IMPACT OF CHILDHOOD IMMUNISATION WITH A PNEUMOCOCCAL
CONJUGATE VACCINE IN NORWAY

Didrik Frimann Vestrheim

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Norwegian Institute of Public Health
Division of Infectious Disease Control

and

University of Oslo
Faculty of Medicine
The peculiarities of Pneumococcus are yielding a generous return to the investors and speculators who have cast in their resources with its lot, resulting in the accumulation of a store of solid bullion for the scientist and for mankind

Benjamin White,
The biology of pneumococcus, 1938
Acknowledgements

When the decision to introduce the 7-valent pneumococcal conjugate vaccine (PCV7) in the Norwegian Childhood Immunisation Programme was made in springtime of 2006, I had just recently started working at The Norwegian Institute of Public Health (NIPH). I am very grateful that I was given the opportunity to join the PCV7 surveillance group and to perform the studies described in this thesis. I want to thank Ingeborg S. Aaberge, director at the Department of Bacteriology and Immunology, for giving me this opportunity, for letting me into the fascinating world of pneumococcolgy, and for heaps of encouragements along the way.

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Oslo, December 2011
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<td>acute otitis media</td>
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<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
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<td>CAP</td>
<td>community-acquired pneumonia</td>
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<td>CbpD</td>
<td>choline binding protein D</td>
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<td>CC</td>
<td>clonal complex</td>
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<td>CFR</td>
<td>case fatality ratio</td>
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<td>CI</td>
<td>confidence interval</td>
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<td>ChoP</td>
<td>phosphorylcholine</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CSP</td>
<td>competence stimulation peptide</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>DCC</td>
<td>day-care center</td>
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<td>DDD</td>
<td>defined daily doses</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>IL</td>
<td>interleukin</td>
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<td>IPD</td>
<td>invasive pneumococcal disease</td>
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<td>IR</td>
<td>incidence rate</td>
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<td>IRR</td>
<td>incidence rate ratio</td>
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<td>Hib</td>
<td><em>Haemophilus influenzae</em> type b</td>
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<tr>
<td>LytA</td>
<td>pneumococcal autolysin</td>
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<td>MIC</td>
<td>minimum inhibitory concentration</td>
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<td>MLST</td>
<td>multilocus sequence typing</td>
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<td>MSIS</td>
<td>Norwegian Surveillance System for Communicable Diseases</td>
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<td>NCKP</td>
<td>Northern California Kaiser Permanente</td>
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<td>NIPH</td>
<td>Norwegian Institute of Public Health</td>
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<td>NORM</td>
<td>Norwegian Surveillance System for Antimicrobial Resistance in Microbes</td>
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<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>Acronym</td>
<td>Term</td>
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<tr>
<td>PAMP</td>
<td>pathogen-associated molecular pattern</td>
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<td>PBP</td>
<td>penicillin binding protein</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PCV</td>
<td>pneumococcal conjugate vaccine</td>
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<td>PCV7</td>
<td>7-valent pneumococcal conjugate vaccine</td>
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<td>PMEN</td>
<td>Pneumococcal Molecular Epidemiology Network</td>
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<td>PNSP</td>
<td>penicillin non-susceptible pneumococci</td>
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<td>PPV</td>
<td>pneumococcal polysaccharide vaccine</td>
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<tr>
<td>PPV23</td>
<td>23-valent pneumococcal polysaccharide vaccine</td>
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<tr>
<td>PspA</td>
<td>pneumococcal surface protein A</td>
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<td>PspC</td>
<td>pneumococcal surface protein C</td>
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<tr>
<td>PRR</td>
<td>pattern recognition receptor</td>
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<tr>
<td>RCT</td>
<td>randomised clinical trial</td>
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<td>rPAF</td>
<td>platelet activating factor receptor</td>
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<td>ST</td>
<td>sequence type</td>
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<td>SYSVAK</td>
<td>Norwegian National Immunisation Register</td>
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<td>The Institute for Genomic Research</td>
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<td>TLR</td>
<td>Toll-like receptor</td>
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1. Background

*Streptococcus pneumoniae* is a major cause of severe human disease. Approximately 14.5 million episodes of severe pneumococcal disease were estimated to have occurred in 2000 (196). Each year 1.6 million people die from serious pneumococcal disease, including approximately 1 million children aged < 5 years (260). This accounts for around 11% of all deaths among children, the majority occurring in developing countries (196).

Primary prevention of pneumococcal infection by vaccination is a favourable approach to limit morbidity and mortality. The first trial of a pneumococcal vaccine was conducted one century ago (261), but different immunisation strategies are continually being explored. The first vaccine indicated for children, a 7-valent polysaccharide-protein conjugate vaccine (PCV7), was licensed in the United States in 2000. The recommended vaccination schedule consisted of 3 primary immunisations during the first year of life (at 2, 4 and 6 months) and a booster dose given during the second year of life (12-15 months), a 3+1 dose schedule (5).

Both clinical trials and post-marketing surveillance of PCV7 have demonstrated a remarkable reduction of invasive pneumococcal disease (IPD) among immunised children (17,257). Declining incidence rates of IPD have also been described in age-groups not targeted by vaccination (159,257). This herd effect results from the impact of vaccination on nasopharyngeal colonisation (45,181); as the pool of circulating virulent bacteria is lessened, transmission of these pathogens is subsequently reduced, resulting in a decline of infections in all age groups.

Currently available pneumococcal vaccines offer protection against a subset of pneumococcal serotypes responsible for the greatest disease burden in industrialised countries; the pneumococcal serotypes may be categorised as vaccine serotypes and
non-vaccine serotypes. Mass vaccination results in an immunological pressure on the pneumococcal population, leading to a reduced reservoir of vaccine serotypes. However, an expansion of non-vaccine serotypes may occur. This phenomenon, termed serotype replacement, has been observed in studies of nasopharyngeal carriage (127), and also in surveillance of IPD following the introduction of PCV7 (115). Hence, the overall reduction in pneumococcal disease is tempered, to a certain extent, by an increase in IPD caused by non-vaccine serotypes.

PCV7 was introduced in the Norwegian Childhood Immunisation Programme in July 2006. An immunisation schedule with reduced number of doses was chosen, including two primary immunisations at age 3 and 5 months, and a booster dose administered at 12 months of age; a 2+1 dose schedule. This schedule had not previously been implemented and evaluated in a national immunisation programme elsewhere.

The aim of this thesis is to describe the changing epidemiology of IPD in Norway following introduction of a pneumococcal conjugate vaccine. A pivotal part is the estimates of vaccine effectiveness following the three-dose immunisation schedule, compared to the four-dose schedule that was originally licensed. The direct effect of vaccination on IPD among children was estimated in Paper I, while the indirect effect, or herd effect, was explored in Paper II. In order to describe the impact of the immunisation programme on the pneumococcal reservoir, two nasopharyngeal carriage studies were performed among healthy children attending day-care centres; a baseline was established in 2006 (Paper III), and in 2008 post vaccination changes could be evaluated (Paper IV). The population biology of colonising pneumococci was approached further by an analysis of the gene encoding the competence stimulating peptide (CSP), in order to explore the impact of competence induced fratricide during pneumococcal co-colonisation (Paper V).
The methods used in this thesis range from infectious disease epidemiology using population-based surveillance data, cross-sectional sample collection from children in day-care centers (DCCs), to phenotypic characterisation and molecular studies of pneumococcal clonality and population biology.

This study of the impact of PCV7 in Norway contributes to research on pneumococcal diseases and to our understanding of pneumococcal population evolution. Furthermore, it may be of help for decision-makers when planning future implementation of pneumococcal immunisation programmes both in Norway and in other settings.
2. Introduction

2.1 The pneumococcus

Pneumococcal infections are caused by *S. pneumoniae*, the pneumococcus. The normal habitat of the pneumococcus is the human nasopharynx, from where it can proceed to various organs and cause a range of infectious diseases, or it can transmit to other hosts and eventually be eliminated (20). High rates of pneumococcal carriage are observed among children, and asymptomatic children are regarded as the pneumococcal infectious reservoir. Following colonisation in the nasopharynx the bacterium may infect the respiratory tract mucosa, and it is one of the most common etiologies in acute otitis media (AOM), sinusitis and pneumonia. From localised infections the bacterium can invade the bloodstream or other normally sterile sites, resulting in IPD such as bacteremia, sepsis or meningitis (Figure 1).

![Diagram of infectious disease entities caused by *S. pneumoniae*](image)

**Figure 1.** Infectious disease entities caused by *S. pneumoniae*
2.1.1 Description of the species and pneumococcal serotypes

*S. pneumoniae* was identified as a pathogen in 1881, when Sternberg in the United States and Pasteur in France independently isolated diplococci from rabbits that had been inoculated with human saliva. However, the bacterium had been recognized by Klebs already in 1875, in secretions from patients with pneumonia (256). In 1886 the bacterium was called “pneumokokkus” by Fränkel. In the same year it was named *Diplococcus pneumoniae* by Weichselbaum, a name that remained until reclassification as *S. pneumoniae* in 1974 (51).

Traditional identification of *S. pneumoniae* is based on morphological characteristics and biochemical tests. The bacterium is a lancet-shaped gram-positive diplococcus, a facultative anaerobe that grows in a 5 % CO₂ enriched atmosphere. It is alpha-hemolytic, catalase negative, optochin susceptible and bile-soluble (165). The pneumococcus is sheathed by a polysaccharide capsule, although non-encapsulated variants exist. The chemical structure of the capsule gives rise to at least 93 antigenically different serotypes (28,113,139,202). When grown on blood agar, capsulated strains will appear as round, flat, smooth colonies that often have a central pitting; some strains, typically serotype 3 and 37, yield mucoid colonies. Noncapsulated strains grow as small, “rough” colonies (165) (Figure 2).
Figure 2. Colony morphology of *S. pneumoniae*. Two morphological variants can be seen in the picture, a smooth colony with central pitting and a mucoid colony. The picture shows a serotype 19F strain (smooth, central pitting) and a serotype 3 strain (mucoid) recovered from a sample obtained in the 2006 carriage study (Paper III) and plated on Columbia horse blood agar containing 5.0 µg/ml gentamicin.

The scientific history of the pneumococcus from its first description until the mid 1930’s was thoroughly reviewed by White in 1938 (256), and more briefly by Watson in the 1990’s (249). Already in 1897, Bezançon and Griffon proposed that “there exist several races of pneumococci that behave as though different microbes” (256). They observed “agglutination” when patient sera were incubated with pneumococcal strains. This phenomenon was further elaborated by Neufeld with the development of the Quellung, or capsular swelling, test in 1902, and the description of serotypes I and II in 1910 (249,256). By the identification of serotypes, specific pneumococcal serum therapy was made possible as a treatment option for pneumococcal infections, based on the knowledge that type-specific serum are usually protective. By 1932, 32 serotypes had been described (36), and during the 1940’s the number of identified
pneumococcal serotypes increased rapidly. At the same time an increasing number of specific therapeutic antisera became commercially available, and the development of pneumococcal vaccines composed of serotype-specific polysaccharide began (166).

A nomenclature for pneumococcal serotypes was developed at Statens Serum Institut in Copenhagen, Denmark. In this system the serotypes are grouped into serogroups, containing 1 to 5 antigenically related serotypes (113). The serogroups are numbered 1 to 48 (groups 26 and 30 are non-existing), and the serotypes within each group are identified by a letter (F for the serotype described first, and alphabetically starting from A for subsequent types).

Traditionally, pneumococcal isolates are serotyped by the capsular swelling test (165); serotype-specific antisera and a suspension of the bacterial specimen are mixed on a slide, covered and examined under the microscope with oil-immersion. In a positive reaction the capsule appears enlarged, due to a reaction between the type-specific serum and the capsular polysaccharide. Identification of the serotype is made by pool-sera, group-sera, and for serogroups with more than one serotype, with factor-sera.

Multiplex assays using monoclonal and polyclonal pneumococcal antibodies have been developed, enabling high-throughput serotyping of pneumococci (263). Recently, new techniques based on amplification of serotype-specific genes of the capsule locus by the polymerase chain reaction (PCR) have been introduced (200).

The distribution of pneumococcal serotypes isolated from IPD patients vary in different geographical locations (104), and shifts in serotype prevalence have been observed over time (64,102,136). Furthermore, certain serotypes are overrepresented among IPD cases compared to those in asymptomatic carriers; from this knowledge, the concept termed “serotype-specific invasive potential” has been inferred
(24,96,228). The structure and composition of the capsule, resulting in different properties for adherence and immune responses, are thought to be the major factors contributing to these observed epidemiological differences in virulence and invasive potential (251,252).

2.1.2 Interaction of the microbe and the host

2.1.2.1 Adherence and virulence

The pneumococcus is an extracellular pathogen, and the first step of interaction with the host is via adherence of the bacterium to nasopharyngeal epithelium. In addition to the polysaccharide capsule, a wide range of surface-exposed proteins have been described as contributing to adherence. Other proteins will promote invasion, balancing inflammation and evasion from innate and adaptive immune responses, as reviewed by Bergmann and Hammerschmidt (15) (Figure 3).

