Respiratory Tract Infections: Aspects of Aetiology, Virulence, and Communicable Disease Control

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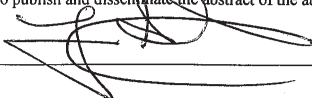Institutionen för Medicin Karolinska Solna,
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Abstract

The paediatric nasopharyngeal flora is regarded as the largest reservoir for *Streptococcus pneumoniae*, and the carrier state is always antecedent to infection and a prerequisite for dispersion of these bacteria. Pneumococci are the predominant aetiology of bacterial respiratory tract infections and a major cause of morbidity and mortality, in the most severe cases due to invasive pneumococcal disease (IPD; mainly sepsis and meningitis). The development and spread of resistant pneumococci are facilitated in day care centres (DCCs), which constitute an optimal environment for these processes. In Sweden, penicillin non-susceptible pneumococci (PNSP) have remained relatively uncommon, an important aspect considering that penicillin is the drug of choice for respiratory tract infections. When a tendency towards increasing PNSP was noted in Skåne County in southern Sweden, a DCC intervention program including screening started when an attending child has been found to be PNSP carrier. To restrict dispersion, all carriers were suspended from DCCs until they were declared free from PNSP. Today, there is no scientific proof that such DCC interventions can effectively restrict PNSP dispersion on a community level.

Our retrospective study of the DCC interventions showed that 5% of the children were PNSP carriers during an outbreak. Personnel were rarely carriers (0.4%) and, if so, for only a very short time. PNSP was found a long time after the intervention started in a few children cultured late due to absence from the DCC for other reasons, indicating a long-lasting risk for dispersion. Furthermore, PNSP carriage was observed in a substantial number of children at DCC departments other than the department attended by the index case, indicating that the index case is not always at the centre of an outbreak. There was also significant seasonal variation seen as lower carrier rates after major holidays, indicating that these rates decline when children are not at DCCs. Day care group size and young age proved to be risk factors for pneumococcal carriage. Our findings can support development of future guidelines for managing PNSP outbreaks in DCCs. Eradication therapy of children with prolonged PNSP carriage was effective, but none of the treated children harboured any highly resistant or multidrug-resistant strains.

Our retrospective study of IPD demonstrated that pneumococcal serotypes differ regarding their capacity to cause septic shock and, together with age and co-morbidities, have an important impact on outcome. The primary endpoint in our investigation was septic shock, a state produced by the immune system and triggered by the invading microorganism. This parameter was chosen instead of the case fatality rate (CFR), which is usually studied as outcome but is biased because serotypes with a low CFR infect healthier and younger individuals and vice versa. Septic shock was significantly more common among patients infected with serotype 3 compared to those with serotype 14, a worrisome finding since the effect of the conjugate vaccine on this serotype seems to be uncertain.

Ventilator-associated pneumonia (VAP) is a common infection and complication in intensive care units. We found that the bacterial aetiology in VAP differed depending on whether the patients were receiving antibiotics at the time of the VAP diagnosis. *Pseudomonas aeruginosa* was a surprisingly widespread cause of early-onset VAP, but most of the patients had been treated with antibiotics. There was a trend towards more resistant bacteria in late-onset VAP.

Key words
Streptococcus pneumoniae, day care center, communicable disease control intervention, eradication therapy, invasive pneumococcal disease, serotype 3, aetiology, Ventilator-associated pneumonia

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Respiratory Tract Infections

Aspects of Aetiology, Virulence, and Communicable Disease Control

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Abbreviations

ATS	American Thoracic Society
BAL	bronchoalveolar lavage
BSA	broad-spectrum antibiotic
CAP	community-acquired pneumonia
CDC	Centers for Disease Control and Prevention (Atlanta, GA, USA)
CFR	case fatality rate
ChoP	phosphorylcholine
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	computed tomography
DCC	day care centre
DRSP	drug-resistant *Streptococcus pneumoniae*
ESBL	extended spectrum beta-lactamase
FiO$_2$	fraction of inspired oxygen
HAP	hospital-acquired pneumonia
HCAP	healthcare-associated pneumonia
ICCR	Intensive Care Complication Register
ICU	intensive care unit
IPD	invasive pneumococcal disease
IVAC	infection-related ventilator-associated complication
MALDI-TOF MS	matrix-assisted laser desorption ionization-time-of-flight mass spectrometry
MDR	multidrug-resistant
MHC	major histocompatibility complex

MIC	minimum inhibitory concentration
NIV	non-invasive ventilation
OCCs	oropharyngeal and cutaneous commensals
PBP	penicillin-binding protein
PcG	pencillin G
PCR	polymerase chain reaction
PCT	procalcitonin
PEEP	positive end-expiratory pressure
$PNSP_{0.5}$	penicillin non-susceptible pneumococci; PcG MIC of ≥ 0.5 mg/L
$PNSP_2$	penicillin non-susceptible pneumococci; PcG MIC of ≥ 2 mg/L
PNSP	penicillin non-susceptible pneumococci; PcG MIC of ≥ 0.125 mg/L
PSB	protected specimen brush
PspA	pneumococcal surface protein A
PspC	pneumococcal surface protein C (can be referred to as CbpA or SpsA in other literature)
RCCDC	Regional Centre for Communicable Disease
SRGA	Swedish Reference Group for Antibiotics
SSPIP	South Swedish Pneumococcal Intervention Programme
TBA	tracheobronchial aspirate
TMP-SMX	trimethoprim-sulfamethoxazole
UTI	urinary tract infection
VAC	ventilator-associated condition
VAP	ventilator-associated pneumonia

Summary

The paediatric nasopharyngeal flora is regarded as the largest reservoir for *Streptococcus pneumoniae*, and the carrier state is always antecedent to infection and a prerequisite for dispersion of these bacteria. Pneumococci are the predominant aetiology of bacterial respiratory tract infections and a major cause of morbidity and mortality, in the most severe cases due to invasive pneumococcal disease (IPD; mainly sepsis and meningitis). The development and spread of resistant pneumococci are facilitated in day care centres (DCCs), which constitute an optimal environment for these processes. In Sweden, penicillin non-susceptible pneumococci (PNSP) have remained relatively uncommon, an important aspect considering that penicillin is the drug of choice for respiratory tract infections. When a tendency towards increasing PNSP was noted in Skåne County in southern Sweden, a DCC intervention program including screening started when an attending child has been found to be PNSP carrier. To restrict dispersion, all carriers were suspended from DCCs until they were declared free from PNSP. Today, there is no scientific proof that such DCC interventions can effectively restrict PNSP dispersion on a community level.

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Populärvetenskaplig sammanfattning (Summary in Swedish)

Barn är ofta bärare av luftvägsbakterien pneumokocker i de övre luftvägarna och det är barnen som anses vara den största reservoaren för bakterien i vårt samhälle. Vanliga pneumokockinfektioner såsom öron-, bihåle- eller lunginflammation föregås av att man bär på bakterien. De flesta barn som är bärare blir dock inte sjuka. Vuxna är mer sällan bärare av bakterien.

Pneumokocker som har utvecklad nedsatt känslighet mot penicillin kallas PNSP och utvecklas och sprids mycket effektivt i förskolemiljöer. Barnen på förskola får ofta antibiotika (så att de bakterier som har plockat upp resistensegenskaper från omgivningen gynnas), de bär på pneumokocker länge och vistas i trånga utrymmen tillsammans med många andra barn, vilket ger pneumokocken perfekta förutsättningar att spridas. PNSP är mycket vanligt i större delen av världen men i Skandinavium har klarat av att hålla förekomsten av PNSP på en relativt låg nivå. Detta är viktigt för att vi skall kunna behålla vanligt penicillin som behandling för våra vanliga luftvägsinfektioner.

När det på 1990-talet noterades en ökning av PNSP i Skåne startade Smittskyddsenheten ett projekt med ett antal åtgärder (interventioner) för att minska spridningen av PNSP på förskolor. När ett förskolebarn har visat sig ha PNSP i luftvägarna vidtas en serie åtgärder: En screening görs på barn i det första barnets närmiljö och på förskolan för att fånga nya bärare av PNSP. Vidare stängs alla barn, som är bärare, av från förskolan och får inte komma tillbaka förrän de förklarats fria från PNSP. Interventionerna görs för att förhindra vidare spridning.

Det saknas fortfarande vetenskapliga studier som visar att metoden är effektiv ur ett samhällsperspektiv. Vi har därför studerat utfallet av de förskoleinterventioner som gjorts i samband med PNSP-utbrott under en tioårsperiod i Skåne. Vi hittade PNSP hos fem procent av alla barn som screenades men det visade sig att det var mycket ovanligt hos personalen. Dessutom var personal som drabbades, bärare av PSNP under en mycket kort tid. Vi hittade PNSP hos barn som återvände till förskolan efter en längre tids frånvaro på grund av andra orsaker. Detta indikerar att risken för spridning av PNSP är långvarig om ingen intervention genomförs. Det var det relativt vanligt att

vi hittade PNSP även på andra avdelningar. Därför tror vi att man borde genomföra screeningen så snart man kan och direkt inkludera hela förskolan. Vi fann också signifikanta variationer i hur många barn som var bärare av pneumokocker beroende på tid på året. Det ser ut som att andelen bärare går ner efter stora helger och lov då barnen inte är på förskolan, vilket skulle tala för att frånvaro från förskolan minskar antalet bärare av pneumokocker. Det visade sig även att barn i som gick i större grupper på förskolan var bärare av pneumokocker i högre utsträckning.

I en annan studie vi genomfört har vi sett att det går bra att behandla bort bärarskap av PSNP med antibiotika. Dessa barn var inte infekterade utan behandlingen genomfördes av sociala och ekonomiska skäl för att de skulle bli av med sitt bärarskap. Vår förhoppning är att våra vetenskapliga resultat skall ligga till grund för riktlinjer för hur man skall hantera PNSP på ett så bra sätt som möjligt.

Pneumokocksjukdomar är en vanlig orsak till sjuklighet och dödlighet i hela världen. De flesta dödsfallen av pneumokocksjukdom sker i utvecklingsländer i så kallad invasiv pneumokocksjukdom (IPD), vilket oftast utgörs av blodförgiftning (oftast i samband med lunginflammation) och hjärnhinneinflammation. Det finns idag 93 kända olika typer av pneumokocker, de kallas serotyper och de klassas efter hur kapseln runt bakterien ser ut. Det finns idag ett vaccin som ges till barn riktad mot de vanligaste serotyperna.

Vi har studerat invasiva pneumokocksjukdomar, dvs infektioner som framför allt spritt sig till blodet. Resultaten stödjer teorin att finns en skillnad mellan olika serotyper när det gäller att orsaka svår sjukdom. Vissa serotyper drabbar oftare äldre patienter med underliggande kroniska sjukdomar medan andra serotyper oftare drabbar relativt friskare och yngre patienter.

I tidigare studier har det visats att vissa serotyper orsakar högre dödlighet och sjukare patienter vid invasiv infektion. Då dessa serotyper också drabbar patienter som är äldre och sjukare i grunden så blir jämförelsen lite haltande även om man i vissa studier försökt att kompensera för dessa skillnader i statistiska analyser. Vi valde därför att studera i vilken utsträckning patienterna fick septisk chock (svårbehandlad cirkulationssvikt i samband med blodförgiftning) – ett sjukdomstillstånd som orsakas av kroppens eget immunförsvar. Hypotesen var att vissa serotyper skulle ge mer chock än andra. Det visade sig att serotyp 3 gav signifikant mer septisk chock jämfört med serotyp 14 även om det drabbade äldre och sjukare patienter med svagare immunförsvar. Generellt visade det sig att de mest kapslade serotyperna gav svårare sjukdom. Att serotyp 3 gav mest chock är lite oroväckande då det nya vaccinet inte ännu på ett övertygande sätt visat att det har en bra skyddseffekt mot serotyp 3.

Respiratorassocierad lunginflammation är den vanligaste infektionen på en intensivvårdsavdelning. Då patienterna är svårt sjuka är det svårt att ställa denna diagnos och samtidigt är det viktigt att ge rätt typ av behandling tidigt för att minska risken för dödsfall. Dessutom är resistenta bakterier och bakterier som normalt inte orsakar lunginflammation mycket vanliga i denna miljö, vilket ytterligare försvårar valet av antibiotika.

De flesta studier angående detta har genomförts i Västeuropa eller i Nordamerika, där bekymret med resistenta bakterier är betydligt större. Det är viktigt att kunna identifiera patienter som kan få en smalare antibiotika behandling och vice versa. Vi har därför studerat vilka bakterier som orsakar respiratorassocierad lunginflammation beroende på vilken fas av vårdförloppet patienten befinner sig och om patienten redan står på antibiotika.

Vi fann att den bakteriella orsaken skilde sig åt beroende på om patienten hade fått antibiotika eller inte. Det var mer samhällsrelaterade bakterier (dvs bakterier som kan ge lunginflammation även ute i samhället) om de inte fått antibiotika. Vi såg vidare en trend mot mer resistenta bakterier om patienten hade legat i respirator i mer än en vecka. Den svårbehandlade bakterien Pseudomonas aeruginosa var, lite oväntat, vanligt förekommande i ett tidigt skede under respiratorbehandlingen men nästan alla dessa patienter hade fått antibiotika. Vi hade inga bakterier med extended spectrum betalactamses (ESBL) och heller inga meticillin resistenta Staphylococcus aureus (MRSA), vilka är vanliga i andra länder. De resistenta bakterier vi fann visade sig också ha en, relativt sett, lägre grad av resistens vid en jämförelse med Västeuropeiska och Nordamerikanska studier.

Introduction

Bakground

History

At the dawn of civilization, our ancestors regarded disease as a consequence of the intervention of spirit forces, and this was later translated into terms of divine punishment or "devil theory". Interestingly, the Chinese and early Greeks shared the concept of disharmony as a cause of disease.

The germ theory of disease was the first hypothesis in this context that could provide scientific proof. This theory may have had its beginnings in ancient Egypt, which left many records of the malign power of a substance called "*ukhedu*". Notably, according to the descriptions, *ukhedu* behaved in a manner remarkably like bacteria, and it was usually dormant but could migrate through the body and cause disease. In 1560, "*De contagione et contagiosis morbis*" was published by Girolamo Fracastoro, who was a physician and professor of philosophy at the University of Padua in Italy. He thought that imperceptible particles were the source of contagion, and that they could be transmitted through the air and had the power of rapid self-multiplication. Antonie van Leeuwenhoek, the father of microbiology, later described these particles as living single-cell organisms, which he originally referred to as animalcules in 1688. Using handcrafted microscopes, he was also able to report that animalcules could be killed with vinegar and heating [1].

The aetiology of pneumonia

Streptococcus pneumoniae (pneumococcus) was one of the first pathogens to be isolated and characterized, and this was done in the late 19th century. Edvin Klebs probably described the pneumococci in 1875 [2], although these bacteria were first isolated, described, and cultured in 1881 by Louis Pasteur [3] and Georg Sternberg [4] working independently. A few years later, Fraenkel concluded that Pasteur, and Sternberg had described the same bacteria, which were the cause of lobular pneumonia [2]. In the 19th and early 20th centuries, pneumococci were responsible for the aetiology of 50–90% of the cases of pneumonia [5-7], and pneumonia was a feared disease at that

time. In 1901, Sir William Osler [8] wrote the famous words "the most widespread and fatal of all diseases, pneumonia, is now Captain of the Men of Death" in his book entitled "The Principle and Practice of Medicine" [8]. Today, in the era of antibiotics, pneumonia is still a common cause of death, especially among the elderly. The time-honoured saying that "pneumonia is the old man's best friend" is still used when an old and sick person with limited quality of life dies of pneumonia. The pneumococcus is the subject for study in Paper I-III.

When 20-100 million people were killed by the Spanish flu in 1918, the world began searching for the aetiology. Many contemporaneous investigators erroneously believed that bacteria was the cause of influenza. Richard Pfeiffer incorrectly concluded already in the 1889-1892 pandemic that the cause was Pfeiffers bacillus or Bacillus influenzae, now known as Heamophilus influenzae [9]. However, the virus that actually caused the Spanish flu remained undetected for another decade, and we now have proof that most of the deaths that occurred during the pandemic were due to secondary pneumococcal pneumonia [6, 7].

The discovery of penicillin

Sir Alexander Fleming discovered penicillin in 1928, but he found it difficult to refine the mould to produce a usable drug and failed to present convincing clinical results regarding its effects. Fleming had to abandon his trials in 1940, but in the same year the Oxford researchers Howard Florey and Ernst Boris Chain took up the work again and were successful in treating mice. In 1941, the first patient was treated with penicillin and started to improve, but nonetheless died due to shortage of the antibiotic [1]. These researchers continued their studies, and penicillin was mass-produced a few years later by large pharmaceutical companies and with supplementary funding from the US and British governments. At the end of the Second World War, there was sufficient capacity to produce larger amounts of penicillin, and the drug was crucial for treatment of soldiers with infections.

Fleming, Florey, and Chain received the 1945 Nobel Prize in Physiology and Medicine for their work on penicillin. Indeed, penicillin is one of the greatest discoveries of our time, and it has dramatically changed the rate of survival from severe infections [10]. In the United States alone, antibiotic treat-

Figure I. Advertisment for penicillin during the Second World War.

ment has increased the average life expectancy by ten years [11]. The difference in mortality in invasive pneumococcal pneumonia after the introduction of penicillin is shown in figure II.
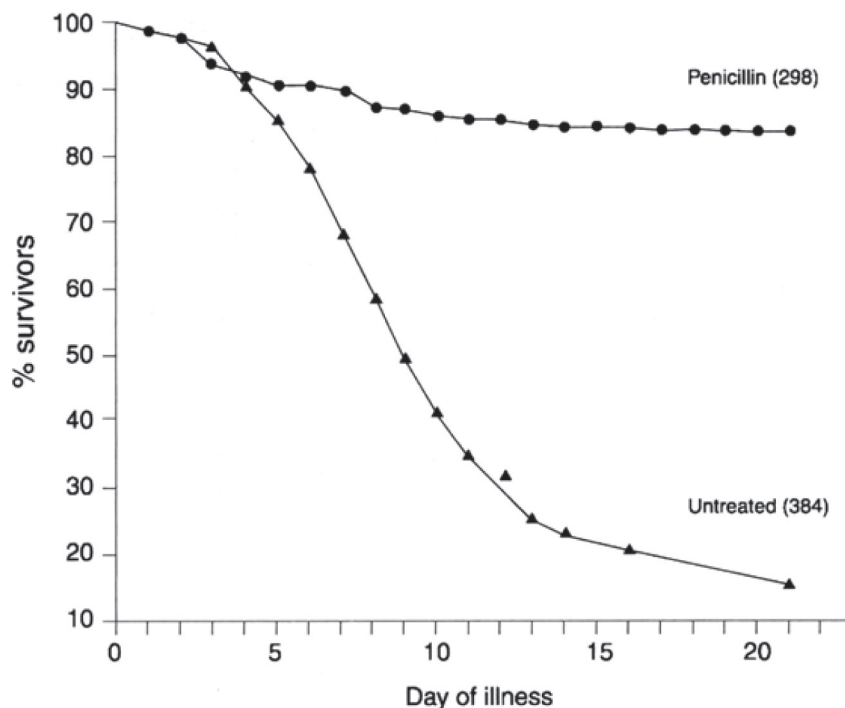
Figure II. Mortality in invasive pneumococcal pneumonia in the pre and post-antibiotic era From: Austrian et al, J Ann Intern Med 1964;60;759-76

The evolution of resistance

Resistance to antimicrobials is a very old and natural property of microorganisms, as demonstrated by the recent discovery of highly resistant and multi-resistant bacteria in a region of the Lechuguilla Cave (New Mexico, USA) that has been isolated from humans for over four million years [12]. Moreover, there is a growing body of evidence implicating organisms in the environment as reservoirs of resistance genes, and the selective pressure from the use of antibiotics promotes the dispersion of these genes.

The existence of antibiotic-resistant pneumococci is now an established fact. Since the introduction of penicillin, many other antibiotics have been marketed and used in medical treatment around the world, but this has always been associated with development of resistance in bacteria. At present, the flow of new antibiotics is rather slow, and the United Nations has proclaimed resistance to antibiotics to be a serious threat to humankind [13].

The modern patient

During the 20th century there was a tremendous improvement of sanitary and living conditions in the industrialized world. Meantime were medical treatment and vaccines developed. This has led to a longer life expectancy and many people can today survive despite multiple and more severe comorbidities [14].

Immunosuppressive therapy has also been introduced for a wide variety of diseases, and has rendered the patients more vulnerable to infections, often presented with vague symptoms in this group of patients. In addition, more antibiotic treatment and more extensive contacts with the health care system has led to numerous different aetiological agents, but pneumococcus has maintained its position as the most common cause of community-associated pneumonia [15]. The aetiology of pneumonia differs depending on whether the infection is acquired in the community, in a primary health care facility, or in a hospital. More advanced health care settings with weaker and more vulnerable patients, more invasive treatments, and higher pressure from antibiotics create a perfect breeding ground for resistant and opportunistic bacteria. This is well illustrated by intensive care patients who develop ventilator-associated pneumonia (VAP), which is the subject of interest in Paper IV [16].

The defence of the airways

The airways have a stunning array of mechanisms to keep the lungs uninfected, even though those organs are constantly exposed to microbes that enter in the inhaled air and by way of microaspiration. However, recent research with culture-independent methods demonstrates that the lungs of healthy never-smokers are inhabited by bacteria and not sterile as we always believed. However, the role of the microbiome of the lung is unclear [17]. A defect in the host's defence mechanisms, excessive numbers of microbes, or introduction of a particularly virulent microorganism can cause an infection in the lower respiratory tract. The defence mechanisms are anatomical, mechanical, and immunological. Those designated immunological comprise both the adaptive and the innate immune responses, which are committed to maintaining sterile conditions in the lower airway.

