)[c]
	3 – 6	8 (25)	9 (31)	10826/11660	1.15 (0.66-2.00)
	7 – 17	0 (3)	1 (13)	29559/29051	4.41 (1.23-15.8)[a]

[a]*p<.05* [b]*p<.01* [c]*p<.001*

The incidence rate per 100,000 children years for each study calendar-year for children less than 18 years of age for acute bronchiolitis, pneumonia and influenza can be seen in Table 17.

Table 17. The incidence rate per 100,000 children years for each study calendar-year for children less than 18 years of age for acute bronchiolitis, pneumonia and influenza.

	2008	2009	2010	2011	2012	2013
Pneumonia	1218	1160	1214	1205	1041	855
Bronchiolitis	187	425	624	671	685	642
Influenza	28	548	2	103	66	110

4.2.2.3 Comparison based on vaccine eligibility

In this sub-analysis, visits by children 3 to 23 months of age were analysed by birth year. During the study period 12,771 children were born in the greater Reykjavík area, 9,798 children were born in 2008 – 2010 and thus vaccine non-eligible (VNEC) and 2,973 children born in 2011 and thus vaccine eligible (VEC).

1,614 children were diagnosed with AOM a total of 2,538 times during the study period, 2,054 times in the VNEC and 484 times in the VEC. Likewise, 711 children were diagnosed with pneumonia a total of 895 times during the study period, 724 times in the VNEC and 171 times in the VEC. For acute bronchiolitis 461 children were diagnosed a total of 561 times during the study period, 389 times in the VNEC and 178 times in the VEC.

Significantly more of the children diagnosed with AOM came only once and fewer came more than twice in the VEC compared to the VNEC. For pneumonia, significantly more children in the VEC were diagnosed exactly two times (Table 18). The mean (median) age of the children diagnosed with AOM did not differ between cohorts, 12.8 months (13) and 13.3 months (13) (p=0.09) for VNEC and VEC, respectively. Likewise, the mean (median) age of the children diagnosed with pneumonia were unchanged between the groups, 14.6 months (15) and 15.3 months (15) (p=0.07) for VNEC and VEC, respectively. However, children in the VNEC diagnosed with acute bronchiolitis were younger than those in the VEC (p=0.001). The mean (median) age of the children were 10.3 months (9) and 12.1 months (12), respectively.

The incidence rate of AOM decreased by 24% (95% CI:13 – 33%), after the initiation of the vaccination, from 120 to 92 visits per 1,000 person-years of

follow-up in the VEC. Likewise, the IR of pneumonia was decreased by 23% (95%CI:5 – 36%) from 42 in the VNEC to 33 per 1,000 person-years of follow-up in the VEC. Conversely, the IR for acute bronchiolitis increased by 53% (95%CI:24 – 90%) from 22 to 34 visits per 1,000 person-years of follow-up (Figure 8).

Table 18. The proportion of children diagnosed once, twice or more than twice for each of the diagnostic groups.

Diagnosis	Number of visits	VNEC	VEC	OR (95% CI)
AOM	**1**	68.1%	77.1%	1.6 (1.2 – 2.1)
	2	15.5%	14.7%	0.9 (0.7 – 1.3)
	> 2	16.4%	8.2%	0.5 (0.3 – 0.7)
Pneumonia	**1**	82.7%	79.7%	0.8 (0.6 – 1.1)
	2	12.4%	17.4%	1.5 (1.1 – 2.1)
	> 2	4.9%	2.9%	0.6 (0.3 – 1.1)
Bronchiolitis	**1**	84.6%	81.0%	0.8 (0.6 – 1.1)
	2	11.3%	13.4%	1.2 (0.8 – 1.7)
	> 2	4.1%	5.6%	1.4 (0.8 – 2.4)

OR: Odds ratio between the VEC and the VNEC. (Acute otitis media (AOM), pneumonia and acute bronchiolitis) for the vaccine non-eligible cohort (VNEC) and the vaccine eligible cohort (VEC).

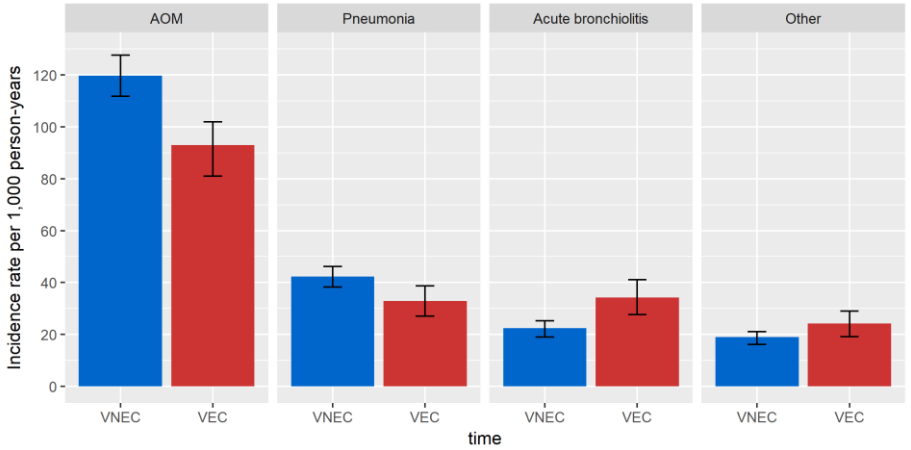

Figure 8. Incidence rate per 1,000 children for AOM, Bronchiolitis (LRTI), pneumonia and other diagnosis in the vaccine non-eligible cohort compared (VNEC) to the vaccine eligible cohort (VEC). Error bars represent 95% confidence intervals.

4.2.3 Impact on hospitalisations (Study IV)

During the study period (2005 – 2015), 53,228 children less than 3 years of age had permanent residence in Iceland at some time. Of those, 1,964 children were excluded, 1,892 because they immigrated to the country after birth and 72 children due to missing information. Therefore, 51,264 children were included in the study. The children were followed for a total of 142,315 person-years, with median follow-up of 1,096 days (range 6 – 1,096), for each child.

A total of 10,520 children had incomplete follow-up due to censoring before their third birthday. Of those, censoring due to emigration occurred in 2,263 children and 23 children died, while the rest, 8,234 were censored at the end of the study period. Birth-cohort size ranged from 4,026 in 2015 to 5,130 children in 2009 and 51.3% of the children were males. 4,842 admissions were registered to the Children's Hospital during the study period, of those 1,703 were for diseases coded by the study's diagnostic groups. For birth cohort 2005, 49.1% of all admissions were due to study diagnosis, while only 27.9% of admissions for children born in 2015 were due to study diagnosis (Table 19). The most common attributable diagnosis were bronchiolitis and bronchitis for children less than one years of age and pneumonia for children one and two years of age. The fluctuations in admissions for each of six different diagnostic clusters by calendar year for the three age groups can be seen in Figure 9.

4.2.3.1 Hospital admissions due to OM and URTI

During the study period, 280 hospital admissions by 256 children were due OM and 131 admissions by 123 children due to URTI. There was no significant change in the crude IR between the cohorts in any age-brackets. The crude IR of admissions for OM was 2.32 per 1,000 person-years for VNEC and 1.45 per 1,000 person-years in the VEC. The parallel crude IR of admissions for bronchiolitis was 0.78 in the VNEC and 1.13 in the VEC (Figure 10 A and B).

There was no significant difference in the mean age of children admitted for OM and URTI, 12.8 months for OM and 13.5 months for URTI (p=0.48). Boys were 57% and 55% of admissions for OM and URTI, respectively. Length of admission was similar, median of 2.0 (IQR 1.0 – 3.7) days and 1.9 (IQR:1.5 – 3.7) days for OM and URTI, respectively. Intensive care unit transfers were rare, only three children admitted due to OM were transferred while five children admitted due to URTI were admitted to ICU. For OM two were transferred due to mastoiditis, and one for unrelated systemic illness. All of the five children with URTI and were transferred to the ICU had laryngitis or laryngotracheitis.

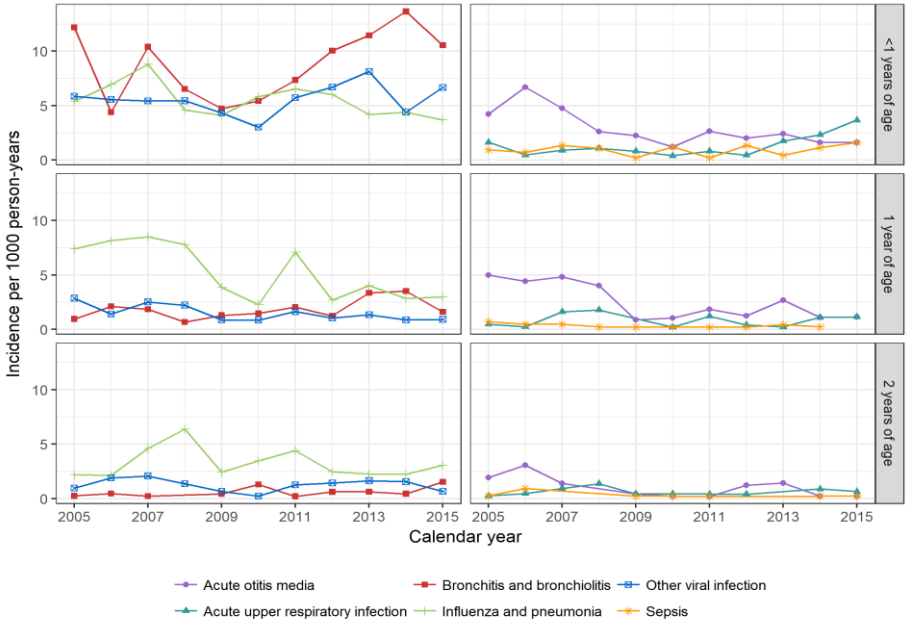

Figure 9. The incidence rate of admissions per 1,000 years of follow-up for each diagnostic cluster and age-group by calendar years.

For the vaccine eligible cohort, the cumulative admission rates per 1000 person-years was significantly lower for OM and significantly higher for URTI, as shown in the Kaplan-Meier curves (Figure 11 A and B, respectively). Similarly, the hazard of admission for OM was 43% lower for VEC than VNEC (HR 0.57, 5%CI:0.43 – 0.73). Conversely, the hazard for URTI admission was 56% higher (HR:1.56, 95%CI:1.11 – 2.19) for children in the VEC (Figure 12).

4.2.3.2 Hospital admissions due to pneumonia and bronchiolitis

During the study period, 660 hospital admissions for 550 children were due to pneumonia and 550 admissions for 508 children due to bronchiolitis. The crude IR of admissions for pneumonia was 4.94 per 1,000 person-years for VNEC and 4.18 per 1,000 person-years in the VEC. The parallel crude IR of admissions for bronchiolitis was 2.94 in the VNEC and 5.23 in the VEC. The highest IR of pneumonia admissions was in 12 – 17 months old children, significantly lower in VEC than VNEC (IRR:0.52, 95%CI:0.35 – 0.77, Figure 10C). Conversely, the highest IR of admissions for bronchiolitis was in children less than six months of age, significantly higher in VEC than VNEC (IRR 1.50, 95%CI 1.23 – 1.84, Figure 10D). For both diagnoses, the IR was unchanged in all other age groups (Figure 10).

Birth-cohort	Children (py), *n*	All cause admissions, *n*	Study admissions, *n*	Proportion due to study diagnosis, %
2005	4,541 (13,278)	446	219	49.1
2006	4,668 (13,658)	415	176	42.4
2007	4,770 (13,985)	423	186	43.9
2008	4,953 (14,472)	442	117	26.5
2009	5,130 (14,965)	484	124	25.6
2010	4,988 (14,593)	384	158	41.1
2011	4,643 (13,638)	392	129	32.9
2012	4,667 (13,750)	576	196	34.0
2013	4,438 (13,033)	472	149	31.6
2014	4,440 (10,916[a])	431[a]	144[a]	33.4
2015	4,026 (6,027[a])	377[a]	105[a]	27.9
Total	51,264 (142.315)	4,842	1,703	

Table 19. Overview of admissions in the study birth-cohorts. The number of all cause admissions for and number and proportion of all cause admissions due to study diagnoses. py: person-years of follow-up. [a]Incomplete follow-up time.

The mean age of children admitted for pneumonia was 13.6 months, significantly higher than the mean age of children admitted for bronchiolitis, 8.0 months (P<.001). Boys were 52% and 53% of admissions for pneumonia and bronchiolitis respectively. Length of hospital stay was similar, but significantly shorter for bronchiolitis than pneumonia (median and IQR of 2.58 (1.7 – 4.0) and 2.84 (1.7 – 4.9), p<0.001). Admission rate to intensive care unit (ICU) was significantly higher for children with pneumonia, 5% compared to 1% for bronchiolitis. There was no significant difference in the length of ICU stay, median and IQR 1.31 (0.72 – 4.12) days for pneumonia compared to 1.16 (0.65 – 1.56) days for bronchiolitis.

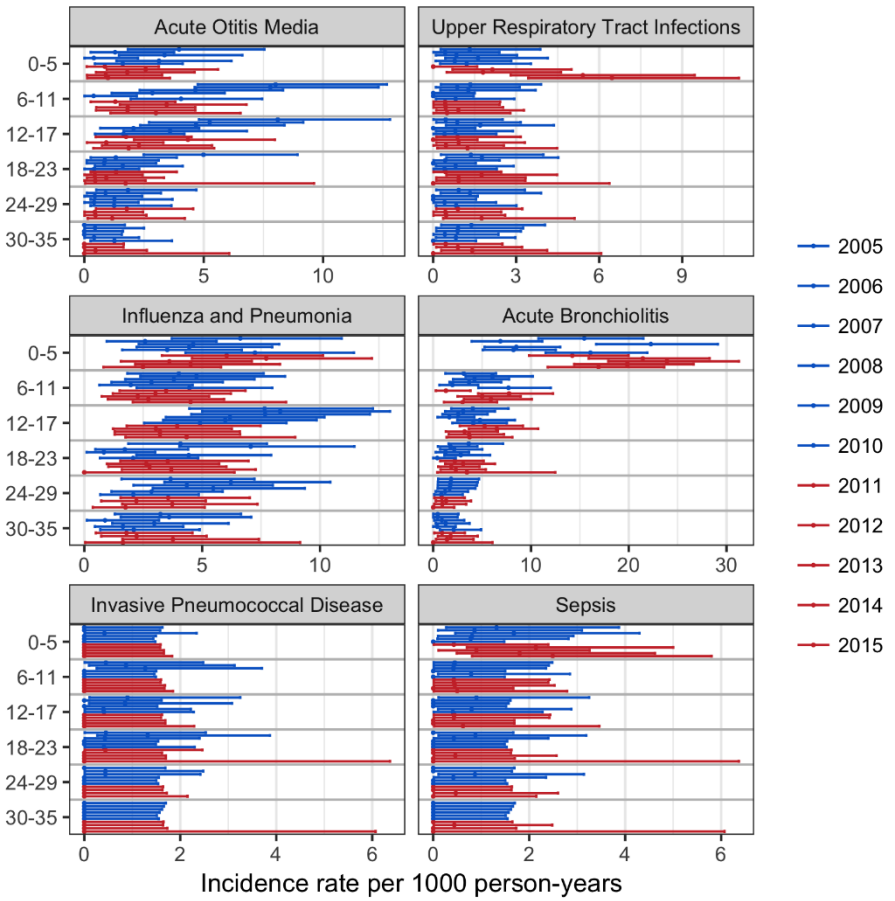

Figure 10. The crude incidence rate per 1,000 person years of follow-up for each of the study diagnostic clusters for each of the study birth-cohorts.

The pneumonia subgroup diagnosis J18 – pneumonia, organism unspecified was the most common diagnosis used for pneumonia and was reduced by 30% between the groups, IRR:0.70 (95%CI:0.56 – 0.88). No significant difference was found between the groups for other sub-group diagnosis of pneumonia. (Table 20). For the vaccine eligible cohort, the cumulative admission rates per 1000 person-years was significantly lower for pneumonia and significantly higher for bronchiolitis, as shown in the Kaplan-Meier curves (Figure 11 C and D, respectively). Similarly, the hazard of admission for pneumonia was 20% lower for VEC than VNEC (HR 0.80, 95%CI:0.67 – 0.95), while the hazard for bronchiolitis admission was 32% higher (HR:1.32, 95%CI:1.14 – 1.53) (Figure 12).

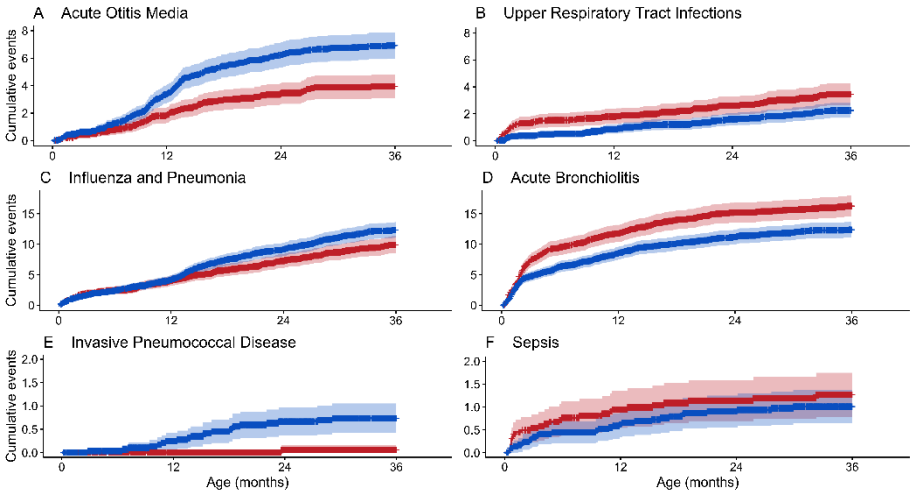

Figure 11. Kaplan-Meier estimator graphs showing the cumulative number of events per 1,000 children followed. Blue line indicates the VNEC and the red line the VEC. Shading indicates 95% confidence intervals.

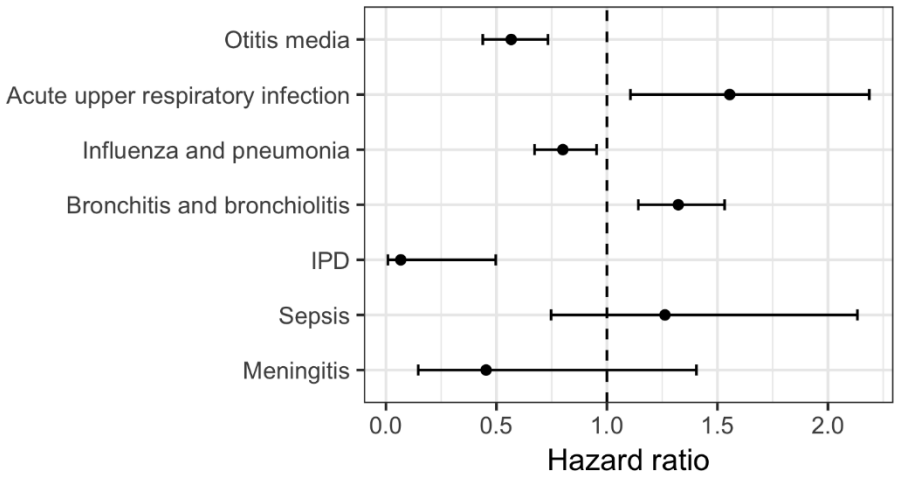

Figure 12. Hazard ratios between VNEC and VEC for each of the study diagnostic groups. HR >1.0 indicates higher hazard in VEC than VNEC, while a HR <1.0 indicates lower hazard in VEC than VNEC. Error bars indicate 95% confidence intervals. Larger intervals indicate less certainity of effect size due to lower number of admissions in that diagnostic cluster.

4.2.3.3 Hospital admissions due to all-cause meningitis, sepsis and IPD

During the study period, 19 admissions for 15 children were due to meningitis

and 63 admissions for 61 children due to sepsis. The crude IR of admissions for meningitis was 16.5 per 100,000 person-years for VNEC and 8.71 per 100,000 person-years in the VEC (Figure 10E). The crude IR of admissions for sepsis were not significantly different, being 38.8 per 100,000 person-years in the VNEC and 52.3 per 100,000 person-years in the VEC (Figure 10F).

In the study birth-cohorts, 37 children were diagnosed with culture confirmed IPD before three years of age. Among those, 23 (59%) were admitted for inpatient treatment. Of the admitted children, nine had a primary discharge diagnosis of meningitis, eight children had pneumococcal sepsis (A40.3) as their primary diagnosis and eight children had pneumonia as their primary diagnosis. A single child had pyogenic arthritis (M00.9) as a diagnosis and another had fever (R50.9). Overall crude IR of both admitted and non-admitted cases was 36.4 per 100,000 person-years in the VNEC, significantly higher than the 1.74 per 100,000 person-years in the VEC. When only including children admitted for IPD, the crude IR for VNEC was 24.7 per 100,000 person-years, significantly higher than the 1.7 per 100,000 person-years in VEC (Figure 10E). Vaccine-type IPD was not found in the Vaccine eligible cohort.

The mean age of children admitted for IPD, meningitis and sepsis were 14.4 months, 9.7 months and 8.4 months, respectively. Boys were 65%, 67% and 57% of admissions, respectively. Length of hospital stay was on median (IQR) 5.8 (4.1 – 6.8), 9.3 (5.4 – 10.7) and 6.1 (3.8 – 9.3) for IPD, meningitis and sepsis admissions, respectively. Admission rate to ICU was high for IPD and meningitis, with 33% of IPD admissions and 55% of meningitis admissions needing ICU care. Lower proportion, 6% of sepsis admissions were transferred to ICU.

For the vaccine eligible cohort, the cumulative admission rates per 1000 person-years was significantly lower for IPD, with no significant difference found for sepsis (Figure 11 E and F, respectively). The hazard of admission for IPD was 93% lower for VEC than VNEC (HR 0.07, 95%CI:0.01 – 0.50). No significant change was noted in the hazard for meningitis and sepsis, HRs:0.45 (95%CI 0.15 – 1.41) and 1.26 (95%CI:0.75 – 2.13), respectively (Figure 12).