![Figure 3](image-url)

**Figure 3.** Schematic model of the pneumococcal outer cell wall and surface-exposed proteins. The pneumococcal cell wall consists of a phospholipid membrane (LM), peptidoglycan (PG), and teichoic acid (TA) and lipoteichoic acid (LTA). An unusual component of the cell wall is phosphorylcholine (PCho), which anchors the choline-binding proteins (CBPs) non-covalently on the cell wall. Virulence proteins of the different classes of pneumococcal surface proteins are depicted. Reprinted from (15) with permission.
The pneumococcal polysaccharide capsule is essential for virulence by inhibiting opsonophagocytosis, and by limiting entrapment in neutrophil extracellular nets and mucus-mediated clearance (8,187,248). With few exceptions, the capsule polysaccharide is negatively charged. Mucus and epithelial cells are also negatively charged due to sialic acid residues, and adhesion is limited by electrostatic repulsion (186). However, sialic acid residues are cleaved by the pneumococcal neuraminidase (NanA), and cleavage of surface-bound serotype-specific IgA by the pneumococcal IgA1-protease results in bound cationic Fab-fragments and enhanced pneumococcal adherence (15,255). Further access to the cell surface might be mediated by a switch in the amount of expressed capsule polysaccharide that has been observed within serotypes, with the least encapsulated variant being more adhesive to epithelium and associated with colonisation; this phenomenon is referred to as opacity phase variation, and is believed to play a central role for invasion of host tissues (254).

Phosphorylcholine (ChoP) is a constituent of the pneumococcal cell wall, and an important mediator of adhesion by interaction with the receptor for platelet activating factor (rPAF) (40). ChoP also binds to C-reactive protein (CRP), an acute phase protein that contributes to the innate immune response by complement activation (235). Furthermore, ChoP serves as the anchor for approximately 10-15 choline-binding proteins, including pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), pneumococcal autolysin (LytA) and choline-binding protein D (CbpD) (15). PspA inhibits complement binding and limits bactericidal activity of apolactoferritin. PspC, a genetically variable surface protein (133), functions as an adhesin by binding to the secretory component of the polymeric immunoglobulin receptor on epithelial surfaces, and as an inhibitor of complement activation by binding to factor H (49,141). The enzymatic activity of LytA results in autolysis with release of pro-inflammatory cell constituents, and this activity is associated with the typical umbilicated morphology of pneumococcal colonies when grown on blood agar (141). The transcription of CbpD has been shown
to be induced by competence stimulating peptide (CSP), and functions in competence induced cell lysis of non-competent cells with release of virulence factors (88,146).

Approximately 20 pneumococcal surface proteins are anchored to the peptidoglycan by an LPXTG amino acid motif recognised by a sortase (15). In TIGR4, a full-genome sequenced pneumococcal strain, 3 of 4 recognised sortase homologues are located within a pathogenicity islet termed rlrA (107). It has been demonstrated that this pathogenicity islet, although not ubiquitously present in clinical isolates, encodes a pneumococcal pilus, pilus I, associated with tissue adherence (12). A second LPXTG anchored pilus, pilus II, that mediates adherence to epithelial cells, has recently been described in a subset of clinical isolates (11). Several proteases, including the neuraminidase NanA and the IgA protease, are also LPXTG anchored proteins (15).

Lipoproteins, of which at least 45 have been described, function in substrate transport and are of importance for virulence (15). The pneumococcal surface adhesin A (PsaA) is a component of a manganese transporter and adds resistance to oxidative stress to pneumococcal virulence (172).

The exotoxin pneumolysin is a well established virulence factor that oligomerises on target cells producing a pore resulting in cell lysis. Pneumolysin inhibits ciliary function, inhibits phagocytic respiratory burst, induces production of cytokines and activates complement and CD4+ T-cells (141,142). A non-hemolytic form of pneumolysin has been described in S. pneumoniae serotype 1 isolated from disease outbreak settings (137).

2.1.2.2 Immune response
The humoral immune response is regarded essential when combating infections caused by extracellular pathogens, and the central role of the humoral immune
response in pneumococcal infection was established by Georg and Felix Klemperer in 1891. They observed that mice could be immunised actively by heat-killed pneumococci and passively by infusion of serum from a previously immunised mouse. At about the same time it was shown that protective serum from immunised mice was not bactericidal, but promoted uptake of pneumococci by phagocytes; see reviews (249,256). Thus, the pivotal part of opsonisation of pneumococci by complement and immunoglobulins and Fc-receptor-mediated uptake by phagocytic cells was established early in research on pneumococcal pathogenesis.

The innate immune system provides a non-specific and immediate response upon challenge with *S. pneumoniae* and may allow the adaptive immune response to develop. The defence against pneumococci includes the complement system, recognition of pathogen-associated molecular patterns (PAMPs), extracellular traps and interleukins (ILs) (203). During initial tissue inflammation, neutrophils are recruited. By activation of the complement system, the bacteria are opsonised and made available for phagocytosis by neutrophils and macrophages. The capsule protects the pneumococcus from complement-mediated opsonophagocytosis by multiple mechanisms, but this property differs between serotypes (131,132). Surface antigens, such as PspC and NanA, contribute to the inhibition of complement activation (203). The innate response of mucociliary clearance and entrapment in neutrophil extracellular nets are countered by the pneumococcal capsule (187,248). CRP and pneumolysin are activators of the innate immune response by interaction with the complement system and stimulating neutrophil influx, respectively (235,246).

As a part of the innate immune system, Toll-like receptors (TLRs) are typical pattern recognition receptors (PRRs) with ability to recognise PAMPs. TLR2 is thought to be a central PRR for gram-positive pathogens, by recognition of lipoteichoic acid in the bacterial cell wall, and may be of importance for clearance of pneumococci (246).
TLR4 is involved in the inflammatory response and resistance to pneumococcal infection due to interaction with pneumolysin (170).

Both antibody and cell-mediated immunity are involved in the adaptive immune response to *S. pneumoniae*. The effect of serotherapy in treatment of pneumococcal infection and the reduction of both carriage and IPD by conjugated pneumococcal vaccines document the protective effect of serotype-specific capsule polysaccharide antibodies (8,47,256,257). Serotype-specific antibodies bound to polysaccharide capsule act as receptor for complement components, and mediate complement- and Fc-dependent opsonophagocytosis. Both capsule polysaccharide and surface proteins elicit antibodies during colonisation (173,212,222,223). However, to what extent serotype-specific antibodies participate in natural development of resistance to pneumococcal colonisation and infection has been questioned (82,161,251).

CD4+ T-cells are of importance for cell-mediated immunity to pneumococcal colonisation and infection. This is clearly demonstrated by the burden and morbidity of pneumococcal disease in HIV-infected patients with low CD4+ cell counts (58,68). In mouse models, CD4+ T-cells have been shown to be essential for protection against colonisation, while antibodies were dispensable (169). IL-17A and neutrophils are required for this immune response, indicating that CD4+ T-cells may interact with pneumococcal antigens and recruit phagocytes via IL-17A (164). Chemotaxis of CD4+ T-cells are stimulated by pneumolysin, and pneumolysin-specific CD4+ T-cells contribute to prevention or clearance of pneumococcal carriage (142,264).

2.1.3 Pneumococcal genetics

*S. pneumoniae* is naturally competent for transformation, i.e. it is able to alter its own genome by uptake of exogenous deoxyribonucleic acid (DNA) and incorporation by homologous recombination. The first evidence for this ability was demonstrated by
Griffith in the 1920’s (86). In these experiments, mice were inoculated subcutaneously with a non-capsulated strain derived from serotype II together with a heat-killed culture of encapsulated serotype I (serotypes I and II correspond to serotypes 1 and 2 in the Danish nomenclature). Subsequently, viable serotype I pneumococci were recovered, indicating that the non-capsulated strain had been transformed into a capsulated strain of a distinct serotype. In the early 1940’s, it was established that the transforming material was DNA (10), a work of fundamental importance for the development of genetic research.

The first complete genome of a *S. pneumoniae* strain that was sequenced, TIGR4, revealed a genome of 2 – 2.1 million basepairs and approximately 2000 genes (240). This strain is a serotype 4 strain which was isolated from an IPD case in Norway in 1987, and has been shown to be highly invasive in a mouse model (2).

*S. pneumoniae* is closely related to commensal streptococci of the oral cavity, and phylogenetic analyses based on sequencing of 16S rRNA-genes reveal that *S. pneumoniae* is clustered together with members of the Mitis group, including *Streptococcus mitis, Streptococcus oralis, Streptococcus infantis* and *Streptococcus pseudopneumoniae*, with more than 99% sequence homology (7,147). Most members of the Mitis group are naturally competent for genetic transformation. The distinction between species is therefore obscured by transitional forms due to horizontal gene transfer between species, leading to “mosaic genes” and, in part, a random distribution of virulence genes within the cluster (90,150).

Competence for genetic transformation in *S. pneumoniae* and related species in the Mitis group is regulated by an extracellular secreted peptide, CSP, encoded by the *comC* gene (108,244). Induction of the competent state occurs when the extracellular concentration of CSP reaches a critical level and is sensed by the two-component regulatory system ComDE, a histidine kinase receptor and a response regulator,
encoded by comD and comE, respectively (109,206). Development of competence in pneumococci occurs during early logarithmic growth in vitro, and in vivo competence might be a stress response caused e.g. by external pH or antibiotics (33,34,211). When competent, the bacterium is able to kill non-competent siblings; it commits fratricide (88). By this mechanism the bacterium is believed to gain access to a wide gene pool during the stress inflicted by selective pressures in vivo (140).

Horizontal gene transfer by homologous recombination is the major source of genetic diversity within pneumococci (67); the recombination rate of S. pneumoniae has been estimated to be three to seven times the mutation rate (73). Genes and chromosomal fragments are constantly being shuffled within the pneumococcal population, resulting in sexual speciation, and a loosely genetically structured population (74).

### 2.1.4 Pneumococcal population biology

Multilocus sequence typing (MLST) is a standardised genetic method for accurate characterisation of pneumococcal isolates in order to describe its molecular epidemiology and population biology (59). In this method, seven house-keeping genes, aroE, gdh, gki, recP, spi, ddl and xpt, which are considered to be under a neutral selective pressure, are characterised by sequencing of an internal gene fragment of approximately 500 base pairs (167). Unique sequences are assigned allele numbers consecutively, and each unique combination of all seven allele numbers is assigned a sequence type (ST) number, marking a clone. Sequence data are entered into a database (www.spneumoniae.mlst.net), permitting global surveillance.

The alleles of one of the house-keeping genes included in the MLST scheme, ddl, have been demonstrated to be highly divergent. The ddl gene is located close to the penicillin-binding protein (PBP) 2b gene (pbp2b), and allele divergence is explained by hitch-hiking of ddl with the pbp2b gene, a gene under antibiotic selective pressure (60); thus, variation in ddl might not be completely neutral.
Data obtained by MLST differentiate pneumococcal strains into clones in a degree that is suitable for different molecular epidemiological purposes. MLST has been used extensively for surveillance of the impact of PCV7 on pneumococcal populations and for identification of possible capsule switch and vaccine escape (13,25,94,97). The technique has also been used in studies aiming at describing the species and its phylogeny (95,150).

By international comparison of pneumococcal strains associated with antimicrobial non-susceptibility it has become evident that a limited number of clones that are geographically widely distributed account for a large proportion of non-susceptible strains. In the Pneumococcal Molecular Epidemiology Network (PMEN), such clones are described and subjected to a standardised nomenclature (www.sph.emory.edu/PMEN). To date 43 clones are proposed as internationally dispersed and described at the PMEN site; the first 16 clones were described by McGee et al. in 2001 (176). In this system, the clones are named by the country in which they were first described, the serotype and the ST. The first clone to be included in this system was a clone described in Spain belonging to serotype 23F and assigned to ST 1, and termed Spain23F-ST1.

2.1.5 Antimicrobial resistance
In the late 1930’s, sulfanilamide and sulfapyridine were introduced as a chemotherapeutic treatment option for pneumococcal infections, followed only a few years later by penicillin. However, antibiotic resistance is closely related to antibiotic consumption (42,218), and resistance to sulfapyridine was observed already in 1943 (242), while penicillin non-susceptibility in a clinical isolate was first described in 1967 (99). Since, antimicrobial resistance has increased worldwide, especially against the two antibiotic classes most frequently used for empirical treatment of respiratory tract infections, β-lactams and macrolides. However, the prevalence of resistant
pneumococci differs by geographic region (214,219), as illustrated for Europe in Figure 4.

![Percentage resistance](image)


Multidrug-resistance, defined as resistance to two or more of penicillin, second-generation cephalosporins, macrolides, tetracycline and trimethoprim/sulphamethoxazol, has been reported in about 40% of respiratory tract samples in a worldwide survey (219). Importantly, IPD caused by penicillin resistant pneumococci is associated with excess mortality (65).