The mechanical, structural, and anatomical defence mechanisms

The nasal mucosa has a ciliated epithelium that captures microorganisms, and this process is facilitated by humidification and secretion of mucus in the upper airway. Furthermore, the turbulence in that location promotes entrapment of microorganisms by the mucosa [5]. Mucus is produced by the epithelial cells and the mechanical clearance of mucus is a very important defence mechanism of the airway. These cells are

regularly exuviated and the bacterial commensal flora in oropharynx is also a competitor for the invader [18]. The binding of microorganisms to the epithelial cells represents a crucial step in colonization that is a prerequisite for infection [19, 20]. The respiratory tract is lined with pseudostratified columnar epithelium in which tight junctions between the cells constitute a mechanical barrier against microorganisms. This epithelium is composed chiefly of ciliated epithelial cells, goblet cells, and basal cells. Mechanically, the epiglottis and cough reflexes are important to maintain the sterile environment in the lungs. If microorganisms do succeed in passing to the lungs, the "mucociliary escalator" helps transport them back to the oropharynx on a mucus "blanket" that is subsequently swallowed or expectorated [21]. The sharp-angled branching of the airways makes it more difficult for microbes to reach the lower part of the respiratory tract [5].

Regulation and navigation of the immune response

Microorganisms express unique molecular structures that bind to pattern recognition receptors (PRPs), such as the Toll-like and NOD-like (Nucleotide Oligomerization Domain) receptors, which are present on respiratory epithelial cells and on alveolar macrophages and dendritic cells located in strategic places in the lungs. The acute phase protein C-reactive protein (CRP) is a soluble form of PRP and facilitates complement activation after binding the microbe (or dead or dying cells). The binding to these receptors induces a cytokine–chemokine cascade that orchestrates the inflammatory response and the crucial recruitment of neutrophils [22]. This cascade is highly complex and involves activation of both pro- and anti-inflammatory response mediators to achieve a balanced response. The intricate control system carries out sterilization of the infected part of the lungs without causing any damage and also elicits a very local reaction that saves the uninfected parts.

Complement and antimicrobial peptides

The mucosa produces for IgA, but IgG and IgM enter the airways mainly via transudation from the blood together with complement factors. The antibodies facilitate complement activation, agglutination, and neutralization by opsonization, and they are also important for clearance of microorganisms (e.g., a new serotype of pneumococci) [20]. The complement system is a collection of blood and cell surface proteins that act as a major primary defense against invading microbes. The complement binds to the microbes, facilitating opsonisation and their subsequent elimination. The complement can be activated by; (i) the classical pathway, triggered by antibody-antigen complexes and is dependent on functional antibodies; (ii) the alternative pathway, directly activated by the pathogen; (iii) the lectin pathway, triggered by human lecitin that binds carbohydrates on bacterial surfaces. All these pathways end up with enzymatic cleavage of C3 and formation of the membrane attack complex (MAC) and lysis of the bacteria.

The classical pathway is considered to be the most important. The more highly invasive serotypes have been found to be more resistant to C3 deposition on the capsule [23].

Epithelial cells, neutophils and monocytes produces an array of antimicrobial peptides, for example defensines that assist in the killing of phagocytosed bacteria. Defensines bind the microbe and form a pore-like membrane defect that allows efflux of essential ions from the microbe. They are also chemotactic for dentritic cells and memory T cells .Defensines have been proven to inhibit growth of pathogens *in vitro* *[24]*. Other antimicrobial peptides are lysozyme and lactoferrin.

The phagocytic cells

Macrophages and neutrophils are phagocytic cells that play an essential role in the pulmonary defence system [25]. If microorganisms reach the alveoli, alveolar macrophages and tissue histiocytes play a major roll in killing the invaders, because physical expulsion is less effective on alveolar level. The alveolar macrophage is the first type of phagocytic cell to meet an invader at the alveolar level [26]. Other key defence cells are the interstitial macrophages in the connective tissue of the lung, which function as phagocytic and antigen-presenting cells. Moreover, in the pulmonary capillaries, there are intravascular macrophages that are prepared to remove invading microorganisms and foreign particles.

The antigen-presenting cells and their interactions with T helper cells

The respiratory epithelium harbours the monocyte-derived dendritic cells that serve to capture antigens in the lungs. These antigen-presenting cells displays foreign antigens with the major histocompatibility complex (MHC) class II proteins on their surface. T helper cells (T_h cells) that show surface expression of the glucoprotein CD4 (CD4+ T cells) interact with the MHC class II molecules. Binding of the MHC class II proteins on the surface of the T_h cells induces the dendritic cells to produce an array of cytokines, which in turn stimulate B cells to differentiate into plasma and memory cells [5]. CD4+ T cells are essential in this context, especially for functioning of the adaptive immune system, and they are also crucial for actions such as the clearance of microorganisms [20]. The dendritic cells can also migrate to lymphoid tissues, where they elicit this T-cell-dependent response.

The lymphocytes

The lymphoid tissue of the lungs harbours uncommitted B and T cells that can differentiate into memory cells and effector cells. This tissue is located primarily in follicles along the bronchial tree and is referred to as bronchus-associated lymphoid tissue (BALT). Intra-alveolar lymphoid cells are activated by antigens, as described above, and

subsequently stimulate migration of memory lymphocytes and antigen-specific T and B cells that act as effector cells in the affected area.

The CD4+ T cells differentiate into three types of cells: memory T_h cells, which are present chiefly in the submucosa where they wait for re-infection with the same microorganism; regulatory T_h cells, which are involved in self-limitation of the immune response; effector T_h cells, which differentiate into T_h1 and T_h2 cells, interacting with macrophages and B-cells respectivly. Upon stimulation, all of these cells release various cytokines that induce different responses leading to either the cell-mediated or humoral immunity.

It is believed that the pulmonary lymphocytes shuttle between the BALT and the lung parenchyma, and, in addition to their functions as antibody-producing and inflammation-mediating cells, they are also assumed to play an important role as cytotoxic T cells. Cell-mediated immunity is necessary for the adaptive immune response, and viruses and intracellular pathogens are cleared through this system.

When does the system fail?

An infection can become established if any part of this complex protective system fails. Such failure can be the result of any of the following: the host defence is defective due to disease; physiological functions for keeping the lungs sterile are unsuccessful due to iatrogenic causes; there is an overwhelming inoculum or introduction of a highly virulent microorganism.

Risk factors for infection

The list of diseases and other factors that have an impact on host defence is long. Conditions or agents that alter consciousness (e.g., stroke and use of sedatives) affect the physiological epiglottis and cough reflexes, which increase the risk of aspiration. Furthermore, abuse of alcohol devitalizes these reflexes and is also associated with increased colonization and altered immune function [27]. Cigarette smoking also has a negative impact on both the mucociliary function and macrophage activity [28].

Tracheostomy and use of oropharyngeal and nasogastric tubes or sedatives are iatrogenic causes that interfere with or bypass the protective functions in the upper respiratory tract. The sicker the patient is, the greater the risk for a super-infection, a common problem in intensive care units (ICUs) [29, 30]. Even widely used medications such as those that alter the gastric pH level [31, 32], and immunosuppressive treatment, used to address an array of diseases give rise to a higher incidence of pneumonia.

Malnutrition caused by undernourishment or disease is another important factor that impairs the cell-mediated immune system and is associated with more severe infections [33]. Viruses and bacteria can predispose to respiratory tract infection with another pathogen by interfering with this system, for example, by damaging the ciliary activity [34] or inhibiting the immune system [35].

Age-dependent factors

Compared to adults, children are more prone to infections because their immune system is immature, and these infections are caused predominantly by viruses and encapsulated bacteria [36]. As children grow, they develop a stronger immunological memory for the antigens they have encountered over the years.

Elderly individuals are at higher risk of pneumonia due to comorbidities, more hospitalizations, and age related impairment of the physiological reflexes and the immune system [37] Immunosenescence, the gradual deterioration of the immune system, causes the most significant and consistent defects in the T cell compartment [38]. Thus, immunosenescence are multifactorial and practically all cell lines are affected; the hemapoietic stem cells are diminished in their self-renewal capacity, probably due to oxidative damage to DNA during life; the cytotoxic effect of Natural Killer (NK) cells decline; The antigen-presenting function of the dentritic cells diminish with profound implications of the adaptive immune response; cells together with a reduced number of antibody producing B-cells.

Colonization of the nasopharynx

The commensals

The commensal flora of the upper respiratory tract in humans consists of a variety of bacteria, including species of the genera *Neisseria* and *Bacteroides*, fusiform bacteria, anaerobic streptococci, and also the alpha haemolytic streptococci, which are considered to constitute the most important group [18]. Colonization of the nasopharynx appears to be a dynamic process that involves acquisition and elimination of various microbes, during which the microorganisms interact with the host's immune system and each other [19]. In a balanced state, this bacterial ecosystem is assumed to be beneficial for the health of the host, for example, by stimulating the immune system and functioning as a protective barrier against invading pathogens [39]. Antibiotic treatment can alter this protective effect [40]. It has been demonstrated that the commensal flora of the upper respiratory tract inhibits the growth of pathogens, both *in vivo* and *in vitro* [41, 42]. Protection from invasion by pathogens is a consequence of the competition for nutrients and receptors in the mucosa, as well as the production of bacteriocins (e.g. pneumocin) and other metabolites that are toxic to competing microorganisms and other pneumococci. The commensal flora helps the host immune system to maintain its guard and stay alert by giving rise to continuous stimulation that induces sustained expression of MHC class II molecules on the surface on antigen-presenting cells. Cross-protective immune factors such as natural antibodies are also activated by the commensal flora [43].

The potential pathogens and their interactions

The potential pathogens *Streptococcus pneumoniae, Moraxella cattarrhalis, Haemophilus influenzae, Neisseria meningitides,* beta-haemolytic streptococci, *Neisseria meningitidis*, and *Staphylococcus aureus* can be members of the commensal flora, especially in children. These bacteria are usually maintained as commensals, apparently causing no harm to the carrier [44, 45], and they interact with each other. *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* have been observed to be positively interrelated in studies of carriage [46-48], and each of these species is negatively associated with carriage of *S. aureus*. On the other hand, when studying colonization in mouse models, both *S. aureus* and *S. pneumoniae* exhibit a synergistic interaction with *H. influenzae* [47]. Interestingly, when the carriage rate of pneumococci decline during childhood, there is a simultaneous increase in *S. aureus* carriage rate, from 10% in the first years of life to a maximum of 50% at the age of 10 years [19]. This is probably due to pneumococcal hydrogen peroxide that may affect growth of more sensitive catalase negative organisms like *S. aureus*. There is also evidence that an established strain of *S. aureus* can prevent other strains of *S. aureus* from colonizing the nasopharynx [47].

Effects of viruses

Local ecological dynamics are also influenced by viruses. In healthy children, viruses are commonly present in the airways and are positively correlated with carriage of the common respiratory bacteria. Furthermore, carriage of influenza virus is strongly associated with carriage of *S. aureus* [48], and, although staphylococcal pneumonia is fairly rare, it occurs more frequently during influenza outbreaks [49]. There is also a marked correlation between influenza and pneumococcal pneumonia [50]. The influenza virus promotes adherence of the bacteria and invasion of the lungs in different ways. Bacterial access to receptors is a key factor, and this may be facilitated when the influenza virus damages the epithelium and thereby exposes or up-regulates receptors, or provokes the epithelial regeneration response to cytotoxic effects. Influenza can also induce neutropenia, which is related to poor outcome, although leukocytosis is seen more often. The double virus–bacteria infection causes an amplification of the inflammatory cascade that probably contributes to the severity of the effects. It is plausible that such infection alters the functional capabilities of neutrophils and macrophages that are necessary for the clearance of bacteria, which include chemotaxis, phagocytosis, and bacterial killing [51].

Risk factors for changes in the nasopharyngeal flora

Environmental factors such as smoking can alter the nasopharyngeal flora. Compared to non-smokers, smokers have a flora that contains fewer aerobic and anaerobic organisms with interfering capability and more potential pathogens. For example, pneumococcal adherence is greater in smokers than in non-smokers. Smoking cessation

decreases the rate of potential pathogens and increases the proportion of interfering commensals [52].

Increased colonization with aerobic Gram-negative bacilli has been documented in elderly persons with comorbidities but is uncommon in healthy individuals in this age group. It has also been observed that healthy people clear Gram-negative bacilli within hours as the result of many factors, the most important being the absence of receptors for Gram negative bacilli [53]. Other factors that have a marked impact on colonization with Gram-negative bacilli are prior use of antibiotics, decreased activity, diabetes and alcoholism, as well as debility, which probably has a more pronounced risk factor than age [54]. The clinical severity of illness is most extensively correlated with aerobic Gram-negative bacilli colonization, perhaps because of impaired oropharyngeal clearance of these bacteria [55]. This is illustrated by research showing that the risks of aerobic Gram-negative bacilli and yeast colonization are significantly higher in patients with a severe form of chronic obstructive pulmonary disease (COPD) than in those with non-severe COPD [56]. High prevalence of aerobic Gram-negative bacilli and MDR pathogens has also been found in colonization studies in children infected with HIV and in newborns admitted to neonatal intensive care units (ICUs) [57, 58]. Several studies have shown that the oral flora of patients in a hospital setting changes dramatically to a predominance of enteric Gram-negative bacilli, staphylococci, and *P. aeruginosa* [59, 60]. Antibiotic treatment is also common in a hospital setting and leads to a shift in the oral flora.

Risk factors for transmission of respiratory pathogens

Respiratory pathogens are easily transmitted trough the expulsion of respiratory droplets or direct contact. Age, attendance at a day care centre (DCC), siblings, underlying diseases, socio-economic status, season, and smoking have been associated with carriage of potentially harmful bacteria and viruses [45]. During hospitalization, potential pathogens can be transmitted by contaminated hands, respiratory instruments, or ambient aerosols [61]. A recent Swedish study showed that day care attendance was associated with significantly higher rates of carriage of *S. pneumoniae, H. influenzae and M. catarrhalis* reflecting the higher transmission of potential pathogens in this environment [62].

Antibiotics and resistance

History

Antibiotics and antibiotic resistance are components of the evolution of microorganisms and the eternal competition between them [12]. The principle of antibiosis was

anticipated by Louis Pasteur and Jels Francois Joubert in the late 19th century when they noted that anthrax organisms cultured in urine showed very little growth or died when another species of bacteria was added to the culture [1]. Penicillin and later numerous other antibiotics we use were discovered by scientists who were searching for active compounds produced by living organisms, and since then many natural, semisynthetic, and synthetic drugs have been developed and marketed with or without modification of the prototypical molecule [63].

Antibiotic therapy

Antibiotic therapy cures a disease by eradicating the very cause of the condition, and a physician must consider several critical aspects when choosing an appropriate treatment: the likely aetiology based on the clinical information; the probable susceptibility of the infecting organism; whether the antibiotic will reach the site of infection; a number of host factors that affect the pharmacokinetics, such as age, occurrence of earlier adverse events, obesity, pregnancy, and co-morbidities. Furthermore, to be able to use antibiotics with as narrow a spectrum as possible, it is essential to consider the severity of the disease in question. If a patient has a life-threatening condition, using the wrong empirical treatment will obviously have dire consequences, and in such cases a broader regimen can be more suitable [64-66].

Ecological effects of antibiotic therapy

Antimicrobial therapy has serious environmental consequences, the severity of which depends on the antibacterial spectrum and pharmacokinetics of the given drug. Humans harbour an abundant commensal bacterial flora in the nasopharynx and the gastrointestinal tract, as well as other sites, and the higher the concentration of an antibiotic is in these locations, the more the natural bacteriological flora will be affected. Penicillin V is the drug recommended for treatment of respiratory tract infections in Sweden. This water-soluble antibiotic appears in a low concentration in the saliva, and hence it has a more limited impact on the commensal flora compared to lipid-soluble antibiotics such as trimethoprim-sulfamethoxazole, macrolides, and tetracyclines.

Antibiotic resistance

The emergence of antibiotic resistance seems to be an inevitable consequence when a new antimicrobial agent is introduced, and it is a well-established fact that high antibiotic pressure leads to more antibiotic resistance. Genetic variability is crucial for the evolution of bacteria and their ability to adapt to changing environmental conditions. Resistance to antimicrobials can be acquired in several ways, such as by point mutations on a microevolutionary or macroevolutionary level when a large sequence of DNA is

moved from one location of a bacterial chromosome or plasmid to another. Bacteria can also gain resistance from foreign DNA in the environment, for instance originating from plasmids or other bacteria. The mechanisms of resistance can be divided into four categories: decreased permeability of bacterial membranes, antibiotic efflux, altered target sites, and inactivating enzymes [67].

Populations that have genes for antibiotic resistance proliferate and are spread vertically to subsequent generations of the same bacteria, as well as horizontally to related and unrelated species and genera [68]. However, in some cases, carriage of resistance genes comes at the cost of fitness, but there are bacterial species that can repress the gene expression when it is not needed and hold it in reserve in the absence of antibiotic pressure [69]. When a resistant clone has appeared in a geographical region or in a hospital, maintaining high antibiotic pressure favour the microbes that are resistant to these agents. Good hygiene routines within the healthcare system can help reduce the spread of resistant bacteria.

Community-acquired pneumonia

The incidence of community-acquired pneumonia (CAP) is approximately 1% in the developed world and is correlated with age, being higher in the elderly and in young children [70, 71]. Spindler and colleagues [72] report a mortality rate of 4.3% among patients with pneumonia who were hospitalized in a clinic for infectious diseases in Sweden in 2010. In many cases, pneumonia can probably be regarded as a sign of failing health, because, for other hospitalized patients within the same age group, the mortality been proven to be lower after hospital discharge [73]. A large number of microorganisms can cause pneumonia, but the dominant pathogen is *Streptococcus pneumoniae*. In a clinical setting, it is often impossible to make a rapid diagnosis, but the physician must nonetheless decide what empirical treatment is most appropriate. To make that choice, it is necessary to consider local antibiotic resistance, current epidemiologal situation, disease severity and underlying comorbidities in the patient to be treated. Today, an increasing number of patients live longer and have more and often serious co-morbidities that require extensive contact with the healthcare system. Patients in this group have less distinct symptoms and more often show a shift in causative organisms from the traditional respiratory tract pathogens [5].

Clinical presentation

Patients with classical CAP present with a sudden onset of chills followed by fever, productive cough, and pleuritic pain, and most exhibit some combination of these as indicated: fever in 68–78%, chills in 40–70%, cough in 80–90% that is productive in 60–80%, and chest pain in 30–46% [5]. Non-respiratory symptoms are also common:

fatigue in 91%, anorexia in 71%, sweats 69%, and nausea in 41%. Older age is associated with these less pronounced symptoms [74].

Respiratory rate is an important parameter that is used extensively, especially in developing countries, where it is part of a simpler algorithm used to diagnose pneumonia. Tachycardia is also a common finding. Rales are noted on auscultation of the lung in 78% of the patients and signs of consolidation in 29%. Sputum is often thick and purulent, and can be rust coloured [5].

Atypical pneumonia syndrome was first described in 1938 by Reimann [75]. Patients with this condition have an atypical clinical picture that starts with a mild respiratory tract infection followed by more traditional symptoms of pneumonia, often without sputum production. In many cases, the aetiology of atypical pneumonia differs from that of classical pneumonia.

Laboratory findings

Laboratory results can strengthen the clinical diagnosis. An elevated white blood cell count is common, and leukopenia is a poor prognostic sign [10]. Biomarkers are often used, but there is no "golden bullet" that can distinguish between viral and bacterial pneumonia, and it appears that the accuracy is too low to safely withhold antibiotic therapy if there is a risk of pneumonia. C-reactive protein (CRP) is recommended in the Swedish CAP guidelines and has been proven to be an independent marker of the severity of infection [72, 76]. Procalcitonin (PCT) has been widely studied over the last decade, and there is a growing body of evidence to support the use of this protein in the community [77]. Schuetz *et al.* [78] found that using PCT to guide initiation and duration of antibiotic treatment in patients with respiratory infections was not associated with higher mortality rates or treatment failure, but it did significantly reduce antibiotic consumption across different clinical settings. Nevertheless, the mentioned observations may not be relevant in Sweden, because all of the cited studies were performed in countries that have a different tradition of antibiotic use.

Radiological examination

Chest radiography findings consistent with pneumonia together with the clinical features of the disease are considered to be the gold standard for identifying patients to participate in clinical trials [79]. Abnormal chest radiographs indicating pneumonia can distinguish a patient population that might benefit from antibiotic treatment from a population that will not. The infiltrate pattern that is observed cannot determine the aetiology, but it can be of some diagnostic help. Most lobar pneumonias are pneumococcal, but, conversely, most pneumococcal pneumonias are not lobar. Bilateral diffuse infiltrates are often noted when the cause is a virus, legionella, or mycoplasma, but these agents can also create a consolidated X-ray image [5]. Computed tomography (CT) can be useful in some clinical situations. High-resolution CT is a superior method for

characterizing lung infections and can increase the number of CAP cases confirmed by imaging and also improve the accuracy compared to classical chest radiography [80]. The more critically ill the patient, the harder it is to interpret the results of chest radiography, because there are many different causes of pathological findings in the X-ray image, such as atelectasis, emphysema, chemical pneumonitis, asymmetric cardiopulmonary oedema, pulmonary embolism, cryptogenic organizing pneumonia, pulmonary contusion, pulmonary haemorrhage, and drug reactions [61, 81].

Aetiology of CAP

CAP is caused primarily by pneumococci often followed by *H. influenzae,* as well as a large number of other microorganisms, and the reported aetiologies vary between different studies and in different settings [15, 82-84]. Atypical pneumonia is caused chiefly by *Mycoplasma pneumoniae*, which can account for 10–30% of all CAP cases, followed by *Legionella pneumophilia* in 2–8% of the CAP cases involving hospitalization. *M. pneumoniae* is epidemic every 2–6 years, and this species is the predominant cause of pneumonia in younger individuals but is also found in elderly patients [85]. Other pathogens that can give rise to atypical pneumonia are *Clamydophila pneumoniae*, *C. psittaci, Pneumocystis jiroveci, Mycobacterium tuberculosis*, and viruses [5]. The aetiology of CAP often involves viruses, which were found in 29% of the CAP patients included in a study recently conducted in Sweden [15]. Atypical agents can also induce a classical acute pneumonia, and hence it is not possible to predict an atypical aetiology at the onset of disease.