Table 20. Comparison of the number and incidence rates of children and incidence rate rations in the Vaccine non-eligible cohorts vs Vaccine eligible cohorts for subgroups of pneumonia. The ICD codes with their subclasses were included.

	Vaccine non-eligible cohorts		**Vaccine eligible cohorts**		
Sub-group diagnosis	**n**	**IR (95%CI)**	**n**	**IR (95%CI)**	**IRR (95%CI)**
J10: Influenza with pneumonia, virus identified	5	0.06 (0.02 – 0.14)	1	0.02 (0.00–0.10)	0.30 (0.01 – 2.65)
J11: Influenza with pneumonia, virus not identified	1	0.01 (0.00–0.07)	0	0.00 (0.00–0.06)	0.00 (0.00 – 57.76)
J12: Viral pneumonia	68	0.80 (0.62 – 1.01)	48	0.84 (0.62 – 1.11)	1.05 (0.71 – 1.53)
J13: Pneumonia due to *S. pneumoniae*	3	0.04 (0.01 – 0.10)	3	0.05 (0.01 – 0.15)	1.48 (0.20 – 11.06)
J15: Bacterial pneumonia, not elsewhere classified	90	1.06 (0.85 – 1.30)	55	0.96 (0.72 – 1.25)	0.91 (0.64 – 1.28)
J16: Pneumonia due to other infectious organisms	3	0.04 (0.01 – 0.10)	2	0.03 (0.00 – 0.13)	0.99 (0.08 – 8.62)
J17: Pneumonia in diseases classified elsewhere	1	0.01 (0.00 – 0.07)	1	0.02 (0.00 – 0.10)	1.48 (0.02 – 116.25)
J18: Pneumonia, organism unspecified	234	2.75 (2.41 – 3.13)	111	1.94 (1.59 –2.33)	0.70 (0.56 – 0.88)

5 Discussion

5.1 Main findings

- The vaccine impact on carriage rate of vaccine type pneumococci was high, 94% in vaccine eligible children and 56% in older, non-vaccine eligible children. Serotype replacement was noted with no significant change in overall carriage rate of pneumococci.
- The proportion of children that carried PNSP was unchanged after the vaccination. However, the children in the VEC were more likely to carry low MIC PNSP while the children in the VNEC, were more likely to carry high MIC PNSP. The children in VEC were also significantly less likely to carry pneumococci non-susceptible to other antimicrobials, being multi resistant or being resistant against all tested antimicrobials in the study.
- The PHiD-CV vaccine impact was demonstrated on various pneumococcal infectious diseases.
- For AOM, primary care visits were reduced by 22% and emergency department visits by 24%, with no clinically significant change in hospital admissions in relation to the introduction of the vaccine. Additionally, fewer children were diagnosed with AOM before their third birthday and fewer children were diagnosed with recurrent disease.
- For pneumonia, a 23% reduction in emergency department visits vs found and a 20% reduction in hospitalizations.
- Admissions due to invasive pneumococcal diseases were reduced by 93%, with no vaccine type IPD detected in vaccine eligible children.
- Significant increases were found in visits and admissions due to infections more likely to be caused by viruses.
- The high impact of the vaccination against all strata of pneumococcal disease in Iceland are indicative of massive success of the implementation of PHiD-CV in Iceland.

5.2 Impact on the nasopharyngeal carriage (Study I)

The vaccine impact on nasopharyngeal carriage of vaccine type pneumococci was great, 94% (95%CI: 91 – 96%), and most vaccine serotypes were not isolated in VEC. The combined prevalence of vaccine associated serotypes 6A and 19A was also significantly decreased by 33% (95%CI: 1 – 55%) while a non-significant decrease for individual VAT serotypes was found. There was no change in the total carriage rate of pneumococci, with an equivalent increase in NVT pneumococci following reduction in VT pneumococci. In the study on herd effect, a considerable vaccine impact was also found on carriage of both VT and VAT pneumococci, 56% (95%CI: 44 – 65%) and 33% (7 – 51%), respectively, with no change in total carriage rate of pneumococci due to replacement. These results are in line with most other studies on pneumococcal carriage which have shown high vaccine impact, both in direct and herd effect analysis and replacement with NVT pneumococci (Adegbola, 2014; Desai, 2015; Dunais, 2015; Hamaluba, 2015; Mehr, 2012; Pavia, 2009; Plosker, 2014; van Hoek, 2014; Wang, 2017).

In contrast to other vaccine serotypes, no change was noted in the prevalence of 19F carriage in the herd effect analysis, despite high impact in the direct impact analysis. This warrants further attention. In a sub-analysis we examined if older non-vaccine eligible children were carrying 19F at more than one sampling occasion i.e. a year later. We found that 7 out of the 40 children that carried 19F in the post-vaccination period, did so at more than one sampling occasion. This could indicate that carriage of 19F was maintained in the older children, despite increasing herd effect from the younger vaccinated children. In a study on antimicrobial non-susceptible pneumococci in Israel, 19F was one of the most commonly carried serotypes prior to introduction of PCV-13, despite years of PCV-7 vaccination. However, after introduction of PCV-13 the prevalence was reduced (Dagan, 2014), perhaps indicating that additional protection was relayed by cross-protection from vaccination against serotype 19A. Other explanation is that eradication of 19F needed a longer period of vaccination than other serotypes, possibly due to persistence in carriage. Additionally, in an abstract presented at ISPPD-10 (Glasgow, 2016) the carriage of 19F was shown to linger in older children in Israel, perhaps due to waning immunity in older children (Althause, 2016). It is unlikely that mucosal immune response is different to serotype 19F than to other serotypes.

In NSCMID 2018, follow-up of the current study was reported, elucidating that in 2017 and 2018, no 19F was found in the day care centres participating in the yearly surveillance of pneumococcal carriage and no VT pneumococci

was found in 2018 (n=500 a year) (Erlendsdóttir, 2018). This supports the hypothesis that eradicating 19F takes longer than most other VT.

The total carriage rate of pneumococci in Iceland is higher than reported in most studies from HIC, closer to what is reported in LMIC (Adegbola, 2014; Desai, 2015; Mehr, 2012; Pavia, 2009; Wang, 2017). As discussed in some detail in Chapter 5.2.1, the incidence of AOM is also high in Iceland and the same factors discussed there, such as that most children in Iceland attend day care may apply for carriage as well. Other factors are also worth discussing in some detail. Iceland is an island situated in the middle of the Atlantic Ocean, with a subarctic climate. Both summers and winters are mild, with average winter (November – March) temperatures in Reykjavik close to freezing point (0°C) (Icelandic Meteorological office, 2012). In addition, in those weather conditions day cares and schools in Iceland rarely close, opting rather to keep children inside, further increasing crowding conditions inside the DCCs. This further increases the spread of pneumococci and other pathogens between the children. As discussed in Chapter 5.3, with high employment of both sexes in Iceland, children might return to DCCs and schools earlier than would be expected when one parent is not employed outside the home. The high carriage rate can also partly be explained by the current studies design, as all samples were taken in March, coinciding with or following yearly epidemics of viral infections, which increase carriage of pneumococci (Ampofo, 2008; T Heikkinen, 2003; Lai, 2016; Peltola, 2004). Additionally, all samples were plated within 4 – 6 hours and detection was performed by the same, experienced scientist the whole study period that might also be a factor in the consistently high carriage reported in the study.

In a recent systemic review on disease potential of common serotypes, serotypes 23B, 6C, 19F, 16F, 15BC, 15A, 10B, 23F, 10A, 6B, 6A, 22F were all found to have invasiveness indexes lower than 1.0, i.e. a low risk for invasive disease. Conversely, serotypes 1, 5, 12F, 7F were found to have indexes higher than 1.0 (Balsells, 2018). That is reassuring, as none of the serotypes found to be more invasive were found in our study after the vaccination, with many of the most common serotypes cultured in the post vaccination period having low indexes. Reports from this study is also in line with other studies conducted prior to the introduction of PCV, discussed in detail in a Chapter 1.3.2.

The most common serotypes found in our DCC study after the vaccination were 15, 11, 23B, 23A and 6C (in descending order), all showing large significant increases in nasopharyngeal carriage after the vaccination. Many of

those serotypes have also been reported in serotype replacement in other countries using the higher valent vaccines (Dagan, 2014; Desai, 2015; Richter, 2014; Vesikari, 2016).

In this study, a large reduction was found in the carriage of multi-resistant pneumococci and pneumococci non-susceptible to the five tested antimicrobials. However, no change was noted in the overall prevalence of PNSP, despite the serotypes causing the majority of PNSP in the pre-vaccination period having disappeared. When stratifying the MIC values of the isolates to low MIC (0.094 – 0.5) and high MIC (>0.5) it was found that most of the PNSP isolates in the post vaccination period exhibited low MIC values, albeit defined as PNSP. Most of the PNSP isolates from the pre-vaccination period had high MIC values. This result was highly appreciated, as this info helps providers choose narrow spectrum antimicrobials when treating infections. However, continued surveillance has demonstrated that the MIC values of the replacing serotypes, mainly 11A and 35B appear to be increasing (Erlendsdóttir, 2018). Decrease in carriage of pneumococci non-susceptible to antimicrobials have been widely reported after the introduction of PCV (Dagan, 2008; Desai, 2015; Dunais, 2015; Gounder, 2015). Similar to our findings the rate of non-susceptibility of the replacing non-vaccine serotypes is generally lower. Additionally, the non-susceptible NVT are more likely to exhibit lower MIC values, thus more likely to be classified as intermediate resistant than completely resistant (Dagan, 2008; Dunais, 2015; Gounder, 2015). However, of increasing concern is the emergence of penicillin and multi-drug resistant NVTs threatening to offset the reduction in antimicrobial non-susceptible pneumococci found after the introduction of PCV (Kawaguchiya, 2017; Neves, 2017; Richter, 2014). As antimicrobial therapy selects for non-susceptible pneumococci (discussed in Chapter 1.2.2), diligent antimicrobial prescriptions remains as important as ever.

Despite the worrying increases in PNSP in Iceland and worldwide (Kawaguchiya, 2017; Neves, 2017; Richter, 2014), the isolation of pneumococci resistant against high-doses of penicillin is still rare in Iceland. In the opinion of the author, empiric treatment for mild paediatric infection in Iceland should still be managed with watchful waiting followed by medium to high doses of narrow-spectrum penicillins, reserving the use of broad-spectrum agents to severe infections and in treatment failure.

5.3 Vaccine impact on infections (Studies II, III and IV)

5.3.1 Acute otitis media

Following introduction of the vaccine in Iceland, numerous changes in the epidemiology of acute otitis media have been observed.

5.3.1.1 Incidence of AOM in primary care (Study II)

The crude overall incidence rate of primary care visits due to AOM in the current study was 41.7 per 100 person-years of follow up among children less than three years of age. The highest incidence was reported in children one to two years of age, 57 in the VNEC and 52 per 100 person-years in the VEC. The mean number of visits per child was 1.61 in the VNEC and 1.37 for the VEC.

The reported incidences of AOM can vary up to 10-fold between other studies conducted in MHIC (Taylor, 2012). Iceland rates among of the most highly developed countries in the world, excelling at most measurements of quality of health care and prevention (Barber, 2017). Despite this the incidence of AOM reported here and in other studies from Iceland (Gudnason, 2012) is higher than in most other studies from MHIC (Gisselsson-Solen, 2017; Liese, 2014; Marchisio, 2012; Monasta, 2012; Sáez-Llorens, 2017; Sartori, 2017; Taylor, 2012; Tregnaghi, 2014), but lower than others, mainly from the USA (Marom, 2014; Paradise, 1997; Taylor, 2012; Teele, 1989). There are many possible reasons for the high incidence in Iceland and varying incidence between other studies.

As OM is generally a mild, benign disease, both cultural and economic factors could be at play affecting how often and fast a doctor is consulted. Iceland has the highest employment rate in Europe for persons 20 – 64 years of age for both sexes. Compared the European Union averages of 71.1%, 76.9% and 65.3% for total employment, male and female employment, respectively, the total employment rate in Iceland was 87.8%, with 91.1% of males and 84.4% of females being employed outside the home in 2016 (Eurostat, 2017). This, in conjunction with free primary care and easy access for children might encourage parents in Iceland towards seeking health care early for mild disease, increasing reported incidence. Additionally, with both parents employed, pressure to return children back to day care, even before fully recovered from illnesses, can cause more children to get infected, which again increases the true incidence.

Icelandic children start day care at young age and the OECD ranks Iceland second in formal day care attendance with 60% enrolment for children <2 years of age (OECD average: 35%), attending for an average of 38 hours a week (OECD average: 30 hours) (Organization for Economic Cooperation and Development, 2016). As both average day care attendance and length of stay increase risk of OM (Baraibar, 1997; Daly, 2000; Gudnason, 2012; Paradise, 1997; Ramakrishnan, 2007) it could be one of the factors explaining the high incidence of OM. On the other hand, the incidence of AOM in the US is even higher compared to Iceland, with low enrolment in formal day care (Organization for Economic Cooperation and Development, 2016). Obviously, there are many factors involved.

The winters in Iceland can be cold which could increase crowding in day care centres, further facilitating spread of pathogens. As corroboration, the carriage rate of pneumococci in Iceland, from nasopharynx collected in March each year, is higher than in most other HIC, discussed in Chapter 1.2.

Each new episode of AOM increased the hazard for contracting additional episodes. Thus, children that had more episodes had higher risk of contracting additional episodes compared to children with fewer prior episodes. This is in line with other studies that indicate that having AOM can have detrimental impact on the mucosal defences of the middle ear, causing increased risk of infection compared to a middle ear without damage (Dagan, 2016; de Hoog, 2016; Teele, 1989; Todberg, 2014).

5.3.1.2 Vaccine impact on epidemiology of AOM in primary care (Study II)

The vaccine impact on the number of primary care AOM episodes was 22% (95%CI:12 – 31%), with reduction found in all age groups following the vaccination. More VEC children had no visits due to AOM compared with VNEC, and children that were ever diagnosed with AOM had fewer episodes, 1.37 vs 1.61 per child. Moreover, fewer children had recurrent AOM.

Examining the trend in visits due to AOM prior to the initiation of the vaccination showed that only the 2007 birth-cohort had significantly different hazard of AOM visits than the reference birth-cohort, 2010. The 2007 birth cohort had a HR of 1.07 (95%CI:1.02 – 1.12). Conversely, every VEC had a significant less hazard of having AOM episodes, ranging from 0.78 – 0.89). Altogether, at the end of the study period in 2016, the absolute number of prevented AOM episodes in vaccine eligible children less than 3 years of age were 4,187 (95%CI:3363 – 5011). In this study each episode of AOM resulted

in on average 1.26 physician visits and thus the number of averted physician visits is likely more than 5,000 in the first 4 years and 9 months of the study.

The randomized controlled trials (RCTs) conducted on PCV reported high efficacy against vaccine type, total pneumococcal and all cause AOM, although with highly variable effect sizes (Eskola, 2001; Fireman, 2003; O'Brien, 2008; Prymula, 2006; Sáez-Llorens, 2017). As AOM is caused by various microbes (Dagan, 2016), the efficacy in RCTs was, as expected highest for vaccine type AOM and lowest for all-cause AOM.

The efficacy of PCV against vaccine serotype pneumococcal AOM has been reported to be high, with effect sizes reported to be 57% – 69.9% (Eskola, 2001; O'Brien, 2008; Prymula, 2006; Sáez-Llorens, 2017). For any serotype pneumococcal AOM the effect sizes were slightly lower, ranging from 34 – 62.9% (Eskola, 2001; Prymula, 2006; Sáez-Llorens, 2017). Finally, efficacy against all cause AOM showed mixed result, -0.4 – 33.6%, with confidence intervals often traversing zero (Eskola, 2001; Fireman, 2003; O'Brien, 2008; Prymula, 2006; Sáez-Llorens, 2017) (Table 21).

Table 21. The efficacy of PCV against acute otitis media as shown in Randomized Controlled Trials.

	Efficacy of RCTs against acute otitis media (95% CI)			
Study	n	VT pneumococcal	Pneumococcal	All cause
Eskola, 2001	1,662	57% (44–67%)	34% (21–45%)	6% (-4–16%)
Prymula, 2006	4,968	58% (30–87%)	52% (37–63%)	34% (21–44%)
O'Brien, 2008	856	64% (-34–90%)	N/A	-0.4 (-19–16%)
Sáez-Llorens, 2017	7,359	70% (30–87%)	56 (22–75%)	24% (9–37%)
Fireman 2003	37,868	N/A	N/A	8% (5–10%)

Eskola et al., O´Brien et al. and Fireman et al. studies were conducted on PCV-7. Prymule et al. study was conducted on PHiD-CV11, a vaccine precursor of PHiD-CV10 and Sáez-Llorenz on the PHiD-CV10. VT: Vaccine type.

Observational studies which employ less stringent diagnostic criteria for AOM than the RCTs have shown varying effect sizes of PCV on all-cause AOM after introduction. In PCV naive populations, the impact effect sizes ranges from 14% to 43% (Fitzwater, 2012; Magnus, 2012; Taylor, 2012). There are many reasons for different effect sizes between studies, including different and varying diagnostic criteria within and between studies, serotype distribution and secular trends in pneumococcal serotypes and respiratory viral infections. The data presented in this study reduces this risk by observing the whole

population in the primary care setting with regards to the most general definition of all-cause physician diagnosed OM and evaluating the vaccine effectiveness against multiple different metrics of OM epidemiology.

When regarding vaccine impact on otitis media, serotype replacement must be considered. Unlike the effect on carriage after the introduction of the meningococcal and *H. influenzae* B protein conjugate vaccines (Robbins, 1996) the introduction of PCVs does not reduce the total carriage rate of pneumococci. Serotypes that used to be rare, have increased greatly, following the eradication of vaccine serotypes. This has both been seen after introduction of PCV in PCV-naive populations, such as in this study and when converting to higher valent vaccines (Dagan, 2014; Desai, 2015; Richter, 2014; Vesikari, 2016). Therefore, continued surveillance of serotype prevalence in carriage, non-invasive and invasive disease is crucial.

As discussed in detail in Chapter 1.3, serotypes have different propensities to cause disease. Therefore, replacement with serotypes less likely to cause disease is the reason for the important impact of PCV despite full overall pneumococcal replacement in the nasopharynx. Next generation of vaccines must continue to consider the difference in disease potential of pneumococcal serotypes and clones.

Additionally, in a poster from ISPPD-11 from our study group the median age at first visit for AOM was delayed by 122 days comparing VNEC and VEC, from 686 to 808 days at first diagnosis (Sigurdsson, 2018a). This further emphasises the impact of the vaccine on AOM in children.

It is generally accepted that prior to PCV, pneumococci were the cause of around 30-35% of episodes of AOM (Leibovitz, 2004; Palmu, 2004; Van Dongen, 2013), with higher proportion in more severe infections (Leibovitz, 2004; Palmu, 2004). It has also been established that pneumococci are more common in early episodes, with less virulent organisms such as *H. influenzae* being increasingly more common in repeated and chronic disease (Dagan, 2016). Early infection likely inflicts more damage to the immature middle ear mucosa, facilitating recurrence of otitis. This is supported by the fact that observational studies have linked contracting otitis at a young age to an increased risk of recurrent episodes (Dagan, 2016; de Hoog, 2016; Teele, 1989; Todberg, 2014). A recent study demonstrated that for children less than two, a linear correlation was found between age at first infection and risk of recurrent OM. The children had a 6 – 9% increased risk for each month younger they were diagnosed with the first AOM (de Hoog, 2016). Similarly, in another study children were seven times more likely to develop recurrent

disease if they were younger than one year when first infected (Macintyre, 2010). Therefore, the vaccination against pneumococci, in which early episodes are averted or delayed, the risk of further episodes by *S. pneumoniae* or different pathogens is decreased, causing the long-term impact to be higher than the predicted impact only on vaccine type pneumococci.

When stratified by number of AOM episodes in this study, the hazard of the first two episodes of AOM for each child were reduced in VEC compared with VNEC. Children with no previous episodes were 16% less likely to be diagnosed with their first AOM in the VEC than in the VNEC. Similarly, children in the VEC with one previous visit for AOM, were 5% less likely to be diagnosed with a second episode (HRs:0.84 (95%CI:0.82 – 0.86) and 0.95 (95%CI:0.93 – 0.98), respectively). For children with two or more episodes, no difference in hazard of subsequent visits was found between the groups. The effect, of the vaccination may therefore mainly be conveyed through the prevention of the first two episodes. Children that have experienced two episodes, regardless of vaccine status are in equal risk of having additional episodes, further strengthening the hypothesis that recurrent infections, disrupt the mucosal defence in the middle ear and increases the risk of recurrent AOM.

Fewer children were diagnosed early with OM in the VEC than in the VNEC. It is therefore unsurprising that the proportion of children with recurrent OM in this study is lower after the vaccination, with only 7.4% of the VEC group compared to 9.3% in the VNEC suffering recurrent disease. Having children with recurrent and severe OM decreases parental Quality of Life (Greenberg, 2003; Holl, 2015), increases the risk of invasive procedures such as tympanocentesis or tympanostomy tube insertion (Gisselsson-Solen, 2017; Greenberg, 2003; Whittemore, 2013). Most children with complex OM have no long-term sequela. There are some evidence that a small subgroup of children with the most severe, longstanding disease, concurrent with previous developmental difficulties and lack of in-home support, might have permanent reduction in some measurements of hearing, speech and intellect, although those results are conflicting (Hall, 2014; Mortensen, 2013; Roberts, 2004), discussed in more detail in Chapter 1.4.8. Therefore, the reduction found in complex OM in this study might have implication far beyond factors measurable in this study.

5.3.1.3 Impact on paediatric emergency department visits due to AOM (Study III)

Outpatient paediatric emergency department visits due to AOM were significantly reduced in the vaccine eligible cohort compared to the vaccine

non-eligible cohort. The study was conducted early following the introduction of the vaccine.

In the main analysis, visits due to AOM three years prior to the initiation of the vaccination (2008 – 2010) were compared to visits three years after the initiation of the vaccination (2011-2013) stratified by age groups. The incidence was the highest in children one year of age, decreasing by 26% (IRR: 0.74, 95%CI: 0.66 – 0.83) after the vaccination. A 15% non-significant reduction was noted in children two years of age, despite only few children in the age-groups being vaccine eligible. No change was noted in children 3 – 6 years and 7 – 17 years of age, although visits in those age groups were few. Interestingly, no change was noted in the youngest age group, those less than one year of age.

