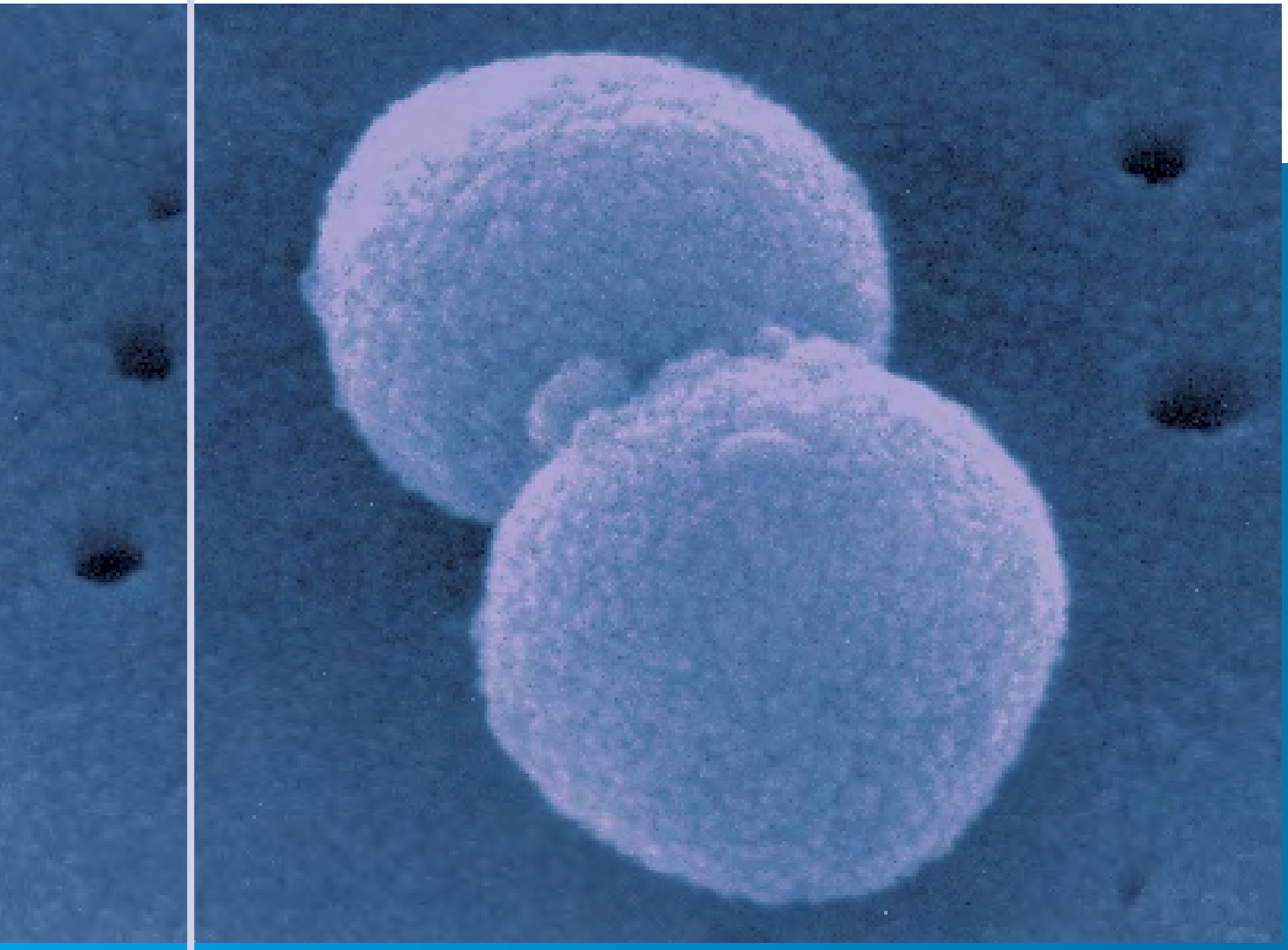
Memoria presentada por **Adoración Navarro Torné**
para optar al título de Doctora en Medicina por la Universidad Autónoma de Barcelona



EUROPEAN SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE. EPIDEMIOLOGY, SEROTYPE DISTRIBUTION AND ANTIMICROBIAL RESISTANCE PATTERNS

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A mis padres, de quienes he aprendido a luchar y a levantarme una y otra vez. Ellos guiaron mis primeros pasos hacia la ciencia.

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Si no conozco una cosa, la investigaré.
Louis Pasteur

La ciencia siempre vale la pena porque sus descubrimientos, tarde o temprano, siempre se aplican.
Severo Ochoa

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Abbreviations

ACIP	Advisory Committee on Immunization Practices
ANSORP	Asian Network for Surveillance of Resistant Pathogens
AOM	acute otitis media
AST	antimicrobial susceptibility testing
CAP	community-acquired pneumonia
CbpA	choline binding protein A
CDC	Centers for Disease Prevention and Control
CFR	case fatality rate
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CSF	cerebrospinal fluid
CTX	cefotaxime
DALY	disability-adjusted life years
DNA	deoxyribonucleic acid
DSN	dedicated surveillance network
ECDC	European Centre for Disease Prevention and Control
EARS-Net	European Antimicrobial Resistance Surveillance Network
EEA	European Economic Area
EQA	External Quality Assurance
ERY	erythromycin
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EU-IBIS	European Union Invasive Bacterial Infections Surveillance Network
GP	general practitioner
HIV	human immunodeficiency virus
HPA	Health Protection Agency (London)
IgG	immunoglobulin G
IgM	immunoglobulin M
IPD	invasive pneumococcal disease
IRAK-4	interleukin-1 receptor-associated kinase 4
LHU	local health unit
MDR	multidrug resistance
MIC	minimum inhibitory concentration
MLSB	macrolide, lincosamide and streptogramin B
mRNA	messenger ribonucleic acid
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	Member States

NEMO	NF-kappa B Essential Modulator
NIP	National Immunisation Programme
OMV	outer membrane vesicle
OPA	opsonophagocytic killing activity
PBP	penicillin-binding protein
PCho	phosphoserylcholine
PCR	polimerasa chain reaction
PCV	pneumococcal conjugate vaccine
PCV7	hepta-valent pneumococcal conjugate vaccine
PCV10	10-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PCV15	15-valent pneumococcal conjugate vaccine
PEN	penicillin
PI-1	pili 1
PI-2	pili 2
PPV	pneumococcal polysaccharide vaccine
PS	polysaccharides
PsaA	pneumococcal surface antigen A
PspA	pneumococcal surface protein A
PspC	pneumococcal surface protein C
rRNA	ribosomal ribonucleic acid
RSV	respiratory syncytial virus
SCID	severe combined immunodeficiency disease
SIR	susceptible, intermediate, resistant classification (antimicrobial susceptibility to penicillin)
SXT	sulfamethoxazole/trimetoprim
TESSy	The European Surveillance System
USA	United States of America
VPD	vaccine-preventable disease
WHO	World Health Organisation

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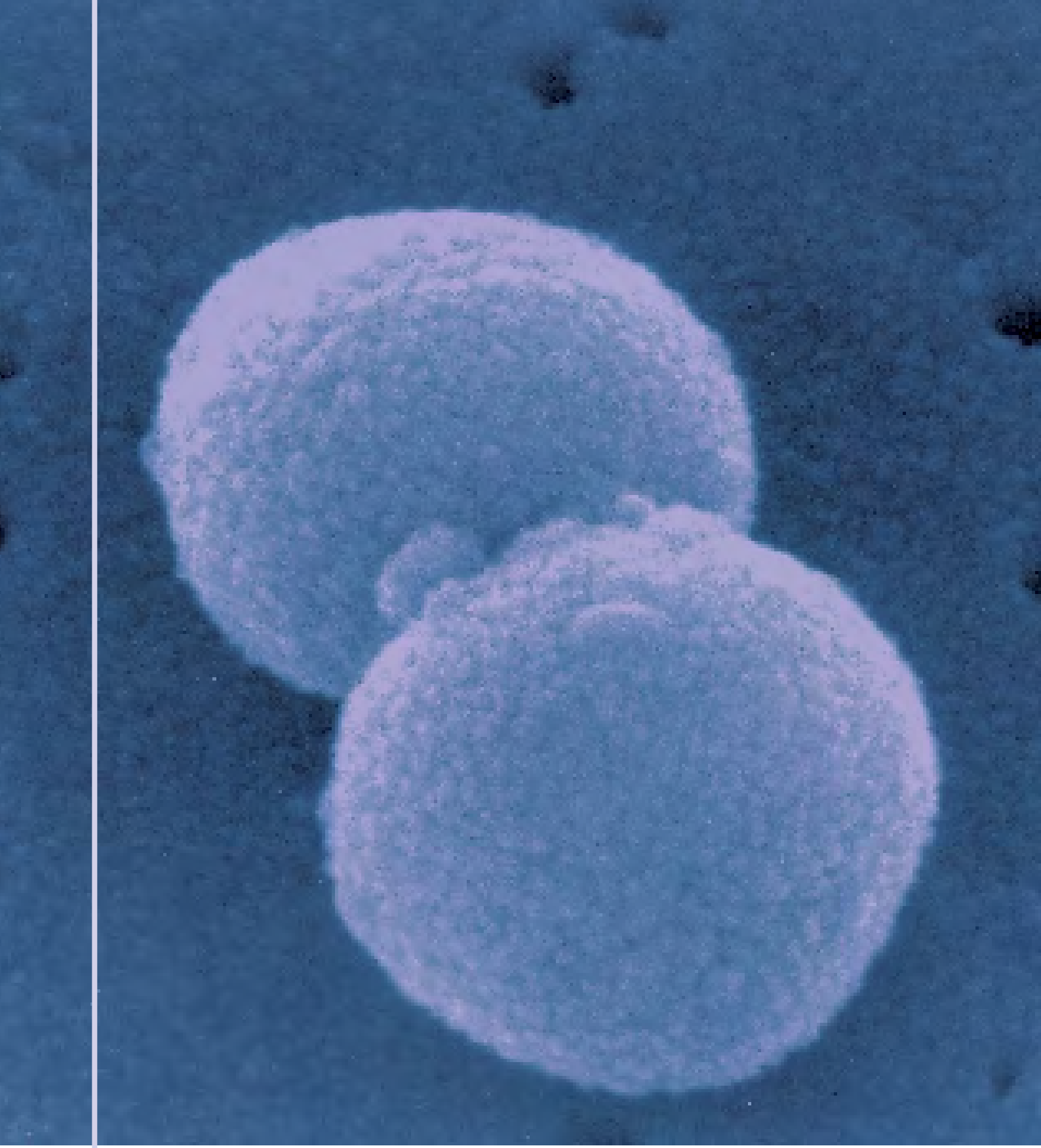

1. INTRODUCTION

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1.1. *Streptococcus pneumoniae*. Microbiological characteristics

Streptococcus pneumoniae is an invasive, gram-positive, extracellular bacterial pathogen. There are several components that play a role in the pathogenesis of the pneumococcus. The pneumococcal **surface** is covered by a **polysaccharide capsule** that consists of teichoic acid covalently linked to a peptidoglycan backbone on the outer surface of the cell wall (1). The peptidoglycan has the classical gram-positive composition of N-acetylglucosamine, N-acetylmuramic, and a lysine-containing stem peptide. On the contrary, the teichoic acid is unusual and contains a ribitol phosphate backbone and covalently attached phosphoserylcholine (PCho) (2). PCho serves as the anchorage platform for many pneumococcal proteins to the bacterial surface. The surface composition varies between two different phenotypes: transparent and opaque colony types (3). The transparent phenotype is prevalent in the nasopharynx and expresses less capsule and less pneumococcal surface protein A (PspA) but more choline binding protein A (CbpA) and major autolysin (LytA). In contrast, the opaque phase predominates in the blood and contains more capsular polysaccharide and PspA and less CbpA. The opaque forms also produce a greater biofilm and are more invasive in the lungs and brain. The precise mechanism of this phase variation is unclear but it helps evading host defences such as C-reactive protein (CRP) and leukocytes in blood, while it contributes to the attachment to host cells in the nasopharynx (3).

The **polysaccharide capsules** are antiphagocytic, impeding the access of leukocytes to complement fixed on the underlying cell wall (3). The capsule locus shows a similar organisation in all strains with genes encoding a specific capsule type surrounded by genes common to all types.

The capsular locus is transcribed as a single operon and all strains exhibit a similar organisation with the genes coding for a specific capsule flanked by other genes common to all strains. Multiple polymorphisms in the capsular operon result in serologically distinct variants (or serotypes), of which there are currently 94 described. The serotypes vary markedly in carriage rates, disease potential (invasiveness, lethality), geographical distribution and the prevalence of antimicrobial resistance (4).

In 1996 and thus before the introduction of pneumococcal conjugate vaccine (PCV), a comprehensive analysis of 13 databases and more than 7,000 samples of invasive pneumococcal disease (5) was performed. As regards age distribution, it showed that the risk for serogroups 6, 14, 18, 19, and 23 declined from childhood over time. According to this study, the decrease was more pronounced for serotype 14, whereas it was gradual for serogroup 18. The risk for serotype 1 decreased with age and for serotypes 3 and 8 it increased during middle age (5). In contrast, serotypes 7 and 23 were frequent in young adults. As regards geographical distribution, the study found that serotypes more related to nasopharingeal carriage such as 19 and 24 are more frequently isolated in Europe and North America, while serotypes 1 and 5 are more predominant in South America but were particularly uncommon in the United States of America and Canada (5). Serotype 1 is also prevalent in many African countries (6, 7).

Only antibodies directed towards the capsular antigen have proven protection. Furthermore, the majority of the capsular composition is polyssacharidic and thus they are T-independent antigens unable to elicit a T-dependent response, meaning that there is no immunogenic memory or booster response. This fact has governed the design of pneumococcal vaccines.

Pili are multimeric filamentous surface structures attached to the wall. Two pathogenicity islets encoding pili (pili 1 or PI-1 and pili 2 or PI-2) are involved in adhesion. PI-1 has been shown to influence colonisation, virulence, and the inflammatory response in mouse challenge models (3).

The role of several pneumococcal proteins in the pathogenicity of pneumococcal infections is being elucidated, namely pneumolysin, PspA, pneumococcal surface protein C (PspC), pneumococcal surface antigen A (PsaA), neuraminidase enzymes and histadine-triad. Proteins are therefore targets for a new generation of protein-based vaccines (1).

The pneumococcus is a highly recombinant species. The paradigm for defining invasive strains so far has been the capsule's composition. Despite multiple studies, it has not been identified as a protein or variant allele that specifically differentiated invasive strains from carriage strains (8). By using the whole-genome sequencing, there might be opportunities to define invasive genotypes and phenotypes beyond the capsule and evolving invasive genotypes towards less invasive strains by the acquisition of certain genes. The identification of these proteins conferring invasiveness may allow its utilisation as vaccine candidates (8).

Pneumococci exist as biofilms within the nasopharynx, a growth phenotype characterised by surface attachment, integration within an extracellular matrix, and antimicrobial resistance. Experimental evidence indicates that biofilm pneumococci are attenuated compared with their planktonic forms. This attenuated phenotype corresponds with observations that biofilm pneumococci elicit significantly less cytokine and chemokine production from host cells than their planktonic forms. Pneumococci within biofilms have a decreased metabolism, less capsular polysaccharide, and a reduced production of the pore-forming toxin pneumolysin. Biofilm pneumococci are predominately in the transparent phenotype, which has elevated cell wall phosphorylcholine, an adhesin subject to C-reactive protein mediated opsonisation. The mechanisms that govern the transition from the biofilm form to the invasive one and the routes to disease still remain unknown (9, 10) but deserve further investigation.

A prerequisite to initiate severe and invasive infections is the ability of the bacteria to adhere to host cells. Adherent molecules are key for the host-pathogen interactions. These bacterial components are usually surface structures that facilitate adherence to host cells or host serum proteins of the extracellular matrix. Enzymes such as neuroaminidase promote biofilm formation and the development of otitis media (3). Hyaluronidase helps spread bacteria in the tissue and other molecules interact with extracellular matrix proteins such as fibronectin and plasminogen, escaping the host inflammatory response.

A relevant aspect of the epidemiology of pneumococcal colonisation is the frequent occurrence of co-infections. Colonisation with pneumococci during influenza leads to a synergistic type-1 interferon response with decreased clearance of bacteria from nasopharynx and increased pneumonia (3).

The pneumococcus is known to be highly transformable and recombining, although this capacity varies among different isolates as it seems that transformation can benefit cells living in stringent environments and can be costly in favourable environments (11). Pneumococci with evidence of large recombination at housekeeping loci are significantly associated with antibiotic resistance (11). Furthermore, the capacity of the pneumococcus to exchange genetic material is not restricted to its own species but this ability is extended to other species of its ecological niche (i.e. oral cavity and nasopharynx). Horizontal gene transfer may explain the numerous virulent genes such as those encoding pneumolysin, mitilysin and neuraminidase A found in other members of the mitis group of Streptococci.

Pneumococcal vaccines have been designed to target the most prevalent serotypes in invasive pneumococcal diseases in children. Despite the fact that conjugate vaccines have proven effective at preventing

invasive disease, the pneumococcus has made evident its ability to circumvent this selective pressure due to its highly variable genome and intra-species diversity. The elimination of previously dominant serotypes due to vaccine selection has led to the extension of non-vaccine serotypes (this phenomenon is known as 'serotype replacement' and it will be developed in more depth in the section on vaccines).