Decreased antimicrobial susceptibility among pneumococci is mainly associated with serotypes that are frequently carried among children, 6A, 6B, 9V, 14, 19F, 19A, and a limited number of clones within these serotypes has been reported to dominate the
global spread of antimicrobial resistant pneumococci (176). Five of these serotypes are included in PCV7, and following wide-spread vaccination a decline of non-susceptible *S. pneumoniae* in invasive disease was observed in the United States (153,234).

2.1.5.1 Resistance to penicillin and β-lactams

Pneumococci with decreased susceptibility to benzylpenicillin and minimum inhibitory concentration (MIC) > 0.64 μg/ml are defined as penicillin non-susceptible pneumococci (PNSP). β-lactamase activity has not been described in *S. pneumoniae*. Decreased susceptibility to penicillin and other β-lactams is caused by structural changes in several PBPs. PBPs are cytoplasmic-membrane proteins involved in peptidoglycan synthesis in the bacterial cell wall. Binding of β-lactams to PBPs inhibits cell wall synthesis, subsequently leading to cell death; i.e. the drugs have bactericidal effect. Reduced affinity of the PBPs to β-lactams leads to reduced antimicrobial activity, and the MIC of the drug increases. Six PBPs have been described in *S. pneumoniae*: 1a, 1b, 2x, 2a, 2b and 3 (91). Alterations leading to low-affinity forms of either PBP2x or PBP2b alone may cause resistance, and these PBPs are termed primary resistance determinants (85). Alterations in PBP1a may lead to high-level resistance when present concomitantly with a low-affinity PBP2x or PBP2b (183).

Point mutations leading to decreased-affinity PBPs are believed to accumulate in commensal streptococci repeatedly exposed to antibiotic selection pressure, e.g. *S. mitis* and *S. oralis*. Subsequently, mosaic genes may arise by homologous recombination within and between species (31,90). The DNA coding sequence of low-affinity PBPs contain segments that are up to 20 % divergent from that of PBP genes in susceptible strains (56).
2.1.5.2 Resistance to macrolides, lincosamides and streptogramin B

The macrolides, lincosamides and streptogramin B, termed MLS₈-antibiotics, interfere with the peptide elongation process in protein synthesis by binding to the large, 50S, subunit of the ribosome in the pneumococcus (239). Resistance to MLS₈-antibiotics may arise by two main mechanisms: target site modification or active drug efflux (154).

Target site modification includes methylation of an adenine residue in the 23S rRNA component of the 50S ribosome subunit by erythromycin ribosome methylation (*erm*) genes, or by mutations in this domain or in ribosomal proteins L4 and L22 (154,237). The most prevalent *erm* determinant, *erm*(B), is found in a wide range of streptococci, enterococci, staphylococci and enterobacteria (154). The result of target site modification is cross-resistance to all MLS₈-antibiotics, either as a constitutive (cMLS₈), or as an inducible phenotype (iMLS₈) where resistance to clindamycin and streptogramins might only be evident after induction with erythromycin (154,253).

Cellular pumps conferring macrolide efflux are encoded by *mef* genes, of which *mef*(A) and *mef*(E) are the most prevalent among *S. pneumoniae*. This mechanism results in the M-phenotype of resistance; non-susceptibility to macrolides only, with MIC-values generally lower than those observed for the MLS₈-phenotype.

The *erm* and *mef* genes are carried on mobile genetic elements, transposons, e.g. *mef*(A) on Tn1207.1 and *mef*(E) on the macrolide efflux genetic assembly (mega) element (53,75). Interestingly, the Tn1207.1 element, carrying the *mef*(A) determinant, is inserted in the *celB* competence gene, and strains harboring this determinant have been shown to be non-transformable (53). In fact, the distribution of the *mef*(A) determinant is highly clonal, restricted to the internationally dispersed England\(^{14}\)-ST9 clone (53).
The prevalence of macrolide resistance genotypes differ geographically; the \textit{erm}(B) genotype conferring the MLS\textsubscript{B}-phenotype dominates in Asia and in Southern and Eastern Europe, while the efflux mechanism encoded by \textit{mef}(A) dominates in Northern Europe and North America, where the overall prevalence of macrolide resistance is generally lower (62). These differences may be due to differences in dominating clones and the selective pressure resulting from overall antimicrobial usage.

The consumption of antibiotics in Norway is low, approximately 16 defined daily doses (DDD) per 1000 inhabitants per day (excluding methenamine) (188). This consumption is comparable to that in the rest of Scandinavia and in the Netherlands, and below the average consumption in Europe (81). The resistance profile of \textit{S. pneumoniae} isolated in Norway is favourable; to date, decreased susceptibility to penicillin has been observed in less than 3 \% of invasive pneumococcal disease isolates (188).
2.2 Pneumococcal infection and epidemiology

2.2.1 Burden of disease and mortality
The World Health Organization (WHO) has estimated that 1.6 million people die annually due to pneumococcal disease; among them nearly 1 million are children aged < 5 years (260). Incidence rates of pneumococcal infections vary greatly, and more than 60 % of deaths related to pneumococcal infections occur in ten countries in Africa and Asia (196) (Figure 5). Pneumonia is the clinical presentation accounting for the greatest burden of mortality and morbidity caused by *S. pneumoniae* worldwide.

![Figure 5. Pneumococcal deaths in children aged 1–59 months per 100,000 children younger than 5 years (HIV-negative pneumococcal deaths only). Reprinted from (196) with permission.](image)

Serious pneumococcal infections, including pneumonia and IPD, occur primarily in young children and the elderly. In the pre-antibiotic era invasive pneumococcal infections were common and with a high mortality. Among patients with confirmed pneumococcal pneumonia at Boston City Hospital in the early 1930’s, the case-fatality ratio (CFR) without serum therapy was 84 % (241). In the late 1950’s, with
penicillin treatment, the CFR declined to 17 % (9). This overall CFR has remained unchanged until the 1990’s and 2000’s (65,101,124). The CFR is highest during the initial five days of illness and it has remained the same from the pre-antibiotic age till present. The CFR of pneumococcal meningitis ranges from approximately 10 % among children in Europe and the United States, to approximately 45 % among children in Africa (101,125,158,205). Notably, *S. pneumoniae* is associated with the highest case fatality of bacterial meningitis in Africa (205).

Although less severe, AOM is exceedingly common among children. It is responsible for substantial costs due to medical visits and parents’ absence from work, and it is a major cause of prescription of antibiotics. Extensive use of antibiotics leads to emergence of non-susceptible pneumococcal strains, of which some have successfully spread globally.

### 2.2.2 Diagnosis of pneumococcal infection

Pneumococcal infection is diagnosed by identification of the bacterium from a site that corresponds with clinical illness. Culture is the preferred method for identification, as this gives the possibility for susceptibility testing and further characterization of the isolate. Both normally sterile sites and non-sterile sites may be sampled for culture.

IPD refers to the identification of pneumococci from a normally sterile site, such as blood, cerebrospinal fluid (CSF), joint fluid, pleural fluid and from the peritoneal cavity, in a clinically ill patient. Growth from blood cultures results from bacteraemia, with or without a clinical focus, while growth from CSF is indicative of pneumococcal meningitis. Growth from a non-sterile site in the airways may represent asymptomatic colonisation, and other etiologies must be considered.
While culture has the advantage of yielding an isolate that can be characterised further, the method does not have optimal sensitivity. Presence of pneumococci may also be proved by antigen detection, e.g. by agglutination of CSF when meningitis is suspected (120), or by immunochromatographic identification of pneumococcal breakdown products in urine as an aid for etiologic diagnosis of non-bacteremic pneumonia (185). By specific PCR targeting genes for pneumolysin, autolysin, etc, pneumococcal DNA may be identified. The sensitivity of PCR methods can exceed culture, and these methods may be useful for rapid diagnosis of meningitis by identification of pneumococcal DNA in CSF (4).

2.2.3 Nasopharyngeal carriage – the pneumococcal reservoir
The pneumococcus is commonly found as a commensal bacterium colonising the nasopharynx of healthy children. This colonisation is believed to be asymptomatic, although symptoms may occur upon acquisition (227). Colonisation is regarded as the first step for symptomatic pneumococcal infection in the host, and it is the reservoir for spread of pneumococci in the community (20). Transmission of the bacterium occurs by the respiratory route, and via fomites; it requires near contact and is facilitated by crowding.

2.2.3.1 Measuring pneumococcal carriage
Carriage of pneumococci is episodic, consisting of acquisition, colonisation and elimination of the bacterium. The duration of colonisation is variable, ranging from days to months, and is dependent on age and serotype; the mean duration of carriage is around 3-4 months (84,118,227). Carriage is detected in healthy individuals by swabbing of the nasopharynx. Carriage studies can be either cross-sectional or longitudinal, i.e. obtaining repeated samples from the same individuals over time. Only longitudinal studies may provide information on transmission dynamics, acquisition rates and duration of carriage. Furthermore, the observed carriage rate is highly dependent on the sensitivity of the sampling technique, conditions for
specimen transport and cultivation, and sensitivity of detection by culture or DNA amplification tests. Traditionally, pneumococcal strains are recovered by culturing the sample on a selective medium containing gentamicin. At any given time one host may be colonised by multiple pneumococcal serotypes, which may be distinguished by different colony morphology. Carriage of multiple strains may also be evidenced by random selection of numerous colonies (103), or by using specific antisera, e.g. in immunoblot (23). Carriage of multiple strains has also been successfully identified using PCR methods and microarray (27).

A transport medium enabling immediate freezing of the sample has been developed in order to facilitate carriage studies in remote areas (191). This method has been standardised, and is recommended by WHO to provide interstudy comparability (192).

2.2.3.2 Risk factors for pneumococcal carriage

Age and exposure to pneumococci in the environment are major determinants for pneumococcal carriage. The cumulative acquisition of pneumococci increases during the first year of life, and all children are believed to have acquired pneumococci repeatedly during childhood. However, marked differences in carriage frequencies are observed between developed and developing countries. In developed countries approximately 90 % of infants have been colonised at least once by 2 years of age (236). The peak prevalence of recorded carriage generally ranges between 40% and 60 %, and decrease below 10 % by the age of 10 years (22,127,130,213,236). Carriage among adults and the elderly is assumed to be well below 10 %, although this has been less extensively studied (130,213,215). Thus, carriage prevalence increase during the first and second years of life, peak in the second or third year of life, and decline gradually thereafter.
In developing countries, however, a 90 % cumulative colonisation of infants is reached by the age three months (83,118). The carriage frequencies observed in developing countries are high, with approximately 90 % carriage among infants and children aged less than 1 year, and the prevalence decline more slowly (83,117,163).

Exposure to carriers represents a risk for being colonised with pneumococci, and studies in families have revealed that infants exposed to older siblings acquire pneumococci earlier (84,130,156,213). DCCs provide ample opportunities for transmission of pneumococci, and DCC attendance is considered a risk factor for colonisation (210,213). Pneumococci may spread like microepidemics within the DCC (217), and may act as a source for transmission to other family members (78). Furthermore, the proportion of children attending DCCs has been modelled to impact the overall frequency of carriage in the community; the more children attending day-care, and for more hours per day, the higher carriage frequency among children both attending and not attending day-care (126).

Factors such as passive smoking, breast-feeding, recent use of antimicrobials, and recent upper respiratory tract infection, have not unequivocally been associated with excess risk of pneumococcal colonisation (210,227).

2.2.3.3 *Serotype-specific carriage*

Differences in acquisition rate, carriage duration, and carriage prevalence are observed for distinct pneumococcal serotypes. The serotypes most frequently identified in carriage studies include serogroups/types 6, 19, 23 and 14, regardless of study setting and geographic location (21,84,118,130,163,213). These serotypes have both high acquisition rates and long carriage duration (84,228). Other serogroups/types that are frequently carried among children include 3, 4, 9, 11, 13, 15, 18 and 33. Non-typeable (NT) pneumococci are also frequently recovered, and may dominate
among adults (213). On the other hand, some serotypes are hardly ever encountered in carriage studies, e.g. 1, 5 and 12F.

2.2.3.4. Serotype-specific invasiveness

Studies have been performed to relate serotype-specific acquisition to invasive disease, by comparing serotype-specific invasive disease incidences with carriage prevalences within comparable populations (24,96,228,230). The invasive disease potential is usually calculated as an odds ratio (OR); the ratio of invasive disease isolates of a given serotype \( a \) among all other IPD isolates \( b \), is divided by the ratio of carried isolates belonging to this serotype \( c \) among all carried isolates \( d \). Hence, the invasive disease potential is \( \frac{ad}{bc} \) (24,230). This measure describes the serotype-specific attack rate, i.e. the risk of invasive disease upon colonisation. These studies indicate that serotypes 1, 4, 5, 7F, 14, 9V, 18C and 19A are associated with a high invasive disease potential, while serotypes 3, 6A, 6B, 15 B/C, 19F and 23F have a low invasive disease potential (24,26,96,226,228).