In the sub-analysis based on vaccine eligibility the first vaccine eligible cohort was followed until two years of age and compared to same aged children in the three previous cohorts. The incidence was reduced by 24% (95% CI: 13 – 33%) from 1198 visits to 915 visits per 10,000 children years of follow-up.

Visits to the paediatric emergency department due to AOM likely represent more severe disease than those that visit other providers such as primary care or private paediatricians. That claim can be strengthened with the fact that the impact found on the IR of the first vaccinated cohort is similar as the 22% impact found five years after the vaccination in the primary care, where the impact for the first cohort was only 12%. This is further established in our study where ceftriaxone use for AOM, a marker for more severe disease or treatment failure, was found to be largely reduced at the Children's Hospital in the VEC, with children one year and two years of age having 56% and 57% lower use of ceftriaxone, respectively. No significant change was noted for children less than one year of age or three years of age, IRRs being 0.6 (95% CI: 0.18 – 1.77) and 0.76 (95%CI: 0.76 – 2.02), respectively (Eythorsson, 2017). This further indicates that more severe cases with treatment failure are cared for at the hospital.

The impact might be expected to be greater with longer follow-up time. That was not the case in another study by the study group with longer follow-up in the same setting (Eythorsson, 2017). In that study the crude IRR was 0.86 (95%CI: 0.81 – 0.91) in children less than four years of age. However, the vaccine impact was calculated from post-vaccine calendar years, with high proportion of children being non-vaccinated, likely restricting effect sizes (Eythorsson, 2017).

5.3.1.4 Admissions due to AOM (Study IV)

In Iceland admissions due to AOM are few, in this study the IR for children less than 3 years of age in VNEC and VEC was only 2.32 and 1.45 per 1,000 person-years, compared to the IR of visits to the paediatric emergency department which was around 100 per 1000 person-years in children less than two years of age. The HR of AOM admissions when comparing the VNEC to VEC was significantly lowered. However, the hazard of admissions for the patients was considerably higher for birth cohort 2005 – 2007 as compared to the last vaccine non-eligible cohort (2010), with no change noted in the hazard for birth cohort 2008 and later. Therefore, the vaccine impact on admission due to AOM found in the study must be interpreted with caution due to the low numbers.

This is discrepant to results from three previous studies which demonstrate reduction in the admissions rates due to otitis media after PCV (Durando, 2009; Gisselsson-Solen, 2017; Tawfik, 2017). However, indications for admissions due to otitis media vary between settings which must be considered when comparing studies. In this context, it may be kept in mind that surgical procedures like tympanostomy tube placements are usually done in private clinics in Iceland and without admission to hospital.

Admission rates due to AOM in the US have been reduced since the introduction of PCV (Tawfik, 2017). For children one year of age the rates were reduced from 22.6 to 8.7 per 100,000 children-years and from 13.7 to 5.6 in children aged two, following the introduction of PCV-13 as compared to rates prior to introduction of PCV-7, 12 years earlier (Tawfik, 2017). In that study, interrupted time series analysis was used. The reduction in the AOM admissions was convincing, both after the introduction of the PCV-7, with further reductions noted following the conversion to PCV-13.

In Sweden, a 42% reduction in AOM admissions were found, comparing admissions rate prior to introduction to PCV-7 to rate after introduction of higher valent vaccines in children less than four years of age. There are other factors that must be considered in the analysis of the Swedish study. There were secular trends clearly visible in both the outpatient and inpatient admissions reported in the study and the largest change in the admission rate was noted in the first year of PCV-7 vaccination (Gisselsson-Solen, 2017). There was no catch-up schedule implemented in Sweden (Berglund, 2014) and had these factors been considered, the point estimate found might have been lower.

In a regional study from Italy, a 36.4% (95% CI: 24.1 – 46.7) reduction in AOM admission after the introduction of PCV-7 was found in vaccine eligible children compared to vaccine non-eligible children (Durando, 2009).

However, similar to our findings no impact was found on admissions due to AOM in a study from Israel, although including fewer children than the other studies cited (Marom, 2017).

When comparing studies conducted in different countries, differences in clinical practice must be considered. When considering admission for children there are several factors that must be evaluated, including the feasibility of strict follow-up, which is influenced by many factors.

In Iceland, children are often observed in the paediatric emergency department, initiating parenteral therapy as needed, before a decision whether to admit the child is taken. Children with severe AOM are often discharged home with strict follow-up in the following days. Ceftriaxone, a parenteral third generation cephalosporin is the drug of choice in severely ill children in Iceland. As it is administered once per day, it is feasible to allow parents to return home overnight, returning the next day for reassessment. Those children are not considered admitted to in-patient wards.

Children admitted with the diagnosis of OM in this study likely represent three diverse patient-groups. The first group contains children, the most severely ill that require in-patient observation and treatment, beyond what is possible at the emergency ward. The second group includes children in which strict follow-up is unfeasibly, due to parental factors such as unreliable providers or being non-residents of the capital area. In the third group are children with underlying comorbidities, which put them at higher risk for adverse effects or those admitted due to other diseases, not included in this study, but have concurrent OM or develop OM during their stay at hospital. Unfortunately, we were unable to differentiate between those groups in this study.

5.3.1.5 Herd effect on AOM (Study II and III)

Strong indications of herd effect on primary care visits for children too young to receive the vaccine according to the paediatric vaccine schedule in Iceland were found. For children <4 months of age a clear reduction was noted in the rate of primary care diagnosed AOM episodes, the IRR for VNEC vs VEC was 0.60 (95%CI: 0.51 – 0.69). Despite few cases in this age-group, the confidence interval is narrow, further strengthening the claim. This is an important finding, as contraction of otitis media in young children can have detrimental impact on

the mucosal defences of the middle ear, causing increased risk for recurrent disease, discussed in more detail in Chapter 5.3.1.2.

A well-being visit is scheduled for all children in Iceland around three months of age, in which all children are given PHiD-CV10 and Pentavac® (Embætti Landlæknis, 2015), unless implicitly rejected by guardians or its administration is contraindicated. Administration of the vaccines are rarely performed prior to the well-being visits unless under special circumstances.

Reports on herd effect on otitis media are scarce (Loo, 2014; Vesikari, 2016), with only one published study that we are aware of which suggests an impact on children too young for vaccination (Ben-Shimol, 2016). In that study, children less than four months of age had significantly fewer positive pneumococcal cultures after the vaccination than same aged children prior to the vaccination. We are unaware of any studies showing decrease in visits due to OM in children in this age group.

There was no evident evidence from visits to paediatric emergency department that would suggest that older, non-vaccinated children experienced herd effect. We did not specifically test for herd effects in older vaccine non-eligible children or children not vaccinated, although eligible in the primary care setting. That would be an interesting next step in elucidating if that effect is present in that age-group. However, as discussed in detail in Chapter 5.3.1.2, herd effect on older children living among younger vaccinated children might not be present due to disruptive effects of early AOM. Our results show that children that have already experienced two episodes of AOM do not benefit further from protection of the vaccine. That suggest that the herd effect might only be present for children living alongside same aged vaccinated children and a subgroup of older children, with intact mucosal defences of the middle ear. Herd effect on carriage of pneumococci is widely reported and is discussed in detail in Chapter 5.2.

5.3.2 Impact on pneumonia (Study III and IV)

Finding the causative agent in community acquired pneumonia, can be challenging, especially in children, as discussed in detail in Chapters 1.1, 1.2 and 1.5. It has been estimated that after PCV, around 9 – 12% of CAP in children are caused by pneumococci (Berg, 2016; Jonnalagadda, 2017). When considering only more severe disease, up to 18% of pneumonia cases and 30% of deaths due to pneumonia, are thought to be caused by pneumococcus (Walker, 2013). These estimates are likely to be influenced by many factors, including the prevalence of pneumococcal carriage, which is high in Iceland,

as elucidated in Study I.

5.3.2.1 Impact on outpatient visits (Study III)

Outpatient paediatric emergency department visits due to pneumonia were significantly reduced after the vaccination. Study III was conducted very early after the introduction of the vaccine, also discussed in Chapter 5.3.1.3.

In the main analysis, visits due to pneumonia three years prior to the initiation of the vaccination (2008 – 2010) were compared to visits three years after the initiation of the vaccination (2011-2013) stratified by age groups. The incidence was the highest in children one years of age, decreased significantly by 23% (IRR: 0.77, 95%CI: 0.66 – 0.90) from 596 to 460 per 10,000 person-years of follow-up. Additionally, visits for children less than one year of age were reduced significantly by 30% (IRR: 0.70, 95%CI: 0.55 – 0.89) from 245 to 172 per 10,000 person-years. No herd effect on visits in older unvaccinated children were noted, as no significant changes were noted in older age groups when comparing the number of visits before and after the vaccination. Had any herd effect developed, it was not detected via the study design this early after the vaccination.

In the sub-analysis based on vaccine eligibility, the first vaccine eligible cohort was followed until two years of age and compared to same aged children in the three previous cohorts. The incidence was reduced by 23% (95% CI: 5 – 36%) from 42 visits to 33 visits per 10,000 children years of follow-up.

Two double blinded RCTs evaluating the PHiD-CV vaccine effectiveness (VE) against pneumonia have been published, one from Finland and one conducted in Latin America (Kilpi, 2018; Tregnaghi, 2014).

In the COMPAS study, carried out in Latin America, the efficacy of the PHiD-CV against pneumonia was demonstrated in a randomized, double-blinded phase III trial. Included were around 24 thousand infants from Argentina, Panama and Columbia that were randomized to receive either PHiD-CV or hepatitis vaccine as control (Tregnaghi, 2014). Presumed bacterial CAP was diagnosed in 3.0% of the control group children and 2.3% in the PHiD-CV group, with estimated vaccine efficacy of 22% (95%CI: 8 – 34%). The efficacy against consolidated CAP was 26% (95%CI: 8 – 40%), diagnosed in 2.0% vs 1.5% of the vaccinated and control groups, respectively. The efficacy was lower for suspected CAP, 6.7% (0.7 – 12%), with 18.6% and 19.8% of the vaccine and control groups diagnosed, respectively (Tregnaghi, 2014).

The FinIP trial, a nation-wide, cluster-randomised, double-blind trial evaluated the vaccine efficacy against pneumonia in Finland (Kilpi, 2018). For children enrolled before the age of 7 months the vaccine efficacy was 27% (95%CI: 14 – 38%) against all-cause hospital diagnosed pneumonia and 45% (95%CI: 26 – 60%) against WHO defined alveolar consolidation or pleural effusion, as read by blinded interpreters (Kilpi, 2018).

Other RCTs evaluating the efficacy of other vaccine formulations have also demonstrated high VE against pneumonia (Black, 2002; Cutts, 2005; Hansen, 2006; Klugman, 2003; Lucero, 2009).

In a study conducted in the Philippines that used an 11-valent vaccine, conjugated to tetanus toxin manufactured by Sanofi Pasteur, more than 12 thousand children were randomized. The VE against radiologically confirmed pneumonia was 23% (-1 – 41%), with significant reduction found for children 3 to 11 months, but not for older children (Lucero, 2009).

Two studies conducted in South-Africa and Gambia using a 9-valent vaccine from Wyeth, showed 20 – 37% VE against first episode of radiologically diagnosed pneumonia (Cutts, 2005; Klugman, 2003). Additionally, the Gambia study demonstrated a 7% VE on clinically diagnosed CAP (Cutts, 2005).

In the Kaiser Permanente trial on effectiveness of the PCV-7, clinically diagnosed pneumonia were reduced by 4.3 % (-3.5 – 11.5%) (Black, 2002) and the VE against radiologically confirmed pneumonia was 17.7% (95%CI: 5 – 29%) and 25.5% (95%CI: 7 – 41%) (Hansen, 2006) using the standardized WHO method (World Health Organization, 2001).

In the current study the reduction was higher than the reported VE against clinically diagnosed pneumonia, more in line with the effect sizes reported for radiologically diagnosed pneumonia in those studies. We did not have access to radiological data, although it is likely that for most of the children diagnosed with pneumonia at the Children's Hospital, the only tertiary paediatric emergency department in the country, would have been evaluated with chest X-rays.

5.3.2.2 Impact on admissions (Study IV)

The admission rate of children was significantly reduced by 20% (5 – 33%) following the introduction of the PHiD-CV in Iceland. confirming a strong vaccine impact. This effect was established despite an increase in bronchiolitis admissions, discussed in more detail in Chapter 5.2.3. In addition to the

vaccine impact estimated, the cumulative incidence was reduced in the vaccinated cohort.

These findings are in good accordance with the reduction found in paediatric emergency department visits due to pneumonia discussed in the preceding chapter.

The impact of RCTs on pneumonia outpatient visits was extensively discussed in Chapter 5.3.2.1. The studies did not report specific results on admissions due to pneumonia, therefore the discussion is focused on pneumonia admissions in post-marketing studies.

In a 2016 systemic review on hospitalisations in Latin America due to pneumonia the effect sizes ranged from 7 – 21% for clinically diagnosed pneumonia and 9 – 38% for x-ray confirmed pneumonia. Included in the study were all studies published from the region regardless if the PHiD-CV or PCV-13 vaccines were used (L. H. De Oliveira, 2016). This is in line with the reduction found in the current study.

In contrast, a study from Quebec, Canada did not find a causal link between the reduction found in admission rates due to LRTI following PCV-7 and conversion to PHiD-CV. In that study the reduction found in LRTI was present from before the PCV-7 was introduced without an obvious change in the trend following PCVs (Anderson, 2017).

5.3.3 Impact on IPD (Study IV)

The incidence of IPD in children was low in Iceland during the study period. The incidence of hospitalized IPD decreased from 24.7 to 1.7 per 100,000 person-years of follow-up and the incidence rate of hospital-diagnosed IPD decreased from 36.4 to 1.7 per 100,000 person-years. The vaccine impact on admissions due to culture confirmed IPD was found to be 93%, (HR 0.07, 95%CI: 0.01 – 0.50). This is a remarkable reduction and the reduction is even higher if the children not admitted had been included in the analysis.

In the Children's Hospital, severely ill children, but not requiring invasive monitoring are often observed at the paediatric emergency department, where treatment is initiated as needed. Generally, children should not be observed for longer than 24 hours at the emergency department before decision on admission or discharge is taken. Therefore, children showing clear clinical improvement in this period and with reliable parents, so that strict follow-up is possible without admission may be discharged rather than admitted if their condition allows. The decision is therefore often made before culture results

are available, explaining why a portion of children with IPD are discharged home from the paediatric emergency department, rather than admitted. Therefore, we made a distinction between children with IPD that were admitted and those who were not.

Following introduction of PCVs, reduction in paediatric culture confirmed IPD has been reported worldwide, with the highest effect sizes for vaccine type IPD (Andrews, 2014; L. H. De Oliveira, 2016; H. Lee, 2014; Plosker, 2014; Poehling, 2006; Weinberger, 2016).

In the current study, the rate of IPD was declining prior to the introduction of PHiD-CV. As IPD is a very rare disease in Iceland, secular trends can have a considerable impact. The trend of IPD in Iceland was recently presented in the 35th meeting of the Nordic Society of Clinical Microbiology and Infectious Diseases (NSCMID) (Erlendsdóttir, 2018). In that study natural fluctuation in the rate of IPD was demonstrated since surveillance started. The prevalence of PNSP and the rate of all-cause meningitis in Iceland, demonstrating similar fluctuations has previously been published (Hjálmarsdóttir, 2014; Snaebjarnardóttir, 2013). Between 2004 to 2010 the prevalence of penicillin non-susceptible 19F increased by more than a factor of five, becoming the dominating serotype by far in non-invasive patient isolates at the start of the vaccination (Hjálmarsdóttir, 2014). Similarly, in our carriage study, 19F was the dominant serotype cultured in healthy carriers. The increase in the prevalence of 19F in both healthy carriers and patient isolates might be one of the reasons for the declining rate of IPD seen in this study between 2005 and 2010, as 19F is recognized as a serotype with low invasiveness potential (Kronenberg, 2006; Sá-Leão, 2011), as discussed in detail in Chapter 1.3.2.

It stands to reason that around the time of the introduction of the PHiD-CV the next peak in the rate of IPD could have been anticipated. It is possible that the introduction of the vaccine interrupted that trend, although we did not perform any specific analysis to further elucidate that idea.

5.3.4 Impact on other respiratory tract infections (Study III and IV)

Other RTIs, which rarely or never are caused by pneumococcus were included in Studies III and IV as comparison to RTIs more commonly caused by pneumococci. Evaluating the incidence of those infections is important as they are an important risk factor for pneumococcal carriage and disease.

The incidence rate of outpatient visits due to bronchiolitis was increased after the introduction of the vaccine. Significant increases were noted in children <1, 1, 2 and 3 – 6 with no change noted in children 7 – 17 years of

age. Similarly, the hazard of admission and cumulative incidence of LRTI was increased in the vaccinated cohorts. In addition, the admission rates, although low for URTI, were slightly increased in the post-vaccination era compared to the pre-vaccination era. In this chapter other LRTI and other URTI will be collectively referred to as viral infections.

Examining the incidence rates for each of the study cohorts reveal an increase in viral infections starting before the vaccination, continuing in the post-vaccination era. There are several factors indicating that the increase in viral infections are indeed a true increase, rather than a shift in coding practices or misdiagnosis.

Firstly, the same diagnostic code system (ICD-10) was used exclusively and have been for several years in the databases used in the study. In 1997 the Directorate of Health recommended that all providers switch from ICD-9 to ICD-10 and likely most providers had gained proficiency in coding with that system by the start of the study in 2005. No change in guidelines on diagnosis of pneumonia were implemented and therefore unlikely that physicians would be more reluctant to diagnose pneumonia than they had been before. However, the Directorate of Health implemented new guidelines in 2009 on diagnosis of AOM (Landlæknisembættið, 2009), reflecting changes in international guidelines developing in the years before. These guidelines might induce a bias in comparison of AOM diagnosis. However, no significant change in AOM diagnosis were noted before the vaccination and more likely that the international guidelines might have been slowly improving Icelandic practice for up to a decade before the official change in guidelines as most Icelandic doctors seek their speciality training in Europe or the US.

Secondly, the increases in viral infections were the most evident in the youngest children and started before the initiation of the vaccination, while the decrease in pneumonia was noted after the vaccination in older children. Admissions due to bronchiolitis were only increased in children less than six months of age, while significant increases in admissions for pneumonia were only found in children 12 – 17 months of age.

Thirdly an increase in bronchiolitis diagnosis have been widely reported in HIC, perhaps due to the spread of a novel RSV strain, genotype ON1 first found in Canada in 2010, subsequently increasingly detected in Europe (Duvvuri, 2015; O'Connor, 2013; Otieno, 2017; Pierangeli, 2014; Yoshihara, 2016). In Iceland, an increase in the positive virology testing, including RSV and human metapneumovirus has been reported in the post-vaccination period (Department of Virology, 2018).

Finally, hospital diagnosed LRTI, especially pneumonia represent a subgroup of children with more severe infections. It is likely that those children would undergo more tests, including x-rays and blood tests such as C-reactive protein, to support the diagnosis. Therefore, the increase in viral infections found in this study is likely due to increased viral load in the post-vaccination period, making the observed reductions found in all aspects of pneumococcal epidemiology, even more impressive.

5.4 Strengths and weaknesses of the study

5.4.1 Nasopharyngeal carriage study (Study I)

The major strength of this study was the large number of pneumococcal isolates collected over a long period of time, including every consenting child in 15 day care centres. The 15 DCCs were equally spread between suburbs in the capital area. The participating DCCs were both small and large, situated in different areas and from newly constructed and older, established neighbourhoods. This allowed the results from this study to be more representative of all children living in the capital area, than if the DCCs included would have been more homogenous. The study groups were well-defined, and the mean and median age of children in the study-groups compared were roughly equal.

In the direct analysis of the impact on carriage, a clear demarcation in the vaccine uptake between the VNEC and VEC was confirmed. This is mainly due to a low uptake in VNEC before the initiation of the vaccine and an immediate high uptake in the VEC (>97% for all birth cohorts (Eyþorsson, 2017)).

All sampling and culturing were done in the same month every year, using the same equipment, methods and protocol. The same experienced biomedical scientist oversaw the study and performed all the serotyping and more complex microbiological analysis during the whole study period.

The main weakness of the study is that the children compared are sampled in different years, and thus subject to external factors such as changes in viral infections, natural serotype fluctuations which can cause a bias in the results. We only included children attending day cares and as such our results are not transferable to children that do not attend day cares. We argue that the number of children between the ages of two and six that do not attend DCCs in Iceland to be too low to be of any significance. Additionally, we only sampled children

from DCCs in the capital area and thus have no information on children living in other areas of the country. This reduces the generality of the study.

5.4.2 Study on primary care physician visits due to AOM (Study II)

In this study we were able to follow every child in the whole country of Iceland during a defined study period, regardless if they sought medical care or not. This allows for an exact person-years of follow up time (denominator) as children individualized statistical data on each child is cross-referenced to the primary health care database. The primary care health database includes complete information on all primary care visits due to AOM from all 69 primary health care centres in Iceland, including an 11-year period in this study. Every provider used the same electronic health record system and used the same coding system from 1997 (Landlæknisembættið, 1997). Converting the children‘s personal identification numbers to unique study numbers, we were able to follow each child on an individual level, regardless of which health care centre they sought medical care from. Repeated visits during the defined episode could be removed and accurate per child analysis performed. All the above permitted the use of survival methods that are usually only possible in prospective studies.

Primary care for children is handled by primary care physicians, not paediatricians in Iceland. As such, most episodes of otitis media are likely seen by a primary care physician, either as the sole provider, before referral or during follow-up. As Iceland is a geographically isolated island with excellent access to health care and follow-up, it is unlikely that a significant number of children sought regular primary care outside of the country. In this study all-cause physician diagnosed OM was used, with no indicator of accuracy or severity of the diagnosis. The awareness of physicians and the public that the vaccine might reduce the number of OM might impact how a perceived OM is approached. Firstly, parents might be more comfortable delaying seeking medical help for mild infections, knowing that the child is vaccinated. Physicians might also be influenced by the fact and when in doubt, might be more likely to diagnose a child with unspecific viral diagnosis rather than OM and consequently delaying antibiotic prescription as parental antimicrobial expectation might be lowered.