Mixed infections are prevalent, and the combination of viruses and pneumococci seems to be the most common finding. Furthermore, these infections can be associated with severe pneumonia [15, 86, 87]. Inasmuch as diagnosis is achieved mainly by conventional microbial methods such as blood, sputum, and nasopharyngeal cultures, many cases of pneumonia are still of unknown origin. When culture of transthoracic needle aspirate is added to the diagnostic protocol, pneumococci are the most common aetiological agents even in this group [88]. Many experts believe that future diagnostic tools will provide faster results and will be applied in closer connection with the clinical setting to better support the choice of adequate empirical treatment.

Streptococcus pneumoniae

Louis Pasteur and George Sternberg independently described the pneumococci in 1881, calling them *Microbe septicemique du* salive and *Mikrococcus Pasteuri*, respectively. In 1926, these bacteria were given the name *Diplococcus pneumoniae* because of their appearance in Gram-stained sputum, but in 1974 they were renamed *Streptococcus pneumoniae* when it was discovered that they belonged to the *Streptococcus* family [89].

The pneumococci play an important part in the history of microbiology. In the late 17th century, the Klemperer brothers discovered that animals were protected from re-challenge with the same strain of pneumococci, and protection could be transferred by infusing serum from an immunized animal. They thought the animals had devel-oped their own protection against a toxin that was referred to as a humoral substance. Neufeldt and Rimpau later described factor(s) in the blood that facilitated phagocy-tosis, a process they termed opsonization from the Greek word for preparing food. These finding were crucial first steps to understanding what we today call humoral immunity [2, 89]. The pneumococci also had a central role in the discovery of DNA. In the 1920s, Griffith described the transfer of capsules from heat-killed pneumococci to unencapsulated strains [90]. Avery, the father of genetics, took up this work a few decades later and showed that DNA is the carrier of genetic information and code for the phenotype [91].

Pneumococci are primarily pathogenic to humans, although colonization and infec-tion have been reported in animals held in captivity [92]. Recently, pneumococci were found to be the probable aetiological agent of sudden deaths in wild chimpanzees in a National park in Côte d'Ivoire, and necropsies of the deceased animals suggested an infection similar to infections observed in humans [93]. However, the results of that study suggest that the pneumococci identified in the chimpanzees were not transferred from humans to the animals.

Pneumococci are the most common cause of pneumonia, meningitis, sinusitis, and otitis media, and in rare cases also endocarditis, septic arthritis, and other infectious diseases. These bacteria also give rise to extensive morbidity and mortality worldwide, and the incidence of pneumococcal disease is highest in children and among the elderly.

> "The worst years are between ten and seventy, after that it gets easier."
> – Magnus Härenstam, a Swedish actor about life, the opposite can be said about pneumococcal diseases.

The burden of pneumococcal disease

The burden of severe pneumococcal disease is enormous in children under five years of age, with an estimated 14.5 million cases in 2000. The same year, approximately 826,000 children died from such disease, and about 91,000 of them were also infected with HIV [94]. The greatest burden is in sub-Saharan Africa and Asia, and 90% of the pneumococcal deaths are due to pneumonia. The risk of dying from pneumococcal dis-ease in childhood is almost 40 times greater in countries that do not use a pneumococ-cal conjugate vaccine (PCV) in a routine immunization programme than in countries that are applying such a schedule. Mortality in children less than five years are presented in figure III. Financial support for vaccination is now offered to low-income countries by the Global Alliance for Vaccines and Immunisation (GAVI), and several African countries have introduced PCV. In high-income countries, children have low mortality

from pneumococcal disease, although there is remarkably high incidence in marginalized indigenous people [95].
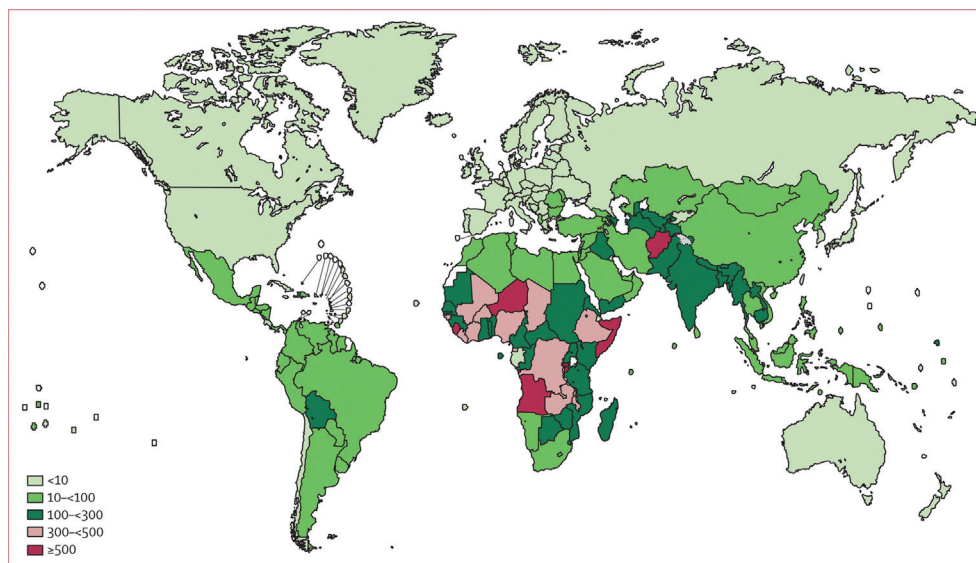
Figure III. Pneumococcal deaths in children aged 1–59 months per 100 000 children younger than 5 years (HIV-negative pneumococcal deaths only). From O´Brien et al, Lancet 2009 [94]. Reprinted with permission from Elsievier.

Considering all types of infections, those occurring in the respiratory tract are the most common cause of death, affecting more than 3.4 million people in 2008 according to statistics provided by the World Health Organization (WHO) [96]. Pneumococcal disease remains a major cause of mortality and morbidity in adults, even in high-income countries [97]. The incidence varies between countries, but the findings consistently show that it increases with age. In the United States, people older than 60 years account for 81.6% of all cases [98]. In a study conducted in the state of Washington [99], the rate of pneumonia was found to be 18.2 per 1,000 person-years among individuals aged 65–69 years but much higher at 52.3 cases per 1,000 person-years among those aged ≥ 85 years, which indicates that one in twenty people in the latter age group will have a new episode of pneumonia each year. Inasmuch as the mean age is increasing sharply in the industrialized world, it can be expected that there will be a rise in the number of cases of pneumonia and accordingly also increased hospital admissions and costs [100]. Hopefully this will be counteracted by the herd effect of the implementation of PCV [101].

Microbiological aspects

Laboratory aspects

The *S. pneumoniae* bacteria are Gram-positive non-motile and non-spore-forming cocci. They do not express catalase, which is an enzyme that is required to neutralize hydrogen peroxide produced by the bacteria, and hence they grow better in the presence of a source of catalase, such as red blood cells. When cultured on blood agar, pneumococci can use the enzyme pneumolysin to oxidize haemoglobin to methaemoglobin, which is seen as greenish halos around the bacterial colonies. This is erroneously referred to as α-haemolysis, because the same phenomenon is observed when these bacteria grow on chocolate agar, a medium in which the red cells are already lysed. Pneumococci can display two different morphologies: umbilicated colonies are most common, and the other type is seen in more encapsulated strains (especially serotype 3), which form mucoid dome-shaped colonies with a larger diameter. Microbiological identification is achieved by use of different reactions: susceptibility to optochin (ethyl hydrocupreine), susceptibility to bile, α-haemolysis on blood agar, and catalase negativity [89]. In some cases, pneumococci die when cultured, possibly because the bacteria are fastidious and have complicated nutritional requirements. Another plausible explanation is that they are lysed by autolysin produced by the bacteria themselves, which in turns releases pneumolysin that kills other bacteria. In these cases, Gram stain from blood culture bottles can detect the pneumococci, but lysed bacteria look more like short, fluffy Gram-negative rods.

The use of polymerase chain reaction (PCR) methods to detect *S. pneumoniae* in respiratory samples is increasing, because PCR offers greater sensitivity compared to conventional culture techniques, and it can also provide positive results even after antibiotic treatment is initiated [102, 103]. Quantitative real-time PCR has shown promise in testing of nasopharyngeal swabs to determine pneumococcal density as a means of predicting pneumococcal aetiology [104]. Antigen-detecting tests are frequently used in clinical practice, because they are fast and have high specificity and hence can support the aetiological diagnosis at a very early stage. A disadvantage of these methods is that false-positive results can be obtained for persons who were recently infected or colonized with *S. pneumoniae*. This suboptimal sensitivity makes it impossible to rule out pneumococcal aetiology if the test results are negative, although it is probably safe to limit treatment to penicillin if the test is positive. Various types of samples can be assayed, and urine is most widely used [105]. Like the PCR methods, the antigen detection tests can identify bacteria even after starting antibiotic treatment. However, a drawback of both these approaches is that they cannot determine antimicrobial susceptibility, and thus they can be considered as supplementary to culture, which will remain the "gold standard" test for diagnosis of pneumococcal infection.

The gold standard of serotyping is the Qeullung reaction, which was first described 110 years ago [106]. In the pre-antibiotic era, this diagnostic test was essential to ascertain specific antiserum (the drug of choice at that time) should be administered to a patient. In this method, serum from rabbits immunized with capsule from a particular

type of pneumococcus is used to determine the serotype. The serum in question stimulates the production of antibodies that cause agglutination, and the bacterial capsule becomes refractile and looks swollen and can thus be detected by phase microscopy. It is this swollen appearance that led to the name Quellung, which is the German word for swelling [89]. The drawbacks of this method are that it is subjective, time-consuming, and expensive. Today, serotyping is motivated mainly in epidemiological studies to ascertain the effects of a vaccine, and it is not used in clinical practice. In the future, serotyping will have to be easier to carry out in large epidemiological studies, especially in developing countries where PCV has been introduced. New methods such as simple latex agglutination kits are now available that have made serotyping easier to a certain extent. PCR tests are also used in some centres. The key limitation of molecular-based assays is the plasticity of the pneumococci, because capsular transformation or point mutations can easily result in serotype misclassification. On the other hand, it seems that PCR techniques are more sensitive than the Quellung reaction, and they can also detect more than one serotype or genotype in a sample [107]. It is possible that assays utilizing high-throughput sequencing technology and/or matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry (MALDI-TOF MS) will be developed in the future that can serve as a novel approach to pneumococcal serotyping [108].

The pneumococcal capsule: the chief virulence factor

The polysaccharide capsule surrounding the cell wall of a bacterium is the most important virulence factor, and it plays a central role in preventing phagocytosis, especially in the absence of anti-capsular antibodies. The capsule also prevents entrapment of pneumococci in the mucus in the airway of the host [20] and is necessary for colonization [109]. It seems that the thickness of the capsule is correlated with the virulence of these bacteria [110]. In mice challenged with a particular capsule type, the amount of specific anti-capsular antibody that is produced corresponds to the level of protection in the animals [111]. Most isolates occur as either of two variant types, one transparent and the other opaque, which have different capacities to escape the defence mechanisms of the host. Pneumococci of the opaque variant have a larger capsule and can probably better avoid entrapment in the mucus, thereby allowing access to the epithelial surfaces. Once the epithelium has been reached, the transparent phase predominates, because such bacteria can adhere more strongly to the mammalian cells as the result of higher expression of certain cell-surface proteins [112]. Strains found in the nasopharynx produce less capsule and are more prone to form biofilms compared to isolates found in the blood stream. Pneumococci of the opaque variant predominate in the invasive phase, since they are better at evading opsonophagocytic killing and exhibit more virulent behaviour, and they are also more lethal when inoculated intraperitoneally, probably partly due to increased capsule production [89, 113]. Unencapsulated pneumococcal strains rarely cause disease, although they have been described to give rise to outbreaks of conjunctivitis. The loss of a capsule has also been shown to render the bacteria essentially avirulent in trials using mice, which is further evidence that the capsule is the principal virulence factor of this pathogen [114].

The chemical composition of the polysaccharide capsule varies greatly, and pneumococci are divided into different serogroups and serotypes based on antigenic differences in the capsule. There are 46 serogroups, 20 of which are assigned to subgroups called serotypes that are designated by a letter (e.g., 6A and 6B). A total of 93 unique serotypes have been described thus far [115]. Pneumococci can shift serotype, a feature that was discovered by Griffth as early as 1928 [90]. Serotype switching has been observed in nasopharyngeal isolates, and it has been postulated that the most optimal environment for this process is in children attending day care centres (DCCs), because carriage is very common in this group [116], although more recent studies indicate that it is the pneumococcal strain rather than capsular type that changes in children [117]. Capsular switching has been observed in multidrug-resistant clones worldwide, perhaps favoured by the selective pressure from PCV [118, 119]. The locus encoding the capsule and the genes for penicillin-binding proteins are located side by side, and it has been suggested that a transformation involving these genes can occur in a natural setting and cause pneumococci to change serotype and acquire β-lactam resistance in a single step [120]. A capsular switch alone might constitute a threat to the efficacy of PCV, but studies indicate that this will not have a significant impact on the incidence of invasive pneumococcal disease (IPD) [121]. Today, serotyping is of substantial interest from an epidemiological standpoint, because each PCV is aimed at specific serotypes, and we can expect that the serotypes now found in the population will be replaced by other serotypes, as has been seen in countries where PCV immunization has been introduced. However, to understand the big picture of pneumococcal epidemiology, it is also important to elucidate antibiotic resistance and genotype surveillance.
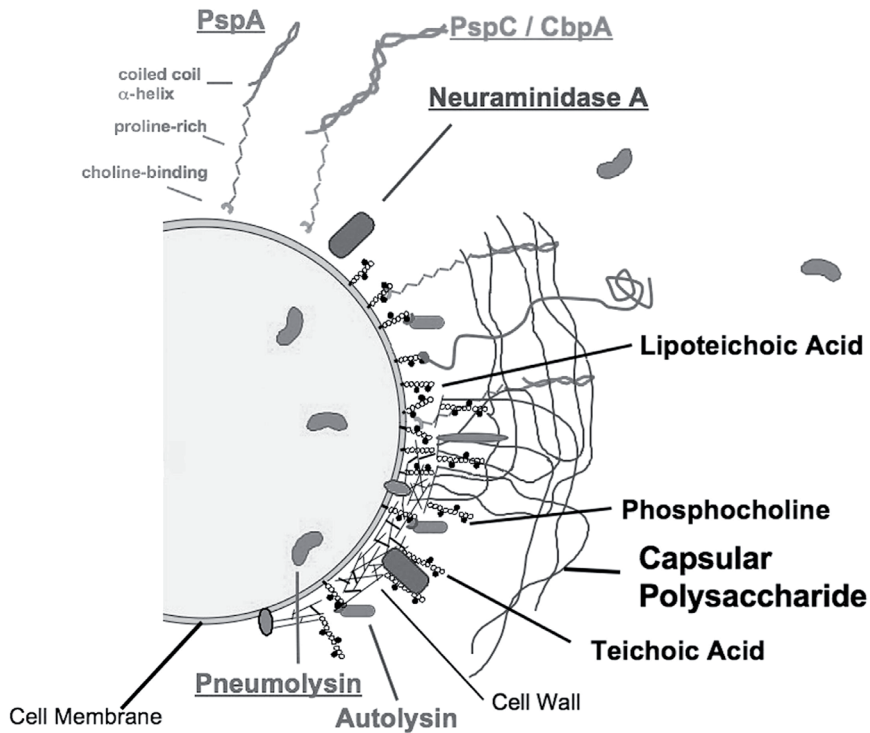
Figure IV. Schematic overview of the major virulence factors of *S. pneumoniae*. From Bhatty *et al*, Alcohol 2011 [122]. Reprinted with permission from Elsivier.

Capsular polysaccharide; prevents entrapment of mucus and phagocytosis and activates complement.

Cell wall polysaccharides (Teichoic and lipoteichoic acid); Activate complement and release of cytochines, interact with CRP.

Pneumolysin; Cytotoxic, activates complement and release cytochines. Responsible for many of the symtoms in pneumococcal disease.

PspA, pneumococcal surface protein A, inhibits phagocytosis and complement activation, binds lactoferrin.

Autolysin; Bacterial disintegration that result in pneumococcal lysis and release of pneumolysin.

Neuraminidase; Biofilm formation, mediates adherence by unmasking receptors.

PspC, pneumococcal surface protein C (also referred to as CbpA or SpsA), inhibit complement and phagocytosis, facilitate penetration of the mucosal barrier.

Phosphorylcholine (ChoP), mediates adherence to the receptor for platelet-activating factor (rPAF).

Non-capsular virulence factors

Trials in mice have shown that it is not only capsule type but also other factors that determine the virulence of pneumococci [123], as shown in figure IV. The cell wall is composed of peptidoglycans, teichoic acid (also called c-polysaccharide), and lipo-teichoic acid, all of which are involved in inducing the innate immunity response. Peptidoglycans form a mesh of glycan chains that are cross-linked by peptide bridges,

and this network of sturdy polymers protects the bacteria from changes in osmotic pressure and plays an important part in determining the shape and formation of daughter cells. The cell wall polysaccharides markedly activate complement and stimulate the release of cytokines. The c-polysaccharide (teichoic acid) reacts strongly with C-reactive protein (CRP), an acute phase protein that is widely used as biomarker in clinical practice, and it is also the target for the antigen detection test. Pili function together with several other classes of surface proteins to facilitate binding of the bacteria to the host cells [124]. Pneumococcal surface protein A (PspA) is required for full virulence, because it binds lactoferrin and inhibits complement activation [125], and pneumococcal surface protein C (PspC) prevents formation of C3b. Bacterial adhesins are also important, for example: phosphorylcholine (ChoP), which mediates bacterial adherence to the receptor for platelet-activating factor (rPAF) and activates host cell signalling through this receptor; PspC, act as an adhesin and is non-covalently anchored to ChoP. PspC binds to human secretory component present on the polymeric immunoglobulin receptor and secretory forms of immunoglobulin. Several exoglycosidases act to remove terminal sugars on human glycoconjugates and thereby unmask receptors that are responsible for adherence and/or providing nutrient source [20]. Neuraminidase cleaves *sialic* acid on host cell surfaces and exposes potential pathogen-binding sites, and it also cleaves the same acid on competing commensal pathogens and thereby renders those microbes more vulnerable to complement-mediated phagocytosis. Moreover, neuraminidase plays an important role in biofilm formation, another way the bacteria can hide from the host defence. Hyaluronidase degrades connective tissue and facilitates the spread of infection, for example, across the blood–brain barrier [126]. Hydrogen peroxide damages host tissue and inhibits the growth of other bacteria.

Pneumococci produce a variety of toxins, among them the autolysin, which is involved in the remodelling of the cell wall structure that occurs during cell division and prevents phagocytosis. As the name indicates, autolysin cause pneumococcal autolysis, during which several virulence factors are released and exposed. The most important and well-studied is pneumolysin. This pore-forming cytolytic toxin is produced by all serotypes of pneumococci, and it is stored in the cytoplasm and released when the bacteria undergo lysis. Pneumolysin is important for colonization, and promotes invasion by activating complement, chemotaxis, and CD4+ cells, and by inducing pro-inflammatory cytokine production and inhibiting ciliary beating. These effects are noted even in sub-lytic concentrations, and strains that express non-cytolytic pneumolysin can cause IPD. Pneumolysin is probably required for spread of the bacteria from the lungs to the bloodstream of the host [20]. Injection of pneumolysin into rat lungs results in the same pathological findings as seen in pneumonia [127]. Furthermore, the absence of pneumolysin in bacteraemia makes the infection pass without any overt symptoms, which can in turn lead to chronic bacteraemia [128].

Carriage

The human nasopharynx is the primary reservoir for pneumococci and also the ecological niche for many other bacterial species. The oropharyngeal flora is established during the first month of life [129], and the first pneumococcal strain is often acquired at around six months of age and can be detected for a mean of about four months [130]. In contrast, infants in certain populations (e.g., Native Americans, Aboriginal Australians, or disadvantaged members of developed societies) are more likely to be colonized with pneumococci, often in high numbers, even during the first few weeks of life [131]. The commensal state gives the bacteria an opportunity to spread within a population, because colonization is commonly followed by horizontal dissemination to persons in the direct environment. Infection usually arises after acquisition of a new pneumococcal strain, and studies have shown that 15% of children who acquire a new strain contract a pneumococcal infection within one month [132]. The disease that is induced is not regarded as very contagious and has little impact on the success of dissemination of a pneumococcal strain. These bacteria are spread via inhalation of airborne droplets or in saliva [133]. The rate of acquisition depends mainly on the age of the host but varies in relation to demographics such as geographical area, genetic background, smoking, socioeconomic conditions, family size (especially the number of older siblings), income, and recent antibiotic use [19]. An optimal environment for horizontal spread of pneumococci is created at DCCs, where young children spend considerable time together, often indoors in a limited space. The colonization rate can be up to 40–60% in toddlers and younger children in DCCs, but declines to 20–40% in healthy children of school age and about 2–9% in healthy adults [40, 89]. A person can harbour several pneumococcal strains at the same time [107, 134], and the duration of carriage depends on both host and bacterial factors. In the host, age is probably the most important factor [135], although immune status and serotype also contribute to a difference in carriage time [136]. In addition, there is seasonal variation in carriage for reasons that remain unclear, which was addressed in one of the present studies (Paper I) under the hypothesis that the carriage rate declines when children are absent from the crowded environment of a DCC.