2.2.3.5 Immune response to carriage

The observed changes in carriage prevalence, peaking in the second or third year of life, and the subsequent reduction in prevalence and duration of carriage (21,121), are likely caused by development of immunity following exposure. The antibody-dependent immune response is believed to play a major role in immunity to pneumococcal carriage. Carriage can induce antibodies against capsular polysaccharides (79,232) and surface proteins (174,212). Based on longitudinal carriage studies acquired immunity to pneumococci is believed to be both serotype-dependent and serotype-independent (82,251); the serotype-independent immunity might be of importance during infancy, before maturation of the immune response to polysaccharide antigens (232). Observations following immunisation with PCV7 clearly indicate that vaccine-induced polysaccharide antibodies lead to a serotype-specific protection against colonisation (45,127,181,193).
2.2.4 Invasive pneumococcal disease

Invasion of a sterile site by pneumococci leading to IPD is believed to occur sporadically, following initial colonisation (20). In children, the risk for invasion appears to be greatest soon after exposure and acquisition of pneumococci in the nasopharynx (84).

The incidence rate (IR) of IPD peaks among infants and children aged less than two years, and among adults aged more than 50 years (Figure 6) (102,204). The peak and subsequent decline of IPD among children is consistent, regardless of serotypes and geography, and is probably caused by maturation of non-serotype specific immune responses to pneumococci (161). In the elderly, both co-morbidity and an age-related dysfunction of the immune system, immune senescence, result in a predisposition to infectious diseases (29,203).

![Figure 6. Incidence rates of invasive pneumococcal disease by age group. Incidence rates in Norway in 2005, the year before introduction of PCV7, are shown.](image-url)
2.2.4.1 Manifestations of IPD

Bacteremia, i.e. isolation of pneumococci in blood culture in a febrile patient, is the disease manifestation associated with the majority of IPD morbidity. Pneumococcal pneumonia is the major primary source of IPD in adults, and bacteremic pneumonia is the most common disease syndrome among IPD cases (144,159). Occult bacteremia, i.e. bacteremia without evident focus, is the dominating disease syndrome among children in the United States, accounting for approximately 60% of IPD cases (19). However, in Western European countries, the infectious focus is identified in the majority of cases and occult bacteremia constitutes 20-30 % of cases (221). This significant difference between Western Europe and the United States is probably due to differences in blood culturing practice; in Europe, blood culturing is primarily performed from hospitalised patients, while in the United States blood cultures are also obtained ambulatory from children with fever and leucocytosis (106).

Meningitis account for approximately 10-20 % of IPD cases, but represents a severe manifestation with higher CFR and long-term sequelae (16,19,32,100). Other IPD manifestations, such as septic arthritis and peritonitis, occur less frequently, although increased rates of pneumococcal empyema have been reported among children in Western Europe since the mid-1990’s (71,198).

2.2.4.2 Geographical differences in IPD

Wide geographical differences in IPD IR have been observed. Among children aged less than 2 years, regional average IRs in Western Europe are between 30 to 40 cases per 100,000 population (138), while in the Unites States the IR in this age group was 188 cases per 100,000 population prior to introduction of PCV7 (257). Even higher IPD rates have been observed in developing countries and among certain subpopulations found to be at excessive risk for IPD, such as Australian aborigines and Alaska natives. The IR among children aged less than 2 years was estimated to
be approximately 450 cases per 100,000 population in a rural area in The Gambia, and similar rates were observed among native children in Alaska (50,197,225).

Less variation is seen across geographical regions for IRs of pneumococcal meningitis, as this disease manifestation is probably diagnosed more uniformly and is less prone to sampling bias. Among children aged < 2 years the IRs range from 4.9 to 14.6 cases per 100,000 population in Western Europe (138) and the IR was estimated to 10.2 cases per 100,000 population in the United States (125). The IRs among Alaska native children < 2 years are 7-8 times higher than in the non-native population, 79.0 vs 10.2 cases per 100,000 population (50), and among children aged < 1 year in The Gambia, a meningitis IR of 148 cases per 100,000 population has been estimated (197).

2.2.4.3 Temporal variation in IPD

Significant changes in IPD epidemiology have been observed over time. In a study of IPD over a 60-year period in Denmark, the IR was shown to be stable during the period 1938-1978 (median IR 2.8 cases per 100,000 population), with a subsequent rapid increase in IPD cases and IR during the 1980’s and 1990’s, levelling off at approximately 20.0 cases per 100,000 population during the period 2000-2005 (101,102). Similar increases in IPD rates during the 1990’s were observed in Finland, Sweden and Norway (77,151,204). On the other hand, rates of pneumococcal meningitis have remained stable over long periods of time (overall median IR 1.3 cases per 100,000 population during the period 1938-2007 in Denmark) (102).

The reason for the increase in IPD rates in Scandinavia during the 1990’s has not been established, and whether the increase was real or due to surveillance bias has been a subject of discussion (77,220). As the increase was seen almost exclusively for bacteremia cases, changed routines for blood culturing practices have been suggested
as explanation; the more blood cultures analysed, the more IPD cases identified (151,220).

2.2.4.4 Risk factors for IPD

As described above, the IR of IPD follows a bimodal age curve; the rates are highest among the very young and the elderly (Figure 6). Racial disparities are evident, e.g. between black and white children in the United States (72,157,189), and between Alaska natives and non-natives (50,225). Low socioeconomic status, defined by income and education, is associated with an increased risk of IPD (72).

Crowding and poor living conditions have also been associated with increased risk of IPD, primarily as a result of outbreaks in institutional settings (122). Transmission and asymptomatic colonisation with pneumococci is facilitated by family exposure (156,163), and the number of siblings in the household and household crowding have been found as risk factors for IPD among young children in case-control studies (119,157). Furthermore, day-care attendance is associated with an increased risk for IPD (157), although this might be restricted to the youngest children soon after enrolment in DCCs (119).

An increased risk for IPD is observed for persons with certain chronic medical conditions, such as functional or anatomic asplenia, chronic obstructive pulmonary disease, diabetes mellitus, cirrhosis, chronic renal failure and HIV-infection (3,65,144,190).

The incidence of invasive pneumococcal infections is at a maximum during the winter season, and this coincides with seasonal epidemics of respiratory syncytial virus and influenza (55,238,250). Influenza infection is believed to predispose to bacterial adherence and pneumococcal pneumonia (175). In case-control studies
preceding influenza, upper respiratory tract infections and AOM are more frequently reported among IPD cases than in controls (157,190,195).

2.2.4.5 *Serotype-specific incidence of IPD*

The capsule polysaccharide is used as vaccine antigen in currently available pneumococcal vaccines, and hence knowledge on serotype distribution is of great importance for the composition of vaccines. Although serotype distribution differs across age-groups, by geography and over time, only a minority of the at least 93 distinct pneumococcal serotypes have consistently been demonstrated to cause the majority of IPD (104).

Studies of serotype distribution among children and adults in different parts of the world have shown that serogroups/-types 6, 14, 19 and 23 dominate among children; serotypes 6B, 9V, 14, 19F and 23F are commonly referred to as the “paediatric serotypes” (66). Prior to implementation of childhood vaccination, the serotypes included in PCV7, i.e. 4, 6B, 9V, 14, 18C, 19F and 23F, were responsible for 80-90 % of IPD cases among children in North America and Australia, 70-75 % in Europe and Africa, and approximately 65 % in Latin America and 50 % in Asia (104,105,138). However, the IR of IPD caused by PCV7 serotypes peak among children aged 6-24 months, while serotypes 1, 3 and 7F account for a significant proportion of IPD in children aged < 6 months or > 24 months (105,143). Furthermore, serotypes 1, 5 and 7F constitute a significant fraction of IPD cases outside North America (104).

The distribution of pneumococcal serotypes in IPD cases changed during the 20th century in the United States; in the first reviews of pneumococcal serotypes causing IPD, the lower number serotypes, i.e. serotypes 1, 2, 3 and 5, often termed “epidemic” serotypes, dominated both among children and adults (9,64,112). The proportion of IPD caused by PCV7 serotypes increased markedly until the end of the century (64,257).
Outbreaks of IPD have been described both historically and recently, primarily caused by serotypes 1, 2, 3, 5 and 12F (105). Outbreaks typically occur in institutions, such as jails (122), or other closed communities or population subgroups (46,216). The incidence of e.g. serotype 1 and 12F disease varies over time, possibly due to population-wide epidemics (102). An epidemic due to serotype 1 pneumococci occurred in Scandinavia during the 1990’s (102,114,204), and large-scale outbreaks of serious meningitis with a high CFR caused by serotype 1 pneumococci have been described in Burkina Faso and Ghana in the early 2000’s (155,262).

2.2.5 Invasive pneumococcal disease in Norway

IPD has been notifiable to MSIS since 1977. Prior to 1993 only septicaemia and meningitis cases were reported. Since 1993 the case definition has included clinically ill patients with isolation of S. pneumoniae from a normally sterile site. The number of reported cases from 1977 to 2005, prior to vaccine introduction, is shown in Figure 7. During the years 1989 to 1996 the annual number of reported cases increased from approximately 200 to more than 850 cases. A new increase started in 2002, and the highest number of notified cases, 1126 cases, was recorded in 2004.
Figure 7. Cases of IPD notified to MSIS per year, 1977 to 2005.

A Norwegian study of IPD in the period 1995 to 2001 demonstrated that PCV7 serotypes constituted 72.7 % of IPD cases among children less than 2 years, and 58.7 % of IPD cases among adults aged more than 65 years (204).

Notably, parallel with the increase in IPD incidence during the first half of the 2000’s the proportion of strains non-susceptible to erythromycin increased rapidly. This increase was related to increased prevalence of a single clone with low-grade resistance to erythromycin, the England14-ST9 clone (231). This clone expresses a serotype 14 capsule, one of the serotypes included in PCV7.
2.3 Prevention of pneumococcal infection

2.3.1 Pneumococcal vaccine history
The first clinical trial of a pneumococcal vaccine was conducted in 1911, among gold and diamond miners in Witwatersrand, South Africa (261). In this trial, immunisation was performed with killed whole-cell pneumococci, and a moderate, although disputable, efficacy was observed.

During the interwar period, the knowledge on pneumococcal capsules and serotypes increased (36), and the capsular polysaccharide became a candidate for serotype-specific vaccines. The first clinical trial of a pneumococcal polysaccharide vaccine (PPV), a four-valent vaccine formulation, was reported in 1945. This study demonstrated protection against vaccine serotype pneumococcal pneumonia, with a vaccine efficacy of 84 % (166). Interestingly, immunisation with polysaccharide was also shown to decrease carriage of vaccine serotype pneumococci, although it is possible that this observation was, in part, caused by temporal shifts in epidemiology (166). In 1946 a 6-valent PPV was licensed in the United States by Squibb & Sons. However, use of this vaccine was limited, probably due to the concurrent introduction of sulphonamide and penicillin; the vaccine was withdrawn in 1954 (168).

During the late 1960’s and 1970’s new studies on polyvalent PPV formulations were conducted (229). A 14-valent PPV was licensed in the United States in 1977, and became available in Norway from 1979. This was succeeded by licensure of a 23-valent PPV formulation in the United States in 1983 and in Norway in 1984 (1,63). The 23-valent PPV (PPV23) has been recommended for adults aged ≥ 65 years in Norway since 1996 (123).
The first pneumococcal vaccine indicated for use in children, PCV7 (Prevenar®, Wyeth/Pfizer), was licensed in the United States in 2000, and in Norway in 2001. During the 2000’s, PCV7 has been implemented in a number of national immunisation programmes in developed countries. Recently, PCVs with increased valency have been licensed, the 10-valent PCV (Synflorix®, GSK) in 2009, and the 13-valent PCV (Prevenar13®, Wyeth/Pfizer) in 2010, adding serotypes 1, 5 and 7F, and 1, 3, 5, 6A, 7F, and 19A to the PCV7 serotypes, respectively. At present, the conjugate vaccines are only indicated for use in children, and PPV23 is the only licensed vaccine for use in adults and the elderly. However, the protective efficacy of PCV13 against community-acquired pneumonia (CAP) and IPD in adults will be assessed in a randomised clinical trial (RCT) study conducted in the Netherlands, the CAPITA study (89). This study is estimated to be completed in 2011, and licensure for use of PCV13 in adults aged 50 years and older is pending.

2.3.2 Pneumococcal polysaccharide vaccine and polysaccharide-conjugate vaccine

Anti-polysaccharide antibodies elicited by immunisation with PPVs or PCVs offer protection against the homologous serotype by opsonising the bacterium. Hence, complement- and Fc-dependent opsonophagocytosis is promoted (6). The repeated carbohydrate epitopes of purified polysaccharide antigens activate B-cells directly without interaction with T-cell help, and are referred to as T-cell independent antigens (247). In the immune response to polysaccharide mainly IgM antibodies are elicited; the immunologic memory is poor, and Ig-class switching and somatic hypermutation is limited (80,243). Furthermore, children aged less than 2 years respond poorly to polysaccharide antigens and PPV is poorly immunogenic in infants and children, an age group carrying a high disease burden (54,247).