During the study period, parents could seek medical care from specialist providers without referral from primary care physicians. We were unable to include visits to private paediatricians and ENT specialists due to coding differences. Theoretically, this could have caused a bias in the study due to

possible changes in health seeking behaviour. In this context it can be kept in mind that all visits to private paediatricians for children <18 years of age increased from 43 thousand visits a year in 2005 to 59 thousand in 2015. Corresponding number of all visits to ENT specialists for all age-groups were 33 thousand in 2005 and 44 thousand in 2015. This might indicate that more children were diagnosed by specialists rather than primary care physicians. However, the number of urgent care visits at the largest private paediatric clinic in Iceland was largely unchanged during the study period, with around 11,000 visits per year (personal correspondence with head of the largest private paediatrician clinic). This clinic primarily serves children with urgent or acute problems as an out of hours service. This indicates that the increase was not due to urgent visits such as acute infections, rather an increase in other non-emergent visits. Additionally, the increased number of paediatrician's and ENT specialist's visits are in line with changes in total visits to private specialists which increased from 490 thousand in 2005 to 711 thousand in 2015. The increase in visits to private specialists is larger than might be suggested only by population growth and is part of a larger shift of out-patient care from public hospitals to specialists offices in Iceland (McKinsey & Company, 2016). We therefore maintain that the number of children seeking specialist treatment did not substantially affect the number of children included in our study and did not change over the study period. Additionally, we argue that most children managed by specialist are seen at some point by primary care physicians, either prior to referral or during follow-up.

5.4.3 Hospital visits and admissions (Study III and IV)

The main strength of Studies III and IV are well defined study groups and distinct difference in vaccine uptake between the VNEC and VEC in the cohort part of the studies. Another strength is the fact that the paediatric emergency department at the Children's Hospital, Landspítali University hospital serves as the primary hospital for more than 80% of children in the country (statice.is). It is the only tertiary children's hospital in the whole country and children from other parts of the country are routinely transferred if specialized assessment and treatment is needed. Thus, being a single centre study is a strength as the same procedures and the same staff handle all children included in the study, eliminating bias caused by different practices between hospitals. Additionally, clinical practices were mostly unchanged during the study period and the same coding system (ICD-10) was used exclusively. The hospital operates the only specialized paediatric emergency department in the country and is one of two hospitals with paediatric inpatient wards, although some smaller hospitals have the capacity to observe children at hospital with mild diseases. This allows

capture of most or all severely ill children in the whole country.

The main weakness of this study is the lack of a reliable comparison in the incidence of other infections. Natural fluctuation in the incidence of respiratory tract infections can cause a bias in all epidemiological studies on RTIs. Including data on unrelated infections, such as infectious gastroenteritis might have allowed us to evaluate if there had been large systemic changes, such as restricted access, restriction in admission or a general change in health seeking behaviour. We do however argue that there has been no systemic change during the study period, as overall visits and admission at the Children´s Hospital rose during the study period.

6 Conclusions

The nasopharyngeal carriage of vaccine type pneumococci was reduced by 94% in vaccine eligible children following the introduction of PHiD-CV in the paediatric vaccination schedule in Iceland. No change was found in the total carriage of pneumococci due to replacement of non-vaccine serotypes. The replacing serotypes were more susceptible to penicillin and a lower proportion was non-susceptible to erythromycin, combined erythromycin and penicillin, co-trimoxazole, multi-resistant or resistant to all tested antimicrobials. Serotype 19F was the most common PNSP and multi-resistant isolate in the VNEC while 15 and 6C were the most common serotypes in children in VEC. Similarly, the carriage of vaccine serotypes was reduced by 56% in older, non-vaccinated children indicating herd effect of the vaccination. The unvaccinated children sampled in the post vaccination period were less likely to carry pneumococci non-susceptible to co-trimoxazole and were less likely to carry multi-resistant pneumococci or pneumococci resistant to all tested antimicrobials. 19F was the most common PNSP and multi-resistant serotype in both the pre-vaccination and post-vaccination groups. The serotypes carried by children after the vaccination are less invasive than the vaccine serotypes. This answers the research questions raised in aim I

The incidence of paediatric emergency department visits due to pneumonia was reduced by 23% in children <2 years of age in the VEC compared to the VNEC. Likewise, the visits due to AOM were reduced by 24%, while visits due to acute bronchiolitis were increase by 53% in the same period. This answers the research questions raised in aim II

Primary care visits in the whole country due to AOM were reduced by 22% for children less than three years of age in the VEC compared to the VNEC. The vaccine impact was conveyed through the prevention of the first two episodes of AOM, with no significant impact on subsequent visits in children already with two prior episodes. Fewer children in VEC were diagnosed with AOM before the age of three, and those diagnosed were older at the first otitis media and experienced fewer episodes. Fewer children were diagnosed with recurrent AOM. This answers the research questions raised in aim III

Admissions to the Children's Hospital due to pneumonia were reduced by 20% and at the same time as admissions due to bronchiolitis were increased by 32% in children less than three years of age in VEC compared to the VNEC.

Admissions due to IPD were reduced by 93%. No clinically significant change was noted in admissions for AOM, sepsis, meningitis or URTI. This answers the research questions raised in aim IV

All the aims put forward in this thesis were met.

A massive amount of data was collected in this study. Many further research questions rose during my four years of PhD work. The next steps for the author and other members of the study group will be to address those questions using the data already gathered.

- Has the age at first visit due to AOM changed (preliminary results presented at ISPPD-11, Melbourne, Australia).
- Does the age at first visit due to AOM affect the risk of recurrent AOM and the total number of visits due to AOM.
- Does the age at first visit due to AOM affect the risk of tympanostomy tube insertion.
- Does inserting a tympanostomy tube in a child affect the risk of further episodes of acute and chronic otitis media and number of antimicrobials prescribed.
- What is the proportion of each RTI that is treated with antimicrobials and has there been a change since the introduction of the vaccination.
- How much variance is there in the proportion of infections that are treated with antimicrobials between individual doctors and health care centres.
- Does feedback on individual physicians prescribing habits or specific physician directed interventions affect the quality of antimicrobial prescriptions.
- Can an intervention directed at patients improve the quality of antimicrobial prescriptions.
- Does carriage of specific serotypes or lack of carriage affect the risk of that child seeking health care for pneumococcal infections.
- Does antimicrobial use affect the risk of contracting a new infection in a period after completion of the antimicrobial

The main conclusion of this study was that after implementing PHiD-CV into the childhood vaccination schedule, the burden of pneumococcal disease in Icelandic children has decreased remarkably. Nasopharyngeal carriage of VT pneumococci was eliminated. Outpatient visits due to OM has been reduced by 24%, visits and admissions due to pneumonia by 23% and 24%. Moreover, culture confirmed invasive pneumococcal diseases have been reduced by

94%. This study further proves that introducing pneumococcal vaccines in previously vaccine naïve societies to be highly effective in preventing VT carriage and disease caused by pneumococci.

References

Acuin, J. (2004). *Chronic suppurative otitis media - Burden of Illness and Management Options*. Geneva. Retrieved from http://www.who.int/pbd/publications/Chronicsuppurativeotitis_media.pdf

Adegbola, R. A., DeAntonio, R., Hill, P. C., Roca, A., Usuf, E., Hoet, B., & Greenwood, B. M. (2014). Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: a systematic review and meta-analysis. *PloS One*, *9*(8), e103293. https://doi.org/10.1371/journal.pone.0103293

Adriaenssens, N., Coenen, S., Versporten, A., Muller, A., Vankerckhoven, V., Goossens, H., & ESAC Project Group. (2011). European Surveillance of Antimicrobial Consumption (ESAC): quality appraisal of antibiotic use in Europe. *The Journal of Antimicrobial Chemotherapy*, *66 Suppl 6*(SUPPL. 6), vi71-77. https://doi.org/10.1093/jac/dkr459

Ahmad, H., & Chapnick, E. K. (1999). CONJUGATED POLYSACCHARIDE VACCINES. *Infectious Disease Clinics of North America*, *13*(1), 113–133. https://doi.org/10.1016/S0891-5520(05)70046-5

Ahmed, S., Shapiro, N. L., & Bhattacharyya, N. (2014). Incremental Health Care Utilization and Costs for Acute Otitis Media in Children. *Laryngoscope*, *124*, 301–305. https://doi.org/10.1002/lary.24190

Althause, B. M., Dagan, R., & Givon-Lavi, N. (2016). Identification of latent reservoirs of pneumococcal conjugated vaccine (PCV) serotypes after extended vaccination. In *ISPPD-10*. Glascow, Scotland.

Ampofo, K., Bender, J., Sheng, X., Korgenski, K., Daly, J., Pavia, A. T., & Byington, C. L. (2008). Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics*, *122*(2), 229–237. https://doi.org/10.1542/peds.2007-3192

Anderson, G., Deceuninck, G., Zhou, Z., Boucher, F. D., Bonnier Viger, Y., Gilca, R., & De Wals, P. (2017). Hospitalisation for lower respiratory tract infection in children in the province of Quebec, Canada, before and during the pneumococcal conjugate vaccine era. *Epidemiology and Infection*, *145*(13), 2770–2776. https://doi.org/10.1017/S0950268817001510

Andrews, N., Waight, P. a., Burbidge, P., Pearce, E., Roalfe, L., Zancolli, M., … Goldblatt, D. (2014). Serotype-specific effectiveness and correlates of protection for the 13-valent pneumococcal conjugate vaccine: A

postlicensure indirect cohort study. *The Lancet Infectious Diseases*, *14*(9), 839–846. https://doi.org/10.1016/S1473-3099(14)70822-9

Arai, J., Hotomi, M., Hollingshead, S. K., Ueno, Y., Briles, D. E., & Yamanaka, N. (2011). Streptococcus pneumoniae isolates from middle ear fluid and nasopharynx of children with acute otitis media exhibit phase variation. *Journal of Clinical Microbiology*, *49*(4), 1646–1649. https://doi.org/10.1128/JCM.01990-10

Arason, V. A., Kristinsson, K. G., Sigurdsson, J. A., Stefánsdóttir, G., Mölstad, S., & Gudmundsson, S. (1996). Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. *BMJ (Clinical Research Ed.)*, *313*(7054), 387–391. https://doi.org/10.1136/bmj.313.7054.387

Arason, V. A., Sigurdsson, J. A., Erlendsdottir, H., Gudmundsson, S., & Kristinsson, K. G. (2006). The Role of Antimicrobial Use in the Epidemiology of Resistant Pneumococci: A 10-Year Follow Up. *Microbial Drug Resistance*, *12*(3), 169–176. https://doi.org/10.1089/mdr.2006.12.169

Arbique, J. C., Poyart, C., Trieu-Cuot, P., Quesne, G., Carvalho, M. da G. S., Steigerwalt, A. G., … Facklam, R. R. (2004). Accuracy of phenotypic and genotypic testing for identification of Streptococcus pneumoniae and description of Streptococcus pseudopneumoniae sp. nov. *Journal of Clinical Microbiology*, *42*(10), 4686–4696. https://doi.org/10.1128/JCM.42.10.4686-4696.2004

Austin, D. J., Kristinsson, K. G., & Anderson, R. M. (1999). The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(3), 1152–1156. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=15366&tool=pmcentrez&rendertype=abstract

Austrian, R. (1981). Pneumococcus: The first one hundred years. *Reviews of Infectious Diseases*, *3*(2), 183–189. https://doi.org/10.1093/clinids/3.2.183

Austrian, R. (1982). Some obvervations on the pneumoncoccus and on current status of pneumococcal disease and it's prevention. *Pneumoncoccus and the Pneumococcal Vaccine*, *3*(April), 191–207.

Austrian, R., & Gold, J. (1964). Pneumococcal Bacteremia with Especial Reference to Bacteremic Pneumococcal Pneumonia. *Annals of Internal Medicine*, *60*(5), 759–776. https://doi.org/10.7326/0003-4819-60-5-759

Balsells, E., Dagan, R., Yildirim, I., Gounder, P. P., Steens, A., Muñoz-Almagro, C., … Kyaw, M. H. (2018). The relative invasive disease potential of Streptococcus pneumoniae among children after PCV

introduction: A systematic review and meta-analysis. *Journal of Infection*. https://doi.org/10.1016/J.JINF.2018.06.004

Baraibar, R. R. (1997). Incidence and risk factors of acute otitis media in children. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *3 Suppl 3*, S13–S22. https://doi.org/10.1016/S1198-743X(14)64947-8

Barber, R. M., Fullman, N., Sorensen, R. J. D., Bollyky, T., McKee, M., Nolte, E., … Murray, C. J. L. (2017). Healthcare Access and Quality Index based on mortality from causes amenable to personal health care in 195 countries and territories, 1990–2015: a novel analysis from the Global Burden of Disease Study 2015. *The Lancet*, *390*(10091), 231–266. https://doi.org/10.1016/S0140-6736(17)30818-8

Barnett, M. L., Linder, J. A., Mölstad, S., Engström, S., Lannering, C., Melander, E., & Hedin, K. (2014). Antibiotic Prescribing to Adults With Sore Throat in the United States, 1997-2010. *JAMA Internal Medicine*, *174*(1), 138. https://doi.org/10.1001/jamainternmed.2013.11673

Baxby, D. (1999). Edward Jenner's Inquiry; a bicentenary analysis. *Vaccine*, *17*(4), 301–307. https://doi.org/10.1016/S0264-410X(98)00207-2

Ben-Shimol, S., Givon-Lavi, N., Leibovitz, E., Raiz, S., Greenberg, D., & Dagan, R. (2016). Impact of Widespread Introduction of Pneumococcal Conjugate Vaccines on Pneumococcal and Nonpneumococcal Otitis Media. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *63*(5), 611–618. https://doi.org/10.1093/cid/ciw347

Berg, A. S., Inchley, C. S., Aase, A., Fjaerli, H. O., Bull, R., Aaberge, I., … Nakstad, B. (2016). Etiology of Pneumonia in a Pediatric Population with High Pneumococcal Vaccine Coverage: A Prospective Study. *The Pediatric Infectious Disease Journal*, *35*(3), e69-75. https://doi.org/10.1097/INF.0000000000001009

Bergenfelz, C., & Hakansson, A. P. (2017). Streptococcus pneumoniae Otitis Media Pathogenesis and How It Informs Our Understanding of Vaccine Strategies Otitis Media: Definitions and Complications. https://doi.org/10.1007/s40136-017-0152-6

Berglund, A., Ekelund, M., Fletcher, M. A., & Nyman, L. (2014). All-cause pneumonia hospitalizations in children <2 years old in sweden, 1998 to 2012: impact of pneumococcal conjugate vaccine introduction. *PloS One*, *9*(11), e112211. https://doi.org/10.1371/journal.pone.0112211

Berndsen, M. R., Erlendsdóttir, H., & Gottfredsson, M. (2012). Evolving epidemiology of invasive Haemophilus infections in the post-vaccination era: results from a long-term population-based study. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *18*(9), 918–923.

https://doi.org/10.1111/j.1469-0691.2011.03700.x

Bjarnason, A., Lindh, M., Westin, J., Andersson, L. M., Baldursson, O., Kristinsson, K. G., & Gottfredsson, M. (2017). Utility of oropharyngeal real-time PCR for S. pneumoniae and H. influenzae for diagnosis of pneumonia in adults. *European Journal of Clinical Microbiology and Infectious Diseases*, *36*(3), 529–536. https://doi.org/10.1007/s10096-016-2829-z

Bjarnason, A., Westin, J., Lindh, M., Andersson, L.-M., Kristinsson, K. G., Löve, A., … Gottfredsson, M. (2018). Incidence, Etiology, and Outcomes of Community-Acquired Pneumonia: A Population-Based Study. *Open Forum Infectious Diseases*, *5*(2), ofy010. https://doi.org/10.1093/ofid/ofy010

Black, S. B., Shinefield, H., Fireman, B., Lewis, E., Ray, P., Hansen, J. R., … Edwards, K. (2000). Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J*, *19*(3), 187–195. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10749457

Black, S. B., Shinefield, H. R., Ling, S., Hansen, J., Fireman, B., Spring, D., … Hackell, J. (2002). Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatr Infect Dis J*, *21*(9), 810–815. https://doi.org/10.1097/01.inf.0000027926.99356.4c

Blaschke, A. J. (2011). Interpreting Assays for the Detection of Streptococcus pneumoniae. *Clinical Infectious Diseases*, *52*(Supplement 4), S331–S337. https://doi.org/10.1093/cid/cir048

Bluestone, C. D., Stephenson, J. S., & Martin, L. M. (1992). Ten-year review of otitis media pathogens. *The Pediatric Infectious Disease Journal*, *11*(8 Suppl), S7-11. https://doi.org/10.1097/00006454-199208001-00002

Bogaert, D., De Groot, R., & Hermans, P. W. M. (2004, March). Streptococcus pneumoniae colonisation: The key to pneumococcal disease. *Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(04)00938-7

Bogaert, D., Keijser, B., Huse, S., Rossen, J., Veenhoven, R., van Gils, E., … Sanders, E. (2011). Variability and diversity of nasopharyngeal microbiota in children: A metagenomic analysis. *PLoS ONE*, *6*(2), e17035. https://doi.org/10.1371/journal.pone.0017035

Bordley, W. C., Viswanathan, M., King, V. J., Sutton, S. F., Jackman, A. M., Sterling, L., & Lohr, K. N. (2004). Diagnosis and Testing in Bronchiolitis. *Archives of Pediatrics & Adolescent Medicine*, *158*(2), 119. https://doi.org/10.1001/archpedi.158.2.119

Browning, G. G., Rovers, M. M., Williamson, I., Lous, J., & Burton, M. J. (2010). Grommets (ventilation tubes) for hearing loss associated with otitis media

with effusion in children. *Cochrane Database of Systematic Reviews*, (10), CD001801. https://doi.org/10.1002/14651858.CD001801.pub3

Brueggemann, A. B., Griffiths, D. T., Meats, E., Peto, T., Crook, D. W., & Spratt, B. G. (2003). Clonal relationships between invasive and carriage Streptococcus pneumoniae and serotype- and clone-specific differences in invasive disease potential. *The Journal of Infectious Diseases*, *187*(9), 1424–1432. https://doi.org/10.1086/374624

Bryant, K. A., Block, S. L., Baker, S. A., Gruber, W. C., Scott, D. A., & PCV13 Infant Study Group. (2010). Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine. *Pediatrics*, *125*(5), 866–875. https://doi.org/10.1542/peds.2009-1405

Butler, J. C., Shapiro, E. D., & Carlone, G. M. (1999). Pneumococcal vaccines: history, current status, and future directions. *The American Journal of Medicine*, *107*(1), 69–76. https://doi.org/10.1016/S0002-9343(99)00105-9

Cabral, C., Ingram, J., Lucas, P. J., Redmond, N. M., Kai, J., Hay, A. D., & Horwood, J. (2016). Influence of Clinical Communication on Parents' Antibiotic Expectations for Children With Respiratory Tract Infections. *Annals of Family Medicine*, *14*(2), 141–147. https://doi.org/10.1370/afm.1892

Carvalho, M. d. G. S., Tondella, M. L., McCaustland, K., Weidlich, L., McGee, L., Mayer, L. W., … Sampson, J. S. (2007). Evaluation and Improvement of Real-Time PCR Assays Targeting lytA, ply, and psaA Genes for Detection of Pneumococcal DNA. *Journal of Clinical Microbiology*, *45*(8), 2460–2466. https://doi.org/10.1128/JCM.02498-06

Casey, J. R., & Pichichero, M. E. (2004). Changes in frequency and pathogens causing acute otitis media in 1995-2003. *The Pediatric Infectious Disease Journal*, *23*(9), 824–828. https://doi.org/10.1097/01.inf.0000136871.51792.19

Catterall, J. R. (1999). Streptococcus pneumoniae. *Thorax*, *54*(10), 929 LP – 937. Retrieved from http://thorax.bmj.com/content/54/10/929.abstract

Cauchemez, S., Temime, L., Valleron, A.-J., Varon, E., Thomas, G., Guillemot, D., & Boëlle, P.-Y. (2006). S. pneumoniae transmission according to inclusion in conjugate vaccines: Bayesian analysis of a longitudinal follow-up in schools. *BMC Infectious Diseases*, *6*(1), 14. https://doi.org/10.1186/1471-2334-6-14

Center for Disease Control and Prevention. (2017). *Invasive Pneumococcal Disease | 2017 Case Definition*. Atlanta. Retrieved from https://wwwn.cdc.gov/nndss/conditions/invasive-pneumococcal-disease/case-definition/2017/

Chalmers, J. D., Singanayagam, A., Akram, A. R., Mandal, P., Short, P. M.,

Choudhury, G., … Hill, A. T. (2010). Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis. *Thorax*. https://doi.org/10.1136/thx.2009.133280

Chevallier, B., Vesikari, T., Brzostek, J., Knuf, M., Bermal, N., Aristegui, J., … Schuerman, L. (2009). Safety and Reactogenicity of the 10-Valent Pneumococcal Non-typeable Haemophilus influenzae Protein D Conjugate Vaccine (PHiD-CV) When Coadministered With Routine Childhood Vaccines. *The Pediatric Infectious Disease Journal*, *28*(Supplement), S109–S118. https://doi.org/10.1097/INF.0b013e318199f62d

Chi, F., Leider, M., Leendertz, F., Bergmann, C., Boesch, C., Schenk, S., … Hakenbeck, R. (2007). New Streptococcus pneumoniae clones in deceased wild chimpanzees. *Journal of Bacteriology*, *189*(16), 6085–6088. https://doi.org/10.1128/JB.00468-07

Clark, J. E. (2015). Determining the microbiological cause of a chest infection. *Archives of Disease in Childhood*, *100*(2), 193–197. https://doi.org/10.1136/archdischild-2013-305742

Collins, D. A., Hoskins, A., Bowman, J., Jones, J., Stemberger, N. A., Richmond, P. C., … Lehmann, D. (2013). High Nasopharyngeal Carriage of Non-Vaccine Serotypes in Western Australian Aboriginal People Following 10 Years of Pneumococcal Conjugate Vaccination. *PLoS ONE*, *8*(12), e82280. https://doi.org/10.1371/journal.pone.0082280

Cook, R. J. (Richard J., & Lawless, J. F. (2007). *The statistical analysis of recurrent events*. Springer.