Pneumococcal colonization of a host is facilitated by several virulence factors (Figure IV). The capsule is necessary for colonization [109], and that process is also aided by phase variation, adhesins, pili, exoglycosidases, neuraminidases, and hydrogen peroxide (see above). The acquisition of a new pneumococcal strain triggers an immune response including influx of neutrophils, and results in mild rhinitis. The neutrophils recruited are initially unable to clear the bacteria due to the presence of the protective capsule. It has long been assumed that eradication of pneumococci requires opsonization achieved by a serotype-specific antibody in combination with complement, resulting in enhanced phagocytosis of the bacteria. It has been shown that carriage of these pathogens induces the production of both mucosal and systemic immunoglobulins that are primarily strain and type specific. However, recent data obtained using mice models emphasize the importance of acquired CD4+ T cell-mediated immunity. Normal clearance during

carriage is probably accomplished by resident and recruited monocytes/macrophages and antimicrobial peptides in secretions [112], and that deduction is supported by the knowledge that, compared to vaccination, colonization induces a relatively small number of antibodies. The decline in carriage rate after childhood is widely observed among the different serotypes, suggesting that exposure during prior colonization events does not lead to immunity in a serotype-specific manner [20]. The host response regulates the trafficking pathogens in the upper respiratory tract, and colonization is a dynamic process in terms of turnover of colonizing species and pneumococcal serotypes [45]. An adequate immune response will eliminate the colonization and prevent re-colonization with the same pneumococci [137]. A strain is generally carried transiently for weeks to months before it is cleared, with shorter duration in adults. Pneumococcal disease cannot occur without preceding nasopharyngeal colonization [19]. Disease is prevented by the host's immunological response to the colonization, and it is likely that antibodies against the capsular polysaccharide of a colonizing organism will appear before an infection is established [89].

Serotype-specific differences

Brueggeman *et al.* [138] conducted a large meta-analysis and compared isolates from carriage and IPD in children and found that serotypes differ widely with respect to their invasive potential. The most invasive serotypes and serogroups (1, 5, and 7) were the least commonly carried, and the most frequently carried (3, 6A, and 15) were the least likely to cause invasive disease. Furthermore, these properties seemed to show stronger correlation with the serotype than the genotype [139, 140]. More heavily encapsulated serotypes are more resistant to neutrophil killing and are therefore more successful colonizers. Replacement after PCV immunization tends to favour the types of capsules that have fewer carbons and low energy expenditure per repeat unit. It has been suggested that this is a biological principle that explain part of the patterns of serotype replacement [141]. There is a significant inverse correlation between invasive disease and carriage rate [138] but also an inverse association between disease severity and invasive capacity. It appears that pneumococcal serotypes with a low invasive potential infect the older population and induce IPD with more severe outcome. Sjöström *et al.* [142] classified the more invasive serotypes as primary pathogens and the serotypes with less invasive potential as opportunistic pathogens that cause infections in older patients with more co-morbidities. Several investigators have pointed out that the host factors are most important in determining the severity and outcome of IPD [143, 144]; whereas others have found that the bacterial serotype is associated with more severe outcome and mortality even after adjusting for relevant host factors [145-148]. Varying virulence of serotypes is supported by studies in mice [149] and seems to be related to the size of the capsule [109]. A meta-analysis performed by Weinberger *et al.* [110] provided evidence to support the theory that serotype is an independent risk factor for worse IPD outcome, and also showed that serotypes that have a thicker capsule *in vitro*

(measured by digital fluorescence microscopy) are more frequently associated with a fatal outcome. The diseases induced by pneumococci in humans are caused by the host immune system, and thus a more virulent serotype should lead to a more severe disease in terms of septic shock. To address that hypothesis, we investigated the frequency of septic shock in relation to pneumococcal serotype (Paper III). Serotypes associated with more severe outcomes preferably infect an older immunosenescent population with more co-morbidities, and serotypes with less severe outcome preferably infect younger and healthier patients with a well-functioning immune system. These aspects influence the outcome and can disguise a true pathophysiological difference between serotypes.

The distribution of pneumococcal serotypes varies widely geographically and over time [150, 151]. In addition, serotypes probably exhibit differences in tropism. For example, serotypes 6, 10, and 23 consistently occur more often in cerebrospinal fluid cultures, whereas the reverse is true for serotypes 1, 4, and 14. Also, serotypes 1 and 3 have been found to be connected extensively with complicated pneumonia and also, but less frequently with peritonitis. The caveat to these findings is that age and antibiotic resistance are often relevant co-factors and young children are more prone to meningitis, complicated form of pneumonia, and/or resistance [152]. Epidemics of pneumococcal pneumonia were common in the pre-antibiotic era, and serotypes 1, 2, and 5 were the predominant causes of those outbreaks, which often affected military recruits, miners, prisoners, and other people living in crowded environments. Today, although such outbreaks do still occur, they are rare, no doubt due to improved socioeconomic conditions and the availability of antibiotics [151].

Pneumococcal serotypes that are better adapted to colonize the nasopharynx of young children are more likely to acquire resistance, because they are carried for longer periods [135] and are also more likely to be exposed to antibiotics, which gives them better opportunities to acquire resistance genes from other species present in the nasopharynx [153]. For instance, the commonly carried serotypes 6A, 6B, 9V, 14, 19A, 19F, and 23 F are more often resistant to antibiotics, whereas serotype 1 is rarely found in the nasopharynx and seldom shows resistance. Serotypes 3, 18C, and 15A are exceptions to this pattern in that they are prevalent colonizers but are rarely resistant [151]. Furthermore, the resistance is associated with genotype, as illustrated by data demonstrating that penicillin-resistant pneumococci in the United States belong to only a few different clones [154].

Antimicrobial resistance in *Streptococcus pneumoniae*

Resistance to penicillin, the drug of choice in Sweden for respiratory tract infections, is due to an alteration of the structure of penicillin-binding proteins (PBPs) that lowers the affinity of these molecules for penicillin. The pneumococci exhibit enormous diversity and adaptability, to a large degree facilitated by active import of DNA and extensive genomic repeats that greatly increase the likelihood of intra- and interspecies homologous recombination [155]. Some of these genes have probably been acquired

from the closely related alpha haemolytic streptococci [156]. This substantial plasticity enables the pneumococci to adapt to a high antibiotic pressure through increasing resistance. Plasmids are common carriers of resistance in other species but to date have not been detected in any resistant pneumococci. In these bacteria, resistance genes are normally acquired from other pneumococci or from other related species through a process called transformation. Perhaps is the quorum sensing system, triggered by the bacterial density responsible for the lysis of non-compentent siblings and the following release of DNA [157]. In contrast to many other bacteria, such as *S. aureus* and Gram-negative species, the pneumococci have remained susceptible to almost all antibiotics for decades. A strain of penicillin-non-susceptible pneumococci (PNSP) was first described in Australia in 1967, and these bacteria were also resistant to erythromycin [158]. Since then, there has been a dramatic rise in the incidence of PNSP, drug-resistant *S. pneumoniae* (DRSP), and multidrug-resistant *S. pneumoniae* (MDRSP), and today most of the pneumococcal isolates in many countries are MDRSP. In Europe the proportion of PNSP ranges from below 1% to almost 50% as shown in figure V.
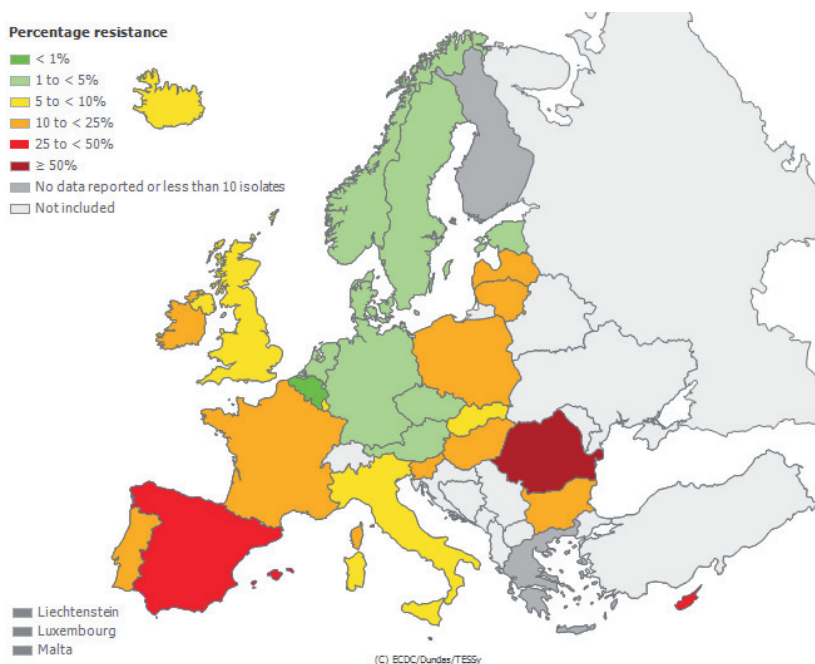
Figure V. Proportion of PNSP isolates in Europe 2011 from countries participating in EARS-NET, reprinted with permission from European Centre for Disease Control (ECDC).

The β-lactam antibiotics exert their bactericidal effect through inhibition of the microbial cell wall biogenesis. This is achieved when β-lactam binds to PBPs, which are peptidoglycan transpeptidase/carboxypeptidase enzymes that catalyze the terminal stage of synthesis of peptidoglycan (murein), the major component of the cell wall. There

are several different PBPs, and they are expressed solely by bacteria. The effect and spectrum of a β-lactam antibiotic is determined by the extent to which it binds to PBPs. Most bacterial species that are resistant to β-lactam antibiotics secrete or contain β-lactamases, which are enzymes that cleave the β-lactams. Pneumococci do not express these enzymes, and hence they have different mechanisms of resistance to β-lactam that consist of multiple mutations within several PBPs. Changes in the penicillin-binding domain of the PBPs are leading to a decreased affinity for β-lactams. Pneumococci produce six types of PBPs, of which those designated 1a, 2b, and 2x are the most important, and 2a, 1b, and 3 have only been described in rare cases. PBP 2b does not interact with cefotaxime over a wide concentration range, and this antibiotic leads to much slower lysis and killing compared to penicillin. Thus it seems that PBP2b is associated with bacterial lysis, an assumption that is supported by apparent tolerance observed in high-level penicillin-resistant strains that usually have low affinity for PBP 2b appear to be tolerant. Perhaps this is an advantage over the wild-type strains, even in the absence of antibiotics. There is evidence that some pneumococci with altered PBPs primarily express branched peptides in cell wall synthesis, whereas sensitive strains chiefly produce linear stem peptides in that context. This is interesting considering that the proteins MurM and MurN have been found to be involved in synthesis of short branched peptides in the bacterial cell wall [159]. It is proven that, inactivation of MurM caused the bacteria to produce cell walls that did not contain branched proteins, which resulted in nearly total collapse of resistance. The reason for this effect is obscure, and data suggest that other, as of yet unknown, factors are involved.

The clinical significance of PNSP in the outcome of pneumococcal pneumonia has been a subject of controversy. In a meta-analysis published in 2006 [160], it was concluded that PNSP was associated with higher mortality rates, although discordant antibiotic therapy was not the cause for the increased mortality in that study since most patients were given broad-spectrum antibiotics. The higher mortality noted in that investigation might have been associated with other factors, such as different bacterial virulence, co-morbidities and disease severity. Another more recent study indicated that PcG can provide good results, even at an MIC ≥ 2 µg/mL [161], indicating that high-dose parenteral PcG is still effective against pneumococcal pneumonia. By comparison, it has been reported that PNSP that cause meningitis are associated with treatment failure and delayed sterilization in patients given PcG [162]. All of the cited studies focused mainly on hospitalized patients who were treated parenterally. Due to the decreased bioavailability of many oral β-lactam antibiotics, treatment failure can be expected and has been reported [163, 164]. The Swedish Reference Group of Antibiotics (SRGA) recommends amoxicillin up to MIC = 2 mg/L [165] based on pharmacokinetic calculations.

Macrolides act bacteriostatically by binding the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome and blocking the elongation step of protein synthesis. There are essentially two mechanisms of resistance: active efflux and target site modification. Active efflux is mediated by an energy-dependent membrane transport protein, which is encoded by the *mef* gene located on a conjugative transposon. This is referred

to as the M-phenotype and gives rise to a low level of resistance. Treatment failure associated with these strains is not well documented [166]. The erythromycin ribosomal methylase (*erm*) mediates the target site modification by adding a CH_3 group to an adenine residue on the 23S rRNA. This methylation blocks the binding site of macrolides, lincosamides, and streptogramins, and the created phenotype, which is called MLSB and can be inducible or constitutive. In experiments *in vitro*, the strains with inducible MLSB appear to be clindamycin sensitive but erythromycin resistant when tested separately, whereas a D-shaped zone occurs around the clindamycin disc if they are tested side by side. These strains are associated with a major risk of treatment failure if clindamycin is used. Therefore, they are reported as resistant to clindamycin. The *erm* gene is also located on a transposon. A third mechanism of macrolide resistance is emerging, which is caused by nucleotide mutations in the 23S rRNA and amino acid substitutions in the ribosomal proteins. This can involve resistance to macrolides, lincosamides, ketolides, and streptogramins [163].

Tetracyclines block the attachment of aminoacyl-tRNA to ribosomes and thereby inhibit bacterial protein synthesis. Pneumococci can produce the ribosomal protection proteins Tet(M) and Tet(0), which are assumed to induce the detachment of tetracycline from the bacterial ribosome. The genes encoding these proteins are located on transposons. Fluoroquinolones bind and inhibit the enzymes DNA gyrase and topoisomerase IV, that normally fulfil the task of unwinding double-stranded DNA into a single stranded structure. This action stops the bacteria from replicating, because it blocks complementary base pairing and halts the synthesis of mRNA. Resistance to fluoroquinolones is caused by efflux and/or by mutations in the quinolone resistance-determining regions (QRDRs) of the genes coding for the enzymes. The impact from efflux is probably limited and does not render the bacteria highly resistant. The mutations occur in a stepwise fashion and can either arise spontaneously or be transferred from foreign genetic material. Studies have shown that quinolone resistance is seldom transferred horizontally [167].

Trimethoprim-sulfamethoxazole (TMP-SMX) inhibits successive steps in the folate synthesis pathway, and the two antibiotics have a synergic effect when used in combination. Resistance to TMP-SMX is acquired through mutations in the bacterial genome, and it is common in MDRSP.

Resistance in the population is a function of the selective pressure of antibiotics and dispersion. This has been found in epidemiological studies conducted on national, community, and individual levels, as illustrated by an investigation demonstrating a correlation between antibiotic prescribing and the incidence of PNSP in different residential areas in the city of Malmö, Sweden [168]. The success of a resistant clone also depends on the balance between the advantage of the resistance and the cost of fitness incurred by the new property [169, 170]. Furthermore, the prevalence of DRSP exhibits seasonal variation, with a higher level in the winter months when the selective advantage of resistance is greater due to more extensive use of antibiotics [171]. The majority of resistant clinical isolates belong to a small number of highly successful clones, some of which have spread globally [163]. The tasks of characterizing, standardizing, classifying,

and naming these clones are managed by the Pneumococcal Molecular Epidemiology Network (PMEN) [172]. For surveillance, it is not appropriate to consider serotype as equivalent to sequence type (clone), because capsular variants of clones exist.

Risk factors for pneumococcal disease

There are many predisposing factors for pneumococcal disease, the most important of which being high or low age (discussed in the section on defence of the airways) [173]. Pneumococcal infection is often the result of a predisposing viral infection, and many viruses themselves exert effects that can promote a superinfection, as already mentioned in earlier sections. Viral infections cause oedema and obstruction of the eustachian tube into the pharynx or the ostium of a paranasal sinus. The normal clearance of pneumococci from these niches can fail and thus result in a clinically recognizable infection. Similarly, chronic or acute damage to ciliated bronchial cells and/or increase in production of mucus can diminish the clearance of inhaled or aspirated microorganisms and lead to infection. An infection can also become established in individuals who aspirate pharyngeal contents or have diminished mechanisms of lower airway clearance, or in persons who are exposed to a high inoculum of organisms [89]. Several different co-morbidities can make people more prone to acquire any infection, as discussed in previous chapters. The list of these concomitant conditions is very long and essentially includes the same groups as those considered to have an indication for pneumococcal vaccination [174], the most well-known of which are conditions that affect the innate and/or the acquired immune system, such as asplenia and haematological disorders like myeloma and chronic lymphatic leukaemia. Other significant risk factors are diabetes and autoimmune, neurological, renal, cardiovascular, liver, and pulmonary diseases [173]. Socioeconomic risk factors include alcohol abuse, smoking, poverty, race, malnutrition, and overcrowding. Patients with a defective blood brain barrier and CSF leakage are also at risk of meningitis [175].

Invasive pneumococcal disease (IPD)

Pneumococci can act as invasive pathogens that penetrate the mucosal barrier. A plausible explanation for this behaviour is that cbpA interacts with polymeric immunoglobulin receptors on the surface of epithelial and mucosal cells, which leads to endocytosis, transport across the cell, and release through the inner cell membrane [89]. Pneumolysin is probably also required for spread from the lungs to the bloodstream [20]. IPD is defined as infection of any normally sterile body site, i.e. blood and/or cerebrospinal, pleural, pericardial, synovial, or vitreous fluid, and even fluids extracted from deep tissues under sterile conditions. The transition from asymptomatic carriage to invasive disease occurs via direct extension from the site of colonization or direct haematogenous spread. The most common foci for infection are pneumonia followed by meningitis, although IPD can accompany septic arthritis or otitis media and can also

occur in the absence of any focal findings. An unknown focus is associated with a more severe outcome [146].

As discussed in the section on serotype-specific differences, serotypes vary regarding their capacity to cause IPD. An interesting hypothesis to explain this disparity was recently put forward by Melin *et al.* [176], who found that that the primary pathogens (e.g., serotypes 1 and 5) are more resistant to complement and require a higher concentration of capsule antibodies to be susceptible to opsonophagocytic killing compared to the opportunistic serotypes (e.g., 6B and 23F), which are associated with a more severe disease outcome. When pneumococci spread to the blood, antibodies specific for the capsule play an important role, as illustrated by the results of trials using mice and the effects of vaccines directed against the capsule. Opsonized bacteria in the blood are most effectively killed in the marginal zone of the spleen, where B cells and macrophages gather waiting to eliminate invading microorganisms. Human neonates lack the marginal zone of the spleen and are thus more prone to develop IPD [177], and asplenia is a well-known risk factor for invasive infections with encapsulated bacteria. Antibodies directed towards anti-capsular structures can also be protective to some extent, as has been shown for pneumolysin [178], pspA [179], and immunization against autolysin and neuraminidase has been found to provide modest protection in experimental animals [89].

In this context, it can also be noted that the risk of IPD is increased in young children enrolled at DCCs, as well as in non-elderly immunocompetent adults living with children attending day care [180].

Pneumococci in day care centres

Nasopharyngeal carriage of pneumococci in children depends primarily on age and other risk factors, as discussed above [19]. Young children in limited and crowded surroundings represent an optimal environment for dispersion of virus and bacteria, and pneumococcal infections are often antecedent to viral infections. Studies of a dutch cohort has [181] shown that the relative risk of pneumococcal colonization was 1.6 to 3.4-fold in children attending DCCs as compared to those cared for at home, and they also found increased genetic clustering among the DCC isolates, which supports the theory of horizontal dispersion. Many other investigators have reported similar findings. In a recent study conducted in the post-conjugate vaccine era, Ercibengoa *et al.* [107] applied PCR-based technology to investigate children and found that 81.9% were colonized with pneumococci, and 43.6% carried more than one serotype of these bacteria. DCC attendance is a risk factor for developing acute otitis media and pneumococcal pneumonia [182, 183], and for acquiring resistant pneumococci [184]. Clusters of IPD caused by multi-resistant strains have been described in DCCs [185], and the following factors in these settings facilitate the development and spread of resistant organisms [186]: large numbers of children and a higher probability of physical interaction with pneumococcal carriers; frequent close person-to-person contact; wide use of antimicro-

bial medications. Similarities of serotypes and genotypes of DRSP found in children at DCCs and in association with disease worldwide strongly suggest that the nasopharynx of children is an important global ecological reservoir of DRSP and may also play a critical role as the optimal anatomical site for the evolution of these bacteria [187].

Pneumococcal vaccines

As mentioned, the bacterial capsule constitutes the major virulence determinant, because it serves as a physical barrier and also interacts with complement in a manner that allows circumvention of the host anti-microbial defence. Specific host antibodies directed towards the capsule have key functions in protection against all encapsulated bacteria. Evaluation of the immune response after vaccination was previously achieved by performing enzyme-linked immunosorbent assay (ELISA) to measure the level of antibodies produced. However, that technique is no longer considered the most reliable, and the gold standard today is instead opsonophagocytic assay [188], which is a qualitative method that determines the level of antibody-dependent killing of bacteria.

Pneumococcal polysaccharide vaccine (PPV) containing purified capsular polysaccharides from the 23 most common serotypes isolated from patients with IPD has been available since 1986. PPV generates a T-cell-independent response and do not stimulate memory B cells. Therefore, re-vaccination with a PPV does not elicit a booster response, but rather induces hyporesponsiveness [177]. The mechanisms of hyporesponsiveness are not known, although there is speculation that subsequent stimulation with polysaccharides provokes a T-cell-independent response that stimulates but does not replenish immune memory cells, resulting in an overall depletion of the memory cell pool and a weaker reaction to re-exposure to the same polysaccharide. Both infection with and carriage of pneumococci have been shown to lead to serotype-specific hyporesponsiveness upon subsequent vaccination [189]. This effect is also seen when PCV is administered shortly after PPV, and therefore it has been proposed that PCV should be given first if immunization with both types of vaccine is planned [174, 190]. The clinical effect of PPV is controversial, and the results of clinical trails are conflicting, especially regarding the protection that is provided against pneumococcal pneumonia. Most experts agree that there is an effect on IPD incidence in a healthy adult population but not in risk groups or the very old [174, 191]. These observations are also supported by a Cochrane report published in 2009 [192] concluding that PPV does influence IPD incidence, but not with respect to all-cause mortality or all-cause pneumonia.