The process of substituting the genes encoding one type of capsule with genes encoding for another is known as 'serotype' or 'capsular switching'. This ability is of concern as vaccine serotype to non-vaccine serotype switches may allow certain strains causing invasive disease to escape vaccines and become more virulent.

1.2 Interaction with other pathogens

Streptococcus pneumoniae is a common inhabitant of the human nasopharynx. Colonisation of the nasopharynx is a necessary, although not sufficient, previous step to pneumococcal disease. In the nasopharynx, the pneumococcus shares the anatomical and physiological niche with other bacterial and viral occupants, namely members of the families Moraxellaceae, Streptococcaceae, Corynebacteriaceae, Pastereullaceae (including the genus Haemophilus) and Staphylococcaceae. Intraspecies and interspecies interactions impact pneumococcal carriage. Bacterial interplay between the pneumococcus and other bacterial species influence carriage prevalence, virulence and biofilm formation (12). The pneumococcus is endowed with a plethora of cellular components that are useful both during carriage and disease. There have been speculations about an inverse relationship between the rise in community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) infections and the implementation of PCVs. Some studies have demonstrated an inverse correlation between the carriage of *S. pneumoniae* and the carriage of *S. aureus* (13). However, studies have failed to demonstrate an association between pneumococcal carriage and MRSA carriage. As regards *H. influenzae* and *Moraxella catarrhalis*, most studies found a positive association between the presence of these two microorganisms in the nasopharynx and the presence of *S. pneumoniae* (13).

Recent studies have revealed that *S. pneumoniae* takes part in interspecies biofilms that confer the pneumococcus protection against antibiotics through passive transference of ß-lactamase produced by resistant *H. influenzae* (14). Pneumococcal vaccination has been postulated to be associated with an increase in non-typeable *H. influenzae* in acute otitis media aetiology (15, 16).

The pneumococcus also shares this ecological niche with respiratory viruses. Bacterial co-infection with influenza viruses has been well documented (17), and increased morbidity and mortality during influenza pandemics (H1N1) has been proven in different settings (18, 19). A number of studies have revealed that viral infection can increase bacterial adhesion to the respiratory epitelium and distort the innate immune response expediting bacterial invasion and impeding clearance (18, 20).

Increased colonisation rates have been described in the presence of other viruses such as respiratory syncytial virus (RSV) in children during RSV epidemics (21) and have also been associated with human metapneumovirus (22).

Conjugate pneumococcal vaccines reduce the nasopharyngeal carriage and disease of vaccine serotypes and thus may also modify the interactions of the pneumococcus with other commensals, either bacteria or viruses. Monitoring the carriage of different components of the nasopharyngeal ecosystem remains very relevant as upper respiratory tract infections and pneumonia constitute a major global burden.

1.3 Host-pathogen interactions

After the implementation of routine childhood immunisation, elderly people are the most susceptible age group to pneumococcal disease in the developed world (23). There are several factors that add to increased susceptibility in the elderly, such as impaired immune function (24). Simell et al. (24) found that IgG antibody concentrations to pneumococcal protein antigens and the IgM antibody concentrations to pneumococcal capsular polysaccharides decline notably with age, particularly in women. These findings are consistent with the increase of pneumococcal diseases in the elderly and suggest an altered immune system. However, the functionality of these antibodies in the elderly remains to be elucidated. The progressive decline of the immune functions with age is known as 'immunosenescence' and has clinical consequences in the susceptibility to infections and other conditions such as cancer. Cellular senescence is an age-related event that contributes to the concept of 'inflammaging'. Chronic inflammation and cellular senescence also affect the susceptibility and response of elderly people to pneumococcal infections (25).

1.4 Epidemiology and burden of the pneumococcal disease

Pneumococcal disease is caused by *Streptococcus pneumoniae* whose natural reservoir is the human nasopharynx. From this niche it can be transmitted to other individuals through respiratory droplets. Pneumococci are carried asymptomatically in the nasopharynx and only a small number of carriers develop disease. Pneumococcal disease can be grouped into invasive disease and non-invasive (also named mucosal) disease. From the nasopharynx, pneumococci may spread into the paranasal sinuses causing sinusitis, into the middle ear cavity causing otitis media, or into the lungs causing pneumonia. Pneumococci, most likely through microaspirations, enter the lungs causing pneumonia that may lead to empyema (the collection or gathering of pus in the pleural cavity) and eventually bacteraemia. Symptoms of pneumococcal pneumonia are characteristic: high fever, chills, malaise, unproductive cough, shortness of breath, chest pain that exacerbates with breathing, and local or diffuse opacity of the affected area in the radiological examination. This is usually referred to as 'typical pneumonia'.

In addition to bacteraemia, pneumococci may cause other systemic infections that are life-threatening, such as meningitis due to haematological dissemination from a focus, i.e. otitis, mastoiditis or sinusitis. More rarely, pneumococcus is responsible for infections in other sites such as joints, bones and soft tissues (1).

Invasive disease refers to the isolation of *S. pneumoniae* or the detection of its antigens from sterile sites (26). The highest burden of pneumococcal disease corresponds to non-invasive disease. Pneumonia can be invasive (bacteraemic) or non-invasive. Some studies have suggested that for every bacteraemic case there are three non-bacteraemic infections (27). Notably, the clinical and economic burden of invasive pneumococcal disease remains particularly high in adults (28).

A number of factors predispose to pneumococcal infections. Defective antibody formation (either primary as in, for example, congenital agammaglobulinemia or secondary as in multiple myeloma); defects in complement, neutropenia; asplenia; alcoholism, malnutrition; diabetes; excess exposure to the pathogen as in day-care centres or military camps (29).

Pneumococcal pneumonia is the leading cause of childhood death in developing countries (30). Moreover, *Streptococcus pneumoniae* is a leading cause of meningitis and sepsis in children worldwide. *S. pneumoniae* causes over 800,000 deaths in children under five years old, 11% of all deaths in this age group (31).

The World Health Organisation (WHO) estimates that pneumonia/lower respiratory infections cause 230,000 deaths (2.3% of total deaths) in the European region and represent 2.2 million disability-adjusted life years (DALY) (1.5% of total DALYs lost in the European region) (31). In the European Union (EU) in 2011, the total

estimated annual economic burden of pneumonia/acute low respiratory infections amounted to €46 billion, both direct (healthcare) and indirect (lost production). Of these, the direct costs are estimated at €2.5 billion. DALYs lost were estimated at €43.5 (32).

In adults, the burden of non-invasive pneumococcal disease is mainly attributable to community-acquired pneumonia (CAP) (33, 34). Pneumococcal disease poses an important morbidity and mortality burden to elderly patients with concurrent conditions, despite the implementation of childhood immunisation programmes (34, 35). In Europe, CAP short-term mortality (in hospital or 30-day mortality) has been reported between <1% and 48% (34). This great variability can be explained by multiple factors such as demographic differences, concurrent conditions, differences in healthcare and time to follow-up. The case fatality rate (CFR) for patients with invasive pneumococcal disease (IPD) ranges from 11 to 30% in the western world (33).

As regards non-invasive pneumococcal disease among children, acute otitis media (AOM) is the most common clinical manifestation of pneumococcal infection and the most common outpatient diagnosis resulting in antibiotic prescriptions in that group. This leads to antimicrobial resistance due to the elevated number of prescriptions (36) and severe sequelae, particularly deafness and suppurative complications (37). AOM incidence has been estimated at 10.85% (709 million cases each year) worldwide with 51% of these occurring in children below five years (37). AOM-related hearing loss has a prevalence of 30.82 per 10,000. Each year 21,000 people die due to complications of otitis media worldwide (37).

Several studies have addressed the distribution of pneumococcal serotypes by age, sex or geography. Invasive pneumococcal disease distribution by age usually depicts a U-shaped curve, with the highest incidence in children under five years of age and adults of 65 years or older. Seasonal patterns have been described and some studies have found differences in distribution of invasive pneumococcal diseases. Bacteraemic pneumococcal pneumonia occurs predominantly in winter, apparently due to increased susceptibility to infection prompted by viral pathogens, whereas seasonal variations of invasive non-pneumonia infections, more frequent in autumn, seem to be related to seasonal variations in nasopharyngeal carriage and thus transmission of the pathogen (38).

1.5 Diagnostic

S. pneumoniae is a gram-positive coccus that occurs in pairs (diplococcus) and reproduces in chains in a liquid medium. This bacterium is catalase negative and produces haemolysin that splits haemoglobin into a green pigment producing the phenomenon called □-haemolysis where, in blood agar, colonies are surrounded by a characteristic green halo. The microorganism growth is inhibited by ethyl hydrocupreina (optochin) and is soluble in bile salts. Therefore these four reactions characterise the pneumococcus, apart from its distinct flame or lanceolate shape: □-haemolysis of blood agar, catalase negativity, susceptibility to optochin and solubility in bile salts. However, some strains have been recently described to be resistant to optochin and therefore the diagnostic relies mainly on bile solubility (29).

1.5.1 Serotyping methods

A variety of laboratory methods are used to serotype strains, such as Quellung, Pneumotest®, slide agglutination, latex agglutination, co-agglutination, multiplex PCR, and gel diffusion.

Quellung reaction is an immunological reaction in which antibodies bind to the capsule of certain capsulated microorganisms. The antibody reaction allows these species to be visualised under a contrast phase microscope. If the reaction is positive, the capsule becomes opaque and appears to enlarge. 'Quellung' is the German word for swelling and describes the microscopic appearance of pneumococcal or other bacterial capsules after their polysaccharide antigen has combined with a specific antibody. The antibody usually

comes from serum taken from an immunised laboratory animal (usually rabbit for pneumococcus). As a result of this combination and the precipitation of the large, complex molecule formed, the capsule appears to swell because of increased surface tension, and its outlines become clearly delineated.

When specific anti-pneumococcal antibodies, either on their own or coupled to latex particles or staphylococci via protein A, are mixed with pneumococci of the corresponding capsular type, an agglutination reaction occurs. This agglutination is visible to the naked eye. This is the principle of slide agglutination, latex agglutination and co-agglutination methods.

Pneumotest® is a commercial application of the latex slide agglutination method (SSI, Denmark).

Multiplex PCR is a molecular method based on the amplification of specific deoxyribonucleic acid (DNA) sequences. It enables causative microorganisms and/or serotype-specific genes to be identified with a high degree of sensitivity and specificity. Unfortunately, this surveillance did not collect data on protocols or gens investigated and therefore molecular characterisation cannot be described.

Gel diffusion is a simple precipitation assay that consists of evaluating the precipitin reaction in a clear gel, seen when an antigen placed in a hole in the gel (usually agarose) diffuses evenly into the medium. An obvious ring forms where the antigen meets the antibody. In the case of pneumococci, specific antisera against capsular antigens are used, allowing the identification and serotyping of a particular pneumococcal strain.

1.5.2 Antimicrobial susceptibility testing

As regards antimicrobial susceptibility testing, the antimicrobial gradient diffusion method is based on the principle of establishing an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility. The Etest® is a commercial version. It employs thin plastic test strips impregnated with a dried antibiotic concentration gradient and marked on the upper surface with a concentration scale. After overnight incubation, the tests are read by viewing the strips from the top of the plate. The minimum inhibitory concentration (MIC) is determined by the intersection of the lower part of the ellipse-shaped growth inhibition area with the test strip.

The broth dilution method procedure involves preparing two-fold dilutions of antibiotics in a liquid growth medium dispensed in test tubes. The lowest concentration of antibiotic that prevented growth represents the MIC. This method can be done on a 'miniature' (broth microdilution) scale using microtiter plates.

The use of instrumentation can standardise the reading of end points and often produces susceptibility test results more quickly than manual readings because sensitive optical systems enable the detection of subtle changes in bacterial growth.

1.6 Antimicrobial resistance

Antimicrobial resistance of *S. pneumoniae* has been evolving over time due to the widespread use of antibiotics and pneumococcal conjugate vaccines. The first strains moderately resistant to penicillin were isolated in Australia in 1964 and then in Papua New Guinea. In 1977, the first strains highly resistant to penicillin were described in South Africa. In Spain between 1979 and 1981, 9% of strains causing invasive pneumococcal disease were resistant to penicillin. By the 1990s, penicillin-resistant clones had spread throughout Europe and globally, together with escalating resistance to macrolides and other antibiotic classes (39), including multidrug resistance (MDR) (resistance to at least three classes of antibiotics). At that time, six international clones were responsible for most of these resistant strains (serotypes 6A, 6B, 9V, 14, 19F, 23F). At present, resistant rates are high in Spain, France, and Eastern European countries, and in the United

States of America (USA), South Africa, and Central and South America. The introduction of the heptavalent pneumococcal conjugate vaccine (PCV7), comprising the most common serotypes causing paediatric invasive pneumococcal disease, has largely contributed to the decrease in the incidence of IPD caused by vaccine serotypes, as well as the prevalence rates of antimicrobial resistance. However, the introduction of PCV7 has led to the emergence of non-vaccine serotypes, some of them particularly resistant, such as 19A, which have spread worldwide. The introduction of 13-valent pneumococcal conjugate vaccine (PCV13) with six additional serotypes including 19A might be able to control the emergence and spread of this serotype and will certainly affect the epidemiology of pneumococcal diseases and antimicrobial resistance.

In *S. pneumoniae* resistance to ß-lactam antimicrobials derives from alterations in the penicillin-binding proteins (PBPs), the cytoplasmic membrane-associated proteins that are primary targets of this antimicrobial class. ß-lactam antimicrobials bind to the cell wall synthesising enzymes (so-called penicillin-binding proteins or PBPs) and interfere with the biosynthesis and remodelling of the bacterial cell wall during the cell growth and division. Six PBPs have been described in *S. pneumoniae*: 1a, 1b, 2x, 2a, 2b and 3 (40). This resistance is thought to be the result of intraspecies and interspecies gene transfer particularly from *Streptococcus mitis* and *Streptococcus oralis*. Mutations have been discovered in the gene encoding the penicillin-sensitive transpeptidase domain of PBP (41). There are degrees of resistance ranging from low-level clinical resistance (intermediate) to full resistance. Usually, infections other than meningitis caused by intermediate resistant strains are often successfully treated with high doses of benzyl penicillin or aminopenicillins. Penicillin resistance is due to the incorporation of penicillin's low-affinity *pbp* genes through transfers from other bacteria such as viridans group streptococci. These transfers are followed by recombination into the chromosomal gene.

ß-lactam non-susceptibility (either intermediate or resistant) rates have increased worldwide during the 1990s and 2000s. The European Antimicrobial Resistance Surveillance Network (EARS-Net) report shows that in 2013 in Europe, penicillin non-susceptibility rates ranked from 1.1% in the Netherlands to 40.0% in Cyprus (42). For the period 2010-2013, significantly increasing trends were observed for Belgium, Denmark, Germany, Italy, Poland, Sweden and the United Kingdom. Significantly decreasing trends were reported for Czech Republic, Hungary, France, Slovenia and Portugal.

The Active Bacterial Core Surveillance Program in the USA showed an increase in the rate of penicillin non-susceptibility (MIC ≥0.12 mg/L) from 21.6% in 1996 to 25.9% in 2000; after the introduction of PCV7, it decreased again to 21.6% in 2004 (43). Another study showed a fluctuation in the rate of penicillin resistance from 15.6% in 1996 to 23.2% in 2000 and to 16.9% in 2008. The SENTRY Antimicrobial Surveillance Program (44) study revealed that the rate of penicillin non-susceptibility in the USA had increased from 3.2% in 1998 to 11.7% in 2011, according to the revised breakpoints for penicillin (susceptible ≤2, intermediate 4, resistant ≥8 mg/L for non-meningeal isolates). The Asian Network for Surveillance of Resistant Pathogens (ANSORP) study performed in 2000-2001 showed very high rates of penicillin resistance in many Asian countries: Vietnam (71.4%), Korea (57.8%), Hong Kong (43.2%) and Taiwan (38.6%) (44). In seven Latin American countries, a study revealed a global rate of penicillin non-susceptibility of 30.7%, ranging from 25% in Mexico to 2.8% in Venezuela (45). In another study, Southern and Eastern Mediterranean countries reported 26% overall of penicillin non-susceptibility, with the highest rates reported in Algeria (44%) and Lebanon (40%) (45).

Resistance to cephalosporins and carbapenems has been described but remains sporadic since these antibiotic classes display great activity against pneumococci.

Macrolide resistance has increased in most parts of the world due to the use and overuse of antibiotics, as macrolides are usually prescribed for upper respiratory tract infections, the clinical entities with the highest number of antibiotic prescriptions (46).

Macrolide, lincosamide and streptogramin are antimicrobials that bind to a ribosomal subunit, inhibiting the initiation of messenger ribonucleic acid (mRNA) binding and hence inhibiting protein synthesis. Macrolide resistance in *S. pneumoniae* is predominantly mediated by two mechanisms (47):

» The acquisition of an erythromycin methylation gene erm(B) results in a post-transcriptional modification of the 23S subunit of ribosomal ribonucleic acid (rRNA), which blocks the binding of the macrolide to the ribosome. This results in macrolide-lincosamide-streptogramin resistance MLSB and often entails high-level resistance (MICs > 128 mg/L). The erm(A) gene is rarely found in pneumococci.