Costelloe, C., Metcalfe, C., Lovering, A., Mant, D., & Hay, A. D. (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ*, *340*. Retrieved from http://www.bmj.com/content/340/bmj.c2096.long

Crawford, B., Hashim, S. S. M., Prepageran, N., See, G. B., Meier, G., Wada, K., … DeRosa, M. (2017). Impact of Pediatric Acute Otitis Media on Child and Parental Quality of Life and Associated Productivity Loss in Malaysia: A Prospective Observational Study. *Drugs - Real World Outcomes*, *4*(1), 21–31. https://doi.org/10.1007/s40801-016-0099-9

Croxtall, J. D., & Keating, G. M. (2009). Pneumococcal Polysaccharide Protein D-Conjugate Vaccine (Synflorix™; PHiD-CV). *Pediatric Drugs*, *11*(5), 349–357. https://doi.org/10.2165/11202760-000000000-00000

Cutts, F. T., Zaman, S. M., Enwere, G. C., Jaffar, S., Levine, O. S., Okoko, J. B., … Adegbola, R. A. (2005). Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet*, *365*(9465), 1139–1146. https://doi.org/10.1016/s0140-

6736(05)71876-6

Dagan, R., Juergens, C., Trammel, J., Patterson, S., Greenberg, D., Givon-Lavi, N., … Scott, D. A. (2014). Efficacy of 13-Valent Pneumococcal Conjugate Vaccine (PCV13) Versus That of 7-Valent PCV (PCV7) Against Nasopharyngeal Colonization of Antibiotic-Nonsusceptible Streptococcus pneumoniae. *The Journal of Infectious Diseases*, *211*(7), 1144–1153. https://doi.org/10.1093/infdis/jiu576

Dagan, R., & Klugman, K. P. (2008). Impact of conjugate pneumococcal vaccines on antibiotic resistance. *The Lancet Infectious Diseases*, *8*(12), 785–795. https://doi.org/10.1016/S1473-3099(08)70281-0

Dagan, R., Pelton, S., Bakaletz, L., & Cohen, R. (2016, April). Prevention of early episodes of otitis media by pneumococcal vaccines might reduce progression to complex disease. *The Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(15)00549-6

Daly, K. A., & Giebink, G. (2000). Clinical epidemiology of otitis media. *The Pediatric Infectious Disease Journal*, *19*(5).

Daly, K. A., Pirie, P. L., Rhodes, K. L., Hunter, L. L., & Davey, C. S. (2007). Early otitis media among Minnesota American Indians: The little ears study. *American Journal of Public Health*, *97*(2), 317–322. https://doi.org/10.2105/AJPH.2004.052837

de Hoog, M. L. A. A., Fortanier, A. C., Smit, H. A., Uiterwaal, C. P. M. P. M., van der Ent, C. K., Schilder, A., … Bruijning-Verhagen, P. (2016). Impact of Early-Onset Acute Otitis Media on Multiple Recurrences and Associated Health Care Use. *The Journal of Pediatrics*, *177*, 286-291.e1. https://doi.org/10.1016/j.jpeds.2016.06.066

De Oliveira, L. H., Camacho, L. A. B., Coutinho, E. S. F., Martinez-Silveira, M. S., Carvalho, A. F., Ruiz-Matus, C., & Toscano, C. M. (2016). Impact and Effectiveness of 10 and 13-Valent Pneumococcal Conjugate Vaccines on Hospitalization and Mortality in Children Aged Less than 5 Years in Latin American Countries: A Systematic Review. *PLOS ONE*, *11*(12), e0166736. https://doi.org/10.1371/journal.pone.0166736

Deceuninck, G., De Serres, G., Boulianne, N., Lefebvre, B., & De Wals, P. (2015). Effectiveness of three pneumococcal conjugate vaccines to prevent invasive pneumococcal disease in Quebec, Canada. *Vaccine*, *33*(23), 2684–2689. https://doi.org/10.1016/j.vaccine.2015.04.005

Denapaite, D., & Hakenbeck, R. (2011). A New Variant of the Capsule 3 Cluster Occurs in Streptococcus pneumoniae from Deceased Wild Chimpanzees. *PLoS ONE*, *6*(9), e25119. https://doi.org/10.1371/journal.pone.0025119

Department of Virology, L. U. H. (2018). *Diagnosis of respiratory viruses (other than influenza) 2011-2017 at the department of Virology, Landspitali,*

University Hospital [In Icelandic]. Reykjavík. Retrieved from https://www.landspitali.is/library/Sameiginlegar-myndir/Klinisk-svid-og-deildir/Rannsoknarsvid/Syklafraedideild/RS-, hMP-, rhino-, entero-adeno- og parainflúensu-veiru greiningar 2011-2017.pdf

Desai, A. P., Sharma, D., Crispell, E. K., Baughman, W., Thomas, S., Tunali, A., … Farley, M. M. (2015). Decline in Pneumococcal Nasopharyngeal Carriage of Vaccine Serotypes After the Introduction of the 13-Valent Pneumococcal Conjugate Vaccine in Children in Atlanta, Georgia. *The Pediatric Infectious Disease Journal*. https://doi.org/10.1097/INF.0000000000000849

Devine, V. T., Jefferies, J. M., Clarke, S. C., & Faust, S. N. (2015). Nasopharyngeal bacterial carriage in the conjugate vaccine era with a focus on pneumococci. *Journal of Immunology Research*, *2015*. https://doi.org/10.1155/2015/394368

Dunais, B., Bruno, P., Touboul, P., Degand, N., Sakarovitch, C., Fontas, E., … Pradier, C. (2015). Impact of the 13-valent Pneumococcal Conjugate Vaccine on Nasopharyngeal Carriage of Streptococcus pneumoniae Among Children Attending Group Daycare in Southeastern France. *The Pediatric Infectious Disease Journal*, *34*(3), 286–288. https://doi.org/10.1097/INF.0000000000000559

Durando, P., Crovari, P., Ansaldi, F., Sticchi, L., Sticchi, C., Turello, V., … Icardi, G. (2009). Universal childhood immunisation against Streptococcus pneumoniae: The five-year experience of Liguria Region, Italy. *Vaccine*, *27*(25–26), 3459–3462. https://doi.org/10.1016/j.vaccine.2009.01.052

Durando, P., Crovari, P., Ansaldi, F., Sticchi, L., Sticchi, C., Turello, V., … Vaccination, P. (2009). Universal childhood immunisation against Streptococcus pneumoniae : The five-year experience of Liguria Region , Italy, *27*, 3459–3462. https://doi.org/10.1016/j.vaccine.2009.01.052

Duvvuri, V. R., Granados, A., Rosenfeld, P., Bahl, J., Eshaghi, A., & Gubbay, J. B. (2015). Genetic diversity and evolutionary insights of respiratory syncytial virus A ON1 genotype: Global and local transmission dynamics. *Scientific Reports*, *5*(1), 14268. https://doi.org/10.1038/srep14268

Eddy, B. (1944). Cross reactions between the several pneumococcic types and their significance in the preparation of polyvalent antiserum. *Public Health Reports*, *59*(15), 485–499. https://doi.org/10.2307/4584847

Embætti Landlæknis. (2015). *Paediatric vaccination schedule in Iceland [In Icelandic]*. Reykjavík. Retrieved from https://www.landlaeknir.is/servlet/file/store93/item27464/Almennar_bolusetningar_barna_eftir_september 2015.pdf

Entwisle, C., Joachim, M., Hill, S., McILgorm, A., Pang, Y., Colaco, C., … Bailey, C. (2018). FUNCTIONAL IMMUNE RESPONSES IN ADULT

SUBJECTS FOLLOWING IMMUNISATION WITH A NOVEL MULTI-ANTIGEN PROTEIN VACCINE-REPORT OF A PHASE I CLINICAL TRIAL. In *ISPPD-11*. Melbourne, Australia.

Epstein, F. H., Tuomanen, E. I., Austrian, R., & Masure, H. R. (1995). Pathogenesis of Pneumococcal Infection. *New England Journal of Medicine*, *332*(19), 1280–1284. https://doi.org/10.1056/NEJM199505113321907

Erlendsdóttir, H. (2018). Carriage and invasive disease post pneumococcal vaccination. In *NSCMID-35*. Reykjavík.

Eskola, J., Kilpi, T., Palmu, A. A., Jokinen, J., Eerola, M., Haapakoski, J., … Makela, P. H. (2001). Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med*, *344*(6), 403–409. https://doi.org/10.1056/nejm200102083440602

Esposito, S., Tansey, S., Thompson, A., Razmpour, A., Liang, J., Jones, T. R., … Principi, N. (2010). Safety and Immunogenicity of a 13-Valent Pneumococcal Conjugate Vaccine Compared to Those of a 7-Valent Pneumococcal Conjugate Vaccine Given as a Three-Dose Series with Routine Vaccines in Healthy Infants and Toddlers. *Clinical and Vaccine Immunology*, *17*(6), 1017–1026. https://doi.org/10.1128/CVI.00062-10

Eurostat. (2017). Employment statistics. Retrieved September 23, 2017, from http://ec.europa.eu/eurostat/statistics-explained/index.php/Employment_statistics

Eythorsson, E., Hrafnkelsson, B., Erlendsdóttir, H., Atli Gudmundsson, S., Kristinsson, K. G., & Haraldsson, Á. (2017). Decreased AOM with Treatment Failure Following Introduction of the Ten-Valent Pneumococcal Haemophilus influenzae Protein D Conjugate Vaccine. *The Pediatric Infectious Disease Journal*, *37*(4), 361–366. https://doi.org/10.1097/INF.0000000000001870

Eyþorsson, E., Erlendsdóttir, H., Kristinsson, K. G., Guðnason, Þ., & Haraldsson, Á. (2017). High uptake of pneumococcal conjugate vaccine (PHID-CV10) in the vaccination program in Iceland. In *ESPID*. Madrid. https://doi.org/ESP17-1267

FDA. (n.d.). Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM 197 Protein) Prevnar ® FOR PEDIATRIC USE ONLY. Retrieved from https://www.fda.gov/downloads/biologicsbloodvaccines/vaccines/approvedproducts/ucm137038.pdf

Fireman, B., Black, S. B., Shinefield, H. R., Lee, J., Lewis, E., & Ray, P. (2003). *Impact of the pneumococcal conjugate vaccine on otitis media.* (2003/01/25). *The Pediatric infectious disease journal* (Vol. 22). The Pediatric infectious disease journal. https://doi.org/10.1097/00006454-200301000-00006

Fitzgerald, D. A., & Kilham, H. A. (2004). Bronchiolitis: Assessment and evidence-based management. *Medical Journal of Australia*, *180*(8), 399–404.

Fitzwater, S. P., Chandran, A., Santosham, M., & Johnson, H. L. (2012). The Worldwide Impact of the Seven-valent Pneumococcal Conjugate Vaccine. *The Pediatric Infectious Disease Journal*, *31*(5), 501–508. https://doi.org/10.1097/INF.0b013e31824de9f6

Flasche, S., Van Hoek, A. J., Sheasby, E., Waight, P., Andrews, N., Sheppard, C., … Miller, E. (2011). Effect of pneumococcal conjugate vaccination on serotype-specific carriage and invasive disease in England: a cross-sectional study. *PLoS Medicine*, *8*(4), e1001017. https://doi.org/10.1371/journal.pmed.1001017

Fleming-Dutra, K. E., Hersh, A. L., Shapiro, D. J., Bartoces, M., Enns, E. A., File, T. M., … E, H. (2016). Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011. *JAMA*, *315*(17), 1864–1873. https://doi.org/10.1001/jama.2016.4151

Fletcher-Lartey, S., Yee, M., Gaarslev, C., & Khan, R. (2016). Why do general practitioners prescribe antibiotics for upper respiratory tract infections to meet patient expectations: a mixed methods study. *BMJ Open*, *6*(10), e012244. https://doi.org/10.1136/bmjopen-2016-012244

Fréttablaðið. (2009, September). Ný bóluefni við eyrnabólgu. *365 Miðlar*, p. 16. Retrieved from http://timarit.is/view_page_init.jsp?pageId=4329985

Friðleifsdóttir, S. 805/138 þáltill.: Bólusetningar barna gegn pneumókokkasýkingum | Þingtíðindi | Alþingi (2009). Reykjavík: Ísland. Retrieved from https://www.althingi.is/altext/138/s/0805.html

Gadomski, A. M., & Scribani, M. B. (2014). Bronchodilators for bronchiolitis. *The Cochrane Database of Systematic Reviews*, (6), CD001266. https://doi.org/10.1002/14651858.CD001266.pub4

García, E., & López, R. (1997). Molecular biology of the capsular genes of Streptococcus pneumoniae. *FEMS Microbiology Letters*, *149*(1), 1–10. https://doi.org/10.1016/S0378-1097(97)00026-8

Gefenaite, G., Bijlsma, M. J., Bos, H. J., & Hak, E. (2014). Did introduction of pneumococcal vaccines in the Netherlands decrease the need for respiratory antibiotics in children ? Analysis of 2002 to 2013 data. *Eurosurveillance*, *19*(44), 20948. https://doi.org/10.2807/1560-7917.ES2014.19.44.20948

Geno, K. A., Gilbert, G. L., Song, J. Y., Skovsted, I. C., Klugman, K. P., Jones, C., … Nahm, M. H. (2015). Pneumococcal capsules and their types: Past, present, and future. *Clinical Microbiology Reviews*, *28*(3), 871–899. https://doi.org/10.1128/CMR.00024-15

Ghosh, P., Lee, S., Kim, B., & Rhee, D. K. (2018). KOREAN RED GINSENG

IMPEDES REACTIVE OXYGEN SPECIES PRODUCTION AND IMPROVES PNEUMOCOCCAL ΔPEP27 VACCINE EFFICACY. In *ISPPD-11*. Melbourne, Australia.

Gisselsson-Solen, M. (2017). Trends in Otitis Media Incidence After Conjugate Pneumococcal Vaccination; A National Observational Study. *The Pediatric Infectious Disease Journal*, *36*(11), 1. https://doi.org/10.1097/INF.0000000000001654

GlaxoSmithKline. (2009). Synflorix, GlaxoSmithKline's pneumococcal vaccine receives European authorisation. *GlaxoSmithKline*. Retrieved from http://www-origin.gsk.com/en-gb/media/press-releases/synflorix-glaxosmithkline-s-pneumococcal-vaccine-receives-european-authorisation/

Gounder, P. P., Brewster, M., Bruce, M. G., Bruden, D. J. T., Rudolph, K., Hurlburt, D. A., & Hennessy, T. W. (2015). Impact of the Pneumococcal Conjugate Vaccine and Antibiotic Use on Nasopharyngeal Colonization by Antibiotic Nonsusceptible Streptococcus pneumoniae, Alaska, 2000-2010. *The Pediatric Infectious Disease Journal*, *34*(11), 1223–1229. https://doi.org/10.1097/INF.0000000000000856

Greenberg, D., Bilenko, N., Liss, Z., Shagan, T., Zamir, O., & Dagan, R. (2003). The burden of acute otitis media on the patient and the family. *European Journal of Pediatrics*, *162*(9), 576–581. https://doi.org/10.1007/s00431-003-1260-5

Greenberg, D., Givon-Lavi, N., Newman, N., Bar-Ziv, J., & Dagan, R. (2011). Nasopharyngeal carriage of individual Streptococcus pneumoniae serotypes during pediatric pneumonia as a means to estimate serotype disease potential. *The Pediatric Infectious Disease Journal*, *30*(3), 227–233. https://doi.org/10.1097/INF.0b013e3181f87802

Griffin, M. R., Zhu, Y., Moore, M. R., Whitney, C. G., & Grijalva, C. G. (2013). U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *The New England Journal of Medicine*, *369*(2), 155–163. https://doi.org/10.1056/NEJMoa1209165

Gritzfeld, J. F., Cremers, A. J. H., Ferwerda, G., Ferreira, D. M., Kadioglu, A., Hermans, P. W. M., & Gordon, S. B. (2014). Density and duration of experimental human pneumococcal carriage. *Clinical Microbiology and Infection : The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, *20*(12), O1145-51. https://doi.org/10.1111/1469-0691.12752

Gudnason, T., Hrafnkelsson, B., Laxdal, B., & Kristinsson, K. G. (2012). Can risk factors for infectious illnesses in children at day care centres be identified? *Scandinavian Journal of Infectious Diseases*, *44*(2), 149–156. https://doi.org/10.3109/00365548.2011.599331

Gudnason, T., Hrafnkelsson, B., Laxdal, B., & Kristinsson, K. G. (2013). Does

hygiene intervention at day care centres reduce infectious illnesses in children? An intervention cohort study. *Scandinavian Journal of Infectious Diseases*, *45*(5), 397–403. https://doi.org/10.3109/00365548.2012.749424

Gudnason, T., Hrafnkelsson, B., Laxdal, B., & Kristinsson, K. G. (2014). Risk factors for nasopharyngeal carriage of Streptococcus pneumoniae and effects of a hygiene intervention: repeated cross-sectional cohort study at day care centres. *Scandinavian Journal of Infectious Diseases*, *46*(7), 493–501. https://doi.org/10.3109/00365548.2014.901553

Hall, A. J., Maw, R., Midgley, E., Golding, J., & Steer, C. (2014). Glue Ear, Hearing Loss and IQ: An Association Moderated by the Child's Home Environment. *PLoS ONE*, *9*(2), e87021. https://doi.org/10.1371/journal.pone.0087021

Hamaluba, M., Kandasamy, R., Ndimah, S., Morton, R., Caccamo, M., Robinson, H., … Pollard, A. J. (2015). A Cross-Sectional Observational Study of Pneumococcal Carriage in Children, Their Parents, and Older Adults Following the Introduction of the 7-Valent Pneumococcal Conjugate Vaccine. *Medicine*, *94*(1), e335. https://doi.org/10.1097/MD.0000000000000335

Hammitt, L. L., Campbell, J. C., Borys, D., Weatherholtz, R. C., Reid, R., Goklish, N., … O'Brien, K. L. (2018). RELATIONSHIP BETWEEN PRE-VACCINATION ANTIBODY LEVELS, POST-IMMUNIZATION IMMUNE RESPONSE AND VACCINE EFFICACY OF AN INVESTIGATIONAL PNEUMOCOCCAL PROTEIN-BASED VACCINE IN A PHASE 2 RANDOMIZED CLINICAL TRIAL. In *ISPPD*. Melbourne, Australia. Retrieved from https://web.kenes.com/klead/ISPPD18AbstractCD/ISPPD2018/data/HtmlApp/main.html#

Hanage, W. P., Auranen, K., Syrjänen, R., Herva, E., Mäkelä, P. H., Kilpi, T., & Spratt, B. G. (2004). Ability of pneumococcal serotypes and clones to cause acute otitis media: implications for the prevention of otitis media by conjugate vaccines. *Infection and Immunity*, *72*(1), 76–81. https://doi.org/10.1128/IAI.72.1.76

Hanage, W. P., Kaijalainen, T. H., Syrjänen, R. K., Auranen, K., Leinonen, M., Mäkelä, P. H., & Spratt, B. G. (2005). Invasiveness of serotypes and clones of Streptococcus pneumoniae among children in Finland. *Infection and Immunity*, *73*(1), 431–435. https://doi.org/10.1128/IAI.73.1.431-435.2005

Hanley, J. A., & Lippman-Hand, A. (1983). If nothing goes wrong, is everything all right? Interpreting zero numerators. *JAMA*, *249*(13), 1743–1745. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6827763

Hansen, J., Black, S., Shinefield, H., Cherian, T., Benson, J., Fireman, B., …

Lee, J. (2006). Effectiveness of Heptavalent Pneumococcal Conjugate Vaccine in Children Younger Than 5 Years of Age for Prevention of Pneumonia. *The Pediatric Infectious Disease Journal*, *25*(9), 779–781. https://doi.org/10.1097/01.inf.0000232706.35674.2f

Harmes, K. M., Blackwood, R. A., Burrows, H. L., Cooke, J. M., Van Harrison, R., & Passamani, P. P. (2013). Otitis media: Diagnosis and treatment. *American Family Physician*, *88*(7), 435–440. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24134083

Hartling, L., Fernandes, R. M., Bialy, L., Milne, A., Johnson, D., Plint, A., … Vandermeer, B. (2011). Steroids and bronchodilators for acute bronchiolitis in the first two years of life: systematic review and meta-analysis. *BMJ*, *342*(apr06 2), d1714–d1714. https://doi.org/10.1136/bmj.d1714

Hava, D. L., & Camilli, A. (2002). Large-scale identification of serotype 4 Streptococcus pneumoniae virulence factors. *Molecular Microbiology*, *45*(5), 1389–1406. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12207705

Heikkilä, P., Renko, M., & Korppi, M. (2018). Hypertonic saline inhalations in bronchiolitis-A cumulative meta-analysis. *Pediatric Pulmonology*, *53*(2), 233–242. https://doi.org/10.1002/ppul.23928

Heikkinen, T, & Chonmaitree, T. (2003). Importance of respiratory viruses in acute otitis media. *Clin Microbiol Rev*, *16*(2), 230–241.