Conjugate vaccines have been used successfully in children to prevent infections with *Haemophilus influenzae* type b, *Salmonella typhi*, and *Neisseria meningitides*. Coupling of bacterial polysaccharide to a carrier protein through conjugation allows the antigen-presenting cells to recognize the polysaccharide and present it to the T helper cells, which in turn stimulates B cells to induce plasma cells and immunological memory [177]. Inasmuch as PCV is essentially a totally different vaccine compared to PPV, it is even effective in children under two years of age and most likely also in some of

the risk groups for pneumococcal infections. This is exemplified by results showing the efficacy of PCV in HIV-positive adults [193]. PCV induces a booster effect upon re-immunization, although possibly with the exception of the reaction to serotype 3, which can instead entail hyporesponsiveness [177]. PCV also has an impact on the colonization rate of the different serotypes it addresses, which contributes to a massive herd effect on a societal level [101]. PCV has reported to prevent pneumococcal pneumonia and acute otitis media [194, 195], and it has been approved for use in adults although there is a debate as to what extent it should be given. Today, there are no official national guidelines in Sweden for PCV immunization for adults in various risk groups, but the guidelines applied in for example Denmark and the United States recommend PCV for patients belonging to groups at risk of pneumococcal infections [174, 196]. Replacement with non-vaccine serotypes in society means that pneumococcal vaccination must be focused on a moving target. Therefore, PCVs including 15 serotypes are under development, and studies aimed at finding protein-based vaccines for the future are in progress.

Nosocomial pneumonia with emphasis on ventilator-associated pneumonia

The concept of nosocomial pneumonia comprises healthcare-associated pneumonia (HCAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP). HCAP is seen in patients subject to any of the following: hospitalized for at least two days within 90 days of the infection, resided in a nursing home or long-term care facility, recently received intravenous antibiotic therapy, given chemotherapy or wound care at some time during the past 30 days of the current infection, or attended a hospital or hemodialysis clinic. HAP is defined as an infection after hospitalization for more than 48 hours. VAP is an infection that occurs in an ICU patient more than 48 hours after endotracheal intubation and start of mechanical ventilation. The guidelines for management of nosocomial pneumonia published in 2005 by the American Thoracic Society (ATS) stipulate that CHAP, HAP, and VAP be treated as one entity and give virtually the same recommendations for all three diagnoses. VAP has been studied from many perspectives over the years, whereas data on HAP in non-intubated patients and HCAP are scarce. Based on extrapolation from investigations of VAP, the ATS advocates that HCAP and HAP should be managed in the same manner as VAP, using the same approach to identify risk factors for infections with specific pathogens [197]. The ATS guidelines have been criticized for simplifying the situation and leading to overuse of antibiotics in patients with HAP and HCAP in light of the extreme heterogeneity of the population of patients affected [198]. Furthermore, clinicians' compliance with the 2005 ATS guidelines has been marginal [199]. Professor Victor Yu discussed this weakness in *The Lancet Infectious Diseases* in 2011, arguing that the fatal flaw is the failure to accurately diagnose HAP and VAP, and that the inability to distinguish between

colonization and infection in respiratory-tract cultures makes the guidelines inherently unstable. Yu indicated that the risk of the ATS recommendations concerns escalating empiricism of antibiotic use for severely ill patients who might not have an infection. He concluded that a vicious circle of antibiotic overuse may result in the emergence of a resistant microflora and unwarranted use of empirical broad-spectrum combination antibiotics accompanied by a rise in mortality.

Must studies on aetiology in these infections are made in Europe or North America and the aetiology can differ, even between hospitals in the same country [200]. Therefore were the objective of the study reported in Paper IV to investigate the aetiology of VAP in a Swedish university hospital.

Incidence and prognosis of VAP

VAP is the most common ICU-acquired infection, with studies indicating incidence ranging from 6% to 52% [201, 202], a huge difference that can be partly explained by the lack of consensus regarding diagnostic criteria and the most appropriate diagnostic method for VAP. An example of the discrepancies is that the incidence of VAP reported in Europe is four times higher than that observed in the United States. Rates of VAP are related to the duration of mechanical ventilation and have been estimated to be 3.3% on day five, 2.3% on day 10, and 1.3% on day 15 [203]. The risk of acquiring VAP seems to be low after ten days of mechanical ventilation [204], and more recent data indicate incidence of VAP ranging from 9 to 31 cases per 1,000 ventilator days [205, 206]. Also, the incidence tends to be higher in surgical ICUs tend than in medical ICUs [207].

The crude mortality rate for VAP ranges from 20% to 60% in different studies depending on the type of patients considered. The aspects of disease severity, diagnostic methods, aetiology, and patient management also differ between investigations [197, 208, 209]. The estimated attributable mortality of VAP is 9% [210]. Data on short- and long-term morbidity are limited, although clinical observations suggest that VAP can significantly alter the quality of life for survivors [16]. Prevention strategies reduce patient mortality and morbidity, and also lower healthcare costs [197, 211]. It has been estimated that an average episode of VAP increases hospitalization by 12 days, use of a ventilator by 6 days, ICU stay by 6 days, and hospital costs from 12,000 to 40,000 USD per episode [197, 208].

Risk factors for VAP

The most important factors contributing to the development of nosocomial pneumonia is the severity of underlying disease and the use of an endotracheal tube [212, 213]. The airway defence mechanisms aimed at preventing an infection are altered in intubated patients. These patients are under extreme stress that changes their immune status. This is illustrated by a study of ICU patients in which those who died from sepsis had

findings consistent with immunosuppression, whereas that was not observed in those who died of non-sepsis aetiologies without hyperinflammation [214]. Other researchers found that critically ill patients had a C5a-mediated neutrophil dysfunction, which was a strong predictor of subsequent acquisition of nosocomial infection [215]. The endotracheal tube prevents effective coughing, and hence the patient cannot protect the lungs from microaspiration of contaminated pharyngeal pathogens. Notably, the harmful effects of an endotracheal tube were demonstrated by Girou *et al.* [216], who found that patients with non-invasive ventilation (NIV) had a significantly lower incidence of VAP compared to those with invasive mechanical ventilation. However, the conclusions drawn by Girou and colleagues have been criticized, because in that study the patients who received NIV were not as ill as those with endotracheal intubation.
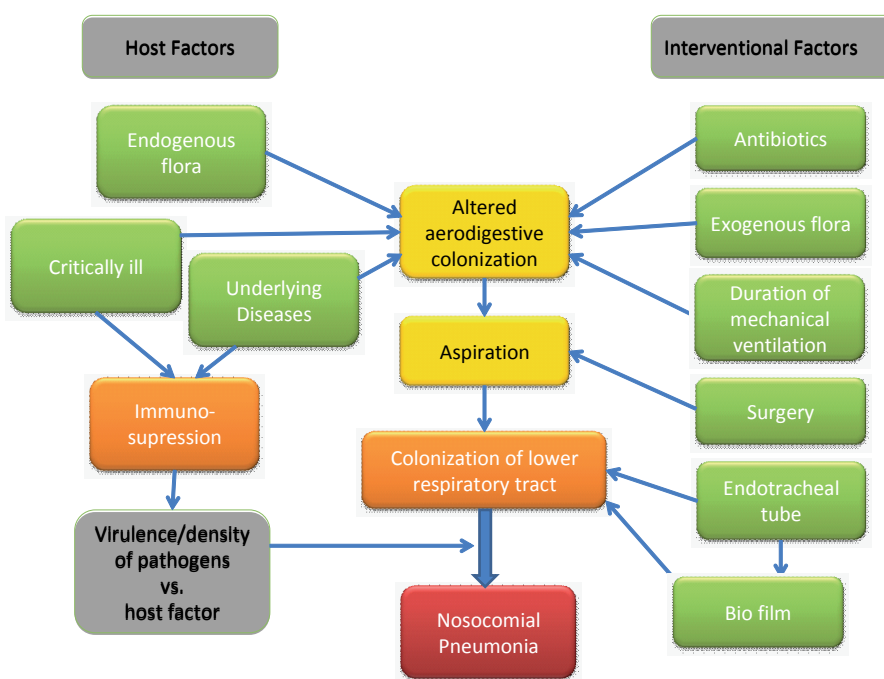
Figure VI Schematic view of the factors involved in the development of VAP.

The list of other factors predisposing to VAP long, and, besides severity of disease, the host factors include old age, presence of comorbidities, organ failure and immunosuppression. Patients admitted to an ICU for post-traumatic or post-surgical care, particularly after burns, are at higher risk of VAP. The more severe disease the patient has, the higher is the risk for colonization with *P. aeruginosa* and MDR pathogens. Interventional factors include tracheostomy, non-supine position, bronchoscopy, enteral feeding, duration of mechanical ventailation, and central vein catheterization [61]. In a study conducted in a tertiary hospital in India, Joseph *et al.* [206] found that

impaired consciousness, tracheostomy, re-intubation, emergency intubation, and naso-gastric tube were risk factors for VAP. Those authors also observed that emergency intubation and intravenous sedatives were specific risk factors for early-onset VAP, whereas tracheostomy and re-intubation were independent predictors of late-onset VAP. The interplay between risk factors for the development of VAP is presented in a simplified schematic manner in figure VI.

Diagnosis of VAP

There is no consensus on how to diagnose VAP. Postmortem examinations have indicated that a diagnosis made using clinical criteria alone leads to 30–35% false-negative and 20–25% false-positive results [217]. The diagnostic value of an abnormal X-ray is questionable, because there are several causes of such findings in a critically ill patient, and this was demonstrated in autopsy studies of this category of patients. In an investigation performed in 1972 [81], air bronchograms proved to be the best basis for diagnosis, correctly predicting 64% of the pneumonias. Surprisingly, 38% of the patients in that study were found to have alveolar haemorrhage, and 29% of that group had had multiple air bronchograms.

Today, securing representative cultures from lower respiratory secretions before starting empiric treatment is common practice worldwide. Many centres use bronchoscopy to acquire samples by bronchoalveolar lavage (BAL) or protected specimen brush (PSB). These samples are routinely analysed quantitatively, since there is a risk of contamination during the bronchoscopy. The levels of contamination are low, and the diagnostic threshold is $\geq 10^3$ CFU/ml for PSB, $\geq 10^4$ CFU/ml for BAL, and $\geq 10^6$ CFU/ml for the non-invasive approach obtained by tracheobronchial aspirate (TBA). Quantitative culture achieves relatively higher predictive values and is now accepted as best practice by most physicians. However, qualitative cultures have a high negative predictive value but the specificity is low [87]. The sensitivity and specificity of the mentioned sampling methods vary between studies (values given respectively): 36–83% and 50–95% for PSB; 39–91% and 66–100% for BAL; 44–87% sensitivity and 31–92% for TBA. Thus some studies report that microorganisms identified in quantitative TBA cultures in many cases do not agree with those found in cultures of pathological samples [218]. Apparently no studies have assessed the diagnostic value of bronchoscopy images, considering whether they reveal normal or inflamed mucosa. Such examination is of course subjective, and the results of a bronchoscopy depend on the experience of the bronchoscopist. However, many ICUs lack expertise in this procedure, which may argue for the use of non-invasive techniques. Bronchoscopy is an invasive procedure that is associated with an increased risk of VAP. The results in the literature are conflicting, and the relative benefits of non-invasive and invasive diagnostic approaches are still unclear.

Gram staining is a useful method for detecting microorganisms. To start with, a sample has to be examined by microscopy to determine its representativeness of the

lower airway. In a postmortem study [219], it was found that microscopy of BAL samples yielding < 50% neutrophils had a 100% negative predictive value for histologically confirmed pneumonia. Gram staining cannot predict the culture results, but it can give some indication of the aetiology. In a study carried out in 2001 [220], correlation of Gram results with BAL cultures was complete in 39% cases, partial in 28%, and absent in 33%.

The utility of the widely used biomarkers CRP and PCT as predictors of VAP has not been demonstrated, because both of those proteins can be elevated in inflammation induced by non-infectious as well as infectious causes [221]. Also, the biomarker type 1 soluble triggering receptor expressed on myeloid cells (sTREM-1) has been studied in BAL specimens and exhaled breath condensate, but unfortunately it has poor discriminatory power.

Patients are diagnosed with VAP despite the lack of a universally accepted definition of the disease. In Sweden, the definition is now divided into verified and suspected VAP in the Registry of Intensive Care Complications [222]. A case of VAP is recorded as confirmed if all three of these criteria are fulfilled: (i) mechanical ventilation ≥ 48 hours; (ii) new or increasing radiological infiltrate consistent with pneumonia; (iii) significant amount of bacteria in quantitative airway secretions (mentioned above). By comparison, a case is registered as suspected VAP if it meets all three of the following criteria: (i) mechanical ventilation ≥ 48 hours; (ii) new or increasing radiological infiltrate consistent with pneumonia; (iii) clinical suspicion of pneumonia based on a body temperature ≥ 38.5 °C or CRP ≥ 100 mg/l. In many other countries, leukocytosis/leukopenia and high or low temperature are used as diagnostic criteria.

Inasmuch as there is no gold standard diagnostic method or valid and reliable definition for VAP, and all existing definitions lack sensitivity and specificity, the Centers for Disease Control and Prevention (CDC) in the United States is working together with several professional societies to launch a totally new approach in 2012 [223]. The aim is to create a surveillance definition algorithm for detection of ventilator-associated events that can identify a broad range of conditions or complications occurring in mechanically ventilated adult patients. It is a surveillance definition and not made to be used in in the clinical care of the patients. Objective, readily available clinical data are being used in order to create an easily applied standard method that will improve surveillance and achieve a high level of coherence between different centres. The new CDC standard does not include X-ray examination of the lungs for the reasons mentioned above. The new surveillance definition can be used in persons who are ≥ 18 years of age, have been intubated and mechanically ventilated for at least 3 calendar days, and are being treated in facilities for acute or long-term acute care or inpatient rehabilitation. The goal is to begin implementing this novel surveillance definition in January 2013.

Patients ≥ 18 years of age who are on mechanical ventilation for ≥ 3 calendar days can be included. Initially, a patient has to have ≥ 2 calendar days of stable or decreasing positive end-expiratory pressure (PEEP) and fraction of inspired oxygen (FiO_2) in the ventilator. Thereafter, the patient can be assigned one of the following diagnoses after evaluation in a ladder-like manner:

1. *Ventilator-associated condition (VAC).* Defined as an increase in FiO_2 or/and PEEP

2. *Infection-related ventilator-associated complication (IVAC).* Defined as high or low body temperature or/and white blood cell count and a new antimicrobial agent(s) started, and is continued for ≥ 4 calendar days.

3. *Possible VAP.* Defined as fulfilling either of the following criteria: (i) representative purulent respiratory secretions, assessed by microscopy and semi-quantitative cultures above threshold if reported; (ii) positive culture (qualitative, semi-quantitative or quantitative) of sputum, endotracheal aspirate, bronchoalveolar lavage, lung tissue, or protected specimen brushing (no threshold)

4. *Probable VAP.* Defined as meeting either of the following criteria: (i) representative purulent respiratory secretions assessed by microscopy and significant levels of bacteria in quantitative culture (same cut-offs as described above); (ii) purulent respiratory secretions with positive pleural fluid culture, positive lung histopathology, or a positive diagnostic test for *Legionella* spp., influenza virus, respiratory syncytial virus, adenovirus, or parainfluenza virus.

The plan is to ensure that all four of these diagnoses will be automatically reported to the surveillance system. It remains to be seen whether Sweden and the rest of the world will adopt this new approach for VAP surveillance.

The aetiology of VAP

The study reported in Paper IV addressed the aetiology of VAP. As discussed above, patients with a severe underlying disease get a subsequent change in bacterial adherence to mucosal surfaces. The potential pathogens gradually create a biofilm on the inner surface of the endotracheal tube to serve as a nidus that is impervious to systemic antibiotics, and the bacteria located within the biofilm are more often less susceptible to antibiotics [224]. The onset of VAP in relation to the start of mechanical ventilation or the beginning of the illness is an important determinant of the likely pathogen. Previous antibiotic therapy, co-morbidities, and immunosuppression are also important to predict the pathogen. In simpler terms, the bacteria can belong to the "normal pathogens", that is, those that normally colonize healthy humans, such as *S. pneumoniae, H. influenzae, S. aureus*, and *M. catarrhalis* in the throat and *Escherichia coli* carried in the gut. The longer a patients is treated in an ICU, and the greater the severity of his/her illness, the higher is the risk of infection with hospital-associated acquired bacteria such as other Gram-negative species or *methicillin-resistant S. aureus* (MRSA). Factors that predispose to infection with MDR pathogens are intravenous antibiotics or chemotherapy within the past 30 days, chronic haemodialysis, residing in a nursing home, and hospitalization for two or more days during the past 90 days [197]. But what are the sources of the infective bacteria? In a study of ICU patients, van Saene *et al.* [225] found that 55% of all infections that occurred within the first week of critical care were primary endogenous infections, that is, they were caused by pathogens that

were already colonizing the patient, which included both the above-mentioned normal pathogens and hospital-associated bacteria. One-third of the infections were secondarily endogenous and with onset after the first week, and these were invariably caused by hospital-associated bacteria. Furthermore,15% of the infections were exogenous, meaning that they were not related to colonization and could occur at any time during the stay in the ICU, and they were also caused by hospital-associated bacteria. The exogenous spread of microorganisms to patients can occur through contaminated hands or respiratory instruments or infective aerosols in the ICU environment, and it can be reduced by good hygiene [61].

The potential pathogens are numerous, and a short review of the most common species associated with VAP is presented below, roughly dividing them into community- and hospital-associated bacteria.

Community-associated Gram-positive bacteria

- *S. aureus* is a frequent colonizer of the skin and mucosa, and it is a successful opportunistic pathogen that is also common in community-acquired infections and can produce a wide variety of diseases.

- *S. pneumoniae* is the most prevalent cause of respiratory infections and often colonizes the nasopharyngeal tract, especially in children.

- *Streptococcus pyogenes* is a ubiquitous species that is the most frequent bacterial cause of acute pharyngitis and also gives rise to a variety of cutaneous and systemic infections.

Community-associated Gram-negative bacteria

- *H. influenzae* is the second most common cause of respiratory infections and is widely carried, especially young children. It is also prevalent in patients with chronic obstructive pulmonary disease (COPD).

- *M. catharralis* often colonizes the respiratory tract and is a common cause of upper respiratory tract infections and can also induce lower respiratory tract infections, especially in COPD patients.

- *E. coli* is the most prominent member of Enterobacteriacae, a family of facultative anaerobic bacteria that are normally found in the human gastrointestinal tract. This species is the most common cause of community-acquired urinary tract infections (UTIs) and bacteraemia, and is also responsible for a variety of nosocomial infections.

Hospital-associated Enterobacteriacae

Enterobacteriacae constitutes a major group in this category of pathogens, and the family members listed below have similar properties and live in the gastrointestinal tract. These microbes can cause community-acquired UTIs and bacteraemia, but are more frequently seen in nosocomial infections. They can arise from the endogenous intestinal flora of hospitalized patients and sometimes cause outbreaks. Species of the genera *Enterobacter, Citrobacter, Serratia*, and *Morganella* carry inducible *ampC* genes that encode resistance to ampicillin and cephalosporins and can be expressed constitutively at high levels after mutations. Members of Enterobacteriacae can also be resistant to many other antibiotics as a result of plasmid-encoded resistance genes, and the greatest threat in that context is related to the extended spectrum beta-lactamases (ESBLs) [226].

- *Klebsiella* spp. are a relatively common aetiology in nosocomial infections and the three species *K. pneumoniae, K. oxytoca* and *K. granulomatis* are most prevalent. These bacteria can also cause pneumonia (Friedländer's disease), wound infections, and cholecystitis, primarily in patients with an underlying disease.

- *E. cloacae, E. aerogenes*, and *E. sakazakii* are responsible for the vast majority of *Enterobacter* infections, and these pathogens are common in ICU patients and in patients that have already been treated with antibiotics.

- *Proteus* spp. give rise to UTIs, often in patients with an underlying urologic condition and an indwelling catheter or functional or anatomical abnormalities. These bacteria tend to cause more severe infections, and they give rise to pyelonephritis more often than other members of Enterobacteriacae.

- *Morganella morganii* is the only species of this genus. It is seldom the cause of nosocomial infections and is usually found in wounds and urine, but can also occur in the lungs. *M. morganii* can cause nosocomial outbreaks.

- *C. freundii* and *C. koseri* are common pathogens in the ICU. The former species is most often found in the urinary tract, and the latter has caused numerous outbreaks of neonatal meningitis.

- *Serratia* spp. cause infections in humans, primarily *S. marcescens*. Unlike other *Enterobacteriacae*, *Serratia* spp. are widespread in the environment but are not generally a component of the human faecal flora, and most infections are acquired exogenously.

Other hospital-associated bacteria

The Gram-positive bacteria MRSA is a common aetiology to VAP in many countries but is a rare cause of VAP in Sweden and is therefore excluded from this list. However,

there are several important Gram-negative bacteria belonging to other families, the most commonly associated with VAP are listed below.

- *Pseudomonas aeruginosa* is primarily encountered as a nosocomial pathogen, which reflects its great propensity to grow in a variety of environments with minimal nutritional components. This species can infect or colonize essentially any part of the body, and it is a common cause of VAP. *P. aeruginosa* also occurs in nature, in otherwise healthy humans (preferably on moist surfaces), and on many man-made surfaces, such as in showers and toilets in hospitals. This species creates a biofilm and often colonizes the lungs of patients with cystic fibrosis or bronchiectasis. Production of biofilm and many other virulence factors by *P. aeruginosa* is mediated by a quorum-sensing system. When the bacteria reach a critical mass, low-molecular-weight mediators of the quorum-sensing response are synthesized and then secreted, diffusing through the cells of the bacterial community to influence gene transcription and expression of virulence factors. *P. aeruginosa* is often MDR, and strains exhibiting resistance to β-lactam antibiotics in combination with fluoroquinolone and aminoglycoside are emerging worldwide. Thus there is an urgent need for new antibiotic treatments focused on *P. aeruginosa*, and, in many countries where MDR strains of this species are more prevalent, physicians are turning back to old drugs such as colistin and polymyxin [227].