» The acquisition of a macrolide efflux system gene mef(A), which encodes an antibiotic efflux pump that results in the excretion of the antimicrobial reducing the intracellular concentration. Other mechanisms include mutations in rRNA (23SrRNA) and ribosomal proteins L4 and L22. In addition to mef(A) efflux pump gene, the variant mef(E) is also expressed (47). These mechanisms conduct to very high MICs and cannot be overcome by increasing the dosage of antimicrobials as opposed to ß-lactam resistance.

Resistance to macrolides and azalides is the most striking problem of in vitro resistance worldwide, particularly in the Asian region (44).

Overall surveillance studies have shown an increase in macrolide resistance rates worldwide. The EARS-Net report reveals that macrolide non-susceptibility ranged from 1.5% in Latvia to 38.1% in Romania. For the period 2010-2013, statistically significant trends were observed in Lithuania, Sweden and the United Kingdom. Dual resistance to penicillins and macrolides ranged from < 0.1% in Estonia and Latvia to 26.7% in Cyprus (42).

In the USA, the SENTRY Antimicrobial Surveillance Program (44) showed that the rate of macrolide resistance has increased from 17.8% in 1998 to 44.8% in 2011. According to ANSORP, in Asian countries the overall erythromycin rate significantly increased from 46.1% in 1996-1997 to 72.7% in 2008-2009. In particular, very high rates were found in China (96.4%), Taiwan (84.9%), Vietnam (80.7%) and Korea (77.7%) in 2008-2009. Recently, prevalence of macrolide-resistant pneumococci expressing both the ermB and mefA genes has increased worldwide (44). In addition to high-level resistance to macrolides those isolates display resistance to multiple antimicrobials (44).

Resistance to fluorquinolones with clinical activity against pneumococci (levofloxacin and moxifloxacin) is mediated by mutations in part C (subunit of topoisomerase IV) and/or gyrA (subunit of DNA gyrase/topoisomerase IV). Additionally, resistance may be conferred by efflux.

The main mechanism of resistance to fluoroquinolones is point mutations producing changes in the quinolone resistance-determining regions of the subunit of DNA topoisomerase IV, as described above. However, resistance can be also acquired by inter- or intraspecies recombination with streptococci of the mitis group (45). The overall resistance of pneumococci to fluoroquinolones remains low, albeit with notable geographic differences.

In Europe in 2013, 4.9% of all reported isolates were resistant to fluoroquinolones (44). Higher rates were detected in some Asian countries (9.1% to levofloxacin in Taiwan and 5.2% in Korea in 2008-2009) (43) and in Canada (7.3% in 2006) (45).

In Europe, the overall prevalence of antimicrobial resistance in *S. pneumoniae* displays geographical differences with lower rates in northern countries than in southern countries (42, 45).

Multidrug-resistant *S. pneumoniae* strains, defined as resistant to three or more antimicrobial classes, have increased worldwide (44). Penicillin resistance is frequently associated with a multiresistant pattern, including macrolides, azalides and cotrimoxazole, apart from ß-lactams. In a survey of 15 countries in Europe in 2004-

2005, 15.8% of pneumococcal isolates were multidrug resistant (47). The emergence of serotype 19A multidrug-resistant clones is of concern. Most of the serotype 19A isolates belong to the clonal complex CC320 that is associated with multidrug resistance. This has increased and these isolates are still predominant after the introduction of PCV13 (44). It is likely that antibiotic pressure and the introduction of pneumococcal conjugate vaccines may be the most important factors for the emergence of multidrug-resistant 19A strains. Since PCV13 includes serotype 19A, a decrement is expected in the clonal spread of multidrug-resistant 19A isolates, although little data is still available. Other non-vaccine serotypes such as 6C, 11, 15A, 33A or 35B may emerge. Extensive drug-resistant serotype 11A isolates as well as serotype 6D multidrug-resistant ST282 have been recently described in Korea (44).

Surveillance and monitoring of emerging serotypes and clones after the introduction of PCV13 is of the utmost importance.

1.7 Vaccines

The history of pneumococcal immunisation goes back to 1911 when a first clinical trial of a pneumococcal vaccine was conducted among the native workers at gold and diamond mines in South Africa. These workers had an extremely high incidence of lobar pneumonia. This vaccine and those developed in the following 30 years were based on killed bacteria (48). In the 1940s, the next generation of pneumococcal vaccines was formulated based on the purified capsular polysacchrides (PS) of the bacteria. These new vaccines were introduced at the same time as the entry onto the market of penicillin and other antimicrobials that were considered to be the definite cure for pneumococcal pneumonia. Therefore, the enthusiasm for the prevention of pneumococcal disease by immunisation vanished and PS vaccines were withdrawn from the market in 1954 (48).

In 1983 the current 23-valent polysaccharide pneumococcal vaccine was licensed in the USA and Canada. It contained serotypes 1, 2, 3, 4, 5, 6A, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33. It was indicated for the immunisation of large at-risk groups and adults over the age of 65 and remains the only vaccine for adult vaccination against pneumococcal infections. The main drawback of the vaccine is the lack of efficacy in children under two years of age because it elicits a T-cell independent response that makes it insufficiently immunogenic in this age group. Moreover, the vaccine is not able to prevent against otitis media or nasopharyngeal carriage.

Children under two years of age present the highest incidence rates for invasive pneumococcal disease, which is an element of its transmission. Therefore a new vaccine capable of eliciting a T-cell-dependent response and thus efficacy in children below two years of age was developed (49). The heptavalent conjugate vaccine (PCV7) contains serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. The conjugate vaccines consist of a conjugate between an antigenic protein and a polysaccharide. Conjugate vaccines prevent against mucosal pneumococcal disease (mainly AOM) and have a spectacular impact on the reduction of nasopharyngeal colonisation. Community protection (or herd immunity) was an added benefit of conjugate vaccines. Furthermore, antibiotic resistance in vaccine serotypes has declined since the introduction of PCV7.

PCV7 was introduced in the USA in 2000. In 2005, the Centers for Disease Control and Prevention (CDC) reported a 77% reduction in overall IPD rates and a 98% reduction in PCV7 serotype disease in children below the age of five, compared to the pre-PCV7 era (50).

Decreases in overall and/or PCV7 serotype IPD cases have been reported in children below two and five years in many countries worldwide (51-56). Moreover, reductions in hospitalisation rates for all-cause pneumonia in children <2 years have also been observed (57). However, the overall proportion of nasopharyngeal carriage in children did not substantially change as conjugate vaccine serotypes were substituted by

non-vaccine serotypes (serotype replacement). Some non-vaccine serotypes became a leading cause of invasive pneumococcal disease, partially offsetting the benefit of PCV7. These changes were also evident in older unvaccinated populations that benefited from the implementation of the vaccine by the reduction of IPD (58). In relation to antimicrobial resistance, the spread of serotype 19A multidrug-resistant strains and the emergence of 35B penicillin-resistant strains have been detected (59) in many settings. This changing serotype epidemiology of pneumococcal infections paved the way for the development of new pneumococcal conjugate vaccines, including 10-valent pneumococcal conjugate vaccine (PCV10) (PCV7 serotypes plus 1, 5, and 7F) and PCV13 (PCV10 serotypes plus 3, 6A, and 19A). The licence for PCV13 was mainly based on serological non-inferiority criteria rather than on efficacy studies. The main objective of IPD surveillance after the introduction of PCV13 was to determine the effectiveness of PCV13 against the seven original serotypes and the six additional ones. Studies in the USA have revealed that the introduction of PCV13 has led to a reduction in overall IPD incidence; also, the incidence of the six PCV13 specific serotypes has declined, even in adult non-vaccinated populations (60). In Europe, recent studies in the United Kingdom have shown decreases in IPD incidence and PCV13-specific serotypes, providing sounded evidence of community protection from these serotypes. However, the British study has gathered evidence of an increase in emerging non-PCV13 IPD in children and older adults (61, 62).

A new 15-valent pneumococcal conjugate vaccine has been developed and recent studies have analysed serotype-specific IgG and opsonophagocytic killing activity (OPA) responses to 15 serotypes included in 15-valent pneumococcal conjugate vaccine (PCV15) (PCV13 serotypes plus 22F and 33F) in healthy adults 18-45 years of age and in toddlers previously vaccinated with PCV7. It has been concluded that PCV15 displays an acceptable safety profile and induces IgG and OPA responses to all serotypes included in the vaccine (63, 64).

Serotype replacement after new higher valency vaccine introduction is likely to occur. Moreover, the total number of strains that can be covered by enhanced valency conjugate vaccines is restricted as PCVs are complex vaccines from the development and manufacturing point of view, since conjugation technology needs to be optimised for every capsular polysaccharide (65). In addition, conjugate vaccines with different polysaccharide components are expensive to produce and might not be affordable for many countries.

New serotype-independent pneumococcal vaccines are being developed based on antibody responses to non-capsular antigens. A serotype-independent protein vaccine would obviate serotype replacement and would confer a broad coverage worldwide (65). Recombinant proteins can be obtained on a large scale at very low cost and are therefore suitable for developing countries.

Overall, a number of candidate pneumococcal proteins have been studied for vaccine possibility:

1. Purified-protein vaccines:
   - » pneumolysin;
   - » pneumococcal surface vaccines: choline-binding proteins, the metal-binding lipoproteins, the sortase-dependent surface proteins and the pneumococcal histidine triad proteins.
2. Combination protein vaccines.
3. Killed whole-cell vaccines: low-cost production and synergistic immunity to multiple pneumococcal targets.
4. Intranasal, live attenuated administration of *S. pneumoniae* strains containing a deletion of genes encoding major virulence factors were able to elicit both systemic and mucosal response (66). These vaccines do not require inactivation or adjuvants and the manufacturing process is less costly than that for whole cell vaccines, but the possibility or reversion of strains to the original virulent state remains a cause for concern.

5. DNA vaccines are low-cost, and easy to manufacture and transport. Nasal delivery of plasmid DNA has rendered poor immune responses, though. It is therefore crucial to find adequate delivery systems for DNA-based vaccines.
6. Antigen delivery vehicles such as outer membrane vesicles (OMVs); nasal delivery of adenoviral vectors encoding PsaA have also elicited an immune response in mice.

In conclusion, the emergence and spread of antimicrobial resistance, the elevated cost of production of conjugate pneumococcal vaccines, the limited serotype coverage of current pneumococcal vaccines, and the subsequent serotype replacement have encouraged researchers to attempt the development of protein-based vaccines, alone or in combination, new routes of administration and delivery systems (such as intranasal needle-less administration) in order to provide new vaccines, with a broader serotype coverage and more affordable pneumococcal vaccines. All these approaches are being explored at present by different research teams.

At the same time progress in genomics and innovative strategies such as reverse vaccinology have changed concepts and design of vaccine development (67, 68). High-throughput DNA sequencing and screening techniques have led to a more comprehensive understanding of both pathogens and human immune response. This systems biology approach and coupled understanding of pathogen and host is supporting the development of new vaccine technologies including the use of new adjuvants to target specific immune responses and new delivery systems and immunisation schemes to maximise vaccine efficacy. Notably, this `personalised vaccinology' approach is particularly relevant for vaccine development for the elderly, characterised for immune dysregulation (or immunosenescence), comorbidities, polymedication, apart from genetic predisposition and gender-related differences that make of this age group a real challenge from the vaccine safety and effectiveness perspective (69).

1.7.1 Vaccine indications

Pneumococcal disease particularly affects children <2 years, individuals at risk due to underlying conditions such as immunosupressed patients, (i.e. human immunodeficiency virus (HIV) and cancer patients), asplenic, and other medical conditions in immunocompetent patients, such as cerebrospinal leaks, alcoholism, etc., and adults aged 65 years or older. Vaccine indications have therefore been issued for these groups.

In the USA, the Advisory Committee on Immunization Practices (ACIP) (70) makes recommendations for immunisation of children and adults.

In Europe, recommendations are usually set by the National Immunisation Programmes (NIPs) and PCV13 has been gradually introduced since 2010, albeit with different schemes, timing and implementation schedules. Routine immunisation schedules in childhood are established by every Member State (Table 1) but there is a certain variation concerning the type of vaccine and schedule across Europe.

Member States have also recommended pneumococcal vaccines outside the routine childhood immunisation programmes (71) for conditions and situations (Annex 2) such as in immunocompromised patients and other at-risk conditions.

In adults, indications are also established as general recommendations (Table 2) or as either age-based or at-risk recommendations (72) (Table 3).

Table 1. Recommended childhood immunisations for pneumococcal disease in Europe

Country	Months												
	2	3	4	5	6	10	11	12	13	14	15	18	23
Austria		PCV		PCV				PCV[1]					
Belgium	PCV		PCV					PCV					
Bulgaria	PCV	PCV	PCV					PCV					
Croatia													
Cyprus	PCV		PCV						PCV[2]				
Czech Republic	PCV10[3]	PCV10[3]	PCV10[3]						PCV10[3]				
Denmark		PCV13		PCV13				PCV13					
Estonia													
Finland		PCV10		PCV10				PCV10					
France	PCV		PCV				PCV						
Germany	PCV	PCV	PCV						PCV			PCV[4]	
Greece	PCV		PCV		PCV				PCV				
Hungary	PCV13[5]		PCV13[5]					PCV13[5]					
Iceland		PCV10		PCV10				PCV10					
Ireland	PCV				PCV			PCV					
Italy		PCV		PCV		PCV							
Latvia	PCV		PCV						PCV				
Liechtenstein	PCV13[6]		PCV13[6]					PCV13[6]					
Lithuania	PCV		PCV						PCV				
Luxembourg	PCV		PCV					PCV					
Malta													
Netherlands	PCV		PCV				PCV						
Norway		PCV13		PCV13				PCV13					
Poland	PCV[7,8]												
Portugal													
Romania	PCV[7,8]		PCV[7,8]							PCV[8]			
Slovakia	PCV	PCV		PCV									
Slovenia		PCV	PCV				PCV						
Spain	PCV[9]		PCV[9]					PCV[9]					
Sweden		PCV		PCV				PCV					
United Kingdom	PCV13		PCV13					PCV13					

1: Earliest, six month after the second dose.
2: Catch-up possible until six years if previous recommended doses were missed.
3: PCV10 can be replaced with PCV13; however the cost of PCV13 is paid by the patient. PCV vaccines can be administered simultaneously with hexavalent vaccine or separately during the first year of life. Three doses at one-month intervals.
4: Number of doses necessary varies according to age. Catch-up (e.g. if previous dosed missed).
5: Mandatory for those born after 30.06.2014 (scheduled at 2, 4 and 12 months).
6: Not part of the basic vaccination plan.
7: Vaccination recommended but not funded by the National Health System.
8: Recommended, but not mandatory.
9: Implementation in Regions due by December 2016.

Source: ECDC, Vaccine schedule, http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx

Table 2. Recommended adult immunisations for pneumococcal disease in Europe

Country	Years									
	2	5	18-49	50	59	60	64	65	85	≥86
Austria							PCV13[1,2,3]			
Belgium			PCV13[4,5]			PCV13[4,6]			PCV13[1,7]	
Bulgaria										
Croatia										
Cyprus						PPSV23[4,8]				
Czech Republic						PPSV23[3,4]			PPSV23[1,3]	
Denmark									PPSV23[1,3,9]	
Estonia										
Finland				PCV10[1,10]						
France										
Germany				Pnc[4,11]				PPSV23[1,12]		
Greece		PCV13[4,13]		PPSV23[4,14]			PCV13[1]			
Hungary								PPSV23[1,3]		
Iceland								PPSV23[1,3,15]		
Ireland									PPSV23[1,16]	
Italy										
Latvia										
Liechtenstein										
Lithuania										
Luxembourg								PPSV23[1,3,17]		
Malta									PCV[1]	
Netherlands										
Norway									PPSV23[1,3,18]	
Poland							PCV[1,3]			
Portugal										
Romania										
Slovakia								PCV[3,4,19]		
Slovenia									PPSV23[1,20]	
Spain									PPSV23[1,21]	
Sweden									PPSV23[1,3]	
United Kingdom									PPSV23[1]	

1: General recommendation.
2: If no previous vaccination, one dose of pneumococcal polysaccharide vaccine (PPSV23) after one year. If previous vaccination with PPSV23, one dose of PCV13 two years later. If previous dose of PCV13, one dose of PPSV23 two years later.
3: Vaccination recommended but not funded by the National Health system.
4: Recommendation for specific groups only.
5: Adults from 19 to 50 years of age with increased risk of pneumococcal infection. Vaccination with PCV13, followed by PPSV23 after at least eight weeks. PPSV23 every five years.
6: Adults from 50 to 65 years of age comorbidity. Vaccination with PCV13, followed by PPSV23 after at least eight weeks.
7: Healthy adults from 65 to 85 years old. Vaccination with PCV13, followed by PPSV23 after at least eight weeks.
8: Vaccines only given on specific indications.
9: PCV13 also recommended. For recommendations from Statens Serum Institut for vaccination of people within at-risk groups refer to http://www.ssi.dk/English/News/

EPI-NEWS/2012/No%2051b%20-%202012.aspx (the English version). There are no official recommendations from the Danish Health and Medicines Authority for use of PPV23 or PCV13, but there is, however, reimbursement for defined at-risk groups.
10: Recommended but not free of charge. For more information, please refer to http://www.thl.fi/fi_FI/web/rokottajankasikirja-fi/pneumokokkikonjugaattirokotukset
11: For people with specific chronic disease. One dose of either PCV13 or PPV23. in some cases, further doses may be necessary.
12: One dose recommended. Booster only for specific indications.
13: In previously unvaccinated children or children previously vaccinated with PCV7 or PCV10 vaccine.
14: One or two doses for high-risk groups only PCV13 + PPSV23.
15: One dose every ten years (every five years for those with conditions putting them at risk of severe disease), polysaccharide vaccine.
16: The vaccine is free of charge, but administration fees may be charged to patient (based on income and eligibility for free healthcare).
17: At-risk groups should have a booster dose every five years.
18: One dose if not vaccinated in the previous ten years. Reimbursed for some at-risk groups.
19: Recommended only.
20: PCV13 can be used. Self-paid. Further information on pneumococcal disease vaccination policy available at http://www.ivz.si/cepljenje/strokovna_javnost/navodila_in_priporocila?pi=18&_18_view=item&_18_newsid=2230&pl=253-18.0
21: Revaccination only if high risk condition (asplenia, chronic kidney disease, nefrotic syndrome and immunosuppression).