Heikkinen, Terho, & Järvinen, A. (2003). The common cold. *The Lancet*, *361*(9351), 51–59. https://doi.org/10.1016/S0140-6736(03)12162-9

Heiskanen-Kosma, T., Korppi, M., & Leinonen, M. (2003). Serologically indicated pneumococcal pneumonia in children: a population-based study in primary care settings. *APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *111*(10), 945–950. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14616546

Henrichsen, J. (1999). Typing of Streptococcus pneumoniae: past, present, and future. *The American Journal of Medicine*, *107*(1), 50–54. https://doi.org/10.1016/S0002-9343(99)00100-X

Hill, P. C., Townend, J., Antonio, M., Akisanya, B., Ebruke, C., Lahai, G., … Adegbola, R. A. (2010). Transmission of Streptococcus pneumoniae in Rural Gambian Villages: A Longitudinal Study. *Clinical Infectious Diseases*, *50*(11), 1468–1476. https://doi.org/10.1086/652443

Hjálmarsdóttir, M. Á., Kristinsson, K. G., Hjalmarsdottir, M. A., Kristinsson, K. G., Hjálmarsdóttir, M. Á., & Kristinsson, K. G. (2014). Epidemiology of penicillin-non-susceptible pneumococci in Iceland, 1995-2010. *Journal of Antimicrobial Chemotherapy*, *69*(4), 940–946. https://doi.org/10.1093/jac/dkt470

Hjálmarsdóttir, M. Á., Quirk, S. J., Haraldsson, G., Erlendsdóttir, H., Haraldsson, Á., & Kristinsson, K. G. (2017). Comparison of Serotype Prevalence of Pneumococci Isolated from Middle Ear, Lower Respiratory Tract and Invasive Disease Prior to Vaccination in Iceland. *PLOS ONE*, *12*(1), e0169210. https://doi.org/10.1371/journal.pone.0169210

Hjaltested, E. K. R., Bernatoniene, J., Erlendsdottir, H., Kaltenis, P., Bernatoniene, G., Gudnason, T., … Kristinsson, K. G. (2003). Resistance in Respiratory Tract Pathogens and Antimicrobial Use in Icelandic and Lithuanian Children. *Scand J Infect Dis*, *35*, 21–26. https://doi.org/10.1080/0036554021000026996

Högberg, L., Geli, P., Ringberg, H., Melander, E., Lipsitch, M., & Ekdahl, K. (2007). Age- and serogroup-related differences in observed durations of nasopharyngeal carriage of penicillin-resistant pneumococci. *Journal of Clinical Microbiology*, *45*(3), 948–952. https://doi.org/10.1128/JCM.01913-06

Holl, K., Rosenlund, M., Giaquinto, C., Silfverdal, S.-A., Carmona, A., Larcombe, J., … Liese, J. G. (2015). The Impact of Childhood Acute Otitis Media on Parental Quality of Life in a Prospective Observational Cohort Study. *Clinical Drug Investigation*, *35*(10), 613–624. https://doi.org/10.1007/s40261-015-0319-1

Howie, S. R. C., Morris, G. A. J., Tokarz, R., Ebruke, B. E., Machuka, E. M., Ideh, R. C., … Adegbola, R. A. (2014). Etiology of Severe Childhood Pneumonia in The Gambia, West Africa, Determined by Conventional and Molecular Microbiological Analyses of Lung and Pleural Aspirate Samples. *Clinical Infectious Diseases*, *59*(5), 682–685. https://doi.org/10.1093/cid/ciu384

Howitz, M. F., Harboe, Z. B., Ingels, H., Valentiner-Branth, P., Mølbak, K., & Djurhuus, B. D. (2017). A nationwide study on the impact of pneumococcal conjugate vaccination on antibiotic use and ventilation tube insertion in Denmark 2000-2014. *Vaccine*, *35*(43), 5858–5863. https://doi.org/10.1016/j.vaccine.2017.09.006

Huang, S. S., Finkelstein, J. A., Rifas-Shiman, S. L., Kleinman, K., & Platt, R. (2004). Community-Level Predictors of Pneumococcal Carriage and Resistance in Young Children. *American Journal of Epidemiology*. https://doi.org/10.1093/aje/kwh088

Hussain, M., Melegaro, A., Pebody, R. G., George, R., & Edmunds, W. J. (2005). A longitudinal household study of Streptococcus pneumoniae nasopharyngeal carriage in a UK setting. *Epidemiology and Infection*, 891–898.

Hyams, C., Camberlein, E., Cohen, J. M., Bax, K., & Brown, J. S. (2010). The Streptococcus pneumoniae capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. *Infection and Immunity*,

78(2), 704–715. https://doi.org/10.1128/IAI.00881-09

Icelandic Meteorological office. (2012). Climatological data. Retrieved September 2, 2018, from http://en.vedur.is/climatology/data/

Jakobsen, H., Saeland, E., Gizurarson, S., Schulz, D., & Jónsdóttir, I. (1999). Intranasal immunization with pneumococcal polysaccharide conjugate vaccines protects mice against invasive pneumococcal infections. *Infection and Immunity*, *67*(8), 4128–4133. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10417183

Jauneikaite, E., Tocheva, A. S., Jefferies, J. M. C., Gladstone, R. A., Faust, S. N., Christodoulides, M., … Clarke, S. C. (2015, June). Current methods for capsular typing of Streptococcus pneumoniae. *Journal of Microbiological Methods*. https://doi.org/10.1016/j.mimet.2015.03.006

Javouhey, E., Barats, A., Richard, N., Stamm, D., & Floret, D. (2008). Non-invasive ventilation as primary ventilatory support for infants with severe bronchiolitis. *Intensive Care Medicine*, *34*(9), 1608–1614. https://doi.org/10.1007/s00134-008-1150-4

Jefferies, J. M., Macdonald, E., Faust, S. N., & Clarke, S. C. (2011). 13-valent pneumococcal conjugate vaccine (PCV13). *Human Vaccines*, *7*(10), 1012–1018. https://doi.org/10.4161/hv.7.10.16794

Jin, P., Xiao, M., Kong, F., Oftadeh, S., Zhou, F., Liu, C., & Gilbert, G. L. (2009). Simple, accurate, serotype-specific PCR assay to differentiate Streptococcus pneumoniae serotypes 6A, 6B, and 6C. *Journal of Clinical Microbiology*, *47*(8), 2470–2474. https://doi.org/10.1128/JCM.00484-09

Johnson, R. T. (1996). Acute Community-Acquired Sinusitis. *Clinical Infectious Diseases*, *23*(September), 219–226.

Jokinen, J., Rinta-Kokko, H., Siira, L., Palmu, A. A., Virtanen, M. J., Nohynek, H., … Nuorti, J. P. (2015). Impact of ten-valent pneumococcal conjugate vaccination on invasive pneumococcal disease in Finnish children - A population-based study. *PLoS ONE*, *10*(3), e0120290. https://doi.org/10.1371/journal.pone.0120290

Jonnalagadda, S., Rodríguez, O., Estrella, B., Sabin, L. L., Sempértegui, F., & Hamer, D. H. (2017). Etiology of severe pneumonia in Ecuadorian children. *PLoS ONE*, *12*(2), e0171687. https://doi.org/10.1371/journal.pone.0171687

Kadioglu, A., Weiser, J. N., Paton, J. C., & Andrew, P. W. (2008). The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. *Nature Reviews Microbiology*, *6*(4), 288–301. https://doi.org/10.1038/nrmicro1871

Kalin, M. (1998). Pneumococcal serotypes and their clinical relevance. *Thorax*, *53*(3), 159–162. https://doi.org/10.1136/THX.53.3.159

Kalin, M., & Kristinsson, K. G. (1999). Drug resistance in Streptococcus pneumoniae: Epidemiology and control. In R. G. Finch & R. J. Williams (Eds.), *Bailliere's Clinical Infectious Diseases* (Vol. 5, pp. 203–219). Baillière Tindall.

Kaur, R., Czup, K., Casey, J. R., & Pichichero, M. E. (2014). Correlation of nasopharyngeal cultures prior to and at onset of acute otitis media with middle ear fluid cultures. *BMC Infectious Diseases*, *14*(1), 640. https://doi.org/10.1186/s12879-014-0640-y

Kaur, R., Morris, M., & Pichichero, M. E. (2017). Epidemiology of Acute Otitis Media in the Postpneumococcal Conjugate Vaccine Era. *Pediatrics*, *140*(3), e20170181. https://doi.org/10.1542/peds.2017-0181

Kawaguchiya, M., Urushibara, N., & Kobayashi, N. (2017). Multidrug Resistance in Non-PCV13 Serotypes of *Streptococcus pneumoniae* in Northern Japan, 2014. *Microbial Drug Resistance*, *23*(2), 206–214. https://doi.org/10.1089/mdr.2016.0054

Keith, E. R., Podmore, R. G., Anderson, T. P., & Murdoch, D. R. (2006). Characteristics of Streptococcus pseudopneumoniae isolated from purulent sputum samples. *Journal of Clinical Microbiology*, *44*(3), 923–927. https://doi.org/10.1128/JCM.44.3.923-927.2006

Kieninger, D. M., Kueper, K., Steul, K., Juergens, C., Ahlers, N., Baker, S., … Scott, D. A. (2010). Safety, tolerability, and immunologic noninferiority of a 13-valent pneumococcal conjugate vaccine compared to a 7-valent pneumococcal conjugate vaccine given with routine pediatric vaccinations in Germany. *Vaccine*, *28*(25), 4192–4203. https://doi.org/10.1016/J.VACCINE.2010.04.008

Kilian, M., Poulsen, K., Blomqvist, T., Håvarstein, L. S., Bek-Thomsen, M., Tettelin, H., & Sørensen, U. B. S. (2008). Evolution of Streptococcus pneumoniae and Its Close Commensal Relatives. *PLoS ONE*, *3*(7), e2683. https://doi.org/10.1371/journal.pone.0002683

Kilpi, T. M., Jokinen, J., Puumalainen, T., Nieminen, H., Ruokokoski, E., Rinta-Kokko, H., … Palmu, A. A. (2018). Effectiveness of pneumococcal Haemophilus influenzae protein D conjugate vaccine against pneumonia in children: A cluster-randomised trial. *Vaccine*. https://doi.org/10.1016/j.vaccine.2018.08.020

Klebs, E. (1875). Beiträge zur Kenntniss der pathogenen Schistomyceten. VII Die Monadinen [Signs for Recognition of the Pathogen Schistomyceten]. *Arch. Exptl. Pathol. Parmakol.*, *4*(5/6), 408–488. https://doi.org/10.1007/BF01976922

Klugman, K. P., Madhi, S. A., Huebner, R. E., Kohberger, R., Mbelle, N., & Pierce, N. (2003). A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *The New England Journal of Medicine*, *349*(14), 1341–1348.

https://doi.org/10.1056/NEJMoa035060

Knol, M. J., Wagenvoort, G. H. J. J., Sanders, E. A. M. M., Elberse, K., Vlaminckx, B. J., de Melker, H. E., & van der Ende, A. (2015). Invasive pneumococcal disease 3 years after introduction of 10-valent pneumococcal conjugate vaccine, the Netherlands. *Emerging Infectious Diseases*, *21*(11), 2040–2044. https://doi.org/10.3201/eid2111.140780

Koch, R. (1881). Mittheilungen aus dem Kaiserlichen Gesundheitsamte, *1*(1), 1:48.

Köndgen, S., Calvignac-Spencer, S., Grützmacher, K., Keil, V., Mätz-Rensing, K., Nowak, K., … Leendertz, F. H. (2017). Evidence for Human Streptococcus pneumoniae in wild and captive chimpanzees: A potential threat to wild populations. *Scientific Reports*, *7*(1), 14581. https://doi.org/10.1038/s41598-017-14769-z

Kristinsson, K. G. (1995). Epidemiology of penicillin resistant pneumococci in Iceland. *Microbial Drug Resistance*, *1*(2), 121–125. https://doi.org/10.1089/mdr.1995.1.121

Kristinsson, K. G. (1997). Effect of Antimicrobial Use and Other Risk Factors on Antimicrobial Resistance in Pneumococci. *Microbial Drug Resistance*, *3*(2), 117–123. https://doi.org/10.1089/mdr.1997.3.117

Kristinsson, K. G. (1999). Modification of prescribers ' behavior: the Icelandic approach. *Clin Microbiol Infect*, *5*(4), 43–47. https://doi.org/10.1111/j.1469-0691.1999.tb00856.x

Kronenberg, A., Zucs, P., Droz, S., & Mühlemann, K. (2006). Distribution and Invasiveness of Streptococcus pneumoniae Serotypes in Switzerland, a Country with Low Antibiotic Selection Pressure, from 2001 to 2004. *JOURNAL OF CLINICAL MICROBIOLOGY*, *44*(6), 2032–2038. https://doi.org/10.1128/JCM.00275-06

Kronman, M. P., Zhou, C., & Mangione-Smith, R. (2014). Bacterial Prevalence and Antimicrobial Prescribing Trends for Acute Respiratory Tract Infections. *PEDIATRICS*, *134*(4), e956–e965. https://doi.org/10.1542/peds.2014-0605

Kumar, P. J., & Clark, M. L. (2012). *Kumar and Clark's clinical medicine*. Saunders/Elsevier.

Lai, S. H., Liao, S. L., Wong, K. S., & Lin, T. Y. (2016). Preceding human metapneumovirus infection increases adherence of Streptococcus pneumoniae and severity of murine pneumococcal pneumonia. *Journal of Microbiology, Immunology and Infection*. https://doi.org/10.1016/j.jmii.2014.04.008

Landlæknisembættið. (1997). *Leiðbeiningar um notkun ICD-10*. Reykjavík. Retrieved from http://www.who.org/whosis/icd10/icd10.htm.

Landlæknisembættið. (2009). Klíniskar leiðbeiningar um meðferð bráðrar miðeyrnabólgu. Retrieved September 23, 2017, from https://www.landlaeknir.is/servlet/file/store93/item2547/4241.pdf

Landlæknisembættið. (2017). *Ráðleggingar um meðferð algengra sýkinga utan spítala*. Reykjavík. Retrieved from https://www.heilsugaeslan.is/library/Files/Faglegar-leidbeiningarnar/Meðferð algengra sýkinga 2017.pdf

Lau, W. C. Y., Murray, M., El-Turki, A., Saxena, S., Ladhani, S., Long, P., … Hsia, Y. (2015). Impact of pneumococcal conjugate vaccines on childhood otitis media in the United Kingdom. *Vaccine*, *33*(39), 5072–5079. https://doi.org/10.1016/j.vaccine.2015.08.022

Leach, A. J., Boswell, J. B., Asche, V., Nienhuys, T. G., & Mathews, J. D. (1994). Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian aboriginal infants. *The Pediatric Infectious Disease Journal*, *13*(11), 983–989. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7845752

Lee, C. R., Cho, I. H., Jeong, B. C., & Lee, S. H. (2013, September 12). Strategies to minimize antibiotic resistance. *International Journal of Environmental Research and Public Health*. Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/ijerph10094274

Lee, G. E., Lorch, S. A., Sheffler-Collins, S., Kronman, M. P., & Shah, S. S. (2010). National hospitalization trends for pediatric pneumonia and associated complications. *Pediatrics*, *126*(2), 204–213. https://doi.org/10.1542/peds.2009-3109

Lee, H., Choi, E. H., & Lee, H. J. (2014). Efficacy and effectiveness of extended-valency pneumococcal conjugate vaccines. *Korean Journal of Pediatrics*, *57*(2), 55–66. https://doi.org/10.3345/kjp.2014.57.2.55

Leibovitz, E., Jacobs, M. R., & Dagan, R. (2004). Haemophilus influenzae: a significant pathogen in acute otitis media. *The Pediatric Infectious Disease Journal*, *23*(12), 1142–1152. https://doi.org/10.1097/01.inf.0000148233.57296.90

Leite, L., Goulart, C., Rodriguez, D., & Kanno, A. (2018). RAPID PNEUMOCOCCAL CLEARANCE FOLLOWING CELLULAR AND HUMORAL IMMUNE RESPONSES INDUCED IN THE BRONCHOALVEOLAR FLUIDS BY RECOMBINANT BCG PSPA-PDT PRIME / PROTEIN BOOST. In *ISPPD-11*. Melbourne, Australia.

Liese, J. G., Silfverdal, S. A., Giaquinto, C., Carmona, A., Larcombe, J. H., Garcia-Sicilia, J., … Rosenlund, M. R. (2014). Incidence and clinical presentation of acute otitis media in children aged <6 years in European medical practices. *Epidemiology and Infection*, *142*(8), 1778–1788. https://doi.org/10.1017/S0950268813002744

Liñares, J., Ardanuy, C., Pallares, R., & Fenoll, A. (2010, May 1). Changes in antimicrobial resistance, serotypes and genotypes in Streptococcus pneumoniae over a 30-year period. *Clinical Microbiology and Infection*. Elsevier. https://doi.org/10.1111/j.1469-0691.2010.03182.x

Llull, D., Lopez, R., & Garcia, E. (2001). Genetic Bases and Medical Relevance of Capsular Polysaccharide Biosynthesis in Pathogenic Streptococci. *Current Molecular Medicine*, *1*(4), 475–491. https://doi.org/10.2174/1566524013363618

Loo, J. D., Conklin, L., Fleming-Dutra, K. E., Knoll, M. D., Park, D. E., Kirk, J., … Whitney, C. G. (2014). Systematic review of the indirect effect of pneumococcal conjugate vaccine dosing schedules on pneumococcal disease and colonization. *The Pediatric Infectious Disease Journal*, *33 Suppl 2*(1), S161-71. https://doi.org/10.1097/INF.0000000000000084

Lorente, M. L. L., Falguera, M., Nogués, A., González, A. R., Merino, M. T., & Caballero, M. R. (2000). Diagnosis of pneumococcal pneumonia by polymerase chain reaction (PCR) in whole blood: a prospective clinical study. *Thorax*, *55*(2), 133 LP – 137. Retrieved from http://thorax.bmj.com/content/55/2/133.abstract

Lucas, P. J., Cabral, C., Hay, A. D., & Horwood, J. (2015). A systematic review of parent and clinician views and perceptions that influence prescribing decisions in relation to acute childhood infections in primary care. *Scandinavian Journal of Primary Health Care*, *33*(1), 11–20. https://doi.org/10.3109/02813432.2015.1001942

Lucero, M. G., Nohynek, H., Williams, G., Tallo, V., Simões, E. A. F., Lupisan, S., … Mäkelä, H. P. (2009). Efficacy of an 11-valent pneumococcal conjugate vaccine against radiologically confirmed pneumonia among children less than 2 years of age in the Philippines: A randomized, double-blind, placebo-controlled trial. *Pediatric Infectious Disease Journal*, *28*(6), 455–462. https://doi.org/10.1097/INF.0b013e31819637af

Macintyre, E., Karr, C., Koehoorn, M., Demers, P., Tamburic, L., Lencar, C., & Brauer, M. (2010). Otitis media incidence and risk factors in a population-based birth cohort. *Paediatrics and Child Health*, *15*(7), 437–442.

MacLeod, C. M. (1945). PREVENTION OF PNEUMOCOCCAL PNEUMONIA BY IMMUNIZATION WITH SPECIFIC CAPSULAR POLYSACCHARIDES. *Journal of Experimental Medicine*, *82*(6), 445–465. https://doi.org/10.1084/jem.82.6.445

Magnus, M. C., Vestrheim, D. F., Nystad, W., Håberg, S. E., Stigum, H., London, S. J., … Nafstad, P. (2012). Decline in early childhood respiratory tract infections in the Norwegian mother and child cohort study after introduction of pneumococcal conjugate vaccination. *The Pediatric Infectious Disease Journal*, *31*(9), 951–955. https://doi.org/10.1097/INF.0b013e31825d2f76

Mann, B., Rowe, H., Iverson, A., Poole, A., Tuomanen, E., & Rosch, J. (2018). PCV13 COMBINED WITH PNEUMOCOCCAL FUSION PROTEIN YLN ELICITS MULTI-SPECIES PROTECTION IN AN ACUTE OTITIS MEDIA MOUSE MODEL. In *ISPPD-11*.

Mansbach, J. M., McAdam, A. J., Clark, S., Hain, P. D., Flood, R. G., Acholonu, U., & Camargo, C. A. (2008). Prospective multicenter study of the viral etiology of bronchiolitis in the emergency department. *Academic Emergency Medicine : Official Journal of the Society for Academic Emergency Medicine*, *15*(2), 111–118. https://doi.org/10.1111/j.1553-2712.2007.00034.x

Marchisio, P., Cantarutti, L., Sturkenboom, M., Girotto, S., Picelli, G., Dona, D., … Pedianet. (2012). Burden of acute otitis media in primary care pediatrics in Italy: a secondary data analysis from the Pedianet database. *BMC Pediatrics*, *12*(1), 185. https://doi.org/10.1186/1471-2431-12-185

Marom, T., Israel, O., Gavriel, H., Pitaro, J., Baker, A. A., & Eviatar, E. (2017). Comparison of first year of life acute otitis media admissions before and after the 13-valent pneumococcal conjugate vaccine. *International Journal of Pediatric Otorhinolaryngology*, *97*, 251–256. https://doi.org/10.1016/j.ijporl.2017.04.023

Marom, T., Tan, A., Wilkinson, G. S., Pierson, K. S., Freeman, J. L., & Chonmaitree, T. (2014). Trends in otitis media-related health care use in the United States, 2001-2011. *JAMA Pediatrics*, *168*(1), 68–75. https://doi.org/10.1001/jamapediatrics.2013.3924

McCracken, G. H. (2000). Diagnosis and management of pneumonia in children. *The Pediatric Infectious Disease Journal*, *19*(9), 924–928. https://doi.org/10.1097/00006454-200009000-00036

McKinsey & Company. (2016). *Lykill að fullnýtingu tækifæra Landspítalans*. Reykjavík. Retrieved from https://www.stjornarradid.is/media/velferdarraduneyti-media/media/skyrslur2016/Lykill-ad-fullnytingu-taekifaera-Landspitalans.pdf

Mehr, S., & Wood, N. (2012). Streptococcus pneumoniae - a review of carriage, infection, serotype replacement and vaccination. *Paediatric Respiratory Reviews*. https://doi.org/10.1016/j.prrv.2011.12.001

Melegaro, A., Choi, Y., Pebody, R., & Gay, N. (2007). Pneumococcal Carriage in United Kingdom Families: Estimating Serotype-specific Transmission Parameters from Longitudinal Data. *American Journal of Epidemiology*, *166*(2), 228–235. https://doi.org/10.1093/aje/kwm076

Messmer, T. O., Sampson, J. S., Stinson, A., Wong, B., Carlone, G. M., & Facklam, R. R. (2004). Comparison of four polymerase chain reaction assays for specificity in the identification of Streptococcus pneumoniae. *Diagnostic Microbiology and Infectious Disease*, *49*(4), 249–254.

https://doi.org/10.1016/j.diagmicrobio.2004.04.013

Metlay, J. P., Kapoor, W. N., & Fine, M. J. (1997). Does This Patient Have Community-Acquired Pneumonia? *JAMA*, *278*(17), 1440. https://doi.org/10.1001/jama.1997.03550170070035

Midulla, F., Scagnolari, C., Bonci, E., Pierangeli, A., Antonelli, G., De Angelis, D., … Moretti, C. (2010). Respiratory syncytial virus, human bocavirus and rhinovirus bronchiolitis in infants. *Archives of Disease in Childhood*, *95*(1), 35–41. https://doi.org/10.1136/adc.2008.153361

Mika, M., Maurer, J., Korten, I., Allemann, A., Aebi, S., Brugger, S. D., … Hilty, M. (2017). Influence of the pneumococcal conjugate vaccines on the temporal variation of pneumococcal carriage and the nasal microbiota in healthy infants: a longitudinal analysis of a case–control study. *Microbiome*, *5*(1), 85. https://doi.org/10.1186/s40168-017-0302-6

Millar, E. V., O'Brien, K. L., Zell, E. R., Bronsdon, M. A., Reid, R., & Santosham, M. (2009). Nasopharyngeal Carriage of Streptococcus pneumoniae in Navajo and White Mountain Apache Children Before the Introduction of Pneumococcal Conjugate Vaccine. *The Pediatric Infectious Disease Journal*, *28*(8), 711–716. https://doi.org/10.1097/INF.0b013e3181a06303

Monasta, L., Ronfani, L., Marchetti, F., Montico, M., Vecchi Brumatti, L., Bavcar, A., … Tamburlini, G. (2012). Burden of disease caused by otitis media: Systematic review and global estimates. *PLoS ONE*, *7*(4), e36226. https://doi.org/10.1371/journal.pone.0036226

Morgunblaðið. (2009, September). Hægt að bólusetja gegn lungnabólgu og eyrnabólgu. *Árvakur*. Retrieved from https://www.mbl.is/frettir/innlent/2009/09/22/haegt_ad_bolusetja_gegn_lungnabolgu_og_eyrnabolgu/

Morona, J. K., Miller, D. C., Morona, R., & Paton, J. C. (2004). The effect that mutations in the conserved capsular polysaccharide biosynthesis genes cpsA, cpsB, and cpsD have on virulence of Streptococcus pneumoniae. *The Journal of Infectious Diseases*, *189*(10), 1905–1913. https://doi.org/10.1086/383352

Morrison, K. E., Lake, D., Crook, J., Carlone, G. M., Ades, E., Facklam, R., & Sampson, J. S. (2000). Confirmation of psaA in all 90 serotypes of Streptococcus pneumoniae by PCR and potential of this assay for identification and diagnosis. *Journal of Clinical Microbiology*, *38*(1).