- *Stenotrophomonas maltophilia* is regarded as an important nosocomial pathogen, particularly in ICUs and in patients with prior broad-spectrum antibacterial treatment. These bacteria are intrinsically resistant to most antimicrobial and disinfectant agents, and can be cultured from diverse environmental sources. When considering *S. maltophilia*, it is essential to distinguish between a clinically significant infection and colonization, because the latter is often the case in respiratory secretions. Risk factors for infection are extensive use of broad-spectrum antibiotics, advanced age, mechanical ventilation, and a higher Acute Physiology and Chronic Health Evaluation II (APACHE II) score. Infections can be polymicrobial, partly due to production of at least two inducible β-lactamases, which support the growth of pathogens such as *Serratia marcescens* and *Pseudomonas aeruginosa* even in the presence of broad-spectrum antibiotics. The most common infections in ICUs are pneumonia and bacteraemia; the latter is often associated with the use of a central venous catheter, and hence standard treatment in Sweden is to remove the catheter and administer TMP-SMX. However, particularly during use of TMP-SMX, the results of *in vitro* sensitivity testing can be in conflict with the clinical outcome. Patients with *Stenotrophomonas maltophilia* infections have a high fatality rate, especially if they are given inappropriate treatment, which is unfortunately not unusual [228].

- *Acinetobacter* spp. are ubiquitous opportunistic pathogens that are detected in nearly 100% of soil and water cultures. These bacteria are found on the skin of 25% of healthy ambulatory adults and also bring about pharyngeal colonization in 7% of adults and infants in the general population. Besides being isolated from the mentioned sources, *Acinetobacter* spp. can also survive on dry inanimate objects for

months and are resistant to biocides (e.g., chlorhexidine), which are perfect properties for bacteria that cause nosocomial infections. However, these microbes have only a limited number of virulence factors, which reduces them to the role of opportunists. *Acinetobacter* spp. can colonize as well as cause suppurative infections in almost every organ system, and, similar to *S. maltophilia*, they constitute a challenge with respect to interpreting the significance of the potential pathogens in clinical specimens. Nosocomial pneumonia is the most common infection, and bacteraemia is frequently associated with respiratory tract infections and use of central intravenous catheters, but less often related to urinary tract, wound, skin, and abdominal infections. *Acintebacter* spp. are prevalent in war wounds and can cause infections after head trauma or neurosurgical procedures. *A. baumannii* is the most widespread species in Sweden and is frequently resistant to multiple antibiotic classes. For years, the mainstay of *Acinetobacter* therapy has been β-lactam antibiotics, particularly third-generation cephalosporins, extended-spectrum penicillins, penicillin–β-lactam inhibitor combinations, and carbapenems, often combined with aminoglycosides in more severe infections. *A. baumannii* is now resistant to many classes of antimicrobials and represents an emerging global problem in ICUs [229].

Oropharyngeal and cutaneous commensals (OCCs)

OCCs include alpha-haemolytic streptococci, *Neisseria* spp., and coagulase-negative staphylococci, which were described in previous chapters. The significance of OCCs in VAP is controversial, although, based on the results of a retrospective study, Lambotte *et al.* [230] came to the conclusion that if these pathogens are the only finding in a distal bronchial sample, they may behave as classic nosocomial pathogens. Indeed, these authors found that OCC-VAP constituted 9% of all VAP episodes in their investigation.

Low-virulence pathogens: anaerobic bacteria, enterococci, and Candida spp.

• Anaerobic bacteria can cause aspiration pneumonia in non-intubated patients, and they are found in cultures from distal bronchial samples, albeit almost always in association with aerobic bacteria. The role of anaerobic bacteria is unclear but is probably of little significance [231, 232]. Furthermore, these microbes are found in polymicrobial flora and are susceptible to most antibiotics given to patients with VAP, and thus they have little impact on daily clinical decisions. *Enterococcus* spp. represent an increasing global problem in hospital settings, particularly in severely ill and immunosuppressed patients. The bacteria themselves are not very virulent, and their intrinsic and acquired resistance to antimicrobials are the most important factors for their success in the mentioned patients. Colonization of the respiratory tract in patients with mechanical ventilation is common and has usually already occurred at the time of intubation, and transmission of enterococcal

strains between patients are prevalent [233]. It is difficult to interpret the significance of substantial growth of enterococci.

- *Candida* spp. cause pneumonia that has been described in organ transplant or immunocompromised neutropenic patients, but the role of these bacteria in immunocompetent patients with VAP is not clear. Experts believe that most *Candida* spp. detected in distal bronchial samples are clinically unimportant in immunocompetent patients [232]. Colonization with *Candida* is associated with worse outcome, but it is not known whether this is due to the colonization per se, or if it is simply a manifestation of increased morbidity and mortality [234].

Polymicrobial flora

A polymicrobial flora is present in 30–70% of all VAP patients, but it has not been determined whether this is due to improper sampling or if it can be explained by aspiration of the bacteria. The outcome for this group of patients does not differ from that seen in patients carrying only one microorganism [61, 235].

Treatment of VAP

High suspicion of VAP should be met with a rapid response that includes collecting adequate samples for culture and starting appropriate empirical antimicrobial therapy. This requires local surveillance data, and must also take into account the risk factors for multi-drug resistance and pseudomonas aetiology, as discussed above. Studies have shown that both a delayed start of antibiotic treatment and an inappropriate choice of antibiotics are associated with worse outcome [64, 236]. It is important to de-escalate the treatment as soon as the culture results are available. There is also evidence that a targeted treatment is correlated with less use of antibiotics and does not harm the patients [237]. In France, Leone and co-workers [238] found that de-escalation was feasible in 42% of the patients they investigated. A suitable duration of treatment was not agreed upon until 2003 when, based on a prospective randomized double-blind study published by Chaste *et al.* [239], consensus was reached that uncomplicated VAP should be treated for 8 days if the therapy chosen resulted in adequate clinical response. Cases involving VAP caused by non-fermenting Gram-negative bacilli represent a possible exception to this strategy, because Chastre and colleagues had observed a higher recurrence rate in that group and hence treatment for 14 days is recommended for such aetiologies.

Objectives

The objectives of the research underlying this thesis were as follows:

- To investigate and evaluate the day care centre (DCC) interventions implemented to constrain $PNSP_{0.5}$ dispersion, and to assess pneumococcal colonization data from the included DCCs in relation to seasonal variation, age, and size of day care group.

- To study the outcome of eradication therapy given to children with prolonged naso-pharyngeal carriage of $PNSP_{0.5}$.

- To determine whether there is a serotype-related difference in the incidence of septic shock in patients with invasive pneumococcal disease.

- To assess the bacterial aetiology in patients with VAP in relation to early and late onset of the disease and antibiotic treatment, and to study the incidence of drug-resistant bacteria.

Materials and methods

All of the studies included in this thesis were conducted in in Skåne county situated in southern Sweden.

Study designs

Paper I: A retrospective epidemiological cohort study conducted in the Malmö–Trelleborg area from 2000 to 2010 to investigate DCC interventions aimed at constraining $PNSP_{0.5}$ dispersion.

Paper II: A retrospective study of medical records of children referred to the hospitals in the cities of Malmö and Lund from 1997 to 2011 for eradication therapy of $PNSP_{0.5}$ due to prolonged nasopharyngeal carriage.

Paper III: A retrospective cohort study of the serotype-related incidence of septic shock in patients with invasive pneumococcal disease in Skåne County between 2006 and 2008.

Paper IV: A retrospective cohort study of the aetiology of VAP in a Swedish university hospital 2004–2007.

South Swedish Pneumococcal Intervention Project (SSPIP) (Papers I and II)

The children included in the research reported in Papers I and II were all participants in the South Swedish Pneumococcal Intervention Project (SSPIP). The SSPIP was initiated because the prevalence of PNSP in the early 1990s increased to approximately 10% in the southern parts of Sweden but remained unchanged at a level of around 2–3% in the rest of the country. In the mid 1990s, Baquero *et al.* described an epidemiological model based on experience in several countries suggesting that when PNSP

(MIC $\geq$ 0.125 mg/L) constitute more than 8% of all pneumococci, they can spread rapidly in the population [240]. In response to this, the regional health authorities in Malmöhus County founded the SSPIP in 1995 in an attempt to limit the dissemination of PNSP [241]. All cultures yielding PNSP with a penicillin G (PcG) MIC of $\geq$ 0.5 mg/L ($PNSP_{0.5}$) were reported directly from the microbiology laboratory to the Regional Centre for Communicable Disease Control (RCCDC) in Skåne County. The cut-off at MIC $\geq$ 0.5 mg/L was chosen to ensure a margin of error in relation to the highly resistant strains with an MIC of $\geq$ 2 mg/L. In January 1996, the Swedish Communicable Disease Act made it mandatory to report all cases of $PNSP_{0.5}$.

If a child enrolled at a DCC was found to be a nasopharyngeal carrier of $PNSP_{0.5}$, the intervention including a series of actions was initiated. First, the index case and usually also siblings of that child were suspended from the DCC. Second, to identify asymptomatic carriers, nasopharyngeal swabs were taken from family members and close contacts, as well as from other children enrolled at the same DCC. The screening was performed in different ways depending on how the DCC was organized and to what extent the departments were linked. It always included the department of the index case and sometimes also adjacent departments or all DCC attendees and personnel. If the primary screening at a DCC resulted in contact cases, a second screening was performed. This second screening included the same departments that were screened on the first occasion and was often extended to comprise the entire DCC. During the second screening, the DCC was first closed to all attendees but was later opened again to personnel and children who tested negative for $PNSP_{0.5}$. The decision to extend the original screening or conduct a second screening was made by the Regional Department of Communicable Disease Control in Skåne County in consensus with the head of the DCC in question. Children with $PNSP_{0.5}$ identified by the screening were followed each week with repeated nasopharyngeal swabs and were suspended from the DCC until two consecutive $PNSP_{0.5}$-negative cultures were obtained. Children with duration of carriage of more than two to three months were offered eradication therapy, if there was a socio-economic impact on the children and their families.

The SSPIP also included a campaign to restrict the use of antibiotics, especially prescriptions to children. Information was given to physicians about treatment recommendations and to parents about respiratory tract infections. All children in the county were offered a free return visit within one week to prevent prescribing of antibiotics "in the event of" lack of improvement or deterioration. Furthermore, better feedback was provided regarding statistics on local sales of antibiotics and resistance.

Paper I

This investigation included children aged 0–7 years who were DCC attendees and had been screened within the SSPIP, and also the DCC personnel. The study period was from June 2000 to December 2010, and the DCCs were chosen in the cities of Malmö

and Trelleborg (including surrounding areas), which together had a total population of 346,429 in 2000 and 394,307 in 2010. The following data on each participant were extracted from paper records kept by the Regional Department of Communicable Disease Control in Skåne County: age, gender, number of children enrolled at the DCC department, whether the person attended the same DCC department as the index case, what month and year the screening was performed, if the culture was taken in the first or second screening, culture results (growth or no growth of pneumococci, and MIC of PcG if that value was ≥ 0.5 mg/L), and number of days to the start of screening. Days to screening start were counted from the date of the final results of the index patient's culture to the date when nasopharyngeal swabs from the screening population arrived at the laboratory. In cases of $PNSP_{0.5}$, the following data were recorded: MIC of PcG, resistance to other antibiotics (erythromycin, clindamycin, tetracycline, trimethoprim-sulfamethoxazole [TMP/SMX]) according to the SIR system, serogroup, and duration of $PNSP_{0.5}$ carriage. Duration of carriage was calculated as the number of days from the first nasopharyngeal culture showing growth of $PNSP_{0.5}$ until the first of two consecutive negative cultures.

Paper II

Paper II describes our retrospective study of the medical records of children referred to the Department of Infectious Diseases in either Lund or Malmö, or the Department of Paediatrics in Malmö, due to prolonged nasopharyngeal carriage of $PNSP_{0.5}$. The study period was from November 1997 to January 2011, and all children between the ages of 0 and 10 years at the time of diagnosis were included. The children were treated according to the clinical guidelines applied in Malmö and Lund: amoxicillin was the primary choice if the PcG MIC was < 2 mg/L; at a PcG MIC of ≥ 2 mg/L, erythromycin or clindamycin was recommended if the bacterial strain was susceptible to these antibiotics.

Information was collected regarding how the $PNSP_{0.5}$ were identified, as well as the children's age, sex, antimicrobial treatment, dose, dosage regimen, side effects of the treatment, and duration of carriage. Furthermore, information about $PNSP_{0.5}$ in the family was obtained from the children's medical records using a standard data collection form. During the time the children were followed, cultures of PNSP with a PcG MIC of ≥ 0.25 mg/l were considered to be $PNSP_{0.5}$, because the E-test is only reliable at ± 1 dilution step. Eradication was defined as at least two consecutive negative cultures after treatment, with the second one performed at least seven days after completion of treatment. No more than 2 months were allowed to pass between the two consecutive cultures. Data on nationalities and background were acquired from the Civil Registry for 102 of the 108 children who were referred for eradication therapy.

Paper III

We conducted a retrospective study of cases of invasive pneumococcal disease (IPD) reported from the laboratories of the hospitals in Lund and Malmö (Paper III). IPD is defined as a condition induced by pneumococci acquired from a normally sterile body site, as described in earlier chapters. The clinical microbiology laboratories in Skåne County serve a population of 1.2 million. Due to reorganization of these facilities, isolates from the smallest local laboratory in the city of Kristianstad were lost, and hence data from this location had to be excluded. All IPD cases in Sweden must be reported to the Swedish Institute for Communicable Disease Control (SMI) and thus were available in charts from the laboratories.

The following data were collected from each patient's medical records: age, gender, infection foci, admission to an ICU, mortality after 28 days and after one year, severity of sepsis, co-morbidities, immunosuppressive treatment, and ongoing alcohol abuse or smoking. Co-morbidities were noted when a diagnosis was specified in the records, and the diagnoses included were divided into these categories: heart, lung, haematological, and autoimmune diseases, liver and renal failure, diabetes mellitus, cancer, splenectomy, and HIV. Vaccination data were not available on an individual level, but it was known that the frequency of vaccination in the general population was low during the study period.

Information on co-morbidities and foci of infection was extracted from the medical records and noted. It was not possible to retrospectively classify the degree of underlying disease. Sepsis shock was determined according to the accepted definition provided in the Surviving Sepsis Campaign. Septic shock was defined as severe sepsis with persisting hypotension despite adequate fluid resuscitation with at least 500 ml of intravenous fluid administered over a period 30 minutes. Patients that fulfilled the criteria for septic shock were evaluated, even if they did not fulfil the SIRS criterion. Only objective parameters were included in the analysis, and a lack of parameters was recorded. The examiner was blinded to the grading of sepsis severity.

Paper IV

Paper IV describes our retrospective study of patients diagnosed with VAP and entered in the Swedish Intensive Care Complication Register (ICCR). Patients treated at Malmö University Hospital between January 2004 and September 2007 were included, and their medical records were studied. Data were collected from two ICUs: one a general facility handling both surgical and medical patients, and the other primarily treating medical patients with infectious diseases. As discussed in the introduction, the diagnostic criteria for VAP are still controversial and hence differ between studies in the literature. During the current study period, a patient with VAP had to fulfil the following the criteria to be included in the ICCR: (i) clinical suspicion of pneumonia dur-

ing invasive ventilator support for > 48 h; (ii) new or increasing radiological infiltrate consistent with pneumonia; (iii) body temperature ≥ 38.5 or ≤ 35.0 °C; (iv) leukocyte count > 10 x 10^9 /L or < 3 x 10^9 /L; (v) purulent secretion from the lower airways or isolation of a significant amount of pathogenic bacteria from airway secretions (≥ 10^3 CFU/mL from protected specimen brush [PSB] samples, ≥ 10^4 CFU/mL CFU/mL from bronchoalveolar lavage [BAL] samples, and ≥ 10^6 CFU/mL from tracheal secretions). In January 2008, the requirements for recording VAP in the ICCR were modified and limited to the criteria designated i, ii, and v above, which we chose to use as requirements for inclusion in our investigation (i.e., patients who did not fulfil criterion i, ii, or v were excluded). At the ICUs in Malmö, patients with clinically suspected VAP are routinely subjected to bronchoscopy, and samples are collected with a PSB.

The following data were obtained from the medical records: time of arrival at the hospital, time of intubation, time of VAP diagnosis, discharge from the ICU and death (within 28 days), co-morbidities, diagnosis on admission to the ICU, immunosuppressive treatment, type of culture sample, bacterial species, bacterial resistance, antibiotic therapy given at and after the onset of VAP, and earlier history of antibiotic therapy in the ICU. The empirical treatment was considered adequate if the considered pathogen was susceptible to the empirical antibiotics given. VAP appearing seven days after intubation was classified as a late-onset VAP, and that arising less than seven days after intubation was designated early-onset VAP.

Microbiology methods

In the studies reported in Papers I and II, nasopharyngeal swabs were obtained and cultured according to Swedish standard methods. The samples were grown on blood agar plates, and pneumococci were identified by morphology and by the Optochin test. A disc diffusion test was performed to detect $PNSP_{0.5}$ and resistance towards other antibiotics based on the SIR system, and this was done according to the guidelines of the Swedish Reference Group for Antibiotics and its subcommittee on methodology. The E-test was used to determine the MIC of benzylpenicillin when the inhibition zone around a 1-mg oxacillin disc was < 18 mm. Serogrouping was performed by two different methods at two separate laboratories: at the Department of Clinical Microbiology in Lund, a capsular reaction test was used according to the instructions of the manufacturer (Statens Serum Institute (SSI), Copenhagen, Denmark, a WHO Collaborating Centre for Reference and Research on Pneumococci); at the Swedish Institute for Communicable Disease Control (SMI) in Stockholm, a gel diffusion method was performed using factor serum from SSI for serogrouping, and that technique is authorized by the Swedish Board for Accreditation and Conformity Assessment.

In our third study (paper III), pneumococci were analysed as described above. In a few cases, the diagnosis of IPD was based on PCR results because the bacteria died during culture, as discussed in the section on laboratory identification. The PCR was

performed according to national standards. Serogrouping was done as described in Papers I and II (see above). Serogroups included in the 13-valent conjugate vaccine with subtypes (i.e., 6, 7, 9, 18, 19, and 23) were further analysed and serotyped using the Quellung reaction with antiserum obtained from SSI. In that test, a particular serum stimulates the expression of antibodies on the capsule of the pneumococci, which causes agglutination and renders the capsule refractile and with a swollen appearance, and thus detectable by phase microscopy. Accordingly, the serotype is determined on the basis of the antiserum that causes this capsule reaction. The antisera are obtained from the SSI in Copenhagen.

The PSB samples used in the fourth study (Paper IV) were analysed according to Swedish national guidelines. The brush was cut off, placed in 1.5 ml of peptone–yeast extract–glucose (PYG) medium, and sent to the Clinical Microbiology Laboratory. The national guidelines recommend 1.0 ml of PYG but 1.5 ml is used in Malmö, which also changes the equivalent cut-off for significant growth from $\geq 10^3$ CFU/ml to ≥ 666 CFU/ml. Known volumes (100 µl and 10 µl) of the PYG medium are added to selective and non-selective agar plates. Antibiotic susceptibility testing on relevant pathogens was performed according to the SRGA guidelines. *Candida* spp., anaerobic bacteria, and *Enterococcus* spp. were defined as low virulence species (LVS). *Corynebacterium* spp., α-haemolytic streptococci, coagulase-negative *Staphylococcus* spp., and *Neisseria* spp. were defined as oropharyngeal and cutaneous commensals (OCCs).

Statistics

Statistical analyses were performed using the software R, version 2.14.1 (14), SPSS 18 and 20, Graph Pad or Excel.

In study III were data assessed in two different ways:

1. Serotypes were divided into three different classes depending on their invasive potential in children according to a meta-analysis by Brueggeman *et al.* (3), where carriage rates of serogroups and serotypes were compared with their rates of IPD. Brueggeman selected serotype 14 as a reference since it is a serogroup without subtypes, is among the most prevalent invasive and carriage serotypes, and, finally, shows no evidence of heterogeneity. Moreover, Brueggeman determined Odds ratio (OR) with 95 % confidence interval (CI) compared to serotype 14 and serotypes were divided as having high, intermediate or low invasive potential. Odds ratio >1 was associated with serotypes included in the highly invasive potential serogroups (1, 5 and 7). Odds ratio 0.5-1 included the intermediate invasive potential serotypes (4, 9,14 and 18), and OR<0.5 the low invasive potential serotypes (3, 6, 8, 15, 19, 23 and 33).
2. Serotypes were compared one by one to serotype 14 as a reference based upon the rationale described above.

Dichotomous outcomes were generally analysed using logistic regression, for example to investigate the relation between age as covariate and pneumococcal carriage as outcome (in study I); generalized additive models where used for modelling continuous covariates without assuming linearity and for controls of linearity in models using this assumption. Fisher's generalized exact test was used when a dichotomous outcome was compared between categorical groups, for only two levels of a group the standard exact test was used. For example when serotypes were compared with serotype 14 as a reference in paper III. In this situation Odds Ratio (OR) and the 95% Confidence interval (CI) are calculated. One can assume that we have an increased risk of statistical type I error in these analyses, since I did not do any adjustments for multiple comparisons, however, it is also possible that a greater number of patients would had resulted in more significant differences in the non-significant comparisons. The OR together with the CI can give a rough guidance of the uncertainty of these results.