Source: ECDC, Vaccine schedule, http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx

Table 3. National adult pneumococcal vaccination recommendations in Western Europe

Country (year)[a]	Vaccine	Pneumococcal vaccine recommendation	
		Age-based	At –risk based, with definition of risk
Austria (2014)	PCV13/PPV23	≥50 years	High-risk group (≥6 years): asplenia (anatomical, functional); chronic renal insufficiency; cochlear implant; complement and properdin deficiency; haematopoietic organ disorder; HIV; hypogammaglobulinaemia; immunodeficiency (congenital, acquired); liquor fistula; nephritic syndrome; nephrotic syndrome prior to immunosuppressive therapy; neurological disorder (in children); sickle-cell anaemia; transplantation (organ, subsequent to stem cell transplantation) At-risk group (≥6 years): chronic cardiovascular disease (except hypertension); chronic respiratory disease; cirrhosis; diabetes; metabolic disease; neoplastic disease
Belgium (2013)	PCV13/PPV23	≥65 years	High-risk groups (≥18 years): asplenia; autoimmune disease/immune-mediated inflammatory disease; cochlear implant; haematological cancer; HIV; immunodeficiency; organ transplantation Risk groups (≥50 years): alcoholism; chronic disease (heart, kidney, liver, respiratory); smoking
Denmark (2012)	PCV13	≥65 years	At-risk group (any age): asplenia (functional); cochlear implant; cerebrospinal fluid (CSF) leak; HIV; history of IPD; lymphoma; organ transplantation; splenectomy (completed/planned) At-risk group (18–65 years): chronic disease (heart, kidney, liver, lung); diabetes mellitus
Finland (2013)	PCV13	No	High risk (≥5 years): asplenia (functional, anatomical); cochlear implant; HIV; immunodeficiency (congenital, acquired); liquor fistula; lymphoma; multiple myeloma; nephrotic syndrome; patients treated with systemic corticosteroids or other immunosuppressants; transplantation (organ, tissue)
	PPV23	≥65 years	At risk or in permanent institutional care (≥5 years): chronic disease (cardiac, pulmonary); diabetes (type 1); hepatic insufficiency; patients treated with systemic corticosteroids or other immunosuppressants; renal insufficiency; transplantation (organ, tissue)
France (2013)	PCV13	No	At-risk group (≥2 years): asplenia or hyposplenia; cancer treated by chemotherapy (solid tumour, haematological); cochlear implant or planned cochlear implant; HIV; immunodeficiency (congenital); immunosuppressive therapy, biotherapy, or corticotherapy for autoimmune disease or chronic inflammation; meningeal fistula; nephrotic syndrome; transplantation or waiting for transplantation (organ, haematopoietic stem cell)
	PPV23	No	At-risk group (≥5 years): asthma (severe with continuous treatment); chronic liver disease (alcoholic or non-alcoholic origin); chronic respiratory failure; COPD; cyanotic congenital heart disease; diabetes (not balanced by diet); emphysema; heart failure; kidney failure

Country (year)[a]	Vaccine	Pneumococcal vaccine recommendation	
		Age-based	At –risk based, with definition of risk
Germany (2013)	PCV13	≥60 years	At-risk group (≥2 years): asplenia; autoimmune disease; chronic disease (heart, kidney, respiratory); CSF leak; HIV; immunodeficiency (congenital or acquired); metabolic disease; neurologic disorder; transplantation (organ)
Germany (1982/1998)	PPV23	≥60 years	At-risk group (≥5 years): asplenia; autoimmune disease; cancer (haematological, solid tumour); chronic disease (heart, kidney, liver, respiratory); CNS disease; CSF leak; HIV; immunodeficiency (congenital, acquired); metabolic disease; transplantation (organ)
Greece (2011)	PCV13	>50 years	No
Ireland (2013)	PCV13/PPV23	No	High-risk group (18-64 years): qsplenia, hyposplenia (including splenectomy, sickle-cell disease, haemoglobinopathies, and coeliac disease); cochlear implant (candidates, recipients); complement deficiency (particularly C1-C4); CSF leak (congenital, complicating skull fracture, neurosurgery); immunosuppressive conditions (e.g. some B- and T-cell disorders, HIV infection, leukaemia, lymphoma) and those receiving immunosuppressive therapies; intracranial shunt; post-haematopoietic stem-cell transplant; solid organ transplant
	PPV23	≥65 years	Medium-risk group (18-64 years): chronic heart, lung, or liver disease; chronic renal disease or nephrotic syndrome; diabetes mellitus requiring insulin or oral hypoglycaemic drugs; individuals with occupational exposure to metal fumes (e.g. welders); smokers and alcoholics
Luxembourg (2008)	PPV23	>60years	At risk or in permanent institutional care (≥18 years): alcoholism; asplenia; chronic disease (cardiovascular, renal, respiratory); cochlear implant; CSF leak; diabetes; HIV; liquor fistula; liver cirrhosis; lymphoma; multiple myeloma; nephrotic syndrome; sickle-cell disease; transplantation (organ)
Norway (2013)	PCV13	No	At-risk groups (all ages): asplenia; HIV; stem-cell transplantation Also, considered for the following groups after collective evaluation of risk: B-cell deficiency; cancer (haematological); cochlear implant; CSF leak; transplantation (organ)
	PPV23	≥65 years	At-risk groups (all ages): asplenia; B-cell deficiency; cancer (haematological); cochlear implant; CSF leak; HIV; transplantation (organ, bone marrow)
Sweden (1994)	PPV23	≥65 years	At-risk group (≥2 years): agammaglobulinaemia; alcoholism; asplenia; asthma; autoimmune disease; cancer (haematological, solid tumour); chronic disease (heart, kidney, liver, respiratory); cyanotic heart disease; CNS disease; CSF leak; haemodynamically significant residual lesion after surgery; haemodynamic respiratory insufficiency; history of IPD; HIV; immunodeficiency (primary); intracranial shunt; metabolic disease; SCID; sickle-cell disease and other haemoglobinopathies; transplantation (organ)
United Kingdom (2013)	PCV13	No	At-risk group (≥5 years): severely immunocompromised: genetic disorders severely affecting the immune system (e.g. IRAK-4, NEMO, complement deficiency); leukaemia (acute, chronic); multiple myeloma; transplantation (bone marrow)
United Kingdom (1992/2003)	PPV23	≥65 years	At-risk group (≥2 years): asplenia; asthma (only if high-dose systemic steroids); cancer (haematological, solid tumour); chronic disease (heart, kidney, liver, respiratory); cochlear implant; CSF leak; diabetes (excludes diet controlled); HIV; immunosuppression; sickle-cell disease; transplantation (organ)

CSF: cerebrospinal fluid; CNS: central nervous system; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; IPD: invasive pneumococcal disease; PCV: pneumococcal conjugate vaccine; PPV: pneumococcal polysaccharide vaccine; SCID: severe combined immunodeficiency

[a] Date of implementation of recommendation

Source: Torres A et al. Eur J Clin Microbiol Infect Dis. 2015;34:19-31 (with permission)

1.8 European surveillance of invasive pneumococcal disease

The European Union issued Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998[1], which set up a network for the epidemiological surveillance and control of communicable diseases in the EU. The main aim of this network was to establish cooperation and coordination among Member States with the view to improve prevention and control of the diseases mentioned in the Decision. The network would implement the epidemiological surveillance and an early warning and response system for the prevention and control of these diseases. The list of diseases in this Decision included invasive pneumococcal disease.

The European Centre of Disease Prevention and Control (ECDC) was established in 2005, with the aim of strengthening Europe's defences against infectious diseases. In the ECDC founding regulation (851/2004 EC)[2] it was clearly stated that ECDC should coordinate the activities that have previously been carried out by the dedicated surveillance networks (DSNs).

The long-term surveillance strategy (2014-2020)[3] for the European Union has been published by the ECDC, outlining the future framework for strengthening surveillance at both EU level and in the Member States. General objectives for the surveillance of communicable diseases in the EU have been developed, together with a roadmap for the implementation of this strategy. The surveillance activities of ECDC should aim to reach these objectives by encouraging implementation of EU case definitions, integrating dedicated surveillance networks into the ECDC, and fostering harmonisation of reporting methods, systems and practices in use for surveillance.

The European Surveillance System (TESSy) intends to provide Member States with a one-stop-shop for EU surveillance data. Member States are expected to submit data related to all variables in the dataset that were agreed upon for IPD, both available and relevant, as dictated by Decision 2119/98/EC of the European Commission.

The European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) was, between 1999 and 2007, the DSN in Europe for the surveillance of invasive bacterial diseases caused by *Neisseria meningitidis* and *Haemophilus influenzae*. The network was supported by the European Commission and successfully coordinated by Public Health England (formerly the Health Protection Agency or HPA) in London. The epidemiological activities of the network focused on the collection and analysis of data on invasive meningococcal and *H. influenzae* disease cases, and the evaluation of the impact that vaccination programmes using conjugate vaccines have on the epidemiology of meningococcal disease. The laboratory activities focused on strengthening the laboratory capacity in the Member States (MS) to accurately characterise the isolates of *N. meningitidis* and *H. influenzae*. The EU-IBIS network did not carry out surveillance of invasive pneumococcal disease.

In October 2007, the coordination of the EU's IBD surveillance activities was transferred to ECDC. The surveillance network is now coordinated by the vaccine preventable disease (VPD) group at the Surveillance and Response Support Unit. The ECDC strives to ensure a high quality of IBD surveillance standardised data from all countries in the 28 Member States of the European Union and the three countries of the European Economic Area (EEA).

[1] Available at: http://eur-lex.europa.eu/resource.html?uri=cellar:b97ab1a4-21f5-49de-9964-bc25617d3485.0008.02/DOC_1&format=PDF
[2] ECDC founding regulation. Available at: http://ecdc.europa.eu/en/aboutus/Key%20Documents/0404_KD_Regulation_establishing_ECDC.pdf
[3] Available at: http://ecdc.europa.eu/en/publications/Publications/long-term-surveillance-strategy-2014-2020.pdf

In 2007-2008, the ECDC sponsored a project aiming to describe the surveillance systems for IPD in Europe (73) to map national laboratory performance, and to collect information on vaccination policies and schedules in Member States in order to find common elements for creating the EU system. The project took into account the knowledge acquired from another EU-funded project, Pneumococcal Disease in Europe (Pnc-EURO), which was established to determine the epidemiology of *Streptococcus pneumoniae* in a variety of European countries prior to the large-scale introduction of the new pneumococcal conjugate vaccine PCV7 (74).

In 2008 – after the transition of the EU-IBIS activities to the ECDC – a call for conducting External Quality Assurance (EQA) schemes and training on invasive bacterial diseases (including IPD) was launched. It focused not only on EQAs and training but also on strengthening and harmonising the laboratory capacities in MS and reinforcing the collaboration between laboratories and public health institutes in the EU. With regard to IPD, a survey on the methods used in reference laboratories in MS was conducted. The results helped to map the capacities of the laboratories and constituted the basis for the EQAs run in 2010. During 2012, another ECDC-funded project, the Vaccine European New Integrated Collaboration Effort (VENICE II)[4], collected information on vaccination policies and the impact of pneumococcal vaccination programmes.

In 2010, for the first time, the ECDC and EU Member States undertook the enhanced surveillance of invasive pneumococcal disease.

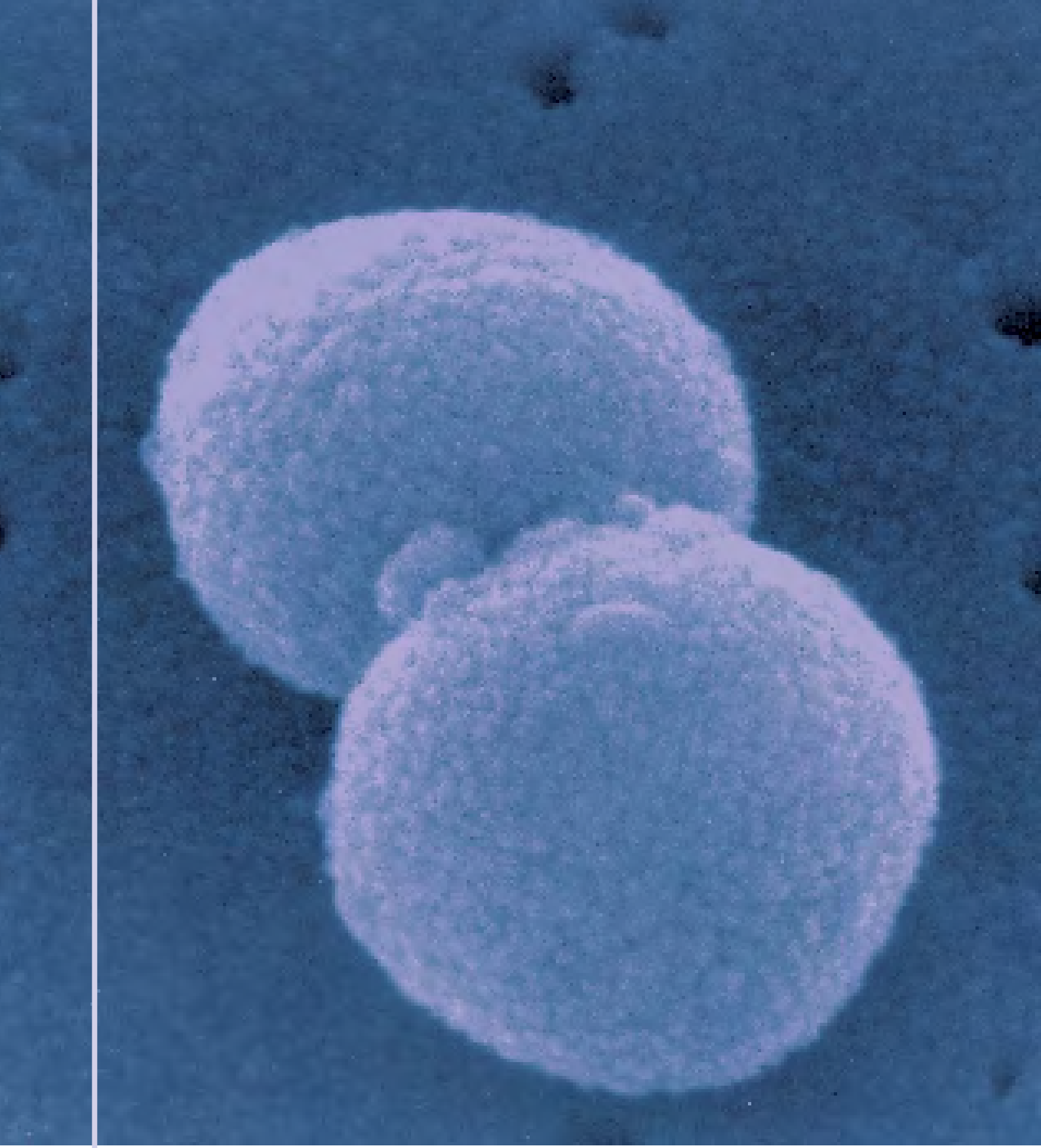
1.9 Aim of the study

Streptococcus pneumoniae is responsible for a considerable burden, in terms of both morbidity and mortality. It causes severe diseases such as invasive pneumococcal disease and pneumonia, and is also the causative agent of upper tract respiratory infections that mainly affect children. The pneumococcus is endowed with a number of virulence factors in addition to the polysaccharide capsule that determine its ability to invade, colonise, and evade host defences. Furthermore, *Streptococcus pneumoniae* has the capacity to perform capsular switching, mainly originating from a recombination of capsular genetic locci. Capsular switching is associated with the emergence and subsequent spread of resistant and multidrug-resistant clones.

The introduction of the first pneumococcal conjugate vaccine (PCV7) in Europe was expected to have an impact on the epidemiology of invasive pneumococcal disease. This made it necessary to study and assess changes in the epidemiology of the disease and its trends, particularly the potential serotype replacement, emergence of new virulent strains, and monitoring of antimicrobial resistance among spreading clones. Moreover, despite the vaccine's introduction, European countries still reported a significant number of cases. Therefore, the enhanced surveillance of invasive pneumococcal disease was warranted to ensure prevention and control of the disease.