There is a lack of high quality RCTs of PPV protective efficacy, and the protection against IPD and CAP is disputed. Based on 10 RCTs, a Cochrane systematic review concluded with an estimated efficacy correlate of 74 % for protection against IPD
among adults (179). Protection against IPD among patients with chronic illnesses could not be demonstrated in this analysis. However, in a recent meta-analysis by Huss et al., the protective effect against IPD was not apparent, probably due to the exclusion of two large RCTs performed prior to 1980 (129). The protective efficacy against pneumonia has been found to be minimal, and results are conflicting (129,179). Furthermore, it appears that the studies of highest quality show the least protective effect (129).

To overcome the poor immune response elicited by PS antigens in children and to stimulate T-cells and induce immunologic memory, vaccine formulations in which the PS antigen is covalently linked to a carrier protein have been developed, i.e. conjugate vaccines. Conjugation to a carrier protein has been tried with other encapsulated pathogens of the respiratory tract, e.g. *Haemophilus influenzae* type b (Hib). Trials of Hib conjugate vaccines conducted in the late 1980’s demonstrated a very high vaccine efficacy against invasive disease (18). The Hib conjugate vaccine was introduced in the Norwegian Childhood Immunisation Programme in 1992, and Hib disease now occurs only sporadically (www.msis.no). However, while invasive disease caused by *H. influenzae* was primarily due to one of its six serotypes, pneumococcal disease is caused by a much higher variety of serotypes. In conjugate vaccines, each polysaccharide must be individually conjugated to the carrier protein. Due to a complex manufacturing process, the number of distinct capsular polysaccharide feasible to combine in a vaccine formulation is limited.

The selection of serotypes to be included in PCV7 was based on surveys of serotype distribution in IPD among children. In a study of a large number of datasets the seven serotypes included in PCV7 were found to be dominant in IPD among children in all regions of the world (104). However, evident from this study were the differences in potential serotype coverage, ranging from less than 50 % of cases in Asia, 60-70 % in Europe to approximately 80 % in North-America and Australia
(104). Thus, the greatest impact of PCV7 in terms of vaccine effectiveness was expected to be in developed countries in North-America and Australia.

2.3.3 Effect and impact of the pneumococcal conjugate vaccine

2.3.3.1 Randomised clinical trials (RCTs)

Between 1995 and 1998 a large double blind RCT was carried out among approximately 38,000 children in Northern California, the Kaiser Permanente study (NCKP) (17). This study was designed to determine the efficacy for prevention of IPD in infants and young children, as well as the safety and immunogenicity of the vaccine. It was the corner stone for licensure of PCV7 by the United States Food and Drug Administration. Another trial designed for this purpose, the Navajo and Apache Native American community randomised study, was still ongoing at the time of licensure of PCV7 (194).

At the final analysis following unblinding in the NCKP study, the vaccine efficacy for protection against vaccine serotype IPD among fully vaccinated children (per protocol) was calculated to be 97.4 % (95 % CI, 82.7 % to 99.9 %) (17). The vaccine efficacy for IPD regardless of serotype was 89.1 % (95 % CI, 73.7 % to 95.8 %). Thus, the vaccine was proven to be highly efficacious in the study population.

In addition to the NCKP and Native American studies, large scale RCTs determining the efficacy of PCVs against IPD have been conducted among children in rural areas of The Gambia (41) and among HIV-positive and HIV-negative children in South Africa (152). The per protocol vaccine efficacy against vaccine serotype IPD was 77 % in the Native American study, 77 % in the study from The Gambia, 85 % in HIV-negative children in South Africa and 65 % among HIV-positive children in South Africa (41,152,194).
The impact of PCVs on mucosal diseases has also been proven by RCTs, although end-points for these outcomes are less clear cut. In the NCKP study a vaccine efficacy of 7.0 % (95% CI, 4 % to 10 %) was found for otitis media episodes (17). In a large RCT performed in Finland, the vaccine efficacy against any episode of AOM was 6 % (95 % CI, -4 % to 16 %), and the vaccine efficacy against AOM caused by a vaccine serotype was 57 % (95 % CI, 44 % to 67 %) (61).

Vaccine efficacy against pneumonia was determined in the NCKP study, the South African study and the study from The Gambia. Whereas the per protocol vaccine efficacy against all cases of clinically diagnosed pneumonia ranged from approximately 4 to 7 % among children in NCKP, South Africa (HIV negative) and The Gambia, the vaccine efficacy against radiographically-confirmed pneumonia ranged from 20 % in NCKP and HIV negative children in South Africa to 37 % in The Gambia.

The effect of PCV on nasopharyngeal carriage has also been evaluated in RCTs, demonstrating that both overall vaccine serotype carriage frequencies and new acquisitions of vaccine serotypes are reduced in the intervention group as compared with controls (45,193,199). These studies indicate that the overall risk of colonisation with vaccine serotypes is significantly lower in immunised children than in the control group. Furthermore, the risk for new acquisition of vaccine serotypes was lower among immunised children, although duration of carriage was unchanged (45,193). From the reduction in carriage of vaccine serotypes observed in these trials, a reduction of vaccine serotypes in the infective reservoir following wide-spread immunisation could be expected. This is the premise for the indirect effect of PCVs.

2.3.3.2 Post-licence surveillance

As PCV7 was first licensed in the United States, the most comprehensive study of PCV7 effectiveness after implementation of routine vaccination to date is based on a
surveillance programme carried out at distinct sites in the United States. In this programme, termed Active Bacterial Core surveillance (ABCs), led by Centers for Disease Control and Prevention (CDC), IPD incidence have been continuously measured in a population of approximately 19 million people in 8 states, and with inclusion of more sites and an increasing study population in recent years. Studies from this surveillance population have demonstrated a rapid and sustained reduction of IPD in all age groups after vaccine introduction compared with the baseline incidence rates observed in 1998 and 1999 (30,257). By 2001, one year after vaccine introduction, the incidence rate of PCV7 serotype IPD among children aged less than two years had declined by 78% (257). An indirect effect was evident already in 2001, and by 2003 the indirect effect was estimated to protect against twice as many IPD cases as the direct effect (30,257).

Data on antimicrobial susceptibility published from the ABCs in the United States have documented a decline of IPD caused by non-susceptible pneumococci following vaccine introduction; by 2004 an 81% decline of PNSP IPD was observed among children and a decline of 49% in the age group 65 years and older, as compared with 1999 (153). Similarly, reductions of macrolide resistant IPD were observed from 1999 to 2002 in a prospective surveillance study carried out in Atlanta; among children less than 2 years old the IR fell by 85% (234).

The impact of PCV on carriage was evident in observational studies comparing carriage prior to and after introduction of routine immunisation with PCV7. Generally, there was a shift from carriage of vaccine serotypes to non-vaccine serotypes, leaving the overall carriage frequency unchanged (127,181). A shift was observed for both immunised and non-immunised parts of the studied populations (92).
2.3.3.3 Serotype replacement

Serotype replacement occurs following shifts from vaccine to non-vaccine serotypes in the carried infectious reservoir, subsequently leading to increased IRs of non-vaccine serotypes in IPD. Serotype replacement in IPD has been documented in post-licensure surveillance in the United States (115,145): four years after vaccine introduction a significant increase of IPD due to non-vaccine serotypes was observed among children aged < 5 years and the elderly aged ≥ 65 years (115). Significant increases in IPD IRs among children were observed for serotype 3, 19A, 22F, 33F and serogroup 15 (115). Among Alaska Native children the IR of IPD caused by non-vaccine serotypes increased by 140 % from pre- to post-vaccine periods (225). The dominating serotype in replacement IPD has been found to be serotype 19A (115,225); from pre-vaccine years to 2003-2004 the IR of serotype 19A among children in the United States increased by 150 %, and the proportion of antimicrobial non-susceptible strains increased (201).

2.3.4 Introduction of pneumococcal conjugate vaccine in the Norwegian Childhood Immunisation Programme

In 2001, PCV7 was licenced in Norway by the Norwegian Medicines Agency, and approved for use in children aged 2 months to 2 years in a 3+1 dose vaccination schedule. In 2004, approval was extended to children aged 2-5 years. In 2003, a committee was appointed by the Norwegian Institute of Public Health (NIPH) in order to prepare recommendations for use of PCV7 in Norway. A report including the committee’s recommendation was delivered to the Ministry of Health in January 2005 (16). In summary, general immunisation for children was recommended, based on the documented efficacy of the vaccine, the knowledge that the majority of children with IPD have no evident risk factors, the potential impact on reduction of antimicrobial resistance and the assumed herd immunity (16).
Serological studies and experiences due to vaccine shortage in the United States provided evidence for protection following immunisation with a reduced dose schedule of 2+1 doses comparable to that of a 3+1 dose schedule (148,258). A Norwegian cost-effectiveness analysis was performed, concluding with overall cost-saving of a 2+1 dose immunisation schedule if indirect costs and herd immunity were included, and assuming the same protection of three and four vaccine doses (259).

In April 2006, a proposal for funding of general childhood pneumococcal immunisation was passed in the Norwegian parliament, Stortinget (St.prp. nr. 39 [2005-2006], Innst. S. nr. 121 [2005-2006]. www.stortinget.no). General immunisation started in July 2006, following a three-dose schedule with vaccine doses administered at 3, 5 and 12 months of age. The vaccine was offered all children born in 2006 and onwards, without further catch-up.

As PCV7 was only approved for a four-dose schedule by the Norwegian Medicines Agency, introduction of the vaccine in a three-dose schedule in the Norwegian Childhood Immunisation Programme warranted thorough surveillance of vaccine effectiveness and vaccine failure. A programme was worked out, based on ongoing surveillance of IPD in the MSIS and the National Reference Laboratory for Pneumococci, and surveillance of immunisations in the Norwegian Immunisation Register (SYsvak), all located at NIPH. The work presented in this thesis was performed as a part of this surveillance programme.
3. Aims and hypotheses

The work presented in this thesis was performed to evaluate the impact of the introduction of PCV7 in the Norwegian Childhood Immunisation Programme on IPD epidemiology and on the pneumococcal reservoir in Norway. An emphasis was made on the effectiveness of the 2+1 dose schedule immunisation programme, as opposed to the 3+1 dose schedule, which was first used in a national immunisation programme in Norway.

Paper I
We performed a population-based cohort study in order to estimate the effectiveness of the 2+1 dose schedule immunisation programme in Norway shortly after introduction of PCV7. In this work we focused on the direct effect among children aged < 5 years.

Paper II
Performed as a continuation of the previous study, we performed a population-based cohort study expanded to include all age groups. The aim of this study was to evaluate the impact of the 2+1 dose schedule immunisation programme on IPD in all age groups and to estimate the indirect effect of the immunisation programme.

Paper III
A cross-sectional study was done to describe the main pneumococcal infectious reservoir, i.e. nasopharyngeal colonisation among children, prior to vaccine introduction. In this study we used a sensitive method collecting nasopharyngeal samples in DCCs and described the population structure in detail on the basis of serotype and MLST data.
Paper IV

The impact of vaccine introduction on carriage of pneumococci was determined by a second collection of nasopharyngeal samples among children in DCCs after introduction of PCV7 in the Childhood Immunisation Programme. Using study III as a baseline we described changes in serotype distribution and clonal shifts.

Paper V

In the carriage studies (Paper III and IV), high proportions of co-colonisation were evidenced. In paper V we used this to explore whether the CSP-pherotype restricts co-colonisation, as an indication for the impact of pherotypes on clonal evolution in vivo.
4. Methodological considerations

4.1 Surveillance of invasive pneumococcal disease
Surveillance of IPD, vaccine uptake and estimation of vaccine effectiveness are considered functions of NIPH according to the law (Law on communicable diseases (Smittenvernloven) § 7-9). Goals for the surveillance of the Norwegian PCV7 vaccine programme effectiveness were set out at the time of vaccine introduction in a document worded by a group appointed by NIPH, “Programplan for overvåkning av systemisk pneumokokksykdom i forbindelse med innføringen av pneumokokk konjugatvaksine i barnevaksinsjonsprogrammet i Norge”. This plan for enhanced surveillance was based on data sources at NIPH, including MSIS, SYSVAK and analyses performed at the National Reference Laboratory for Pneumococci.

4.1.1 IPD case definition
IPD was defined as the identification of *S. pneumoniae* from a normally sterile body site, including positive culture from blood or CSF, or detection of *S. pneumoniae* in CSF by antigen tests or nucleic acid amplification.

4.1.2 IPD notification and surveillance
Since 1977 notification of IPD cases to MSIS has been statutory. Cases are reported by both the diagnostic microbiology laboratory and the clinician, including data on clinical manifestations and previous vaccination.