Mortensen, M., Nielsen, R. B., Fisker, N., & Nørgaard, M. (2013). Hospitalisation with otitis media in early childhood and cognitive function in young adult life: a prevalence study among Danish conscripts. *BMC Pediatrics*, *13*(1), 8. https://doi.org/10.1186/1471-2431-13-8

Mosser, J. F., Grant, L. R., Millar, E. V., Weatherholtz, R. C., Jackson, D. M.,

Beall, B., … O'Brien, K. L. (2014). Nasopharyngeal carriage and transmission of Streptococcus pneumoniae in American Indian households after a decade of pneumococcal conjugate vaccine use. *PLoS ONE*, *9*(1), 3–10. https://doi.org/10.1371/journal.pone.0079578

Murdoch, D. R., Anderson, T. P., Beynon, K. A., Chua, A., Fleming, A. M., Laing, R. T. R., … Jennings, L. C. (2003). Evaluation of a PCR assay for detection of Streptococcus pneumoniae in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. *Journal of Clinical Microbiology*, *41*(1), 63–66. https://doi.org/10.1128/jcm.41.1.63-66.2003

Myint, T. T. H., Christopoulou, D., & Schmitt, H. J. (2015). Pneumococcal serotype evolution in Western Europe. *BMC Infectious Diseases*, *15*(1). https://doi.org/10.1186/s12879-015-1147-x

Nair, H., Watts, A. T., Williams, L. J., Omer, S. B., Simpson, C. R., Willocks, L. J., … Campbell, H. (2016). Pneumonia hospitalisations in Scotland following the introduction of pneumococcal conjugate vaccination in young children. *BMC Infectious Diseases*, *16*(1), 390. https://doi.org/10.1186/s12879-016-1693-x

Naucler, P., Galanis, I., Morfeldt, E., Darenberg, J., Örtqvist, Å., & Henriques-Normark, B. (2017). Comparison of the Impact of Pneumococcal Conjugate Vaccine 10 or Pneumococcal Conjugate Vaccine 13 on Invasive Pneumococcal Disease in Equivalent Populations. *Clinical Infectious Diseases*, *65*(11), 1780–1789. https://doi.org/10.1093/cid/cix685

Navarro, D., García-Maset, L., Gimeno, C., Escribano, A., & García-De-Lomas, J. (2004). Performance of the binax NOW Streptococcus pneumoniae urinary antigen assay for diagnosis of pneumonia in children with underlying pulmonary diseases in the absence of acute pneumococcal infection. *Journal of Clinical Microbiology*, *42*(10), 4853–4855. https://doi.org/10.1128/JCM.42.10.4853-4855.2004

Nelson, A. L., Roche, A. M., Gould, J. M., Chim, K., Ratner, A. J., & Weiser, J. N. (2007). Capsule enhances pneumococcal colonization by limiting mucus-mediated clearance. *Infection and Immunity*, *75*(1), 83–90. https://doi.org/10.1128/IAI.01475-06

Neufeld, F., & Rimpau, W. (1904). Ueber die Antikorper des Streptokokken- und Pneumokokken-Immunserums. *Dtsch. Med. Wochenschr.*, (30), 1458–1460.

Neves, F. P. G., Cardoso, N. T., Snyder, R. E., Marlow, M. A., Cardoso, C. A. A., Teixeira, L. M., & Riley, L. W. (2017). Pneumococcal carriage among children after four years of routine 10-valent pneumococcal conjugate vaccine use in Brazil: The emergence of multidrug resistant serotype 6C. *Vaccine*, *35*(21), 2794–2800.

https://doi.org/10.1016/j.vaccine.2017.04.019

Ngo, C. C., Massa, H. M., Thornton, R. B., & Cripps, A. W. (2016). Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media: A Systematic Review. *PloS One*, *11*(3), e0150949. https://doi.org/10.1371/journal.pone.0150949

NHS, & (UK), C. for C. P. at N. (2008). Respiratory Tract Infections - Antibiotic Prescribing. *NICE Clinical Guidelines*, *69*(July), 1–240. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21698847

Niemelä, M., Uhari, M., Möttönen, M., & Pokka, T. (1999). Costs arising from otitis media. *Acta Paediatrica, International Journal of Paediatrics*, *88*(5), 553–556. https://doi.org/10.1080/08035259950169585

O'Brien, K. L., David, A. B., Chandran, A., Moulton, L. H., Reid, R., Weatherholtz, R., & Santosham, M. (2008). Randomized, controlled trial efficacy of pneumococcal conjugate vaccine against otitis media among Navajo and White Mountain Apache infants. *Pediatr Infect Dis J*, *27*(1), 71–73. https://doi.org/10.1097/INF.0b013e318159228f

O'Brien, K. L., Moulton, L. H., Reid, R., Weatherholtz, R., Oski, J., Brown, L., … Santosham, M. (2003). Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet*, *362*(9381), 355–361. https://doi.org/10.1016/S0140-6736(03)14022-6

O'Brien, K. L., Wolfson, L. J., Watt, J. P., Henkle, E., Deloria-Knoll, M., McCall, N., … Hib and Pneumococcal Global Burden of Disease Study Team. (2009). Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. *Lancet (London, England)*, *374*(9693), 893–902. https://doi.org/10.1016/S0140-6736(09)61204-6

O'brien, T. F. (1997). *The Global Epidemic Nature of Antimicrobial Resistance and the Need to Monitor and Manage It Locally*. *Clinical Infectious Diseases* (Vol. 24). Retrieved from https://academic.oup.com/cid/article-abstract/24/Supplement_1/S2/283543

O'Connor, G., Tariq, M., Greally, P., & Elnazir, B. (2013). The changing epidemiology of the bronchiolitis epidemic in Tallaght hospital. *Irish Medical Journal*, *106*(10), 11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24579414

Oliveira, M. L. S., Monedero, V., Miyaji, E. N., Leite, L. C. C., Ho, P. L., & Pérez-Martínez, G. (2003). Expression of Streptococcus pneumoniae antigens, PsaA (pneumococcal surface antigen A) and PspA (pneumococcal surface protein A) by Lactobacillus casei. *FEMS Microbiology Letters*, *227*(1), 25–31. https://doi.org/10.1016/S0378-1097(03)00645-1

Organization for Economic Cooperation and Development. (2016). OECD

family database: Enrolment in childcare and pre-school, 8. Retrieved from https://www.oecd.org/els/soc/PF3_2_Enrolment_childcare_preschool.pdf

Otieno, J. R., Kamau, E. M., Agoti, C. N., Lewa, C., Otieno, G., Bett, A., … Nokes, D. J. (2017). Spread and Evolution of Respiratory Syncytial Virus A Genotype ON1, Coastal Kenya, 2010–2015. *Emerging Infectious Diseases*, *23*(2), 264–271. https://doi.org/10.3201/eid2302.161149

Pai, R., Gertz, R. E., & Beall, B. (2006). Sequential Multiplex PCR Approach for Determining Capsular Serotypes of Streptococcus pneumoniae Isolates. *Journal of Clinical Microbiology*, *44*(1), 124–131. https://doi.org/10.1128/JCM.44.1.124-131.2006

Palmu, A. A., Herva, E., Savolainen, H., Karma, P., Makela, P. H., & Kilpi, T. M. (2004). Association of Clinical Signs and Symptoms with Bacterial Findings in Acute Otitis Media. *Clinical Infectious Diseases*, *38*(2), 234–242. https://doi.org/10.1086/380642

Palmu, A. A., Rinta-Kokko, H., Nohynek, H., Nuorti, J. P., Kilpi, T. M., & Jokinen, J. (2017). Impact of ten-valent pneumococcal conjugate vaccine on pneumonia in Finnish children in a nation-wide population-based study. *PLOS ONE*, *12*(3), e0172690. https://doi.org/10.1371/journal.pone.0172690

Paradise, J. L., & Bluestone, C. D. (2005). Consultation With the Specialist: Tympanostomy Tubes: A Contemporary Guide to Judicious Use. *Pediatrics in Review*, *26*(2), 61–66. https://doi.org/10.1542/pir.26-2-61

Paradise, J. L., Rockette, H. E., Colborn, D. K., Bernard, B. S., Smith, C. G., Kurs-Lasky, M., & Janosky, J. E. (1997). Otitis Media in 2253 Pittsburgh-Area Infants: Prevalence and Risk Factors During the First Two Years of Life. *Pediatrics*, *99*(3). Retrieved from http://pediatrics.aappublications.org/content/99/3/318.long#T2

Pasteur, L. (1881). Note sur la maladie nouvelle provoquee par la salive d'un enfant mort de la rage. *Bulletin de l'Academie de Medicine (Paris)*, (10), 94–103.

Paterson, G. K., & Orihuela, C. J. (2010, July 20). Pneumococci: Immunology of the innate host response. *Respirology*. Wiley/Blackwell (10.1111). https://doi.org/10.1111/j.1440-1843.2010.01814.x

Pavia, M., Bianco, A., Nobile, C. G. A., Marinelli, P., & Angelillo, I. F. (2009). Efficacy of Pneumococcal Vaccination in Children Younger Than 24 Months: A Meta-Analysis. *PEDIATRICS*. https://doi.org/10.1542/peds.2008-3422

Peltola, V. T., & McCullers, J. A. (2004). Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *The Pediatric Infectious*

Disease Journal, *23*(Supplement), S87–S97. https://doi.org/10.1097/01.inf.0000108197.81270.35

Pettigrew, M. M., Laufer, A. S., Gent, J. F., Kong, Y., Fennie, K. P., & Metlay, J. P. (2012). Upper respiratory tract microbial communities, acute otitis media pathogens, and antibiotic use in healthy and sick children. *Applied and Environmental Microbiology*, *78*(17), 6262–6270. https://doi.org/10.1128/AEM.01051-12

Pichichero, M. E. (1998). Group A Beta-hemolytic Streptococcal Infections. *Pediatrics in Review*, *19*(9), 291–302. https://doi.org/10.1542/pir.19-9-291

Pichichero, M. E., Shelly, M. A., & Treanor, J. J. (1997). Evaluation Of A Pentavalent Conjugated Pneumococcal Vaccine In Toddlers. *The Pediatric Infectious Disease Journal*, *16*(1), 72–74. Retrieved from https://insights.ovid.com/pubmed?pmid=9002106

Pierangeli, A., Trotta, D., Scagnolari, C., Ferreri, M., Nicolai, A., Midulla, F., … Bagnarelli, P. (2014). Rapid spread of the novel respiratory syncytial virus A ON1 genotype, central Italy, 2011 to 2013. *Eurosurveillance*, *19*(26), 20843. https://doi.org/10.2807/1560-7917.ES2014.19.26.20843

Pierce, H. C., Mansbach, J. M., Fisher, E. S., Macias, C. G., Pate, B. M., Piedra, P. A., … Camargo, C. A. (2015). Variability of Intensive Care Management for Children With Bronchiolitis. *Hospital Pediatrics*, *5*(4), 175–184. https://doi.org/10.1542/hpeds.2014-0125

Plosker, G. L. (2014). 10-Valent Pneumococcal Non-Typeable Haemophilus influenzae Protein D-Conjugate Vaccine: A Review in Infants and Children. *Pediatric Drugs*. https://doi.org/10.1007/s40272-014-0089-x

Poehling, K. A., Talbot, T. R., Griffin, M. R., Craig, A. S., Whitney, C. G., Zell, E., … Schaffner, W. (2006). Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA : The Journal of the American Medical Association*, *295*(14), 1668–1674. https://doi.org/10.1001/jama.295.14.1668

Prymula, R., Peeters, P., Chrobok, V., Kriz, P., Novakova, E., Kaliskova, E., … Schuerman, L. (2006). Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both Streptococcus pneumoniae and non-typable Haemophilus influenzae: A randomised double-blind efficacy study. *Lancet*, *367*(9512), 740–748. https://doi.org/10.1016/S0140-6736(06)68304-9

Prymula, R., & Schuerman, L. (2009). 10-Valent pneumococcal nontypeable Haemophilus influenzae PD conjugate vaccine: Synflorix™. *Expert Review of Vaccines*, *8*(11), 1479–1500. https://doi.org/10.1586/erv.09.113

Ralston, S. L., Lieberthal, A. S., Meissner, H. C., Alverson, B. K., Baley, J. E.,

Gadomski, A. M., … American Academy of Pediatrics. (2014). Clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. *Pediatrics*, *134*(5), e1474-502. https://doi.org/10.1542/peds.2014-2742

Ramakrishnan, K., Sparks, R. A., & Berryhill, W. E. (2007). Diagnosis and treatment of otitis media. *American Family Physician*, *76*(11), 1650–1658. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18092706

Raskin, D. M., Seshadri, R., Pukatzki, S. U., & Mekalanos, J. J. (2006). Bacterial genomics and pathogen evolution. *Cell*, *124*(4), 703–714. https://doi.org/10.1016/j.cell.2006.02.002

Reller, L. B., Weinstein, M. P., Werno, A. M., & Murdoch, D. R. (2008). Laboratory Diagnosis of Invasive Pneumococcal Disease. *Clinical Infectious Diseases*, *46*(6), 926–932. Retrieved from http://dx.doi.org/10.1086/528798

Remington, L. T., & Sligl, W. I. (2014). Community-acquired pneumonia. *Current Opinion in Pulmonary Medicine*, *20*(3), 215–224. https://doi.org/10.1097/MCP.0000000000000052

Richter, S. S., Diekema, D. J., Heilmann, K. P., Dohrn, C. L., Riahi, F., & Doern, G. V. (2014). Changes in pneumococcal serotypes and antimicrobial resistance after introduction of the 13-Valent conjugate vaccine in the United States. *Antimicrobial Agents and Chemotherapy*, *58*(11). https://doi.org/10.1128/AAC.03344-14

Rivera-Olivero, I. A., Del Nogal, B., Sisco, M. C., Bogaert, D., Hermans, P. W. M., & De Waard, J. H. (2011). Carriage and invasive isolates of Streptococcus pneumoniae in Caracas, Venezuela: The relative invasiveness of serotypes and vaccine coverage. *European Journal of Clinical Microbiology and Infectious Diseases*, *30*(12), 1489–1495. https://doi.org/10.1007/s10096-011-1247-5

Robbins, J. B., Schneerson, R., Anderson, P., & Smith, D. H. (1996). Prevention of Systemic Infections, Especially Meningitis, Caused by Haemophilus influenzae Type b. *JAMA*, *276*(14), 1181. https://doi.org/10.1001/jama.1996.03540140069031

Roberts, J. E., Rosenfeld, R. M., & Zeisel, S. A. (2004). Otitis Media and Speech and Language: A Meta-analysis of Prospective Studies. *Pediatrics*, *113*(3). Retrieved from http://pediatrics.aappublications.org/content/113/3/e238.long

Roca, A., Dione, M. M., Bojang, A., Townend, J., Egere, U., Darboe, O., … Antonio, M. (2013). Nasopharyngeal Carriage of Pneumococci Four Years after Community-Wide Vaccination with PCV-7 in The Gambia: Long-Term Evaluation of a Cluster Randomized Trial. *PLoS ONE*, *8*(9), 5–9. https://doi.org/10.1371/journal.pone.0072198

Rodriguez, W. J., Gruber, W. C., Groothuis, J. R., Simoes, E. A., Rosas, A. J., Lepow, M., … Hemming, V. (1997). Respiratory syncytial virus immune globulin treatment of RSV lower respiratory tract infection in previously healthy children. *Pediatrics*, *100*(6), 937–942. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9374560

Rose, M., & Zielen, S. (2009). Impact of infant immunization programs with pneumococcal conjugate vaccine in Europe. *Expert Review of Vaccines*, *8*(10), 1351–1364. https://doi.org/10.1586/erv.09.78

Rosenfeld, R. M., Schwartz, S. R., Pynnonen, M. A., Tunkel, D. E., Hussey, H. M., Fichera, J. S., … Schellhase, K. G. (2013). Clinical Practice Guideline: Tympanostomy Tubes in Children. *Otolaryngology -- Head and Neck Surgery*, *149*(1 Suppl), S1–S35. https://doi.org/10.1177/0194599813487302

Rothman, R., Owens, T., & Simel, D. L. (2003). Does this child have acute otitis media? *Jama*, *290*(12), 1633–1640. https://doi.org/10.1001/jama.290.12.1633

Rupp, R., Johnston, W., Hurley, D., Petrecz, M., Li, J., Nolan, K., … Musey, L. (2018). A DOSE RANGING STUDY OF 15-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV-15) IN HEALTHY INFANTS. In *ISPPD-11*. Melbourne, Australia.

Rytel, M. W. (1982). Pneumococcal infections and pneumococcal vaccine: an update. *Infection Control*, *3*(4), 295–298. https://doi.org/10.2307/30146408

Sá-Leão, R., Pinto, F., Aguiar, S., Nunes, S., Carriço, J. A., Frazão, N., … Ramirez, M. (2011). Analysis of invasiveness of pneumococcal serotypes and clones circulating in Portugal before widespread use of conjugate vaccines reveals heterogeneous behavior of clones expressing the same serotype. *Journal of Clinical Microbiology*, *49*(4), 1369–1375. https://doi.org/10.1128/JCM.01763-10

Sáez-Llorens, X., Rowley, S., Wong, D., Rodríguez, M., Calvo, A., Troitiño, M., … Schuerman, L. (2017). Efficacy of 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine against acute otitis media and nasopharyngeal carriage in Panamanian children - A randomized controlled trial. *Human Vaccines & Immunotherapeutics*, *0*(0), 1–16. https://doi.org/10.1080/21645515.2017.1287640

Sartori, A. L., Minamisava, R., Bierrenbach, A. L., Toscano, C. M., Afonso, E. T., Morais-Neto, O. L., … Andrade, A. L. (2017). Reduction in all-cause otitis media-related outpatient visits in children after PCV10 introduction in Brazil. *PloS One*, *12*(6), e0179222. https://doi.org/10.1371/journal.pone.0179222

Satzke, C., Turner, P., Virolainen-Julkunen, A., Adrian, P. V., Antonio, M., Hare, K. M., … O'Brien, K. L. (2013). Standard method for detecting

upper respiratory carriage of Streptococcus pneumoniae: Updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine*, *32*(1), 165–179. https://doi.org/10.1016/j.vaccine.2013.08.062

Schappert, S. M., & Rechtsteiner, E. A. (2006). National Health Statistics Reports, Number 8, (August 6, 2008), *6*. Retrieved from https://www.cdc.gov/nchs/data/nhsr/nhsr008.pdf

Schönberger, K., Kirchgässner, K., Riedel, C., & von Kries, R. (2013). Effectiveness of 2 + 1 PCV7 vaccination schedules in children under 2 years: A meta-analysis of impact studies. *Vaccine*, *31*(50), 5948–5952. https://doi.org/10.1016/J.VACCINE.2013.10.042

Shak, J. R., Vidal, J. E., & Klugman, K. P. (2013). Influence of bacterial interactions on pneumococcal colonization of the nasopharynx. *Trends in Microbiology*, *21*(3), 129–135. https://doi.org/10.1016/j.tim.2012.11.005

Shapiro, E. D., Berg, A. T., Austrian, R., Schroeder, D., Parcells, V., Margolis, A., … Clemens, J. D. (1991). The Protective Efficacy of Polyvalent Pneumococcal Polysaccharide Vaccine. *New England Journal of Medicine*, *325*(21), 1453–1460. https://doi.org/10.1056/NEJM199111213252101

Sharland, M. (2011). *Manual of Childhood Infections*. *OUP Oxford*.