The work carried out and reflected in this thesis was intended to offer an overview of the epidemiology of invasive pneumococcal disease in the EU/EEA countries, to assess the impact of the introduction of the pneumococcal conjugate vaccine (PCV7) and to monitor changes in the epidemiology of IPD and antimicrobial resistance in 2010, when most of the European countries had already implemented PCV7 in their immunisation programmes. Furthermore, the new conjugated vaccines (PCV10 and PCV13) had already been licensed in Europe in 2010 and were to be introduced in national vaccination schemes. Hence, this thesis has the unique feature of presenting the IPD panorama in Europe after the introduction of PCV7 and will serve as baseline for comparison studies after the implementation of PCV10/PCV13 in European countries.

[4] VENICE II. Available at http://venice.cineca.org/



2. OBJECTIVES AND WORKING HYPOTHESIS

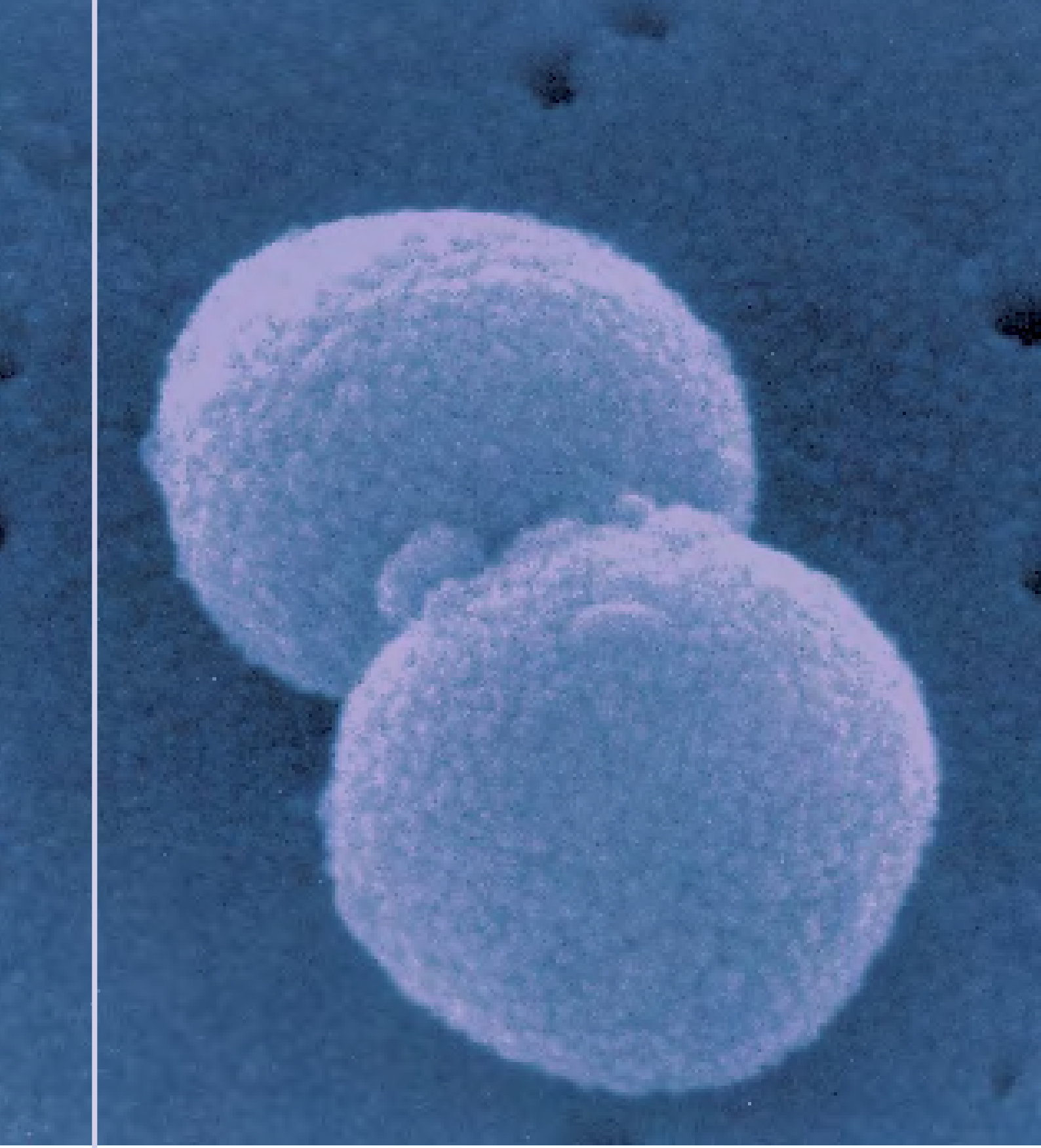
2. OBJECTIVES AND WORKING HYPOTHESIS

2.1 Objectives

1. To determine age-specific notification rates and the burden of invasive pneumococcal disease in the European Union and European Economic Area (EEA) countries.
2. To describe the epidemiology of invasive pneumococcal disease in the European Union and European Economic Area (EEA) countries.
3. To monitor circulating serotypes of *S. pneumoniae* in order to detect emerging strains and serotype replacement in the European Union and European Economic Area (EEA) countries.
4. To describe the antimicrobial resistance patterns of invasive pneumococcal disease in the European Union and European Economic Area (EEA) countries.
5. To analyse risk factors for mortality in invasive pneumococcal disease in the European Union and European Economic Area (EEA) countries.

2.2 Hypothesis

Despite the introduction of the PCV7 in most of the EU/EEA countries, there is still a significant number of notifications of invasive pneumococcal disease. There is a notable variation in notification rates and epidemiological characteristics of invasive pneumococcal disease cases across Europe. The relevant number of invasive pneumococcal disease notifications might be likely due to serotype replacement. There are significant rates of antimicrobial resistance among cases of invasive pneumococcal disease across Europe. Moreover, some of the causing serotypes might be multidrug-resistant (i.e. 19A) and those are more prone to causing more severe clinical entities. In Europe in 2010, the most severe clinical presentations and certain serotypes in IPD are most likely associated with death. Antimicrobial resistance might also play a significant role as risk factor for death in IPD.



3. MATERIAL AND METHODS

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3.1 Study design

The study was conceived as an observational study based on the European population and on data from the national surveillance for IPD in EU/EEA countries in 2010. Approximately, the population coverage of the reported data represented 82% of the total population in the EU/EEA countries in 2010. To respond to the proposed objectives and additional epidemiological questions the enhanced surveillance of invasive pneumococcal disease was undertaken.

3.2 Reporting of invasive pneumococcal disease data in the European Surveillance System (TESSy)

Countries that participated in this surveillance were the Member States of the European Union (EU-27), and Norway and Iceland as part of the European Economic Area (EEA).

National data was uploaded directly by the reporting country into the TESSy database. TESSy is the technical platform for EU/EEA communicable disease surveillance. This database allows web-based data submission, it is password-protected and fully anonymised, curated and maintained by ECDC. A set of validation rules was designed, together with the variables of the dataset. The validation rules facilitate verification of data by an automated procedure. This verification of data during the uploading process enables countries to check their files before submission, thus improving the data's quality.

Together with the data collection, countries were asked to provide a description of their national surveillance systems. The system allows the reporting of aggregate data, although case-based reporting is favoured by the ECDC.

The IPD dataset consisted of a core group of variables, common to all diseases, combined with an enhanced dataset specific for IPD.

3.3 Implementation of EU case definitions

The EU's official 2008 case definition for IPD applied for this surveillance and only confirmed cases of invasive pneumococcal disease should be reported.

Case definition applied and data source

Case definition for invasive pneumococcal disease

Clinical criteria

Not relevant for surveillance purposes

Laboratory criteria

At least one of the following three:

- Isolation of *Streptococcus pneumoniae* from a normally sterile site
- Detection of *Streptococcus pneumoniae* nucleic acid from a normally sterile site
- Detection of *Streptococcus pneumoniae* antigen from a normally sterile site

Epidemiological criteria

N/A

Case classification

- Possible case: N/A
- Probable case: NA
- Confirmed case: Any person meeting the laboratory criteria for case confirmation.

All Member States apart from Bulgaria (aggregate data) reported case-based data. The EU's 2008 case definition was applied by 18 Member States, one country applied the EU's 2002 case definition, while two used the 'Other' (unspecified) case definition. The case definition was unknown for five countries.

With regard to population coverage, at national level France applies a correction factor of 1.61904 to estimate the total number of cases in its national reports (the correction factor has not been applied for this analysis). Greece has a surveillance system with national coverage for meningitis only. The population coverage is not national for Spain and therefore the notification rate needs to be interpreted cautiously. The true notification rate for Spain is probably higher than reported here due to the incompleteness of the data submitted. There is no unique surveillance system in the United Kingdom. The Netherlands did not report adult cases of IPD (all reported cases were under five years of age).

All countries but three reported data from a unique data source (Cyprus, Czech Republic and France submitted data from two different data sources).

According to the data source profiles uploaded by countries, 18 countries had a reconciled notification/laboratory surveillance system (meaning that laboratory data and epidemiological and/or vaccination information are collected and filed together on a case-by-case basis at national level), only six countries had laboratory-based surveillance systems, and only two countries presented data from the notification system.

3.4 Data collection

In 2011, data using the EU's enhanced invasive pneumococcal disease dataset was reported to TESSy for the first time. The collection of 2010 data took place between 4 July and 10 September 2011.

Due to the diversity among national surveillance systems, it was considered important that countries updated the available information on the case definition used, the data sources available in the country, and the characteristics of the surveillance systems (e.g. universal versus sentinel, active versus passive, etc.).

3.5 Study variables

The study variables were grouped into a set of 35 variables (Table 4). This set of variables was implemented into the reporting system and all countries agreed to report on these variables. It included variables related to the codification into the system (technical fields), epidemiological variables and laboratory variables (detailed definition of the variables is included in Annex 3).

Table 4. Overview of set of variables for IPD surveillance

Technical fields	Epidemiological variables	Laboratory variables
1. RecordID	10. DateOfNotification	22. DateOfSpecimen
2. RecordType	11. PlaceOfNotification	23. Specimen
3. RecordTypeVersion	12. PlaceOfResidence	24. Serotype
4. Subject	13. Age	25. TestMethodTyping
5. Status	14. AgeMonth	26. ResultMICValuePEN
6. DataSource	15. Gender	27. ResultMICValueERY
7. DateUsedForStatistics	16. DateOfDiagnosis	28. ResultMICValueCTX
8. ReportingCountry	17. Outcome	29. ResultMICSign_PEN
9. NRLData	18. Classification	30. ResultMICSign_ERY
	19. ClinicalPresentation	31. ResultMICSign_CTX
	20. VaccStatus	32. TestMethodMIC
	21. VaccType	33. SIR_PEN
		34. SIR_ERY
		35. SIR_CTX

3.6 Data quality and completeness of variables

Data was uploaded, validated and approved in TESSy by the EU/EEA countries. Individual datasets were then manually checked, validated and cleaned for inconsistencies and impossible values, and potential double reporting by different data sources within the same country. Data collection comprised cases of IPD notified from 1st January 2010 until 31st December 2010.

In 2010, 21,565 confirmed cases of invasive pneumococcal disease (IPD) were reported by 26 countries, namely Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom. Germany, Liechtenstein, Luxembourg and Portugal did not report data on IPD in 2010.

Data on serotypes were reported by 22 countries: Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Greece, Hungary, Iceland, Ireland, Italy, Lithuania, Malta, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, and United Kingdom.

Data on antimicrobial susceptibility was submitted by 21 countries: Austria, Belgium, Cyprus, Denmark, Estonia, Finland, France, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Norway, Poland, Romania, Slovakia, Slovenia, Spain, and United Kingdom.

All cases considered for inclusion in the analysis were laboratory-confirmed cases.

All countries reported case-based data except Bulgaria, which submitted aggregate data.

Data on age, age month, gender and classification were almost complete. Information on the variable specimen was also nearly complete (1.3% missing) (Table 5)

Table 5. Quality of 2010 data. Distribution of known, unknown, and blank responses per variable for all reported cases of IPD by country, EU/EEA countries* (n=22 667)

Variable**	Known		Unknown		Blank		Overall missing
	N	%	N	%	N	%	%
Age	22601	99.7	0	0	66.0	0.3	0.3
AgeMonth	1471	100	0	0	21196	93.5	93.5
Classification	22666	100	1	0	0	0	0
Clinical Presentation	8449	37.3	14169	62.5	49	0.2	62.7
Gender	22598	99.7	69	0.3	0	0	0.3
Outcome	4638	20.5	17101	75.4	928	4.1	79.5
VaccStatus	1979	8.7	20639	91.1	49	0.2	91.3
VaccType	1919	8.5	7521	33.2	389	1.7	91.5
Serotype	10585	46.7	4839	21.3	7243	32.0	53.3
Specimen	22370	98.7	268	1.2	29	0.1	1.3
ResultMICSign_CTX	5240	23.1	0	0	17427	76.9	76.9
ResultMICSign_ERY	3953	17.4	0	0	18714	82.6	82.6
ResultMICSign_PEN	5244	23.1	0	0	17423	76.9	76.9
ResultMICValueCTX	5252	23.2	0	0	17415	76.8	76.8
ResultMICValueERY	4031	17.8	0	0	18636	82.2	82.2
ResultMICValuePEN	5384	23.8	0	0	17283	76.2	76.2
SIR_PEN	9247	40.8	879	3.9	12541	55.3	59.2
SIR_CTX	6186	27.3	998	4.4	15483	68.3	72.7
SIR_ERY	8382	37.0	929	4.1	13350	58.9	63.0
TestMethodMIC	7730	34.1	107	0.5	14830	65.4	65.9
TestMethodTyping1	9880	43.4	84	0.4	7367	32.3	56.6

*Data in this table represents values before data cleaning and checking for inconsistencies
** Variables defined in the dataset used for the 2010 IPD data collection

Data on vaccination status represented less than 10% of the total reported cases.

Completeness on serotype (53.3% missing) and the test method for serotyping (56.6% missing) were very similar, indicating that the serotyping method is known for almost all cases of serotype reported.

Minimum inhibitory concentration (MIC) data were reported in approximately 20-25% of the total reported cases. The method for determining MIC was reported in approximately 53% of the reported results for MIC (the three antibiotics pooled).

Antimicrobial resistance data expressed as sensitive (S), intermediate (I) and resistant (R) was more complete than when expressed as MIC, especially for penicillin (40.8%) and erythromycin (37.0%).

3.7 Data analysis

IPD surveillance data was uploaded, validated, and approved in TESSy by the Member States' contact points. A verification report produced by TESSy provides an overview of the completeness of data by country. Once the data was submitted, the EU's individual datasets were validated.

The ECDC asked the national experts about potential duplication of data or surveillance restricted to certain age groups. Potential overlapping of the two data sources available at national level was reported by Czech Republic and France, although the extent was difficult to determine. Therefore the following criteria were applied:

- For Czech Republic, only data submitted from the data source 'Laboratory surveillance of invasive pneumococcal infections' (CZ-NRL-STR, combined notification-laboratory data) was considered for the analysis in this report.
- For France, the total number of cases was calculated considering only the data reported by the data source 'Community invasive infections hospitalised' (FR-EPIBAC[5], notification data). Data uploaded from the FR-PNEUMO-NRL (combined notification-laboratory data) data source was taken into account for the analysis of the enhanced variables (clinical presentation, specimen, serotype, and antimicrobial susceptibility data). France IPD surveillance relies on a sentinel network of hospital laboratories, covering at least 75% of acute care activity and the French metropolitan population (the coverage proportion was 75.3% in 2010). Incidence rates are estimated using the population covered by the participating hospitals as a denominator.
- In the Netherlands, IPD is only notifiable for children up to five years of age, and only cases within this age group were reported. The denominators were therefore considered accordingly.

This study includes the total number of reported confirmed cases of IPD, and a description of epidemiological and laboratory variables with appropriate completeness.

3.8 Mortality

The case fatality rate (CFR) was calculated as the proportion of cases with fatal outcomes among those with known outcomes. Cases with the variable 'outcome' reported as 'unknown' or with a missing value were not taken into account in the denominator. There is no common definition of the time at which a fatal outcome is determined; this may add variation to the outcome figures throughout Europe. Acknowledging the differences in IPD surveillance systems and reporting across Europe, CFR was calculated on a country basis. Serotype-specific case fatality rate was calculated following the same rule. Consequently, only cases with known outcomes were considered.

[5] Surveillance des infections invasives à *Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae* (B) et *Streptococcus pyogenes* (A) en France métropolitaine.

3.9 Statistical analysis

General analysis

The notification rate was defined as the number of laboratory confirmed cases of IPD per 100,000 inhabitants. Population data for denominators were retrieved from the European Statistics (EUROSTAT) website[6].

Categorical variables are presented as number of cases and percentages.