4.1.3 Surveillance of serotype-specific IPD
Viable pneumococcal IPD isolates are forwarded to the National Reference Laboratory for Pneumococci at NIPH, confirmed as *S. pneumoniae* and serotyped. The results are reported to MSIS. Starting at the introduction of PCV7, cases notified to MSIS with missing serotype data were identified and missing isolates were requested from the diagnostic laboratory. The proportion of isolates received at NIPH was
approximately 85% in the first half of the 2000’s, and increased to approximately 95%
% after vaccine introduction.

4.1.4 Definition of vaccine failure
Vaccine failure following primary vaccination (i.e. two-dose primary series at 3 and 5
months of age) was defined as IPD caused by a PCV7 serotype occurring more than
two weeks after the second vaccine dose and before administration of the third dose
or before the child had reached an age of 13 months.
Vaccine failure following complete vaccination (i.e. booster dose at 12-13 months of
age after primary series, or two vaccine doses administered with at least two months
interval in the second year of life) was defined as IPD caused by a PCV7 serotype
more than one week after complete vaccination.

4.1.5 Surveillance of vaccine uptake and identification of vaccine failure
Immunisations administered in the Norwegian Childhood Immunisation
Programme are routinely registered in SYVAK. Data on vaccine uptake in cohorts
targeted for vaccination were obtained from SYVAK. Possible cases of vaccine
failure, i.e. IPD caused by a PCV7 serotype in a child born in 2006 and onwards, were
investigated by checking immunisation status in SYVAK.

4.2 Studies of pneumococcal carriage
Nasopharyngeal carriage of pneumococci was examined in two cross-sectional
studies among healthy children attending day-care centres (DCCs). The studies were
performed following a protocol approved by the Regional Committee for Medical
Research Ethics, Southern Norway. Concession for handling of health related
information was obtained from the Data Inspectorate, and the Norwegian
Directorate of Health approved establishing a research bio-bank in relation to the
project. Children were included in the study following informed and written consent
by parents/guardians.
4.2.1 Study population

DCCs in three municipalities in suburban areas around Oslo were contacted and invited to participate in the carriage studies. In 2006 and 2008, respectively, 29 and 27 DCCs agreed to collaborate. Recruitment to the studies was performed after information by letters and leaflets, and information meetings in several DCCs. In both study years, sampling was performed during the autumn months, mainly September and October.

4.2.2. Bacterial specimen collection

A nasopharyngeal bacterial specimen was obtained from the participating children. Sampling was performed by medical doctors; the vast majority (> 95%) of samples was obtained by one medical doctor (DFV). With the participant’s head slightly tipped backwards and the tip of the nose gently lifted, a flexible wire shaft with a rayon bud (Medical Wire & Equipment, Wiltshire, UK) was inserted through a nostril, parallel to the floor of the nasal cavity, until meeting slight resistance at the posterior pharyngeal wall, about one-half to two-thirds of the distance from the nostril to the ear lobe. The swab was rotated and preferentially kept in place for 5 seconds before removal. It was then inserted into a tube containing 1.5 ml of broth from beef infusion, enriched with 5% horse serum and 3.3% defibrinated horse blood (Statens Serum Institut, Copenhagen, Denmark). The swabs were transported to the laboratory and further processed within 3-4 hours.

4.3 Microbiological methods

4.3.1 Culture of S. pneumoniae

Pneumococcal isolates from IPD patients were received at the National Reference Laboratory for Pneumococci. Samples were plated onto Columbia horse blood agar and incubated in 5% CO$_2$ overnight before species confirmation, serotyping and antimicrobial susceptibility testing.
The swabs obtained in carriage studies were transported in serum broth from beef infusion. In the laboratory, the swabs were plated onto a non-selective chocolate agar and a Columbia horse blood agar containing 5.0 μg/ml gentamicin. The swabs were then re-inserted into the respective enrichment broth used for transport. Both plates and broth were incubated in 5 % CO₂ overnight.

All pneumococcal isolates were stored in Greaves medium at -80°C (38).

4.3.2. Serotyping of S. pneumoniae
Presence of pneumococci in nasopharyngeal specimens was detected by direct serotyping of all enrichment cultures, using a commercial kit for latex agglutination (Pneumotest-Latex kit, Statens Serum Institut, Copenhagen, Denmark). By this method, presence of multiple serotypes in one sample is easily detected. Growth on the selective blood agar was examined for α-hemolytic colonies with typical pneumococcal morphology to confirm the findings from the broths. If several serogroups/-types were identified in the enrichment culture, up to 16 colonies were passaged in the attempt to identify the different strains.

Final confirmation of pneumococcal serotype was determined for both carriage and IPD isolates by the Quellung reaction using specific antisera (Statens Serum Institut, Copenhagen, Denmark). Serotype 6C, which was described after the onset of the project (202), was confirmed in all carriage isolates and in selected IPD isolates by re-typing of isolates earlier classified as serotype 6A, using the 6d-factor serum. The more recently described serotypes 6D (139) and 11E (28) were not differentiated from serotypes 6B and 11A, respectively.

4.3.3. Antimicrobial susceptibility testing
The disc diffusion method was applied for antimicrobial susceptibility testing of all IPD isolates prior to 2009. Testing was performed on Mueller-Hinton agar with 5 %
horse blood (before 18.12.2006: Neo-Sensitabs, Rosco Diagnostics, Taastrup, Denmark; after 18.12.2006: BD BBL Sensi-Discs, Becton, Dickinson and Company, Maryland, USA). Screening for non-susceptibility to β-lactam antibiotics was performed with a 1 μg oxacillin disc. The MIC value was determined for IPD isolates with reduced susceptibility to one or more antimicrobial using the antimicrobial gradient strip diffusion method (AB Biodisk, Solna, Sweden). The MIC value was determined for all isolates obtained in the carriage studies. The isolates were tested for susceptibility against penicillin G, cefotaxime, ceftriaxone, erythromycin, clindamycin, and tetracycline, using the pneumococcal strains ATCC 49619 and TIGR4 as quality control strains. Susceptibility was reported according to breakpoints from the Clinical Laboratory Standards Institute (35).

4.3.4 DNA extraction
Genomic DNA was isolated by boiling of a 1 μl loopful of bacteria in 100 μl Tris-EDTA buffer for 10 minutes. After centrifugation at 13000 rpm for 5 minutes, the supernatants were stored at -20°C.

4.4 Genetic analyses

4.4.1 Multilocus sequence typing
MLST was performed on all isolates from the carriage studies. Briefly, internal fragments of seven housekeeping genes (aroE, gdh, gki, recP, spi, ddl and xpt) were sequenced. Alleles and STs were assigned according to MLST database (http://www.mlst.net). Novel alleles and STs were submitted to the curator of the database. Clonal relationships among the strains were visualised using eBURST version 3 (http://eburst.mlst.net). STs sharing 6 of the 7 MLST alleles were named single locus variants (SLVs), and groups of STs connected by SLVs were assigned to clonal complexes (CCs). STs belonging to internationally spread and antibiotic
resistant clones described by the Pneumococcal Molecular Epidemiology Network (PMEN) were identified.

4.4.2 Characterisation of comC alleles and pneumococcal phenotype
Two methods were used for sequencing of the comC gene. In the first method, primers complementary to Arg-tRNA and Glu-tRNA genes located upstream and downstream of the comCDE operon were used for amplification as described by Håvarstein (109). The comC sequence was obtained using an internal sequencing primer, NPARG: 5’-CGAACGGTCGCAGGTTCGAATCCTGCTGGGATC-3’ (L. S. Håvarstein, personal communication). An alternative method, as described by Cornejo (37), was employed if a comC sequence was not obtained by the former method. The comC sequences were translated into amino acid sequences and the corresponding CSP and phenotype were assigned using published sequences (150).

4.5 Statistical analyses

4.5.1 Incidence rates and incidence rate ratios
IRs of IPD were calculated in Microsoft Excel using population figures from Statistics Norway (www.ssb.no) as denominator. IR ratios (IRR) were calculated in Episheet.

4.5.2 Vaccine effectiveness
Vaccine effectiveness was determined for children aged < 5 years in 2007, the first year following vaccine introduction, using the average IR in the two pre-vaccine years 2004 and 2005 as a baseline. Vaccine effectiveness was calculated as:

\[
\text{vaccine effectiveness} = (1 - [\text{IR post-vaccine} / \text{IR pre-vaccine}]) \times 100\%.
\]

4.5.3 Significance testing
Chi-square test, Fisher’s exact test and Student’s t-test analyses were used for significance testing when appropriate. Analyses were performed in SPSS 14.0 for Windows and GraphPad Prism 5.01. P-values < 0.05 were considered significant.

4.5.4 Analysis of population diversity
The genetic diversity in the total population and within each DCC was calculated using Simpson’s index of diversity (D) (224). D is a measure of the probability that two randomly and independent samples from a population will belong to the same group and is defined as 1-λ, where λ = Σ[ni(ni-1)]/N(N-1), ni is the number of isolates with the ith ST in the population, and N is the number of isolates in the population. Confidence intervals (CI) were determined using the method described by Grundmann (87).

The classification index (C) was calculated to compare the ST distribution in the two carriage studies. C is defined as 1-Σ(ρ1ρ2/ρ), where ρ1 and ρ2 is the frequency of the ith ST in populations 1 and 2, respectively, and ρ is the frequency of the ith ST in the combined dataset. If the two populations have identical proportions of each ST the index is 0; as the populations become more dissimilar, the index increases toward 1.
5. Main results

5.1 Direct effect of a 2+1 dose PCV7 immunisation programme (Paper I)
This study was performed to establish an estimate of the effectiveness of the Norwegian PCV7 immunisation programme. This was the first published report on use of three doses of PCV7 in a 2+1 dose schedule in a national immunisation programme.

PCV7 was introduced in the Norwegian Childhood Immunisation Programme in July 2006, and the immunisation programme effectiveness was estimated already by the end of 2007. In the estimates, IPD IR in 2007 was compared with a pre-PCV7 baseline estimated as the average IR of 2004 and 2005. This method was chosen to facilitate comparison of results with estimates from the CDC’s ABC-surveillance in the United States.

A rapid decline in IPD in the birth cohorts targeted for vaccination, i.e. children born in 2006 and 2007, was evidenced with IRRs for PCV7 serotype IPD of 0.08 (95% CI, 0.02 to 0.35) among children aged < 1 year and 0.45 (95% CI, 0.25 to 0.81) among children aged 1 year. The overall immunisation programme effectiveness among children aged < 2 years was estimated to 74% (95% CI, 57 % to 85%) when accounting for missing isolates. No vaccine failure following complete primary or booster immunisation was observed by the end of 2007. Thus, it was concluded that the direct effect of the 2+1 immunisation programme was equivalent to the 3+1 schedule.

A decline of IPD due to macrolide-resistant pneumococci was also documented from baseline years to 2007 with an IRR of 0.31 (95% CI, 0.15 to 0.66) among children aged < 5 years.
5.2 Indirect effect of a 2+1 dose PCV7 immunisation programme (Paper II)
In this study, changes in IPD IRs were calculated for all age-groups from baseline years (2004/2005) to 2008. Declines in PCV7 serotype IPD were observed across all age-groups, indicating an indirect effect of the immunisation programme.

The IR of non-PCV7 IPD increased significantly in the age-group ≥ 65 years, indicating serotype replacement disease. In the overall population aged ≥ 5 years, the serotype-specific IR increased significantly for serotypes 19A, 9N, 33F and 35B.

In order to describe serotype-specific changes following vaccine introduction in relation to long-term trends, fluctuations over a period of 20 years were studied for three serotypes, serotype 1, 14 and 19A. The IR of serotype 1 increased dramatically during the mid-1990’s, and declined prior to vaccine introduction. The IR of serotype 14 increased from 2002 until vaccine introduction, predominantly due to erythromycin-resistant strains. Serotype 14 is a PCV7 serotype, and the IR declined rapidly following vaccine introduction. The IR of serotype 19A was studied over 20 years and compared to the IR of penicillin non-susceptible pneumococci (PNSP) and PNSP serotype 19A. The IR of serotype 19A has increased dramatically following implementation of PCV7 in the United States (180), and penicillin non-susceptibility within this serotype has been proposed as a selective advantage. The data presented in Study II document an increased IR of serotype 19A, with only a minority of isolates being non-susceptible to penicillin and with a relatively stable IR of PNSP serotype 19A.

5.3 Characterisation of pneumococcal carriage at onset of the PCV7 immunisation programme (Paper III)
The main objective of this study was to characterise pneumococcal carriage among healthy children in Norway and to establish a baseline from which PCV7 induced changes in the pneumococcal reservoir could be evaluated. The study was performed
during autumn months of 2006, and 611 children attending DCCs were recruited to participate.

A very high carriage rate was observed; 78.4% of the children were colonised with pneumococci, with a peak prevalence of 88.7% among the 2-year-olds. Furthermore, 12.7% of colonised children carried multiple serotypes. PCV7 serotypes accounted for 44.6% of the isolates. No association between colonisation and risk factors other than age was observed.