In study I and III ordinal outcomes were analyzed with the Kruskal-Wallis exact test if more than two groups were compared, for example the when the significance between the three groups infected with pneumococci with different invasive potential were compared. Post-hoc pairwise comparisons were made according to the Nemenyi-Damico-Wolfe-Dunn method. This method is accounts for the several groups and compensates for the multiple comparisons made such that the probability that any comparisons is falsely identfied as significant is less than 0.05. When only two groups were compared, the exact Wilcoxon-Mann-Whitney test was used.

In study III, we analyzed the annual change of incidence of IPD in Skåne by using a Poisson regression analysis. This was not an important issue in this paper and to try to fit a trend to only three year points can be considered as controversial. Unfortunately, the number of patients in paper III was too small to make a multivariate analysis including all relevant factors such as age and comorbidities.

Ethical considerations

All of our studies were approved by the regional ethics committee for medical research in Lund. In three of the investigations (Papers II–IV), we were given permission to collect data from the patients' medical records. Furthermore, in two of the studies (Papers II and IV), patients were contacted by regular mail and given the option not to participate, but none chose to withdraw. The results presented in the papers cannot be connected to individual patients. All participants were assigned a study code, and the data were analysed without names or personal codes in the form used. We are convinced that the integrity of the participants in our research remained intact.

> *"Doctors are men that prescribe medicines of which they know little, to cure diseases of which they know less, in human beings of whom they know nothing"* – Voltaire

Results

Paper I

Children and personnel at 109 DCCs were screened for $PNSP_{0.5}$. A total of 7,157 individuals (109 index cases) were included in the study: 5,792 children and 1,365 personnel. After exclusion of the index cases, 5% (279) of the children carried $PNSP_{0.5}$ but only 0.4% of the personnel. No significant change in serogroup distribution was observed during the study period, nor was there a trend towards more multi-resistant $PNSP_{0.5}$ or higher MICs among the $PNSP_{0.5}$ isolates. A large proportion (41%) of the $PNSP_{0.5}$ were MDR, defined as non-susceptibility to three or more classes of antibiotics.

The first screening included 207 departments at 109 DCCs, and 218 contact cases of $PNSP_{0.5}$ were identified at 67 (61%) of the DCCs. A second screening was performed at 51% (47) of the remaining DCCs and included 122 departments. Sixty-four contact cases of $PNSP_{0.5}$ in 28 (60%) of the DCCs were identified. Contact cases were found in 50% (75/149) of the departments that had an index case and 34% (61/180) of the departments without an index case. The proportion of $PNSP_{0.5}$ and pneumococcal carriage decreased significantly with age ($p = 0.001$; figure VII).
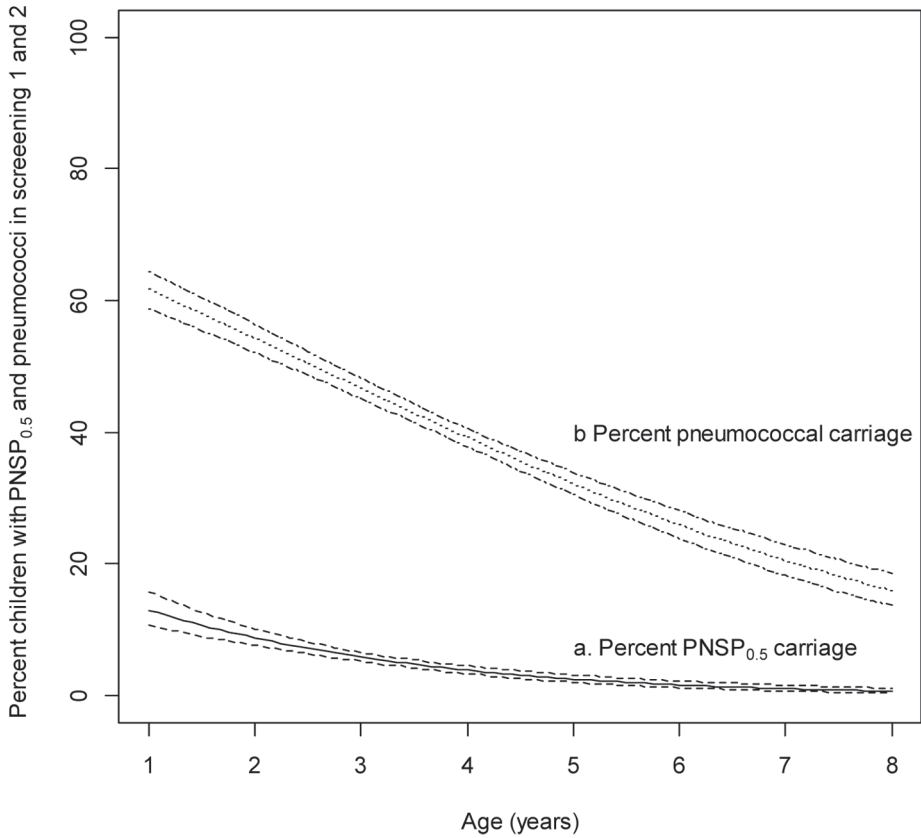
Figure VII. $PNSP_{0.5}$ and pneumococcal carriage (%) in all screened children in our cohort according to age. Dashed curves are approximate 95% confidence limits. (a) The proportion of pneumococcal carriers decreased significantly with age (p < 0.001), and the odds for pneumococcal carriage decreased 73.7% per year of age (95% CI = 70.9–76.6 %), with baseline odds (at age = 3.75 years) of 0.751 (95% CI = 0.710–0.793). (b) The proportion of $PNSP_{0.5}$ carriers decreased significantly with age (p < 0.001), and the odds for $PNSP_{0.5}$ carriage decreased 65.7% per year of age (95% CI = 58.8–71.0%), with baseline odds (at age = 3.75 years) of 0.0504 (95% CI = 0.0441–0.0572). The numbers of children that had to be screened to find one $PNSP_{0.5}$ carrier with a matching serogroup were 15, 18, 28, 61, 83, and 143 at the ages of 1, 2, 3, 4, 5, and 6 years, respectively.

There proportion of contact cases carrying a matching serotype was significantly larger in the first screening (85%) than in the second screening (69%; *p* = 0.012). In the first screening, contact cases with a serogroup matching index were significantly more often enrolled at the same DCC department as the index case than at a different department (93% vs. 71%; *p* = 0.0001)

Median time to the first screening was eight days (range 0–103 days). Median time to the second screening was 15 days (8–91 days). In a few children who had late cultures due to absence from both the DCC and the initial screening, $PNSP_{0.5}$ were found for up to 6 weeks or more after the intervention started. In children younger than 8

years of age, there was significant seasonal variation in pneumococcal carriage, with a marked drop in July and August (Figure VIII) and smaller decreases in January and April.
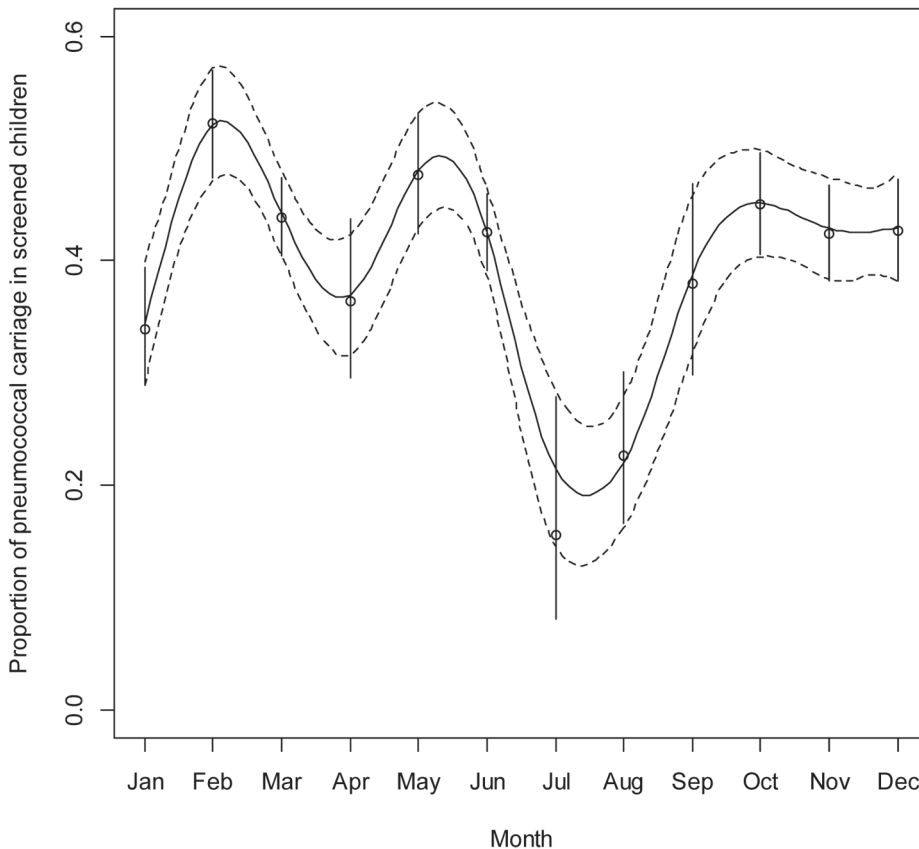

Figure VIII. Seasonal variation in pneumococcal carriage among screened children, presented as proportion of cultures. The carriage rate differed significantly between months.

Children were categorized into groups according to the size of the DCC department: 0–10, 10–15, 16–20, 21–25, 26–35, and 36–50 children. Day care group size was found to be a significant risk factor for pneumococcal carriage ($p = 0.0026$) after adjusting for the risk factors age ($p = 0.001$) and screening occasion ($p = 0.012$). Children in DCC groups of 26–35 attendees were carriers of pneumococci significantly more often than those in groups of 16–20. Furthermore, the pneumococcal serogroups differed regarding their capacity to cause contact cases: compared to serogroup 9, serogroup 6 caused significantly more contact cases with $PNSP_{0.5}$ (OR 1.64, 1.15–2.35).

Paper II

One hundred twenty-five children were referred for eradication therapy, but 17 were excluded for various reasons. In the remaining 108 cases, the colonization with $PNSP_{0.5}$ was identified in the following ways: as a clinical infection in 29; through screening of close contacts that identified $PNSP_{0.5}$ in 58; by screening related to an adoption in 21.

Of the 108 colonized children, 37 (34%) did not receive treatment, because they spontaneously produced two consecutive nasopharyngeal cultures that were negative for $PNSP_{0.5}$ before treatment was initiated. The median time of colonization in the group with spontaneous resolution of the carriage was 73 days (range 7–427 days). The duration of carriage in the children who were treated increased with age, with a median of 71 days in the group aged > 3 years and 124 days in the group aged < 1 year. However, treatment was performed to a greater extent in children under the age of 3 years (69%) compared to those who were older than 3 years (50%). In the 71 cases that did receive treatment, the median duration of carriage was 94 days (3–267 days).

For 52 (48%) of the 108 children, carriage was also found in at least one sibling or parent. Thirty-five (32%) had no family members who were found to be colonized with $PNSP_{0.5}$, and data regarding screening of the family were lacking in 21 cases.

Twenty-five of the 102 children (24%) were adopted and had available data on their nationalities, and an additional 36 (35%) had a foreign background. The most predominant serogroups harboured by those who received treatment were in descending order 9 (n = 13; 18%), 23 (n = 12; 17%), and 6 (n = 9; 11%). In six cases, the subject carried two or three different serogroups.

In the group that were treated, all strains had MIC values of ≤ 2 mg/L. The primary eradication rate was 91.5% (Table I), and there was a trend towards a better rate (p = 0.0762) for rifampicin in combination with either of the intracellularly active antibiotics clindamycin and erythromycin (27/27; 100%), as compared to rifampicin combined with amoxicillin (38/44; 86%). Data on the dosing regimens for amoxicillin were scarce, and, of the 16 cases in which that information was recorded, only four had received amoxicillin three times daily. There were pronounced side effects of clindamycin, and two out of seven patients had to discontinue such therapy. All isolates with a PcG MIC of ≥ 4 mg/L (n = 3) and all isolates found to be resistant to rifampicin (n = 2) were detected in the group of adopted children.

Table I. Antibiotic combinations and outcome

Antibiotic combination	Treated No.	Eradicated No. (%)
Rifampicin + amoxicillin	44	38 (86)
Rifampicin + erythromycin	22	22 (100)
Rifampicin + clindamycin	5	5 (100)
Total	71	65 (91.5)

Paper III

A total of 551 patients were included in this analysis (Paper III), 267 men and 284 women with a median age of 66 years (range 0–101 years). In all, 514 pneumococcal isolates were serotyped. The most abundant serotypes were 14 (12.5%) and 7F (12.2%). The majority of patients suffered from pneumonia (86%), and serotypes 14, 7F, 4, 9V, and 3 represented 49% of the isolates.

The 28-day crude mortality for the patients was 11.4%, and after one year 18.3% had died. Only serotype 3 was associated with a significantly higher mortality (29%) compared to the reference serotype 14 (12%) (Table II). A difference, albeit not statistically significant, was revealed between 28-day mortality in serotype/ serogroups divided into high (6%), intermediate (12%), and low (13%) invasive potential (Table III). Considering the 12 most common serotypes, no mortality was found in patients infected with serotypes 23F, 1, or 19A (Table 2). Eighty-three patients (15%) were treated in an ICU, and 26 (31%) died within 28 days. Unknown foci were significantly more common in the group infected with low invasive potential serotypes (12%, 5%, and 4%, respectively; p = 0.006).

Septic shock was caused significantly more often by serotype 3 (OR 6.83, 1.72–27.08) than by serotype 14 (Table II). In addition, this condition occurred more frequently (finding not statistically significant) in patients who were infected with serotype 19F (OR 4.71, 0.86–25.83), and OR values were also > 2 for serotypes 4, 7F, 8, 9V, and 18C (Table II). Patients with IPD caused by serotype 19F required intensive care significantly more often (OR 6.79, 2.03–22.60) than those with IPD induced by serotype 14 (Table II). Detailed analysis of patients without any co-morbidities showed that septic shock was caused more frequently by serotypes 3, 4, and 19F than by serotype 14, although without statistical significance (p = 0.19, 0.087, and 0.10, respectively). As expected, antimicrobial resistance was very low, and only 3% (n = 16) were infected with PNSP. Patients infected with highly invasive serotypes were relatively young (median age 59 years, range 0–95 years) and had a relatively low incidence of co-morbidities (47%). There were significant differences in age and co-morbidities between the groups categorized according to the invasive potential of the infecting pneumococcal serotype (Table III). Patients infected with pneumococcal serotypes 3, 6A, 18C, and 23 F had co-morbidities significantly more often and also significantly more co-morbidities per individual compared to patients infected with serotype 14. Also, IPD patients presenting with serotypes 1 and 7F were significantly younger than those with serotype 14.

Table II. Characteristics of Patients with IPD Shown by Infecting Serotype and Statistical Comparison in Relation to Infection with Serotype 14

Serotype (n)	Septic shock		28-day mortality		Admitted to the ICU		Any co-morbidity		Two or more co-morbidities per patient		Median age	
	No. (%)	OR (CI)	No. (%)	OR (CI)	No. (%)	OR (CI)	No. (%)	OR (CI)	No. (%)	OR (CI)	Years (range)	p-value
14 (69)	3 (4)	1[a]	8 (12)	1[a]	8 (12)	1[a]	35 (51)	1[a]	10 (14)	1[a]	67 (1–94)	-----
1 (15)	0 (0)	----	0 (0)	----	0 (0)	----	11 (73)	0.35 (0.10–1.29)	0 (0)	----	**49 (3–68)**	**0.005**
3 (38)	9 (24)	**6.83 (1.72–27.08)**	11 (29)	**3.11 (1.11–8.59)**	8 (21)	2.03 (0.69–5.95)	30 (79)	**3.64 (1.46–9.06)**	15 (39)	**3.85 (1.51–9.79)**	74 (0–94)	0.230
4 (58)	6 (10)	2.54 (0.61–10.64)	3 (5)	0.42 (0.11–1.65)	7 (12)	1.05 (0.36–3.08)	28 (48)	0.91 (0.45–1.82)	15 (26)	2.06 (0.84–5.02)	65 (1–91)	0.388
6A (21)	0 (0)	----	3 (14)	1.27 (0.30–5.30)	0 (0)	----	15 (72)	**4.86 (1.29–18.30)**	11 (52)	**5.45 (1.94–15.28)**	73 (4–91)	0.807
6B (31)	1 (3)	0.73 (0.07–7.34)	1 (3)	0.25 (0.003–2.13)	4 (13)	1.13 (0.31–4.07)	20 (65)	1.77 (0.74–4.23)	9 (29)	2.41 (0.87–6.73)	63 (1–97)	0.317
7F (67)	6 (9)	2.16 (0.51–9.03)	4 (6)	0.40 (0.13–1.69)	13(19)	1.83 (0.70–4.76)	31 (46)	0.83 (0.43–1.64)	13 (19)	1.42 (0.57–3.50)	**59 (0–95)**	**0.006**
8 (18)	2 (11)	2.75 (0.42–17.86)	2 (11)	0.95 (0.18–4.93)	2 (11)	1.05 (0.20–5.43)	7 (39)	1.53 (0.53–4.40)	5 (28)	2.27 (0.66–7.76)	64 (49–8)	0.386
9V (40)	4 (10)	2.44 (0.52–11.53)	4 (10)	0.84 (0.23–3.0)	8 (20)	1.91 (0.65–5.55)	26 (65)	1.80 (0.81–4.02)	9 (22)	1.71 (0.63–4.66)	70 (41–97)	0.729
18C (16)	2 (12)	3.14 (0.48–20.59)	4 (25)	2.54 (0.66–9.81)	4 (25)	2.54 (0.66–9.81)	3 (19)[l]	**4.21 (1.10–16.09)**	11 (69)	**12.98 (3.71–45.38)**	64 (0–85)	0.279
19A (7)	0 (0)	----	0 (0)	----	1 (14)	1.27 (0.16–11.96)	6 (86)	5.83 (0.67–51.0)	2 (28)	2.36 (0.40–13.88)	70 (53–91)	0.708
19F (17)	3 (18)	4.71 (0.86–25.83)	4 (24)	2.35 (0.61–8.97)	8 (47)	**6.79 (2.03–22.60)**	4 (24)	3.16 (0.94–10.65)	7 (41)	**4.13 (1.27–13.38)**	64 (14–91)	0.823
23F (25)	0 (0)	----	0 (0)	----	1 (4)	0.32 (0.31–4.08)	21 (84)	5.1 (1.58–16.41)	12 (48)	**5.45 (1.94–15.28)**	73 (23–101)	0.442

Table III. Characteristics of Patients with IPD Grouped According to Invasive Potential of the Infecting Pneumococcal Serotype

Invasive disease potential[a] (no.)	Septic shock no. (%)	28 day mortality no. (%)	Admitted to the ICU no. (%)	Any co-morbidity no. (%)	Two or more co-morbidities per patient no. (%)	Median age no. years (range)
High[b] (93)	7 (8%)	6 (6%)	14 (15%)	44 (47%)	19 (20%)	59 (0–95)
Intermediate[c] (208)	18 (9%)	25 (12%)	30 (14%)	119 (57%)	52 (25%)	67 (0–97)
Low[d] (181)	17 (9%)	24 (13%)	25 (14%)	136 (75%)	74 (41%)	70 (0–101)
p-value[e]	$p = 0.891$	$p = 0.225$	$p = 0.942$	$p < 0.001$	$p < 0.001$	$p < 0.001$

[a]Serogroups were divided into three categories according to invasive potential as described by Brueggeman *et al.* [138], and 69 IPD cases were excluded because the serotype was not included in this classification.
[b]High invasive disease potential includes serotypes in serogroups 1, 5, and 7.
[c]Intermediate invasive disease potential includes serotypes in serogroups 4, 9, 14, and 18.
[d]Low invasive disease potential includes serotypes in serogroups 3, 6, 8, 15, 19, 23, and 33.
[e]Univariate *p*-value for differences among the groups (high, intermediate, and low invasive potential).

Paper IV

During the study period, 109 patients diagnosed with VAP were entered in the ICCR. Forty-four were excluded from the investigation because they did not meet the inclusion criteria, and thus 65 patients (39 men, 26 women) with a median age of 66 years (22–88 years) were enrolled. The median time on ventilator support at the onset of VAP was 6 days (2–43 days), and the median time of hospitalization was 9 days (2–293 days). The median length of hospitalization before intubation was 1 day (0–286 days). Twenty-eight patients were intubated on the day of admission. The crude mortality 28 days after VAP diagnosis was 34% (22 patients: 5 women, 17 men). The median time in hospital after VAP diagnosis was 41 days.

Fifty-three patients (81.5%) had growth of bacteria in the PSB culture. Among the patients with negative PSB cultures, three were positive by tracheal culture and one by blood culture. In total, four patients were positive by tracheal culture and three by blood culture. Fifty of the 53 patients with a positive PSB culture had growth of one or more pathogens, as indicated in Table IV. Three patients had growth of only LVS and/or OCCs in the PSB culture and were excluded from this table. The most common pathogens were as follows (number of positive cultures within parentheses): Enterobacteriacae (28), *Pseudomonas aeruginosa* (13), *Haemophilus influenzae* (12), and *Staphylococcus aureus* (8). A polymicrobial flora with two to four species was found in 29 PSB cultures (55% of the culture-positive PSB samples), and all but one of the findings of LVS and OCCs were part of a polymicrobial flora. No treatment was directed primarily against LVS and OCCs.

We found statistically more *H. influenzae* (p = 0.035) and pathogenic Gram-positive bacteria (p = 0.019) in the group not treated with antibiotics at onset than in the other groups. In the patients who did receive antibiotics at onset, there was a tendency towards more *P. aeruginosa* (p = 0.135). Three of 27 patients without any antibiotics at VAP onset were positive for *P. aeruginosa*, but two of those three had received prior antibiotic treatment (> 24 h before onset. In the group given broad spectrum antibiotics; defined as an antibiotic effective against P. aeruginosa. negative cultures and LVS were significantly more common (p = 0.001). Forty-four patients were diagnosed with VAP prior to the culture results and were given empirical treatment, and 21 patients received their VAP diagnosis after a positive PSB culture.