Risk factors for death from Invasive pneumococcal disease

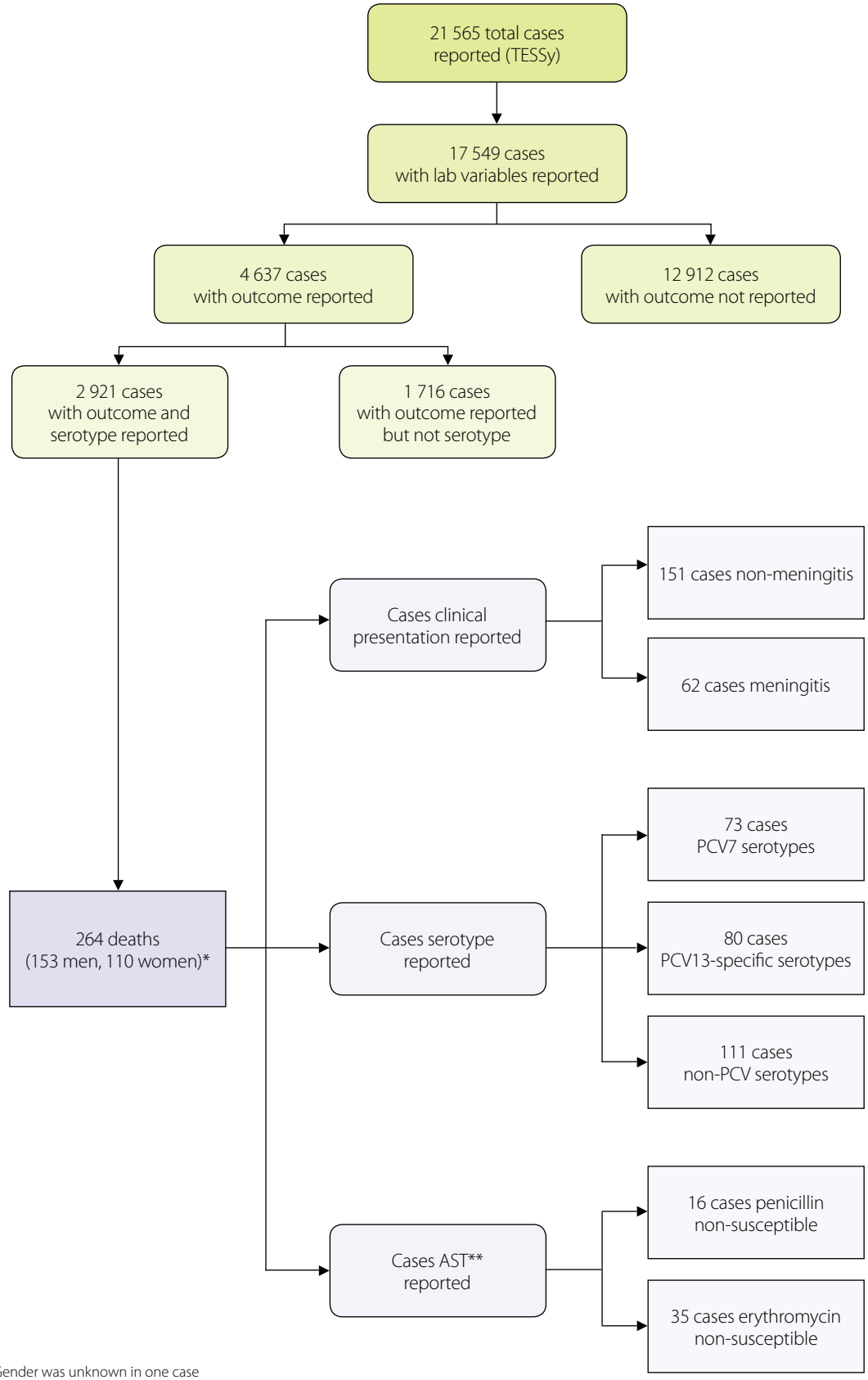
In the study of risk factors for death, the study sample was the sub-sample of cases that had information on both serotype and outcome (Figure 1) and it represents data from 17 European countries (Table 6).

Table 6. Distribution of cases with known serotype and outcome, and CRF by reporting country

Reporting country	Number of cases	Percentage (%)	Number of deaths	CFR (%)
Austria	190	6.5	15	7.9
Belgium	1255	43.0	67	5.3
Cyprus	3	0.1	0	0.0
Czech Republic	242	8.3	43	17.8
Denmark	35	1.2	0	0.0
Greece	20	0.7	1	5.0
Hungary	26	0.9	7	26.9
Ireland	78	2.7	4	5.1
Italy	209	7.2	31	14.8
Lithuania	3	0.1	0	0.0
Malta	7	0.2	0	0.0
Netherlands	45	1.5	4	8.9
Norway	357	12.2	41	11.5
Poland	205	7.0	43	21.0
Romania	21	0.7	2	9.5
Slovenia	224	7.7	6	2.7
Slovakia	1	0.0	0	0.0
Overall	**2921**		**264**	**9.04**

[6] EUROSTAT: http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/

Figure 1. Flow of invasive pneumococcal disease cases through the study



*Gender was unknown in one case
**Antimicrobial susceptibility testing

Countries reported the outcome according to their national surveillance and guidelines. The following age groups were defined for the study: < 5 years, 5-64 years and ≥ 65 years. For the purpose of this analysis, clinical presentation was recoded as 'meningitis' and 'non-meningitis'. The grouping of clinical presentation was done based on a literature review (75) which suggested that presenting meningitis and non-meningitis had different degrees of severity and mortality.

Serotypes were grouped in three categories: PCV7 serotypes (included in PCV7: 4, 6B, 9V, 14, 18C, 19F, 23F), PCV13-specific serotypes (included in PCV13 but not in PCV7: 1, 3, 5, 6A, 7F, 19A) and non-PCV serotypes (not included in any pneumococcal conjugate vaccine).

Antimicrobial susceptibility testing (AST) to penicillin and erythromycin was reported as 'susceptible', 'intermediate' or 'resistant' by the countries according to their national standards and protocols. Therefore, there was not available information on the breakpoints and guidelines used for AST in each country. As an indication, in the European Antimicrobial Resistance Surveillance Network (EARS-Net) report for 2010 (76) 66% of reporting laboratories in Europe used Clinical and Laboratory Standards Institute (CLSI) standards whereas 29% applied the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

For the study, the variable was redefined to include just two categories: 'susceptible' (cases reported as susceptible by the countries) and 'non-susceptible' (intermediate + resistant), both for penicillin and erythromycin. A univariable analysis was performed to identify the factors associated with a fatal outcome. To test the association between age, serotype, clinical presentation, and death, a generalised linear model with robust standards accounting for the country effect was fitted to address heterogeneity as data came from different national surveillance systems, and vaccination policies and practices differ widely across Europe. The role of the variables as potential confounders/modifiers was also addressed in this study. Age was an effect modifier of the association between serotype and risk of death, and thus the analysis was stratified by age group.

The Pearson $\chi 2$ test was used to compare the proportion of deaths by PCV7, PCV13-specific serotypes and non-PCV serotypes, the proportion of deaths in the defined age groups, and between genders, the proportion of deaths by clinical presentation as well as the proportion of deaths in the susceptible and non-susceptible cases, according to antibiotic type.

The Fisher exact test was used to analyse the association between penicillin susceptible/penicillin non-susceptible cases and outcome for the subgroup below five years and non-PCV serotypes, and to assess differences between penicillin susceptible/penicillin non-susceptible cases and outcome for serotype 35B.

Additionally, the associations between each serotype and death were assessed using a generalised linear model with log link function. This analysis was performed for all serotypes that accounted for up to 80% of the cases with fatal outcome (28 serotypes, n>1) and each individual serotype was compared to all the others.

A regression analysis was conducted, including those variables that were significant by the univariable analysis. The factors included in the regression model were those that were found to be significant by univariable analysis and those that had previously been hypothesised to affect the disease fatality. All p values were two-tailed and statistical significance was defined as $p<0.05$.

STATA® 12.0 (StataCorp, College Station, TX, USA) software was used to perform statistical tests and analysis.

3.10 Laboratory methods

Confirmation of an IPD case implies the isolation and/or detection of nucleic acid and/or detection of *Streptococcus pneumoniae* antigens at a normally sterile site.

3.10.1 Serotyping methods

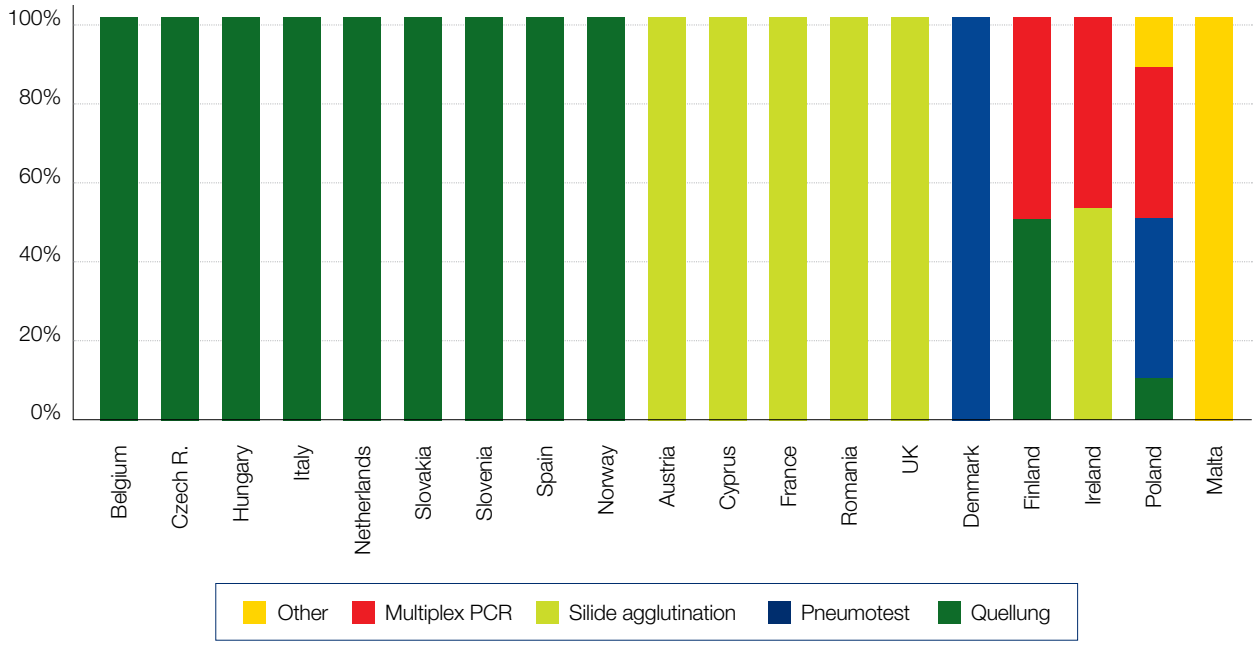
According to the data, Quellung is the preferred technique for serotyping in Europe and was used in 62% of all cases for which serotype was reported, followed by slide agglutination and Pneumotest®.

Of the 9,946 cases for which information on serotype was available, the test method was reported in 9,880 (99.3%) cases (Table 5).

Some cases were reported to the serogroup level (i.e. serogroup 19, serogroup 7). This may indicate that the countries reporting to this level did not have the information available to characterise to the serotype level.

Figure 2 presents the distribution of serotyping methods by country. Finland, Ireland, and Poland used two or more methods for serotyping pneumococcal strains.

Figure 2. Percentage of reported serotyping test methods in reported IPD cases by country, EU/EEA countries, 2010 (n=9,880)



3.10.2 Antimicrobial susceptibility testing methods

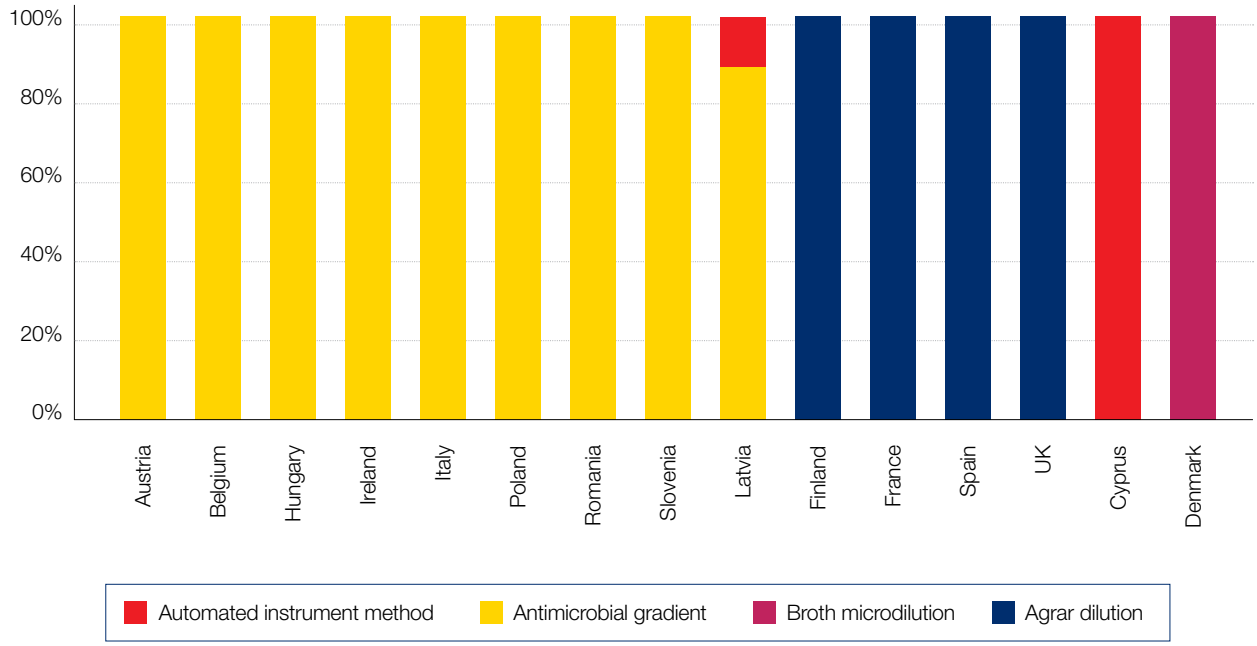
Antimicrobial susceptibility testing was reported by the countries as MIC and some countries also reported by category (susceptible S, intermediate I or resistant R) according to national standards and protocols.

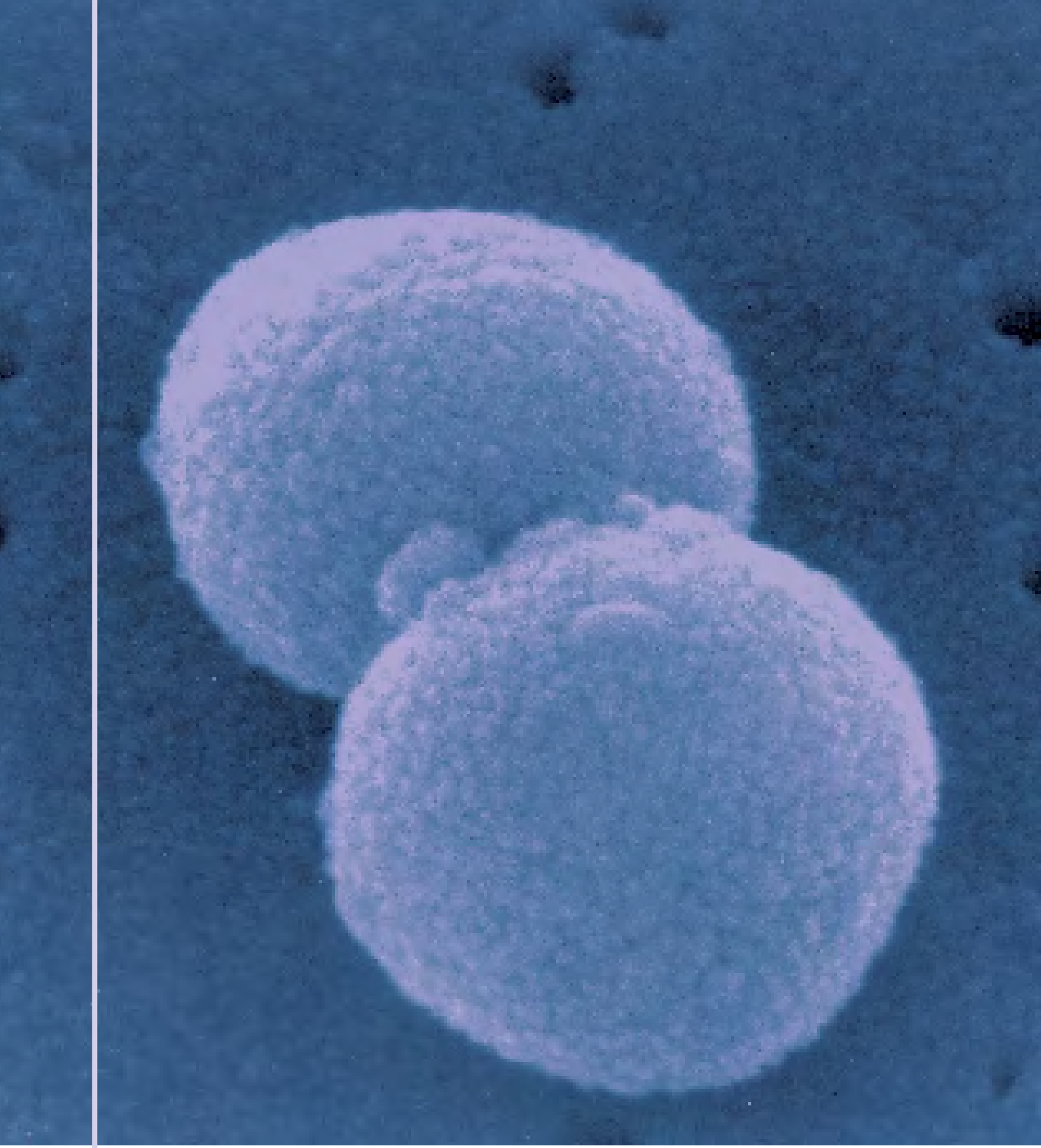
EU Member States reported antimicrobial susceptibility testing results expressed as minimum inhibitory concentration (MIC). Countries that reported data on antimicrobial susceptibility as MIC were: Austria, Belgium, Cyprus, Denmark, Spain, Finland, France (for penicillin and cefotaxime), Hungary, Ireland, Italy, Lithuania (only for penicillin), Latvia, Poland (for penicillin and cefotaxime), Romania and Slovenia. Belgium, France, Slovenia and Spain reported the MIC test method for all the cases where MIC was reported (Figure 3). Data was reported for penicillin (n=5,384), erythromycin (n=4,031), and cefotaxime (n=5,252).