The results of MLST analyses revealed 102 distinct STs within the sample set, of which approximately one third were novel. A small number of STs accounted for the majority of isolates.

The distribution of genotypes within and between DCCs was examined. Half of the STs identified were shared between DCCs and accounted for the majority (83.5%) of isolates. The remaining identified STs were represented by small numbers of isolates and restricted to one DCC. Furthermore, the genotypic diversity within distinct DCC was generally below that in the overall sample set. Thus, the carried pneumococcal population was consisted of both dispersed and highly prevalent clones and of unique clones present in small numbers. The results indicated that clones spread within DCC as microepidemics and that the DCC might be regarded an epidemiological unit.

5.4 Shifts in the pneumococcal reservoir following introduction of PCV7 (Paper IV)

The impact of the PCV7 immunisation programme on pneumococcal carriage was evaluated in a second carriage study performed in 2008, identically to the study performed in 2006 (Paper III). In 2008, 602 children were recruited to participate.
The proportion of children who had been immunised at least twice with PCV7 increased from 3.4% in 2006 to 39.4% in 2008. Overall carriage and rates of colonisation with multiple serotypes remained unchanged from 2006 to 2008; carriage of PCV7 serotypes decreased from approximately 45% in 2006 to approximately 20% in 2008. Increased carriage frequency was observed for 21 distinct non-PCV7 serotypes, and was statistically significant for serotypes 9N, 16F, 24F, 33F and 35B.

The genotype distribution obtained by MLST shifted from 2006 to 2008. The number of distinct STs belonging to PCV7 serotypes decreased from 43 to 26; 27 clones disappeared and the genetic diversity decreased. Within non-PCV7 serotypes the genetic diversity, as measured by Simpson’s index of diversity, remained unchanged; the number of distinct STs increased from 63 to 73 in 2006 and 2008, respectively, including disappearance of 28 STs and emergence of 37 STs. However, among the most rapidly expanding serotypes, a limited number of STs and CCs accounted for the increased number of isolates. Only one possible serotype switch was identified in the 2008 sample set. Thus, the substantial genotypic shift in the pneumococcal reservoir observed two years after introduction of PCV7 was caused by expansion of a limited number of STs and CCs, emergence of new STs within non-PCV7 serotypes, and a concomitant disappearance of clones belonging to PCV7 serotypes.

5.5 Lack of evidence for competition between pneumococcal pherotypes in co-colonisation (Paper V)

In this study a subset of carried isolates obtained in the 2008 carriage study (study IV) was analysed further. The aim of the study was to determine the impact of CSP pherotype on co-existence of pneumococcal strains within the same host. CSP is encoded by the comC gene, and contrasting models for the role of pherotype, either limiting or facilitating genetic diversification within the species, have been proposed. In one of these models the ability of pneumococci to kill non-competent sister cells
(fratricide) belonging to other pherotypes is assumed to result in restriction of genetic diversity between pherotypes. In study V, an in vivo approach was made to test this research question; co-colonising pneumococcal strains were assigned to pherotypes and the pherotype distribution within pairs or triplets of co-colonising strains was evaluated.

CSP pherotype distribution in carried pneumococcal strains had not previously been described. We identified five comC alleles, including a novel allele, coding for the two dominating pherotypes, CSP-1 (62.7%) and CSP-2 (37.3%). The observed distribution of pherotypes in co-colonising strains was compared with the estimated pair-wise probability based on the overall pherotype distribution in the sample set. The observed distribution of pherotypes in sets of co-colonising pneumococcal strains did not differ from the probability estimate. This result indicates that co-colonisation of S. pneumoniae in healthy children is not restricted by pherotype.
6. Discussion

6.1 Measuring the impact of PCVs

6.1.1 Invasive pneumococcal disease
Well-conducted RCTs with measurements of vaccine efficacy provide the best level of evidence when describing the effects of a vaccine. Vaccine efficacy in RCTs is calculated as:

\[
\text{vaccine efficacy} = (1 - \frac{\text{IR in vaccine group}}{\text{IR in placebo group}}) \times 100\%
\]

RCTs of PCVs have been performed in various populations and geographic locations and the trial vaccine formulation was found to be highly efficacious in all these studies (17,41,152,194). However, the vaccine efficacies observed in the studies among Native Americans and in Africa, ranging from 65% to 85%, were somewhat lower than the very high vaccine efficacy of 97.4% observed in the NCKP study (17,41,152,194). Factors influencing the variation of results include differences in the populations studied, the number of vaccine doses administered and identification of cases; the NCKP study was performed in a population without excess IPD burden or risk factors, and four vaccine doses were administered versus three doses in the African studies. Furthermore, the dominating disease syndrome in the NCKP study was occult bacteremia, and it has been proposed that a more effective protection against a mild disease syndrome than more severe disease might partially explain the high vaccine efficacy observed in NCKP.

As the infectious reservoir for S. pneumoniae is the human nasopharynx, and as the response elicited by PCVs reduce acquisition and carriage of vaccine serotypes, the transmission of the bacterium in the population was postulated to be reduced following widespread immunisation, leading to herd effects. Hence, the overall effectiveness of PCVs in a population with widespread use of the vaccine is the sum of the direct effect and the indirect effect due to reduced transmission. In RCTs only
the direct effect can be determined, while indirect effects will increase over time following community-wide immunisation over a certain threshold. The indirect effect on IPD was studied in the cluster randomised trial among Native Americans. Although an indirect effect on nasopharyngeal carriage was observed (177), no significant herd effect on IPD could be measured (182).

The overall impact of vaccine introduction in the community may be assessed as the vaccine effectiveness in population-based surveillance studies; the IR in the pre-vaccine era is compared with the IR following vaccine introduction with calculation of IR ratios. Vaccine effectiveness can be expressed as as:

\[
\text{vaccine effectiveness} = (1 - \frac{\text{IR post-vaccine}}{\text{IR pre-vaccine}}) \times 100\%
\]

Although the level of evidence is highest in RCTs, the external validity may be highest in studies of effectiveness in unselected patients included by community-wide surveillance. However, such studies are unable to control for ecological bias, e.g. temporal trends in serotype specific IPD IRs, and for sampling bias, e.g. case ascertainment and changes in clinical and sampling practices.

Vaccine effectiveness can also be calculated in case-control studies, by comparing the odds of disease caused by vaccine serotypes in vaccinees and unvaccinated individuals, and substituting the IRR by odds ratio. This design may be helpful if population denominators are lacking.

The most extensive surveillance based studies of vaccine effectiveness originate from the ABC-surveillance of IPD in the United States (115,159,208,209,257,258). These studies have documented a rapid and sustained decline in IRs of vaccine serotype IPD, both in the age group targeted by vaccination and in the overall population. Hence, the overall effectiveness is a combination of direct and indirect effects.
Although vaccine effectiveness in Europe could be expected to be comparable to that observed in the United States, certain disparities demanded thorough surveillance, including differences in IPD epidemiology, serotype distribution and immunisation schedule. As licensure of the vaccine was based on a 3+1 dose schedule, there was need for documentation of population wide effects when choosing a 2+1 dose schedule. The vaccine effectiveness of the Norwegian 2+1 dose immunisation schedule among children < 2 years was estimated to be 74% (95% CI, 57 % to 85%) one year after vaccine introduction (Paper I). In the ABC-surveillance in the United States, the estimate performed for the same age group at the same time point following vaccine introduction was 78% (95 % CI, 74 % to 82 %) (257). In Figure 8 the incidence rates of IPD among children less than 5 years in the United States and Norway are shown, including one year of post vaccine surveillance at both sites. The vaccine effectiveness measured at the two sites was equivalent. However, the baseline IR in the United States was considerably higher than in Norway, probably related to the inclusion of occult bacteremias in out-patients. As the vaccine might protect more effectively against milder disease syndromes, the decline in IR, and the IRR, might have been expected to be higher in the United States than in Norway. Thus, one might speculate whether the impact of the immunisation programme actually was higher in Norway than in the United States. The ultimate measure of direct effect is the occurrence of vaccine failures; no vaccine failure was observed in Norway during the first two years following vaccine introduction (Paper I and II). By October 2011 one case of IPD caused by serotype 6B in a child after complete primary immunisation with two vaccine doses is the only vaccine failure recorded in Norway (unpublished data).
**Figure 8.** Incidence rates of invasive pneumococcal disease among children aged less than five years, according to age and year in:

a. the United States; PCV7 introduced in 2000. Reprinted from (257) with permission.

b. Norway; PCV7 introduced in 2006.

The population-based surveillance in the United States was able to document a massive herd effect; significant changes were observed in the population not targeted for vaccination and 69 % of protected vaccine serotype IPD cases were estimated to be due to the indirect effect (30,159,209). Although protection against IPD among adults and the elderly is not the aim of the immunisation programme, the indirect effect has major impact on cost-effectiveness analyses (259). A crude estimate of the indirect effect in Norway revealed that 74 % of the projected number of protected cases of vaccine serotype IPD was in the population aged 5 years or older (Paper II). Thus, a substantial indirect effect comparable to that in the United States was observed using the 2+1 dose immunisation schedule. With these observations, and including indirect costs and herd immunity, the Norwegian immunisation programme is cost-saving according to the cost-effectiveness analysis performed prior to vaccine introduction (259).

Following widespread use of PCV, however, increased IRs of IPD caused by non-vaccine serotypes have consistently been observed by surveillance (69,115,178,208) (Paper II). This will be discussed further in section 6.2.
6.1.2 Nasopharyngeal carriage

In order to understand and explain the impact of PCVs on IPD, studies and descriptions of the carried infectious reservoir is pivotal. Shifts in the reservoir impact the indirect effect on pneumococcal disease, and evolution of the pneumococcus and of the pneumococcal population occurs in the reservoir. The effect of immunisation with PCV is dependent on carriage and transmission routes in the community, as well as the immunisation schedule employed. A variety of studies, both RCTs and observational studies using different methodology and immunisation schedules in different settings, have aimed at describing the impact of PCVs on carriage.

The direct effect of PCVs on carriage has been demonstrated by RCTs (43,193,199). This effect is related to the high concentration of type-specific IgG in serum, probably leaking onto the mucosa and leading to reduced acquisition of vaccine-serotypes and reduced carriage density (43,193). Furthermore, an indirect effect due to reduced transmission of vaccine serotypes has also been documented in double-blind studies (78,193). In these studies reduced carriage was observed among siblings or household members of vaccinated children. However, increased carriage of non-vaccine serotypes have also been shown in RCTs (162,171,199).

Observational studies performed following implementation of routine immunisation can only describe the combined impact on carriage caused by the direct and indirect effect. Such studies have documented decreased carriage of vaccine serotypes, but consistently the carriage of non-vaccine serotypes have been found to increase, leaving the overall carriage frequency unchanged (57,76,128,181).

In Norway, a baseline for nasopharyngeal carriage in the pre-PCV-era was required, and a cross-sectional study was performed in DCCs in the autumn of 2006 (Paper III). Upon follow-up of this study employing the same protocol, a shift towards non-
vaccine serotypes was found in the carried reservoir, consistent with findings in other settings (Paper IV).

Thus, overall reductions of the carried pool of vaccine serotypes were evident following immunisation. However, a substantial serotype replacement was observed. This will be discussed further in the section 6.2.

6.1.3 Antimicrobial resistance

By immunisation with PCVs, an overall reduction in non-susceptibility among pneumococcal strains is expected by two mechanisms: (1) pneumococcal strains with decreased susceptibility to β-lactams and macrolides predominantly belong to vaccine serotypes and are specifically targeted by vaccination; (2) declining rates of pneumococcal disease, both mucosal and invasive, may reduce prescription rates and, consequently, the antibiotic selective pressure. Thus, PCVs might impact both the prevalence of non-susceptible strains and the use of antibiotics.

Surveillance studies in the United States have demonstrated declining IRs of IPD caused by non-susceptible pneumococcal strains following vaccine introduction (153,234). In Norway, the IR of macrolide non-susceptible strains increased rapidly from 2002 to 2005. Shortly after vaccine introduction the IR declined (Paper I), similar to the trend observed in the United States (234). The pre-vaccine increase and post-vaccine decline in macrolide resistance in Norway was confined to low-level erythromycin-resistant strains, the M-phenotype, belonging to serotype 14 and dominated by a single clone, the England14-ST9 clone (231) (Paper II).

Use of antimicrobial agents is a motive force for the spread of non-susceptible strains, and widespread use of PCV7 is expected to reduce the indication for and use of antibiotics in the community. Reductions in antibiotic use among immunised children have been documented in two RCTs, indicating causality (48,70). A
population-based study from the United States demonstrated declining rates for prescription of antibiotics, except azithromycin, from 1996 to 2003, and found an association between high rates of prescription and high rates of IPD caused by non-susceptible strains (116). In Norway, a reduced prescription rate of erythromycin was noted from 2006 to 2008. However, no reduction was observed for other orally administered antibiotics (Paper II). Among children in DCCs, both the number of respiratory tract infections and the use of antibiotics as reported by parents declined following PCV7 introduction (Paper IV).