Shouval, D. S., Greenberg, D., Givon-Lavi, N., Porat, N., & Dagan, R. (2006). Site-Specific Disease Potential of Individual Streptococcus pneumoniae Serotypes in Pediatric Invasive Disease, Acute Otitis Media and Acute Conjunctivitis. *The Pediatric Infectious Disease Journal*, *25*(7), 602–607. https://doi.org/10.1097/01.inf.0000220231.79968.f6

Sigurdsson, S., Erlendsdóttir, H., Quirk, S. J., Kristjánsson, J., Hauksson, K., Andrésdóttir, B. D. I., … Haraldsson, Á. (2017). Pneumococcal vaccination: Direct and herd effect on carriage of vaccine types and antibiotic resistance in Icelandic children. *Vaccine*, *35*(39), 5242–5248. https://doi.org/10.1016/j.vaccine.2017.08.020

Sigurdsson, S., Eythorsson, E., Hrafnkelsson, B., Erlendsdóttir, H., Kristinsson, K. G., & Haraldsson, Á. (2018a). PNEUMOCOCCAL VACCINATION IN ICELAND: DELAYED FIRST PRESENTATION OF AOM. In *ISPPD-11*. Melbourne, Australia.

Sigurdsson, S., Eythorsson, E., Hrafnkelsson, B., Erlendsdóttir, H., Kristinsson, K. G., & Haraldsson, Á. (2018b). Reduction in all-cause acute otitis media in children less than three years of age in primary care following pneumococcal vaccination with PHiD-CV10: A whole population study. *Clinical Infectious Diseases*, *67*(8), 1213–1219. https://doi.org/10.1093/cid/ciy233

Simell, B., Auranen, K., Käyhty, H., Goldblatt, D., Dagan, R., O'Brien, K. L., & O'Brien, K. L. (2012). The fundamental link between pneumococcal carriage and disease. *Expert Review of Vaccines*, *11*(7), 841–855. https://doi.org/10.1586/erv.12.53

Skinner, J., Kaufhold, R., McGuinness, D., McHugh, P., Winters, M., Smith, W., … Musey, L. (2018). IMMUNOGENICITY OF PCV24, A NEXT GENERATION PNEUMOCOCCAL CONJUGATE VACCINE, IN MACAQUES. In *ISPPD-11*. Melbourne, Australia.

Sleeman, K. L., Griffiths, D., Shackley, F., Diggle, L., Gupta, S., Maiden, M. C., … Peto, T. E. A. (2006). Capsular Serotype–Specific Attack Rates and Duration of Carriage of Streptococcus pneumoniae in a Population of Children. *The Journal of Infectious Diseases*, *194*(5), 682–688. https://doi.org/10.1086/505710

Slotved, H.-C., Kaltoft, M., Skovsted, I. C., Kerrn, M. B., & Espersen, F. (2004). Simple, rapid latex agglutination test for serotyping of pneumococci (Pneumotest-Latex). *Journal of Clinical Microbiology*, *42*(6), 2518–2522. https://doi.org/10.1128/JCM.42.6.2518-2522.2004

Smith T, Lehmann D, Montgomery, J., Gratten, M., I.D, R., & M.P, A. (1993). Acquisition and invasiness of different serotypes of Streptococcus pneumoniae in young children. *Epidemiol Infect*, *111*, 27–39. https://doi.org/10.2307/3863747

Snaebjarnardóttir, K., Erlendsdóttir, H., Reynisson, I. K., Kristinsson, K., Halldórsdóttir, S., Hardardóttir, H., … Haraldsson, Á. (2013). Bacterial meningitis in children in Iceland, 1975-2010: a nationwide epidemiological study. *Scandinavian Journal of Infectious Diseases*, *45*(11), 819–824. https://doi.org/10.3109/00365548.2013.817680

Snape, M. D., Klinger, C. L., Daniels, E. D., John, T. M., Layton, H., Rollinson, L., … Pollard, A. J. (2010). Immunogenicity and reactogenicity of a 13-valent-pneumococcal conjugate vaccine administered at 2, 4, and 12 months of age: a double-blind randomized active-controlled trial. *The Pediatric Infectious Disease Journal*, *29*(12), e80-90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21155091

Song, J. Y., Eun, B. W., & Nahm, M. H. (2013, December 1). Diagnosis of pneumococcal pneumonia: Current pitfalls and the way forward. *Infection and Chemotherapy*. https://doi.org/10.3947/ic.2013.45.4.351

Sóttvarnarlæknir. (2010). Bólusetning gegn pneumókokkasýkingum í undirbúningi. Retrieved from https://www.landlaeknir.is/servlet/file/store93/item1846/4476.pdf

Spellberg, B., Guidos, R., Gilbert, D., Bradley, J., Boucher, H. W., Scheld, W. M., … Edwards, J. (2008). The Epidemic of Antibiotic-Resistant Infections: A Call to Action for the Medical Community from the Infectious Diseases Society of America. *Clinical Infectious Diseases*, *46*(2), 155–

164. https://doi.org/10.1086/524891

Stein, K. E. (1992). Thymus-independent and thymus-dependent responses to polysaccharide antigens. *The Journal of Infectious Diseases*, *165 Suppl 1*, S49-52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1588177

Sternberg, G. M. (1881). A fatal form of septicemia in the rabbit produced by subcutaneous injection of human saliva. *Natl. Board of Health Bull.*, *3*(87), 108. Retrieved from http://www.worldcat.org/title/fatal-form-of-septicaemia-in-the-rabbit-produced-by-the-subcutaneous-injection-of-human-saliva-an-experimental-research/oclc/63122948

Stille, C. J., Rifas-Shiman, S. L., Kleinman, K., Kotch, J. B., & Finkelstein, J. A. (2008). Physician Responses to a Community-Level Trial Promoting Judicious Antibiotic Use. *The Annals of Family Medicine*, *6*(3), 206–212. https://doi.org/10.1370/afm.839

Su, W.-J., Lo, H.-Y., Chang, C.-H., Chang, L.-Y., Chiu, C.-H., Lee, P.-I., … Lin, T.-Y. (2016). Effectiveness of Pneumococcal Conjugate Vaccines of Different Valences Against Invasive Pneumococcal Disease Among Children in Taiwan. *The Pediatric Infectious Disease Journal*, *35*(4), e124–e133. https://doi.org/10.1097/INF.0000000000001054

Sun, K., Johansen, F.-E., Eckmann, L., & Metzger, D. W. (2004). An important role for polymeric Ig receptor-mediated transport of IgA in protection against Streptococcus pneumoniae nasopharyngeal carriage. *Journal of Immunology (Baltimore, Md. : 1950)*, *173*(7), 4576–4581. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15383591

Swingler, G., Fransman, D., & Hussey, G. (2003). Conjugate vaccines for preventing Haemophilus influenzae type B infections. In George Swingler (Ed.), *Cochrane Database of Systematic Reviews* (p. CD001729). Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/14651858.CD001729

Syrjanen, R. K., Herva, E. E., Makela, P. H., Puhakka, H. J., Auranen, K. J., Takala, A. K., … Kilpi, T. M. (2006). The value of nasopharyngeal culture in predicting the etiology of acute otitis media in children less than two years of age. *The Pediatric Infectious Disease Journal*, *25*(11), 1032–1036. https://doi.org/10.1097/01.inf.0000241097.37428.1d

Tang, K. L., Caffrey, N. P., Nóbrega, D. B., Cork, S. C., Ronksley, P. E., Barkema, H. W., … Ghali, W. A. (2017). Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *The Lancet Planetary Health*, *1*(8), e316–e327. https://doi.org/10.1016/S2542-5196(17)30141-9

Tawfik, K. O., Ishman, S. L., Altaye, M., Meinzen-Derr, J., & Choo, D. I. (2017). Pediatric Acute Otitis Media in the Era of Pneumococcal Vaccination. *Otolaryngology-Head and Neck Surgery*, *156*(5), 938–945.

https://doi.org/10.1177/0194599817699599

Taylor, S., Marchisio, P., Vergison, A., Harriague, J., Hausdorff, W. P., & Haggard, M. (2012). Impact of pneumococcal conjugate vaccination on otitis media: A systematic review. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *54*(12), 1765–1773. https://doi.org/10.1093/cid/cis292

Teele, D. W., Klein, J. O., & Rosner, B. (1989). Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *The Journal of Infectious Diseases*, *160*(1), 83–94. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2732519

The European Committee on Antimicrobial Susceptibility Testing. (2018). Breakpoint tables for interpretation of MICs and zone diameters. Retrieved from http://www.eucast.org.

Therneau, T. M., & Grambsch, P. M. (2000). *Modeling Survival Data: Extending the Cox Model*. New York: Springer-Verlag. https://doi.org/10.1007/978-1-4757-3294-8

Tlaskalová-Hogenová, H., Štěpánková, R., Hudcovic, T., Tučková, L., Cukrowska, B., Lodinová-Žádníková, R., … Kokešová, A. (2004). Commensal bacteria (normal microflora), mucosal immunity and chronic inflammatory and autoimmune diseases. *Immunology Letters*, *93*(2–3), 97–108. https://doi.org/10.1016/J.IMLET.2004.02.005

Todberg, T., Koch, A., Andersson, M., Olsen, S. F. S. F., Lous, J., Homøe, P., … Casey, J. R. (2014). Incidence of otitis media in a contemporary danish national birth cohort. *PloS One*, *9*(12), e111732. https://doi.org/10.1371/journal.pone.0111732

Tomasson, G., Gudnason, T., & Kristinsson, K. (2005). Dynamics of pneumococcal carriage among healthy Icelandic children attending day-care centres. *Scandinavian Journal of Infectious Diseases*, *37*(6–7), 422–428. https://doi.org/10.1080/00365540510035346

Tregnaghi, M. W. M. M. W., Sáez-Llorens, X., López, P., Abate, H., Smith, E., Pósleman, A., … COMPAS Group. (2014). Efficacy of pneumococcal nontypable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in young Latin American children: A double-blind randomized controlled trial. *PLoS Medicine*, *11*(6), e1001657. https://doi.org/10.1371/journal.pmed.1001657

Usonis, V., Jackowska, T., Petraitiene, S., Sapala, A., Neculau, A., Stryjewska, I., … Holl, K. (2016). Incidence of acute otitis media in children below 6 years of age seen in medical practices in five East European countries. *BMC Pediatrics*, *16*, 108. https://doi.org/10.1186/s12887-016-0638-2

van der Linden, M., Al-Lahham, A., Nicklas, W., & Reinert, R. R. (2009). Molecular characterization of pneumococcal isolates from pets and

laboratory animals. *PloS One*, *4*(12), e8286. https://doi.org/10.1371/journal.pone.0008286

Van Dongen, T. M. A., Van Der Heijden, G. J. M. G., Van Zon, A., Bogaer, D., Sanders, E. A. M., & Schilder, A. G. M. (2013). Evaluation of concordance between the microorganisms detected in the nasopharynx and middle ear of children with otitis media. *Pediatric Infectious Disease Journal*, *32*(5), 549–552. https://doi.org/10.1097/INF.0b013e318280ab45

Van Eldere, J., Slack, M. P. E., Ladhani, S., & Cripps, A. W. (2014). Non-typeable Haemophilus influenzae, an under-recognised pathogen. *The Lancet Infectious Diseases*, *14*(12), 1281–1292. https://doi.org/10.1016/S1473-3099(14)70734-0

van Hoek, A. J., Sheppard, C. L., Andrews, N., Waight, P. A., Slack, M. P. E., Harrison, T. G., … Miller, E. (2014). Pneumococcal carriage in children and adults two years after introduction of the thirteen valent pneumococcal conjugate vaccine in England. *Vaccine*, *32*(34), 4349–4355. https://doi.org/10.1016/j.vaccine.2014.03.017

Ventre, K., & Randolph, A. (2004). Ribavirin for respiratory syncytial virus infection of the lower respiratory tract in infants and young children. *The Cochrane Database of Systematic Reviews*, (4), CD000181. https://doi.org/10.1002/14651858.CD000181.pub2

Verani, J. R., Domingues, C. M. A. S., Moraes, J. C. de, & Brazilian Pneumococcal Conjugate Vaccine Effectiveness Study Group. (2015). Indirect cohort analysis of 10-valent pneumococcal conjugate vaccine effectiveness against vaccine-type and vaccine-related invasive pneumococcal disease. *Vaccine*, *33*(46), 6145–6148. https://doi.org/10.1016/j.vaccine.2015.10.007

Vesikari, T., Forsten, A., Seppä, I., Kaijalainen, T., Puumalainen, T., Soininen, A., … Schuerman, L. (2016). Effectiveness of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D-conjugated vaccine (PHiD-CV) against carriage and acute otitis media-a double-blind randomized clinical trial in Finland. *Journal of the Pediatric Infectious Diseases Society*, *5*(3). https://doi.org/10.1093/jpids/piw010

Vuononvirta, J., Toivonen, L., Gröndahl-Yli-Hannuksela, K., Barkoff, A.-M., Lindholm, L., Mertsola, J., … He, Q. (2011). Nasopharyngeal Bacterial Colonization and Gene Polymorphisms of Mannose-Binding Lectin and Toll-Like Receptors 2 and 4 in Infants. *PLoS ONE*, *6*(10), e26198. https://doi.org/10.1371/journal.pone.0026198

Walker, C. L. F., Rudan, I., Liu, L., Nair, H., Theodoratou, E., Bhutta, Z. A., … Black, R. E. (2013). Global burden of childhood pneumonia and diarrhoea. *The Lancet*, *381*(9875), 1405–1416. https://doi.org/10.1016/S0140-6736(13)60222-6

Wang, L., Fu, J., Liang, Z., & Chen, J. (2017). Prevalence and serotype

distribution of nasopharyngeal carriage of Streptococcus pneumoniae in China: a meta-analysis. *BMC Infectious Diseases*, *17*(1), 765. https://doi.org/10.1186/s12879-017-2816-8

Watson, D. A., Musher, D. M., Jacobson, J. W., & Verhoef, J. (1993). A Brief History of the Pneumococcus in Biomedical Research: A Panoply of Scientific Discovery Description of the Organism and Demonstration of Its Virulence. *Clinical Infectious Diseases*, *17*, 913–924. https://doi.org/10.1093/clinids/17.5.913

Weil-Olivier, C., van der Linden, M., de Schutter, I., Dagan, R., & Mantovani, L. (2012). Prevention of pneumococcal diseases in the post-seven valent vaccine era: A European perspective. *BMC Infectious Diseases*, *12*(1), 207. https://doi.org/10.1186/1471-2334-12-207

Weinberger, R., van der Linden, M., Imöhl, M., & von Kries, R. (2016). Vaccine effectiveness of PCV13 in a 3+1 vaccination schedule. *Vaccine*, *34*(18), 2062–2065. https://doi.org/10.1016/j.vaccine.2016.02.043

Whittemore, K. R. (2013). What is the role of tympanostomy tubes in the treatment of recurrent acute otitis media? *The Laryngoscope*, *123*(1), 9–10. https://doi.org/10.1002/lary.23601

Williams, D. J., Hall, M., Auger, K. A., Tieder, J. S., Jerardi, K. E., Queen, M. A., … Shah, S. S. (2015). Association of White Blood Cell Count and C-Reactive Protein with Outcomes in Children Hospitalized for Community-acquired Pneumonia. *The Pediatric Infectious Disease Journal*, *34*(7), 792–793. https://doi.org/10.1097/INF.0000000000000724

World Health Organization. (2001). *Standardization of interpretation of chest radiographs for the diagnosis of pneumonia in children / World Health Organization Pneumonia Vaccine Trial Investigators' Group*. Retrieved from http://www.who.int/iris/handle/10665/66956

World Health Organization. (2018). *WHO | Pneumococcal conjugate 3rd dose (PCV3) immunization coverage*. *WHO*. World Health Organization. Retrieved from http://www.who.int/gho/immunization/pneumococcal/en/

Yatsyshina, S., Mayanskiy, N., Shipulina, O., Kulichenko, T., Alyabieva, N., Katosova, L., … Shipulin, G. (2016). Detection of respiratory pathogens in pediatric acute otitis media by PCR and comparison of findings in the middle ear and nasopharynx. *Diagnostic Microbiology and Infectious Disease*, *85*(1), 125–130. https://doi.org/10.1016/j.diagmicrobio.2016.02.010

Yoshihara, K., Le, M. N., Okamoto, M., Wadagni, A. C. A., Nguyen, H. A., Toizumi, M., … Yoshida, L.-M. (2016). Association of RSV-A ON1 genotype with Increased Pediatric Acute Lower Respiratory Tract Infection in Vietnam. *Scientific Reports*, *6*(1), 27856. https://doi.org/10.1038/srep27856

Zemlickova, H., Jakubu, V., Urbaskova, P., Motlova, J., Musilek, M., & Adamkova, V. (2010). Serotype-specific invasive disease potential of Streptococcus pneumoniae in Czech children. *Journal of Medical Microbiology*, *59*(9), 1079–1083. https://doi.org/10.1099/jmm.0.018390-0

Zola, T. A., Lysenko, E. S., & Weiser, J. N. (2008). Mucosal clearance of capsule-expressing bacteria requires both TLR and nucleotide-binding oligomerization domain 1 signaling. *Journal of Immunology (Baltimore, Md.: 1950)*, *181*(11), 7909–7916. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19017981

Zuccotti, G., Mameli, C., Daprai, L., Garlaschi, M. L., Dilillo, D., Bedogni, G., … Chiara, V. (2014). Serotype distribution and antimicrobial susceptibilities of nasopharyngeal isolates of Streptococcus pneumoniae from healthy children in the 13-valent pneumococcal conjugate vaccine era. *Vaccine*, *32*(5), 527–534. https://doi.org/10.1016/j.vaccine.2013.12.003

Original publications

Paper I

Sigurdsson, S., Erlendsdóttir, H., Quirk, S. J., Kristjánsson, J., Hauksson, K., Andrésdóttir, B. D. I., Jónsson, A. J., Halldórsson, K. H., Sæmundsson, Á., Ólason, Ó. H., Hrafnkelsson, B., Kristinsson, K. G., Haraldsson, Á. (2017). Pneumococcal vaccination: Direct and herd effect on carriage of vaccine types and antibiotic resistance in Icelandic children. Vaccine, 35 (39).

Paper II

Sigurdsson, S., Eythorsson, E., Hrafnkelsson, B., Erlendsdóttir, H., Kristinsson, K. G., Haraldsson, Á. (2018). Reduction in All-Cause Acute Otitis Media in Children less than 3 Years of Age in Primary Care Following Vaccination With 10-Valent Pneumococcal Haemophilus influenzae Protein-D Conjugate Vaccine: A Whole-Population Study. Clinical Infectious Diseases.

Paper III

Sigurdsson, S., Kristinsson, K. G., Erlendsdóttir, H., Hrafnkelsson, B., Haraldsson, Á. (2015). Decreased Incidence of Respiratory Infections in Children After Vaccination with Ten-valent Pneumococcal Vaccine. Pediatric Infectious Disease Journal, 34 (12), 1385–1390.

Paper IV

Sigurdsson, S., Eythorsson, E., Erlendsdóttir, H., Hrafnkelsson, B., Kristinsson, K. G., Haraldsson, Á. Impact of the 10-valent pneumococcal conjugate vaccine on hospital admissions in children under three years of age in Iceland (submitted)

7 Appendix

7.1 Study I. Questionnaire (In Icelandic)

Rannsókn á útbreiðslu og sýklalyfjaónæmi pnemókokka, Haemophilus influenzae og streptókokka af flokki A í nefkoki hjá börnum á leikskólum vorið 2014

Spurningalisti fyrir foreldra í tengslum við nefkoksræktanir

1. **Nafn barns:** _____ Rannsóknarnúmer:

2. **Kennitala barns:** _____

3. **Nafn leikskóla:** _____

4. **Nafn leikskóladeildar:** _____

5. **Nafn bæjarfélags: Reykjavík ☐, Kópavogur ☐, Hafnarfjörður ☐**

6. **Tekur barn þitt sýklalyf núna**
   já ☐ nei ☐
   a) **ef já, hvaða:** _____
   b) **við hverju var lyfið gefið:** _____

7. **Hefur barnið fengið sýklalyf sl. 30 daga (önnur en í lið 6)**
   já ☐ nei ☐

8. **Hefur barnið fengið sýklalyf þrisvar eða oftar á sl. 6 mán.**
   já ☐ nei ☐

vinsamlegast ath. næstu blaðsíðu

9. **Á síðustu 6 mán., hefur barnið verið greint af lækni með eftirfarandi sjúkdóma:**

   a) **eyrnabólgu**

      1) **já ☐ nei ☐**

      2) **hversu oft: _____**

      3) **hefur barnið einhvern tíma greinst með eyrnabólgu já ☐ nei ☐**

   b) **kinnholubólgu (sinusitis)**

      1) **já ☐ nei ☐**

      2) **hversu oft: _____**

   c) **lungnabólgu**

      1) **já ☐ nei ☐**

      2) **hversu oft: _____**

   d) **annað: _____**

10. **Hefur barnið misst úr daga á leikskólanum vegna veikinda sl. 3 mánuði**

   a) **hversu oft** **_____**

   b) **hve marga daga alls**

Dagsetning svara við þessum spurningum

_____

Kærar þakkir fyrir þátttökuna

7.2 Study I. Informed consent (In Icelandic)

Upplýst samþykki

Ég

_____*,Kennitala*_____

fullgildur forráðamaður/foreldri barns míns, samþykki af fúsum og frjálsum vilja að barn mitt taki þátt í rannsókninni **"Rannsókn á útbreiðslu og sýklalyfjaónæmi pneumókokka, hemophilus og streptókokka af flokki A í nefkoki hjá börnum á leikskólum vorið 2014."**

Ég hef með bréfi þessu verið upplýst(ur) um tilgang og framkvæmd rannsóknarinnar og þær aðgerðir sem henni fylgja. Ég hef fengið tækifæri til að spyrja spurninga um rannsóknina sem hefur verið svarað fullnægjandi. Ég get hætt við þátttöku í rannsókninni hvenær sem er án nokkurra skilyrða eða eftirmála.

NAFN (barnsins)

_____ **Kt.**_____

Dagsetn._____

UNDIRSKRIFT FORELDRIS/FORRÁÐAMANNS

_____

Ég hef með bréfi kynnt tilgang og eðli ofangreindrar rannsóknar og alla áhættu henni samfara.

Undirskrift læknanema/læknis

_____

Kennitala _____

Undirskrift ábyrgðarmanns rannsóknar

_____

Kennitala_____

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