The test method for MIC is reported in 53% of the cases that include information on MIC (pooling the three antibiotics together). Antimicrobial gradient is the preferred method for determining MIC among the countries reporting this method. This method represented 60% of all cases for which MIC was reported. The method is preferred in nine out of 15 countries reporting MIC data. Most of the countries applied a single method for determining MIC.

Information on national standards and methods for antimicrobial susceptibility testing was lacking. As a reference we adopted the European Committee on Antimicrobial Susceptibility Testing's (EUCAST) breakpoints for the analysis.

Figure 3. Percentage of reported MIC test methods among reported IPD cases by country, EU/EEA countries, 2010 (n=7,730)





4. RESULTS

4. RESULTS

4.1 Epidemiology

In 2010, ECDC conducted the first European enhanced surveillance for invasive pneumococcal disease. Twenty-six EU/EEA countries notified 21,565 cases of IPD. Previously to the data collection, I coordinated a project that consisted in the implementation of a programme for harmonisation and standardisation of laboratory methods for characterisation of *S. pneumoniae* and antimicrobial susceptibility testing, the establishment and coordination of the European surveillance network, and the in-house preparatory work, i.e. the creation of the metadataset adapted to TESSy. This preparatory work paved the way for the subsequent coordination of the data collection, data cleaning and data analysis.

4.1.1 Notification rates

In 2010, 21,565 confirmed cases of invasive pneumococcal disease (IPD) were reported to TESSy by the EU/EEA countries. Notification rates ranged from 17.4 per 100,000 (Denmark) to 0.28 (Lithuania). The Nordic countries (Denmark, Finland, Norway and Sweden) presented the highest notification rates, together with Belgium. This statement needs to be interpreted cautiously due to the diversity of surveillance systems and variations in the completeness/representativeness of their data across Europe (Table 7, Figure 4).

Figure 4. IPD cases and notification rates (cases per 100,000) in EU/EEA countries, 2010

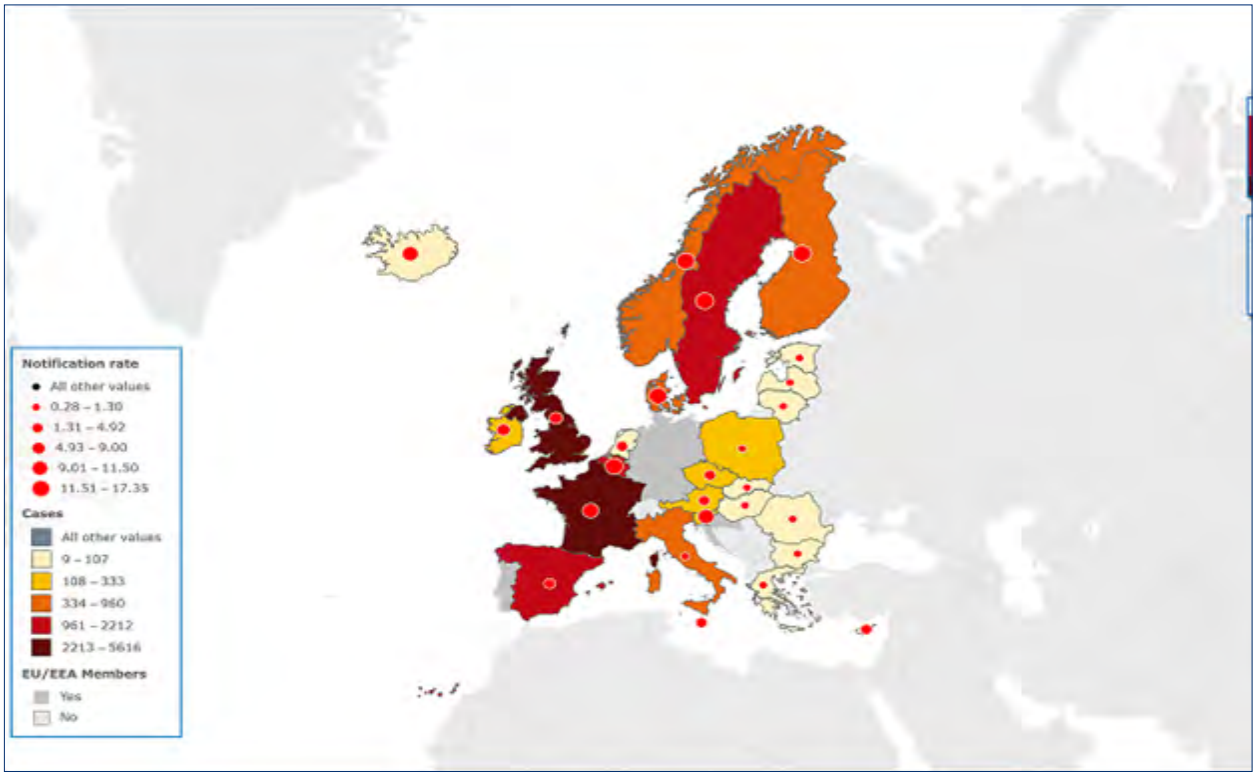


Table 7. Number of reported cases and notification rates of IPD cases in EU/EEA countries, 2010 (n=21,565)

Country	Number of reported cases (N)	Notification rate (cases per 100,000)
Austria	325	3.88
Belgium	1,851	17.08
Bulgaria[a]	26	0.34
Cyprus	23	2.86
Czech Republic	300	2.86
Denmark	960	17.35
Estonia	14	1.05
Finland	836	15.62
France[b]	5,117	10.80
Greece[c]	38	0.34
Hungary	107	1.06
Ireland	304	8.19
Italy	854	1.30
Latvia	16	0.67
Lithuania	9	0.28
Malta	11	2.68
Netherlands[d]	55	4.92
Poland	333	0.89
Romania	80	0.38
Slovakia	18	0.34
Slovenia	224	10.73
Spain[e]	2,212	4.74
Sweden	1,456	14.82
United Kingdom[f]	5,616	9.00
EU total	20,785	5.09
Iceland	32	11.50
Norway	748	16.18
Total	**21,565**	**5.22**

[a] Aggregated reporting
[b] France: no national coverage for IPD (see Methods)
[c] National coverage only for meningitis
[d] Netherlands reports data on IPD only for children up to five years. Notification rate was calculated accordingly.
[e] No national coverage of this surveillance for Spain. Notification rate needs to be interpreted with caution. Notification rate for Spain is probably higher due to the incompleteness of the data submitted.
[f] There is no unique surveillance system in the UK. Data are representative (as submitted by England and Wales, Scotland and Northern Ireland), however surveillance systems might not be identical.

4.1.2 Age and gender

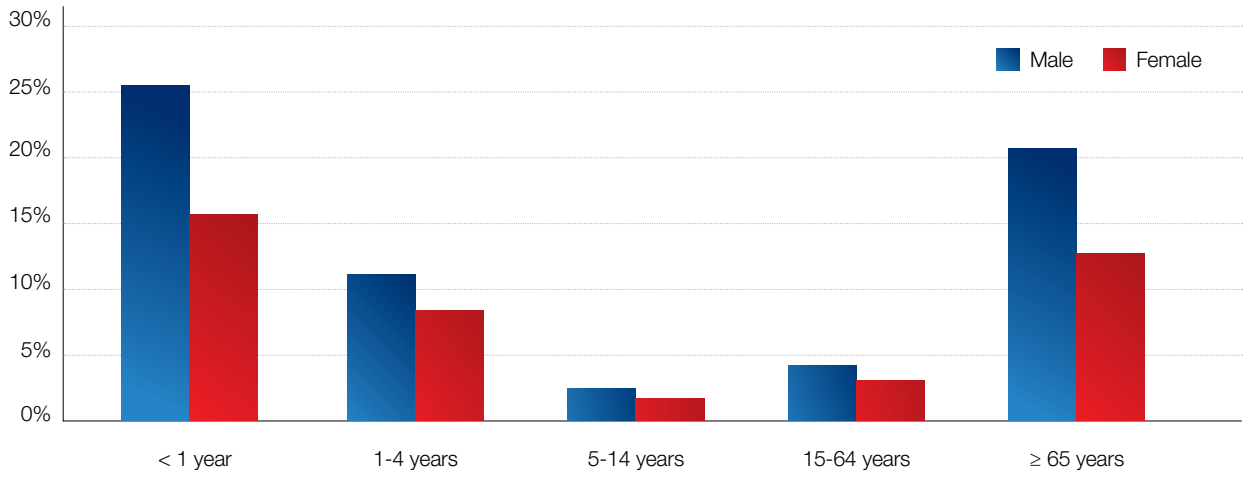
Of the 21,473 reported cases for which age information was provided, 45% (n=9,727) concerned people aged 65 years or older, 42% (n=9,036) concerned adults aged 15 to 64 years and 13% (n=2,710) concerned children 0 to 14 years of age. In the latter group, children aged one to four years accounted for the highest proportion of cases (7%, n=1,444).

The highest notification rates were reported among children below one year (18.5 per 100,000) followed by adults aged 65 years or above (15.6 per 100,000) (Figure 5).

For the Netherlands 50.9% of the cases reported concerned infants under one year of age and 47.3% concerned children aged one to four years. Adult cases were not reported since IPD is only notifiable at national level for children up to the age of five years. Slovenia (20.5%), Slovakia (22.3%), Greece (21.1%), Romania

(25.1%) and Poland (24.1%) reported a significant number of cases in the under-five age group. Cyprus (20%) was the country that reported the highest number of cases in the age group five to 14 years. Estonia and Malta did not report cases among children.

Figure 5. Notification rate (cases per 100,000 population) of reported IPD cases by age group and gender, EU/EEA countries, 2010 (n=21,496)



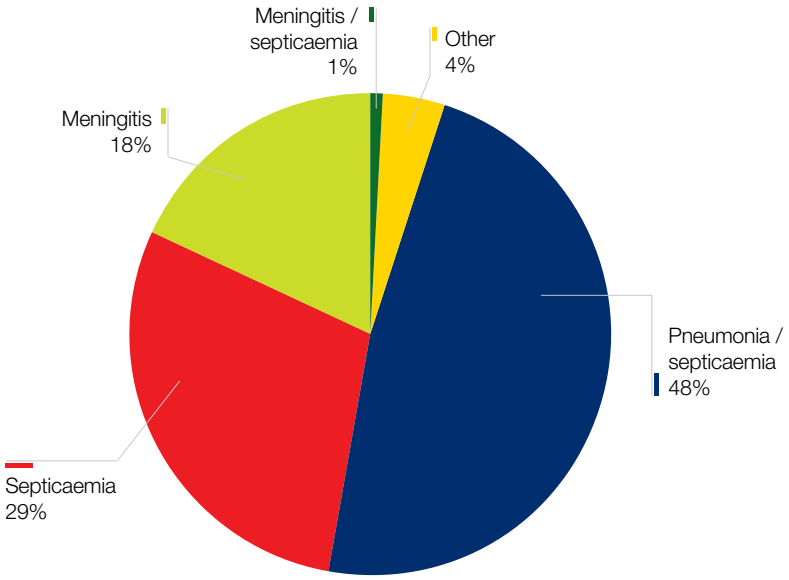
Of the 21,496 reported cases where gender information was specified, 55% (n=11,798) were male and 45% (n=9,698) were female, corresponding to a 1.22:1 male/female ratio.

As regards the distribution of notification rates among genders (Figure 5), male predominance was more evident in children under one year and adults over 65 years. Males showed slightly higher rates than females for all other age groups, although this difference was not statistically significant.

4.1.3 Clinical presentation

The clinical presentation was available in 37.1% (n=7,948) of cases. Non-meningitis was the most frequent clinical presentation for all age groups (Figure 6), particularly pneumonia/septicaemia.

Figure 6. Distribution by clinical presentation of reported IPD cases, 2010 (n=7,948)



4.1.4 Case fatality rate

Twenty countries reported data on outcome but the completeness for this variable differed widely from country to country. Cyprus, Denmark, Lithuania and Malta reported no deaths. The case fatality rate ranged from 0% for Cyprus, Denmark, Lithuania and Malta to 26.9% for Hungary (Table 8). Data on CFR should be interpreted with caution because data for the variable 'outcome' was significantly incomplete (overall missing 79.5%) and there was uncertainty regarding the denominator. The total number of reported deaths was 450.

Overall the CFR was 9.8% and ranged from 0.0% for Cyprus, Lithuania and Malta to 26.9% for Hungary. Nevertheless this data should be interpreted cautiously as the completeness for the variable outcome varied widely from country to country. In children under five years of age, meningitis was the clinical presentation that accounted for the greatest number of deaths while non-meningitis was the major cause of death in the age group ≥ 65 years. The case fatality under 5 years was low (overall 2.4%). Among the age group 5-64 years the overall CFR was 9.1%. Cases aged 65 years and over presented the highest CFR (18.6%).

Table 8. Case fatality rate due to IPD in EU/EEA countries*, 2010 (n=4,596)

Country	No. of cases	No. of cases with known outcome	No. of deaths	CFR (%)	Confidence Interval 95% (%)
Austria	325	218	16	7.3	4.3 - 11.7
Belgium	1851	1255	67	5.3	4.2 - 6.7
Cyprus	23	11	0	0.0	0.0 - 28.5
Czech Republic	300	247	44	17.8	13.3 - 23.2
Denmark	960	35	0	0.0	0.0 - 10.0
Estonia	14	14	1	7.1	0.2 - 33.9
Greece	38	32	4	12.5	3.5 - 29.0
Hungary	107	26	7	26.9	11.6 - 47.8
Ireland	304	93	5	5.4	1.8 - 12.1
Italy	854	605	101	16.7	13.8 - 19.9
Latvia	16	15	1	6.7	0.2 - 32.0
Lithuania	9	8	0	0.0	0.0 - 36.9
Malta	11	11	0	0.0	0.0 - 28.5
Netherlands	55	54	5	9.3	3.1 - 20.3
Norway	748	373	44	11.8	8.7 - 15.5
Poland	333	333	65	19.5	15.4 - 24.2
Romania	80	80	12	15.0	8.0 - 24.7
Slovenia	224	224	6	2.7	1.0 - 5.7
Slovakia	18	16	1	6.3	0.2 - 30.2
United Kingdom	5616	946	71	7.5	5.9 - 9.4

*Outcome not reported by Finland, France, Iceland, Spain or Sweden.

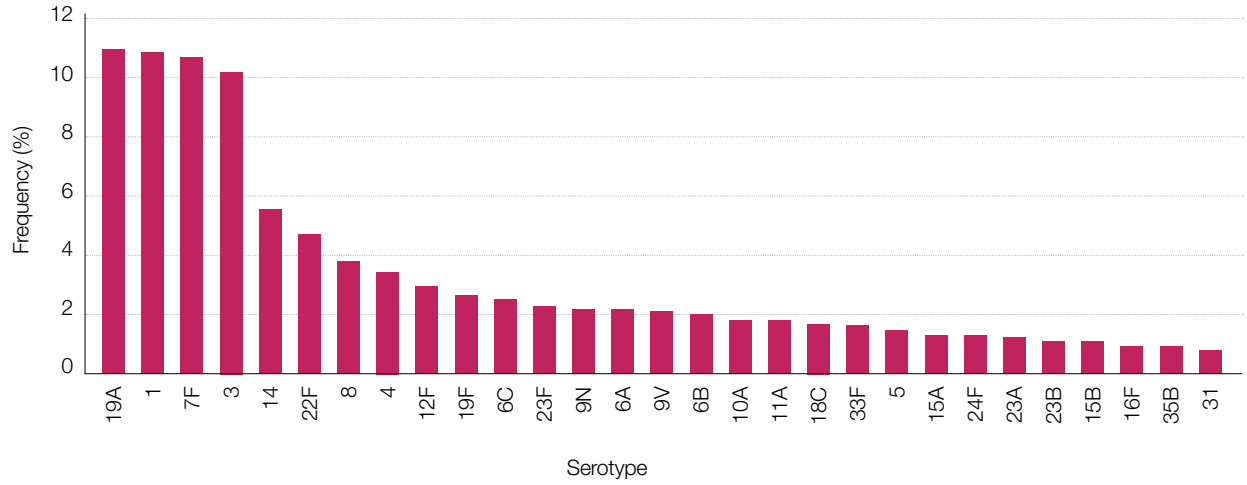
4.1.5 Vaccination status

Vaccination status was known in only 8.7% of the reported cases. Of the 1,979 cases for which vaccination status was reported, only 345 (17.4%) were fully vaccinated, 4.2% partially vaccinated and 78.3% unvaccinated, according to the respective national schedule (Table 5).