Twelve isolates from 12 patients were resistant to one or more antibiotics (Table V). Eleven of those 12 patients were treated with antibiotics at the onset of VAP or had previously received antibiotics. However, earlier or ongoing antibiotic treatment at the onset of VAP was not a statistically significant risk factor for VAP caused by resistant bacteria (p = 0.278). There were 32 late and 33 early VAP cases (with 37 and 66 isolates, respectively). Compared to the late group, the early group had more isolates of *P. aeruginosa* (9 vs. 4), *H. influenzae* (8 vs. 4), and Gram-positive pathogens (8 vs. 5), although these differences were not statistically significant. Furthermore, there were significantly more negative cultures (9 of 32) in the late-onset group compared to the early-onset group (p = 0.022). There was also a trend towards presence of more resistant bacteria

in the late-onset VAP patients (p = 0.061). One patient with late-onset VAP who was earlier treated with a broad spectrum antibiotics had *Stenotrophomonas maltophilia* in the PSB culture. Nine patients had early-onset VAP with *P. aeruginosa*, and they all had a normal resistance pattern.

Table IV. Aetiology in VAP at Malmö University Hospital Malmö 2004–2007. Only bacteria considered to be pathogenic are presented[a]

Pathogen	Number of isolates	Percent of all 65 VAP episodes	Number of mono-/ polymicrobial floras[b]
Gram-negative bacteria	54		
P. aeruginosa	13	20	12/1
H. influenzae	12	18	6/6
Escherichia coli	9	14	4/5
Enterobacter spp.	4	6	4/0
Klebsiella spp.	4	6	3/1
Proteus spp.	3	5	1/2
Morganella spp.	3	5	1/2
Citrobacter spp.	3	5	1/2
Serratia spp	2	3	2/0
Stenotrophomonas maltophilia	1	2	0/1
Gram-positive bacteria	13		
Staphylococcus aureus	8	12	3/5
Streptococcus milleri	3	5	1/2
S. gr B	1	2	0/1
S. pneumoniae	1	2	0/1

[a]Fifty positive PSB cultures with 67 isolates.
[b]Polymicrobial flora: 38 patients had one pathogen, 11 patients had two pathogens, one patient had three, and one patient had four.

Table V. Antibiotic-resistant pathogens cultured from PSB samples from patients with VAP

Pathogen (resistance mechanism)	piz[a]	cxm[b]	ctx[c]	caz[d]	imi[e]	tob[f]	tsu[g]	cip[h]	BSA[i]	Earlier BSA[i]	NSA[j]	Earlier NSA[j]	Day of onset of VAP
H. influenzae (BLNAR[k])	I		S			S	R	S					2
H. influenzae (BLNAR[k])			S			S	S	S				X	8
E. coli	S	R	S	S	S	S	S	S	X				22
E. coli	S	R	S	S	S	S	S	R		X			39
E. coli	R	S	S	S	S	S	R	S	X	X			8
P. vulgaris	S	R	S	S	I	S	S	S			X		6
C. freundii	R	R	R	R	S	S	S	S		X	X		15
K. pneumoniae (AmpC)	R	R	R	R	S	S	S	I	X				16
M. morganii	S	I	S	S	S	S	S	S			X		4
P. aeruginosa	S			R	R	S		R	X				12
P. aeruginosa	R			R	R	S		S	X	X	X	X	15
P. aeruginosa	R			R	R	S		S		X			11

[a]Piperacillin/tazobactam.
[b]Cefuroxime.
[c]Cefotaxime.
[d]Ceftazidime;
[e]Imipenem.
[f]Tobramycin.
[g]Trimetoprim-sulpha.
[h]Ciprofloxacin.
[i]Broad-spectrum antibiotics defined as having an effect on *Pseudomonas*.
[j]Narrow-spectrum antibiotics defined as having no effect on *Pseudomonas*.
[k]BLNAR = beta-lactamase negative, ampicillin resistant.

Discussion

PNSP are now stable at a lower level, perhaps partly due to the DCC intervention

In the mid 1990s when the South Swedish Pneumococcal Intervention Project (SSPIP) was initiated, the threat of a rapidly rising rate of PNSP was imminent. Unfortunately, no randomized trial was started to answer the question of whether a DCC intervention can help diminish or restrict the spread of PNSP in the community. That was not an option in that time, because any measures taken against $PNSP_{0.5}$ were determined by the Swedish Communicable Disease Act, and all $PNSP_{0.5}$ cases had to be treated according to the act. Moreover, the act did not allow the SSPIP to screen for PNSP after the DCC intervention was implemented, or at least that is how the authorities in Skåne County interpreted the communicable disease ruling. Nonetheless, we conducted a large retrospective study (Paper I) in an attempt to evaluate the DCC intervention. Notably, 5% of the screened children were carriers of $PNSP_{0.5}$ and hence were suspended from DCC attendance. Theoretically, this is an excellent way to stop the vicious cycle of dissemination of $PNSP_{0.5}$, and of course another approach would be to decrease the antibiotic pressure (Figure VII). The prescription of antibiotics has been declining significantly since the mid 1990s, from 1.2 to 0.6 prescriptions annually for children under five years of age. Furthermore, improved hygiene measures may also have contributed to less dispersion of PNSP. Consequently, it is impossible to discern the impact of the DCC intervention alone, although it may have played a role in the change from increasing to decreasing and now stable prevalence of $PNSP_{0.5}$ in Skåne County.

> *"The first duties of the physician is to educate the masses not to take medicine"*
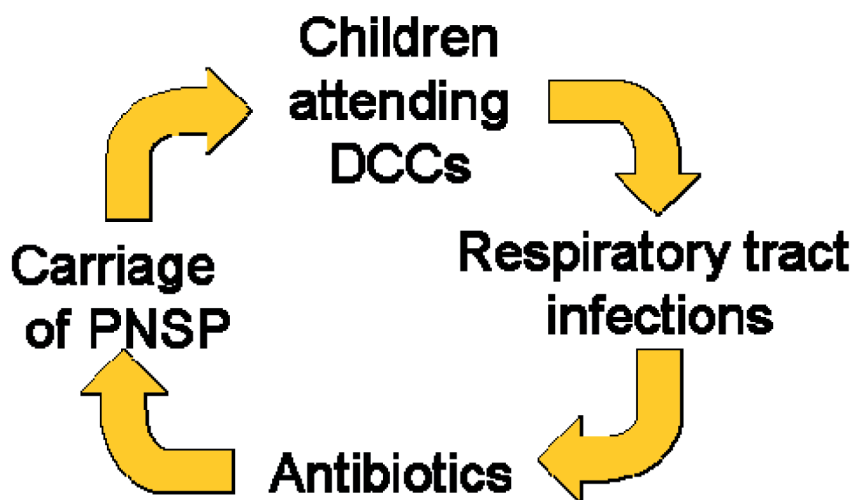> *– Sir William Osler*

Figure VII. The vicious cycle of dissemination of PNSP.

Högberg *et al.* conducted a small study to compare the spread of $PNSP_{0.5}$ in DCCs with or without an active intervention [242]. Due to several important shortcomings in that investigation, it was not feasible to make a uniform comparison. In short, the study was small (48 children in the non-intervention group), there were large differences in the screening done to detect $PNSP_{0.5}$, important since new $PNSP_{0.5}$ constituted the primary outcome.

How should a DCC intervention be performed?

The public health community must decide how important it is to strive to reach a low incidence of PNSP and to what extent measures based on the available data should be implemented. Apparently no randomized trial is planned in Skåne County at present. However, some of the findings reported in Paper I are worth considering in any future DCC interventions. Personnel at the DCCs we studied had a low prevalence of $PNSP_{0.5}$ and a short period of carriage, which argues against including personnel in screening. If they are healthy, members of this group are probably not as contagious as young children are. Our data support the theory to include the whole DCC in the first screening. This is indicated by the observation that the department with the index case was not always the centre of an outbreak, because there was a substantial risk of $PNSP_{0.5}$ in other DCC departments as well. In addition, it was necessary to close more than 50% of the DCCs according to the guidelines and conduct a second screening. Ensuring a rapid start of the intervention is supported by the theory that PNSP carriers remaining in the DCC are a potential spreaders. Moreover, the theory that the spread of PNSP in a DCC can continue for months if no intervention is introduced is corroborated by our

findings of $PNSP_{0.5}$ long after the intervention started in cases that were cultured late for various reasons. Also, the idea that absence of children from their DCC can decrease pneumococcal carriage seems to be confirmed by our results demonstrating significant seasonal variation in carriage, with lower rates after major holidays and other free days. Children obviously transfer pneumococci to their surrounding environment, not only to other children. Interstingly, the holiday spikes of IPD incidence, affecting especially older women probably infected during social gatherings during holidays have declined in the United States since PCV has been introduced [243]. The peak of pneumococci noted in February in our study can probably be partly explained by the influenza season, although the data covered a period of more than 10 years and the time of the annual influenza epidemic varies from year to year.

Eradication therapy is successful in children with prolonged carriage of PNSP

We found that eradication therapy effectively eliminated PNSP when rifampicin was used in combination with amoxicillin, erythromycin, or clindamycin (Paper II). Side effects of clindamycin were common in this study, and two of the seven patients given this antibiotic had to interrupt the treatment. There was a trend towards better outcome with clindamycin and erythromycin than with amoxicillin (p = 0.0762).

This was surprising, since single therapy with amoxicillin previously have been proven to be effective in eradicating penicillin sensitive pneumococci from the nasopharynx [244]. The failures in the amoxicillin group is most likely due to the short time above MIC in the amoxicillin group. The recommended dosing regimen for amoxicillin was twice daily at the beginning of the study period but was changed to three times daily in the middle of the 2010s. The patients' medical records contained little information on dosing.

The literature contains few studies on this subject. One investigation did focus on the outbreak of multidrug-resistant pneumococci that occurred in South Africa in the 1970s and was addressed by use of eradication therapy [245]. It was found that erythromycin alone completely eliminated the pneumococci in 42% of the cases, whereas adding rifampicin increased the success rate to 96%. Therefore, rifampicin in combination with other antibiotics was used in the SSPIP for eradication therapy. A study from the SSPIP on eradication therapy inform 1979 included 39 children and reached a success rate of 97% [246]. Notwithstanding, a proper follow-up is probably essential, considering that it was necessary to perform a second or third culture to identify three of the six children in the our study who were found to have continued carriage of $PNSP_{0.5}$ despite the eradication therapy. The consensus in Sweden has been a follow-up consisting of nasopharyngeal cultures every week starting one week after completed treatment. PCV has been suggested for eradication of pneumococcal carriage but the PCV-induced an-

tibodies does not eradicate the existing carriage, rather reduce the acquisition of new vaccine serotypes [247].

Implications of the new PNSP guidelines

The new guidelines presented by the Swedish National Board of Health and Welfare in May 2012 for the management of PNSP are to be implemented when the PcG MIC is ≥ 2 mg/L (PNSP$_2$). Based on the results presented in Paper I, DCC personnel are not included in the screening outlined in the new guidelines. We found a low rate of PNSP$_2$ in that study, which should lead to a dramatic reduction in the number of interventions in Skåne County. On the other hand, the spread of a successful clone with a higher MIC will have an impact that is more difficult to handle, because it will be associated with the risk of inappropriate empirical treatment. Therefore, the observations discussed above suggest that an intervention should be rapidly initiated and should include the entire DCC in question.

> "He who seeks, let him not cease seeking until he finds; and when he finds he will be troubled, and when he is troubled he will be amazed, and he will reign over the All." – The immortal words of Jesus from the Gospel of Thomas

In our second study (Paper II), nine patients that carried PNSP with a PcG MIC of 2 mg/L were treated successfully, but no patients with a higher MIC value were treated. Importantly, a group of adopted children, mainly from China, carried multidrug-resistant and highly resistant strains. This observation raises the question of whether all newly adopted children should be screened for resistant bacteria to address the potential impact on personal aspects and the communicable disease perspective. Implementation of eradication therapy for multidrug-resistant pneumococci seems to be a more difficult issue, as indicated by the results achieved in the 1970s in South Africa, where the most widely used combination of antibiotics (i.e., rifampicin and fusidic acid) was successful in only 63% of the cases treated.

Decreased prescribing of antibiotics lowers the carriage of PNSP on both an individual and a small community level, as has been shown in Iceland [248]. The work to restrict prescribing of antimicrobials does have an effect and must be continued, even if we have not come very far in an international perspective.

The introduction of pneumococcal conjugate vaccine (PCV) in the immunization program for children in Sweden in January 2009 (Stockholm started already in july 2007) will most likely change the scenario, but it is not a final solution. The most prevalent PNSP serotypes today are of vaccine serotypes. In countries where resistance is more prevalent and PCV is being used, a decline in resistant strains has been observed, although strains showing intermediate resistance are more common among the nonvaccine serotypes [249]. If there is a high level of antibiotic pressure, a new resistant strain will prevail and replace the serotypes that are nearly eliminated by the PCV. One

example of this is the rise of resistant clones of serotype 19A in the United States and South Korea [250, 251]. This serotype has been favoured in South Korea as the result of the antibiotic pressure and in the United States due to a combination of the effect of replacement after PCV and high antibiotic pressure. Serotype shift of a successful clone is also a threath to the effectivness of PCV to restrain PNSP.

Serotype 3 caused significantly more septic shock and higher mortality and the effect of conjugate vaccines on this serotype is uncertain

Patients infected with serotype 3 had significantly more often septic shock than serotype 14. The results reported in Paper III also support the notions that pneumococcal serotypes differ regarding their capacity to cause severe disease, and, together with age and co-morbidities, they have an important impact on outcome. This conclusion agrees with several other studies. Furthermore, it corroborates the theory that pneumococci related to serotypes with intermediate and low invasive potential act as opportunists that constitute the main cause of IPD in patients immunosuppressed by disease or old age, whereas high invasive serogroups function as primary pathogens and more frequently affect younger and healthier individuals [110, 142]. Similar to a Dutch study [146], we found that patients infected with serotypes that have low invasive potential more often presented with unknown foci as a marker of more severe disease. The primary endpoint in our investigation was septic shock, a state produced by the immune system and triggered by the invading microorganism. This parameter was chosen instead of the case fatality rate (CFR), which is usually studied as outcome but is biased because serotypes with a low CFR infect healthier and younger individuals and vice versa. We believe our study design is a way to bypass this bias, and to find a more true picture of the differences in virulence between serotypes.

There are several possible explanations for the difference in virulence between serotypes. An interesting hypothesis was recently put forward by Melin *et al.* [176]. These authors found that that the primary pathogens (e.g., serotypes 1 and 5) are more resistant to complement and require a higher concentration of capsule antibodies to be susceptible to opsonophagocytic killing, as compared to the opportunistic serotypes (e.g., 6B and 23F), which are associated with a more severe disease outcome. Weinberger *et al.* showed that serotypes that are carried more prevalently (i.e., more resistant to neutrophil-mediated killing); have a lower invasive potential and are more heavily encapsulated *in vitro* are associated with a higher case fatality rate [110]. In that study, serotypes 19F and 3 displayed the highest degree of encapsulation *in vitro* and these serotypes were also more extensively associated with septic shock, ICU care and mortality compared to serotype 14 in our study.

Serotype 3 stands out among most of the other pneumococcal serotypes, because it has a large mucoid polysaccharide capsule [20] that inhibits phagocytosis [252]. Thus our observation suggesting that serotype 3 is the most virulent is perhaps not unexpected, but it is worrisome considering that reports have described hyporesponsiveness to serotype 3 after a PCV booster dose, as well as a lack of protection against clinical infection after vaccination [177]. In a recent study [253], no decrease in nasopharyngeal carriage of serotype 3 was seen after vaccination with PCV13. There is presently no strong clinical data available to support the idea that when serotype 3 is included in PCV, this vaccine can protect against this particular serotype.

Antibiotic treatment at the onset of VAP changed the aetiology

We found that the bacterial aetiology of VAP differed depending on whether the patients were on antibiotic treatment at the time of diagnosis and whether they were treated with narrow or broad spectrum antibiotics (Paper IV), and these findings are in agreement with other studies [254, 255]. We also observed significantly more *H. influenzae* (p = 0.035) and Gram-positive pathogens (p = 0.019) in patients who were not treated with antibiotics at the onset of VAP. In addition, patients without antecedent or ongoing antibiotics at VAP onset seemed to be at low risk of infection with resistant bacteria or *P. aeruginosa*. According to the results of this retrospective study, narrow spectrum antibiotics probably constituted adequate empirical treatment in nine out of ten of the VAP patients without antimicrobial therapy at the onset of VAP. On the other hand, patients in this category are very vulnerable and are at large risk if treated inappropriate [64]. Factors such as the severity of disease, the local microbial flora, and the possibility to secure adequate samples for culture must be taken into account when considering the use of an antibiotic that has a narrow spectrum. When culture results are available, de-escalation is recommended to ensure suitable therapy for VAP without overuse of antibiotics [256].

In the group that received broad spectrum antibiotics, there were significantly more negative cultures and more low virulent species compared to what was observed in the other groups (p = 0.001). This discrepancy is not remarkable and was probably due to the antimicrobial selection pressure. Another possibility is that these patients were incorrectly diagnosed with VAP, as has been shown in many cases in autopsy studies [81, 217]. Eleven of the 12 patients infected with resistant bacteria in our study were treated with antibiotics, nine at the onset of VAP and two prior to VAP. However, antibiotic treatment was not a significant risk factor for infection with resistant bacteria, which can probably be explained by the low number of observations and that antibiotic treatment was relatively common even in the group without resistant bacteria. Earlier studies and consensus documents have stipulated that antecedent and ongoing antibiotic therapy represent a risk factor for MDR pathogens [197, 257]. However, we observed

a tendency towards larger numbers of resistant bacteria in late-onset VAP ($p = 0.061$). The onset of VAP counted in days from the start of mechanical ventilation is an important determinant of the likely pathogen, because the longer the time with mechanical ventilation in an ICU, the higher is the risk of MDR and a pathogen that is difficult to eradicate. We classified VAP arising at least seven days after intubation as late onset, a cut-off that was also used in many other contemporary studies. Unfortunately, that choice was a mistake, because that cut-off does not concur with modern guidelines that uses five days as cut-off [61] and makes our data less suitable for comparison with other more recent studies. On the other hand has this cut-off not been validated and is arbitrary [87]. We found that a surprisingly large number of patients with early-onset VAP ($n = 9$, 27%) were positive for *P. aeruginosa*, and eight of those subjects were being or had previously been treated with antibiotics. All of their isolates exhibited a normal resistance pattern. Similar findings, with early *P. aeruginosa* infections were made in a recent Swedish study [258]. According to other investigators [254, 255, 259], earlier or ongoing antibiotic treatment is a stronger risk factor for infection with *P. aeruginosa* than the duration of the ventilator support. We also conclude that none of the patients in our study were positive for ESBLs or MRSA and that most of the resistant strains were relatively benign in an international context [257, 260].

"Medicine is a science of uncertainty and an art of probability"
– Sir William Osler

Conclusions

The main conclusions that can be drawn from the present studies are summarized below. In general, our findings can aid the development of guidelines for management of PNSP. Furthermore, *S. pneumoniae* serotype 3 proved to be the most virulent in cases of IPD, and the results also demonstrated that serotypes behave differently and antimicrobial treatment alters the aetiology of VAP.

Our study of DCC screening during an outbreak of PNSP demonstrated the following:

- Very few personnel were carriers of $PNSP_{0.5}$, and those who were carriers were so for a very short time.

- During outbreak, 5% of the children were carriers of $PNSP_{0.5}$.

- The proportion of children carrying $PNSP_{0.5}$ with the same serotype as the index case was significantly larger in the same DCC department as the index case, indicating that the index case often is in the center of the outbreak.

- $PNSP_{0.5}$ were found in 34% (61/180) of the DCC departments without an index case, indicating that the index case is not always in the centre of an outbreak or that $PNSP_{0.5}$ can spread to other departments as well.

- There was significant seasonal variation in the pneumococcal carriage rate, and furthermore, the carriage rates were lower after major holidays and other free days, indicating that absence from DCC causes the carrier rate to decline.

- The rate of pneumococcal carriage was higher in the larger DCC groups than in the smaller ones, and the carriage rates of both $PNSP_{0.5}$ and pneumococci in general decreased significantly with age.

Our evaluation of the treatment of children with prolonged carriage of $PNSP_{0.5}$ showed that eradication therapy based on a combination of rifampicin and amoxicillin, erythromycin, or clindamycin was effective.

Considering the patients diagnosed with IPD, septic shock was seen significantly more often in those infected with serotype 3 than in those with serotype 14. Also, the results reported in Paper III support the following theories: (i) pneumococcal serotypes differ in their capacity to cause severe disease and, together with age and co-morbidities, have an important impact on outcome; (ii) pneumococci related to serotypes with in-

termediate and low invasive potential act as opportunists that cause IPD mainly in patients immunosuppressed by disease or old age, whereas high invasive serogroups act chiefly as primary pathogens and more frequently affect younger and healthier individuals.

The main findings of our VAP study were as follows:

- The bacterial aetiology of VAP in a Swedish university hospital differed from that indicated by pooled international data.

- Patients who were not treated with antibiotics at the onset of VAP had significantly higher levels of *H. influenzae* and Gram-positive pathogens.

- There was a trend towards higher rates of resistant bacteria in late-onset VAP, but the resistant strains were relatively benign in an international comparison.

- *P. aeruginosa* was a surprisingly common aetiology of early-onset VAP, and most of the patients had been treated with antibiotics.

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> *"It is very expensive to give bad medical care to poor people in a rich country."*
> *– Paul Farmer*

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Sist men inte minst ………………………………………………………………………., För att Du/Ni är så fin människa/or.

> *"Isn't it a bit unnerving that doctors call what they do "practice"?"* *– George Carlin*

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