Although reduced carriage of non-susceptible pneumococci has been demonstrated by RCTs (44,171), population-wide use of PCV7 has not been unambiguously found to reduce the overall carriage of non-susceptible strains; the reduction of PCV7 serotypes is countered by an increased prevalence of non-susceptible non-PCV7 serotypes (127,181). Among children in DCCs in Norway the prevalence of non-susceptible pneumococci was limited prior to vaccine introduction and the majority of isolates belonged to PCV7 serotypes (Paper III). In the follow-up study, reduced carriage was observed for multi-drug resistant and erythromycin-resistant strains, including the disappearance of the England14-ST9 clone (Paper IV).

6.2 Serotype replacement

6.2.1 Serotype replacement in nasopharyngeal carriage

The possibility for serotype replacement and replacement disease was discussed and predicted prior to vaccine introduction (160,233). The observed increase of non-vaccine serotypes in carriage following immunisation with PCV7 may be the result of two distinct phenomena: serotype unmasking or serotype replacement (160). Serotype unmasking refers to a situation of multiple colonisation, in which in the pre-PCV period the vaccine serotypes dominate in density, blurring the recovery of non-vaccine serotypes from nasopharyngeal samples. In the post-PCV period,
however, the density of vaccine serotypes declines, and the higher relative density of non-vaccine serotypes allows their identification, although the frequency of non-vaccine serotypes is unchanged. Serotype replacement represents a true shift in frequencies of colonising serotypes and is believed to arise by (at least) three possible mechanisms: 1) expansion and propagation of non-vaccine serotype clones present in low frequencies in the pre-PCV period; 2) introduction of new non-vaccine serotype clones; or 3) capsule switch, in which a vaccine serotype clone acquires a non-vaccine serotype capsule by horizontal gene transfer of the capsule locus.

In the carriage studies performed in DCCs in Norway, a sensitive sampling method was used, and a high proportion of co-colonisation by multiple strains was observed (Paper III and IV). Hence, true replacement rather than unmasking may be assumed to be the main mechanism leading to increased carriage of non-vaccine serotypes in Norway.

Although capsule switch has been described (25,201), expansion of existing clones has been the predominant finding in studies addressing serotype replacement (94,162,201). Analysis of clonal relationships in carriage following PCV7 introduction in Norway revealed that serotype replacement was dominated by expansion of clones and clonal complexes that were already present prior to vaccine introduction, and that, surprisingly, the genetic diversity was more or less unchanged (Paper IV).

However, it takes time to reach equilibrium following introduction of PCV and other mechanisms for serotype replacement might be evident with longer observation periods. In studies of carriage in Massachusetts, an increased contribution of serotype switching was found on a long-term follow-up post PCV7 introduction (97). From these observations it was postulated that serotype replacement in carriage might be divided into two parts. Initially, a rapid outgrowth of clones already existing in the carried reservoir dominates, but with serotype switching events
occurring. With time, the serotype switched clones may expand and become more prevalent (97).

6.2.2 Serotype replacement IPD

With observational data accumulating from surveillance sites in North-America and Western Europe, it has become evident that IPD due to non-PCV7 serotypes is indeed increasing following widespread immunisation (115,184). Four years after vaccine introduction in the United States a significant increase in non-vaccine serotype IPD was observed among children aged < 5 years and the elderly aged ≥ 65 years (115). Significantly increased serotype-specific IPD IRs among children were observed for serotype 3, 19A, 22F, 33F and serogroup 15 (115). Notably, from pre-vaccine years to 2003-2004 the IR of serotype 19A among children in the United States increased by 150 % and the proportion of antimicrobial non-susceptible strains increased (201).

The IR of serotype 19A has consistently been reported to increase in the post-PCV7 period from several surveillance sites (135,149,180,207). By 2005 serotype 19A had emerged as the most frequent cause of IPD in the United States (180). In addition, serotype 19A is commonly non-susceptible to penicillin and other antimicrobial classes, and accounts for the largest proportion of resistant invasive infections in the United States (180).

Although the magnitude of serotype replacement in IPD varies between study sites, it has been observed to temper the net effect of PCV7 immunisation programmes. Surveillance performed in England and Wales has demonstrated that the overall incidence rate of IPD in the population not targeted by vaccination has remained more or less unchanged (www.hpa.org.uk) (178). In the United States, however, declines in overall IPD IRs were observed in all age groups, and IRs have remained fairly stable since approximately four years after vaccine introduction (208).
The reason why some serotypes appear to be more successful than others in replacement is unknown. One explanation might be the relative invasive potential; serotype 19A is both a successful serotype in colonisation and has a relatively high invasive potential (24). Furthermore, the antibiotic selective pressure has been proposed to act as a promoter for replacement, and that the increased IR of serotype 19A is driven by selection of clones with decreased susceptibility to penicillin. Observations from Norway, where the antibiotic pressure is limited, demonstrate a rapid increase in serotype 19A incidence following vaccine introduction, dominated by penicillin-susceptible strains (Paper II). However, the carriage frequency of serotype 19A did not change from 2006 to 2008 (Paper IV). This finding might indicate a change of the invasive disease potential of circulating serotype 19A strains, but results from further characterisation of serotype 19A strains by MLST has not confirmed this (Vestrheim DF et al., submitted for publication)

Temporal trends have been demonstrated by long-term serotype-specific changes in IR. Such fluctuations might coincide with the shifts caused by widespread immunisation and obscure observations in population-based surveillance studies. The IR of serotype 1, a serotype that is generally susceptible to antimicrobials, has fluctuated dramatically over the past decades (69,102). The massive increase in serotype 1 IPD in Norway during the 1990’s impacted the overall IR of IPD (Paper II). In addition, the increased IR of serotype 14 IPD in Norway in the years prior to vaccine introduction was dominated by erythromycin-resistant strains (Paper II). Although the selective pressure caused by vaccine introduction is presumed to cause rapid and substantial shifts in the pneumococcal population, both temporal trends and antibiotic selection should also be accounted for. Furthermore, other unknown factors for clonal success might be of importance.
6.3 The adaptable pneumococcus

Man is the sole host for *S. pneumoniae*. Transmission of pneumococci is influenced by changes in contact patterns, living conditions and socioeconomic factors (126). The bacterium is challenged by immune responses of the host, and by antibiotic use. Hence, the pneumococcus has to adapt in order to survive.

During the pre-PCV era of the 20th century, significant shifts in the serotypes causing invasive disease occurred. From being dominated by the lower numbered serotypes, i.e. serotypes 1, 2 and 5, in the first half of the century, trends towards higher proportions of less invasive serotypes, e.g. serogroup 6, 19 and 23, were observed (64,102). The epidemic potential of serotypes 1, 2, 3 and 5 is indicated by peaks of IPD incidence and further documented by recent outbreaks of serotypes 1 and 5 (102,155,216). The biological basis for such temporal trends is largely unknown, but might be related to societal and immunological factors in the host population.

The massive challenge imposed on the pneumococcus by the introduction of PCV7 at the turn of the millennium has resulted in substantial serotype shifts in the pneumococcal reservoir, reflected in the strains causing IPD. Although events of serotype switch have been documented (25,39,97), the extent to which recombination and chromosomal plasticity contributes to serotype replacement is unclear. In recent publications from the United States it appears that serotype replacement levels off approximately four to six years after vaccine introduction (14,93,208). However, clonal shifts in the populations might continue, e.g. leading to increased rates of antimicrobial non-susceptibility (14).

The introduction of antimicrobial agents during the 20th century offered new challenges for the pneumococcus, and adaptation by accumulation of resistance mechanisms has occurred. Adaptation may occur as a result of chromosomal
alterations, either by horizontal gene transfer or by accumulation of base substitutions by mutations. The pneumococcus is a recombinogenic bacterium, allowing uptake and exchange of chromosomal fragments. This is typically evidenced as an adaptation to the selective pressure caused by penicillin by changes in genes encoding PBP{s} (60,90).

In a recent study by Croucher et al., the phylogeny of a multidrug resistant clone, the Spain23F-1-ST81 clone, was investigated (39). Evolution of this highly resistant and widely distributed clone occurred over a short time period, and chromosomal changes were evident in resistance determinants, as well as in recombinational hotspots in genes coding for surface proteins. Hence, the pneumococcus has potential for rapid adaptation to both immunological and antimicrobial pressure. However, by studies of pneumococcal clones in the MLST database, presence of certain hyper-recombinogenic clusters in the pneumococcal population has been described, and the potential for adaptation has been suggested to vary between clusters within the species (98).

Several attributes may impact gene flow within the species. One example is the insertion of the defective transposon carrying the mef(A) resistance determinant, Tn1207.1, into a gene associated with recombination, celB; the pneumococcal strains carrying this determinant appears to be highly clonal, belonging to the England14-ST9 clone (52). Another mechanism by which clonal evolution might be affected is through fratricide; killing of non-competent sibling cells by lysins produced by pneumococci in the competent state (88). It has been postulated that bacteria belonging to the same pherotype, i.e. producing and responding to the same CSP, are protected from lytic activity during the competent state due to an immunity protein encoded by comM (111). However, two opposing models have been proposed for the impact of CSP polymorphisms, or pherotypes, on genetic differentiation: 1) limited inter-pherotype recombination induction of competence is restricted to occur within
pherotype (110,134,245); 2) no restriction as competence leads to lysis (fratricide) of cells belonging to a different pherotype due to lack of immunity (140).

As horizontal gene transfer is believed to occur during co-colonisation, we investigated comC polymorphism in sample sets obtained from co-colonised carriers (Paper V). Clonal distribution of the two major pherotypes was evident by MLST and the two pherotypes were randomly distributed among the sample sets. The results of Study V indicate that pneumococcal pherotypes can co-exist during co-colonisation in an asymptomatic host, and apparently are not impacted by fratricide restricted by pherotype. Likely events of intra-host recombination were detected in this study. However, further work is warranted to describe pneumococcal micro-evolution during co-colonisation.
7. Perspectives

A very high effectiveness of the PCV7 immunisation programme has been documented in Norway by surveillance of IPD incidence and vaccine uptake; the IR of IPD has successfully been reduced in the target population. The effectiveness is further documented by the great rarity of occurrence of vaccine failure. Furthermore, an indirect effect has been evidenced by declines of IPD incidence rates in the overall population (Figure 9). This population-based surveillance was enabled by established routines for notification of IPD cases, strain referral and characterisation, a system for registration of immunisations, and by population denominators available from Statistics Norway. Importantly, long-term surveillance prior to vaccine introduction provided a basis for discussion of fluctuations and shifts caused by either the vaccine or by temporal trends.

![Figure 9](image)

**Figure 9.** Cases of IPD notified to MSIS per year, 1977 to 2010. PCV7 was introduced in July 2006, as indicated by the shaded column.

Worldwide, however, the pneumococcus continues to cause substantial morbidity and mortality, especially among children in developing countries. In these countries,
where the IRs and mortality rates of pneumococcal disease are the highest, access to vaccines has been limited. A high benefit of conjugate vaccines is expected, although the impact of different serotype distributions and societal factors found in these settings are difficult to foresee. Hopefully, vaccine introduction might be realised and speeded by help of the GAVI Alliance.

However, high quality IPD surveillance data are lacking in developing countries, and population-based surveillance should be established prior to vaccine introduction in selected sites. Results from poorly conducted surveillance might not be valid, and the balance between vaccine serotype reductions, serotype replacement and temporal trends might easily be misinterpreted by incomplete surveillance.

PCVs do not reduce overall transmission of pneumococci among colonised children. Replacement disease is likely to occur as long as the serotype coverage of available vaccines is limited. Therefore, further research on alternative immunisation strategies should be continued. Possible vaccine candidates might consist of combinations of selected pneumococcal proteins common to all serotypes, or killed whole-cell vaccines.

With widespread use of PCVs, evaluation of new vaccine candidates is becoming problematic. RCTs examining the protective efficacy of IPD are unethical. Studies of non-inferiority based on serological correlates have been performed for the higher valency PCVs. For novel vaccine candidates, impact on carriage studies might be used as a surrogate marker for vaccine efficacy.

The licensure of PCV13 for use in adults is pending. Although the impact of conjugate vaccines among the elderly is uncertain, the disease burden due to pneumococci is large, and the potential for protection is substantial. This potential is
of importance in developed countries such as Norway, where the population of elderly and immunocompromised individuals is increasing.

In conclusion, introduction of PCV7 has had a substantial impact on pneumococcal disease in Norway and, with the transition to PCV13 in 2011, further declines in IPD IRs are expected. However, the greatest disease burden of pneumococci is in developing countries and a substantial reduction in morbidity and mortality may be achieved by introduction of currently available vaccines in these settings.
8. References


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