4.1.6 Serotype distribution and serotype-specific case fatality rate

Of the 21,565 reported confirmed cases of invasive pneumococcal disease, only 9,946 (46.1%) had included information on the isolate serotype. Of these, the ten most common serotypes were 19A, 1, 7F, 3, 14, 22F, 8, 4, 12F and 19F, accounting for 59.8% (n=5,949/9,946) of the typed isolates reported (Figure 7). Detailed information on serotype distribution by country is provided in Annex 4.

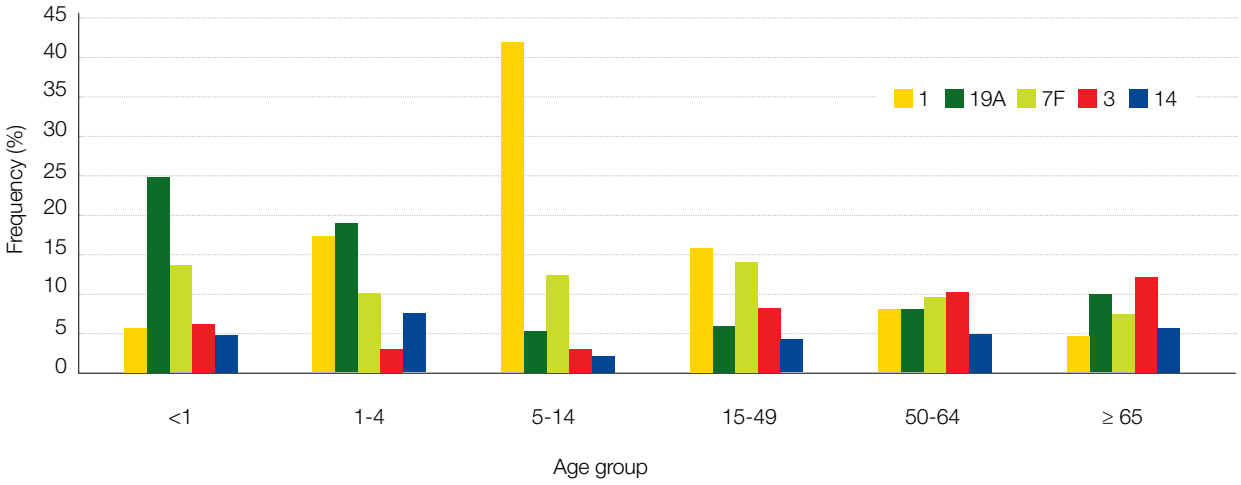
Figure 7. Distribution of reported IPD cases by serotype*, EU/EEA countries, 2010 (n=9,946)



*Distribution of 29 most common serotypes

Serotypes 19A and 7F were the most commonly reported in children <1 year of age, whereas serotypes 1 and 19A were the most frequently reported in the group aged 1-4 years. Among those 15-64 years, serotypes 1, 7F and 3 were predominant while serotypes 19A, 3, 7F and 8 were most common among those aged ≥65 years (Figure 8).

Figure 8. Distribution of the most common serotypes by age

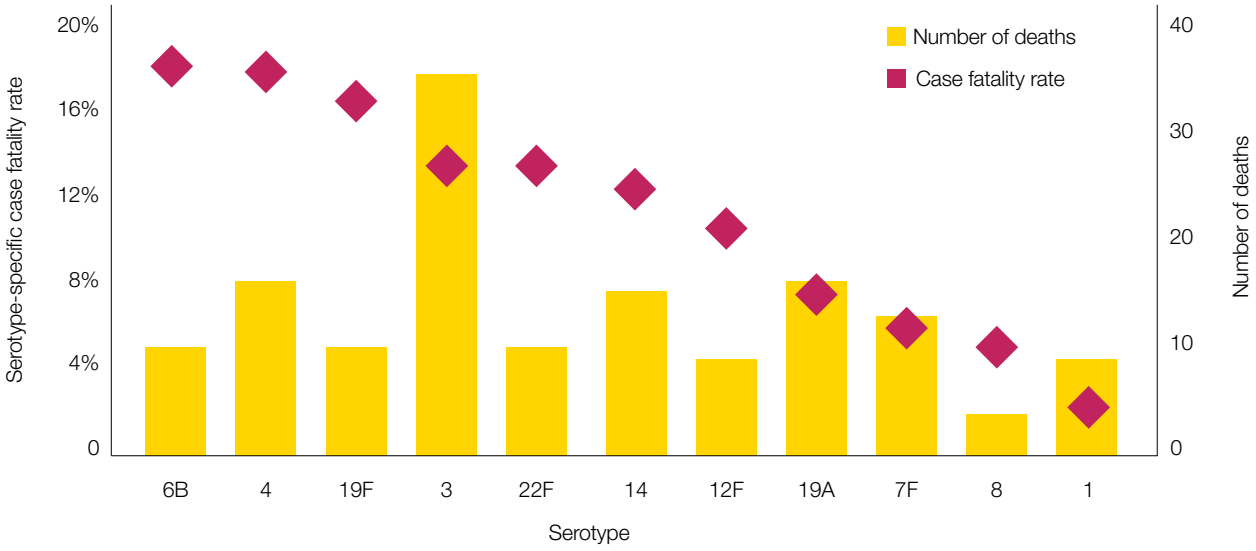


Among the non-PCV serotypes, serotype 22F (426/9,946) accounted for 4.3%, serotypes 8 (343/9,946) for 3.5%, 12F (266/9,946) for 2.3% and 6C (226/9,946) accounted for 2.3% of all serotyped isolates.

Serotype 1 was the most frequent serotype reported among cases presenting with non-meningitis (413/3,588, 11.5%), followed by serotypes 19A, 7F and 3. Similarly serotype 19A was the most frequent serotype reported among cases presenting with meningitis (112/1,075, 10.4%), followed by serotypes 3 and 7F.

In children below five years the serotype with the highest CFR was 10A (16.6%) although serotype 19A caused the highest number of deaths (n=3, serotype-specific CFR 2.8%) in this age group. In age groups 5-64 years and 65 years and over, serotype 3 accounted for the highest number of deaths (n=35, serotype-specific CFR 11.2% and 14.1% respectively) but in both age groups serotype 4 accounted for the highest CFR (21.4% and 14.3% respectively) (Figure 9).

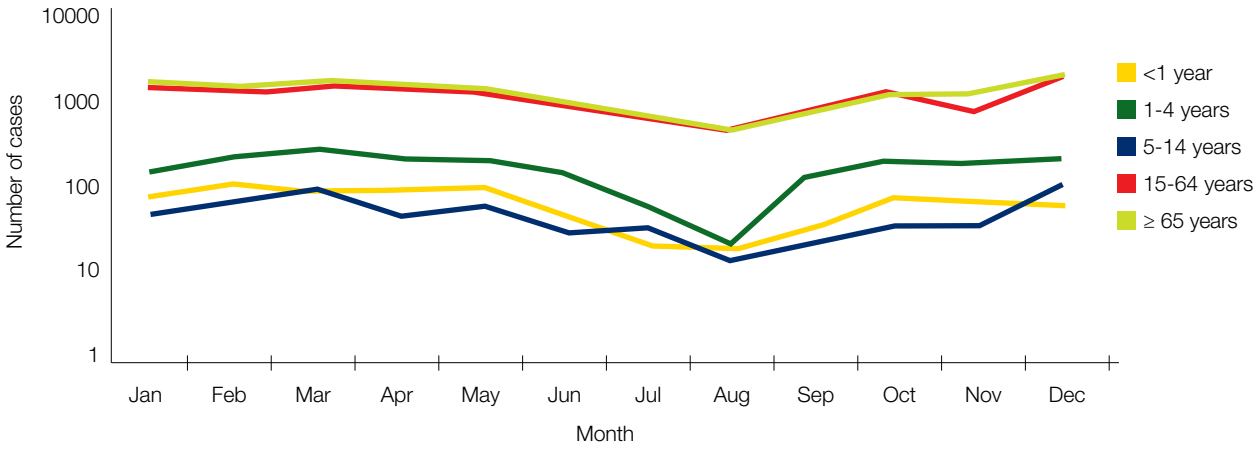
Figure 9. Distribution of reported IPD deaths and case-fatality ratio by serotype, EU/EEA countries, 2010 (n=147*)



*Serotypes distribution refers to the 11 most frequent serotypes that account for 147 deaths

During 2010, the distribution of IPD cases followed a seasonal pattern with a clear increase during the winter months, peaking in December (Figure 10). This sequence was observed both for the total number of cases and for the 'top ten' serotypes.

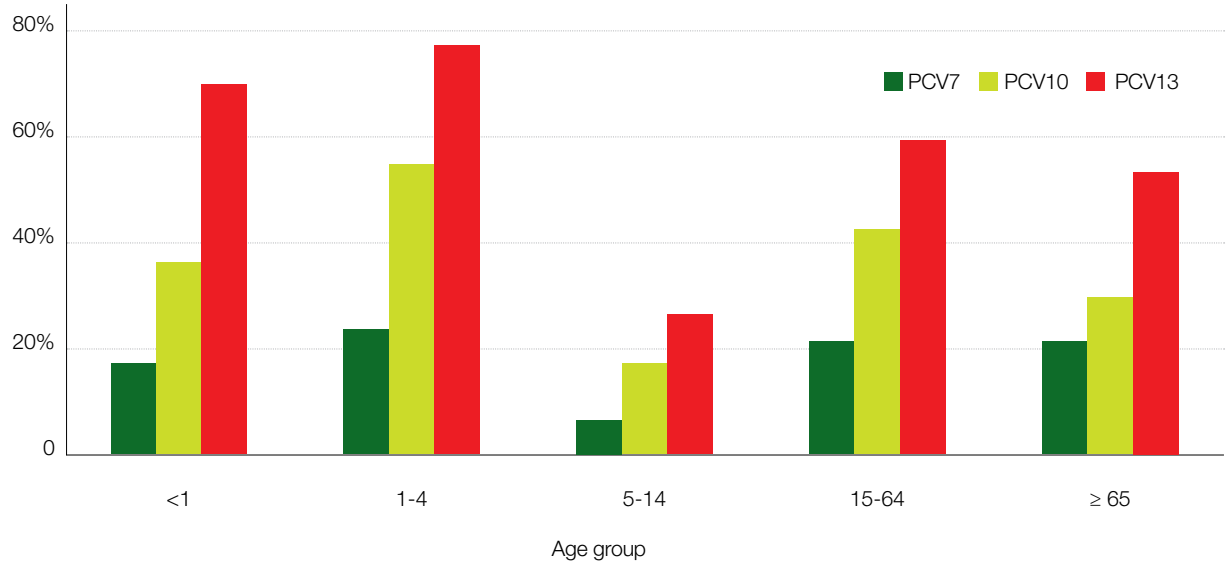
Figure 10. Distribution of reported IPD cases by month and age group, EU/EEA countries, 2010 (n=21,120)



4.1.7 Serotype coverage of pneumococcal conjugate vaccines

Overall, PCV7 serotype coverage among children <5 years in Europe was 19.2%; for the same age group, the serotype coverage for PCV10 was 46.1% and for PCV13 was 73.1% (Figure 11). Among adults, PCV13 serotype coverage was 60.1% for cases from 15 to 64 years, and 53.9% for the elderly (≥65 years).

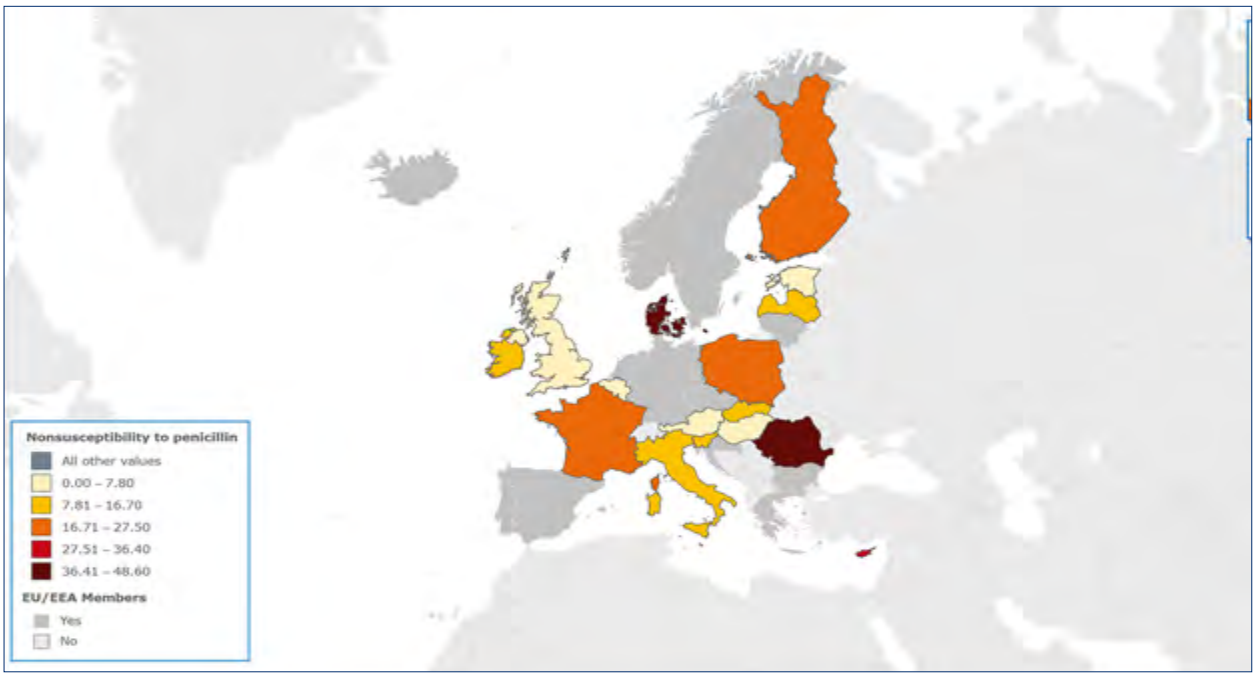
Figure 11. Percentage of cases covered by PCV type and age group, EU/EEA countries, 2010 (n=9,946)



4.1.8 Antimicrobial susceptibility

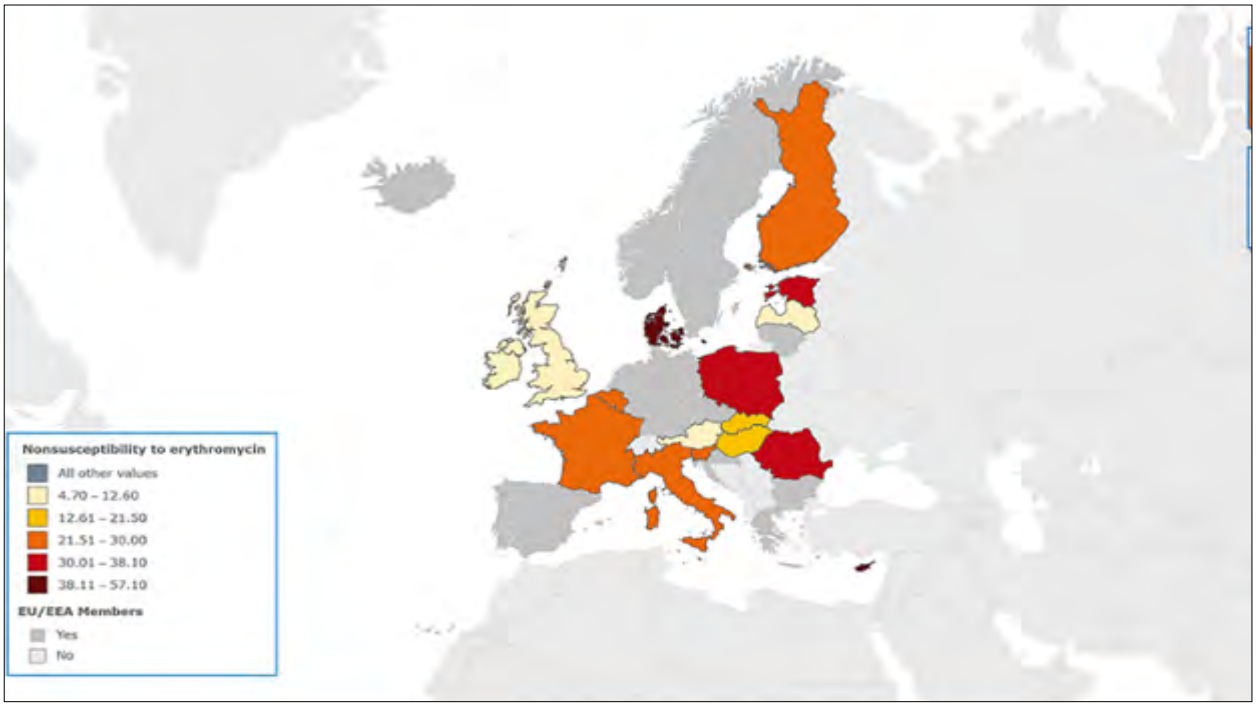
Romania (42.2%), Cyprus (36.4%) and France (27.5%) reported the highest rates of non-susceptibility to penicillin (Figure 12) (Denmark appears in the map with high non-susceptibility to penicillin most likely due to a surveillance artefact because an incomplete reporting of denominators).

Figure 12. Non-susceptibility to penicillin (%) in EU/EEA countries, 2010



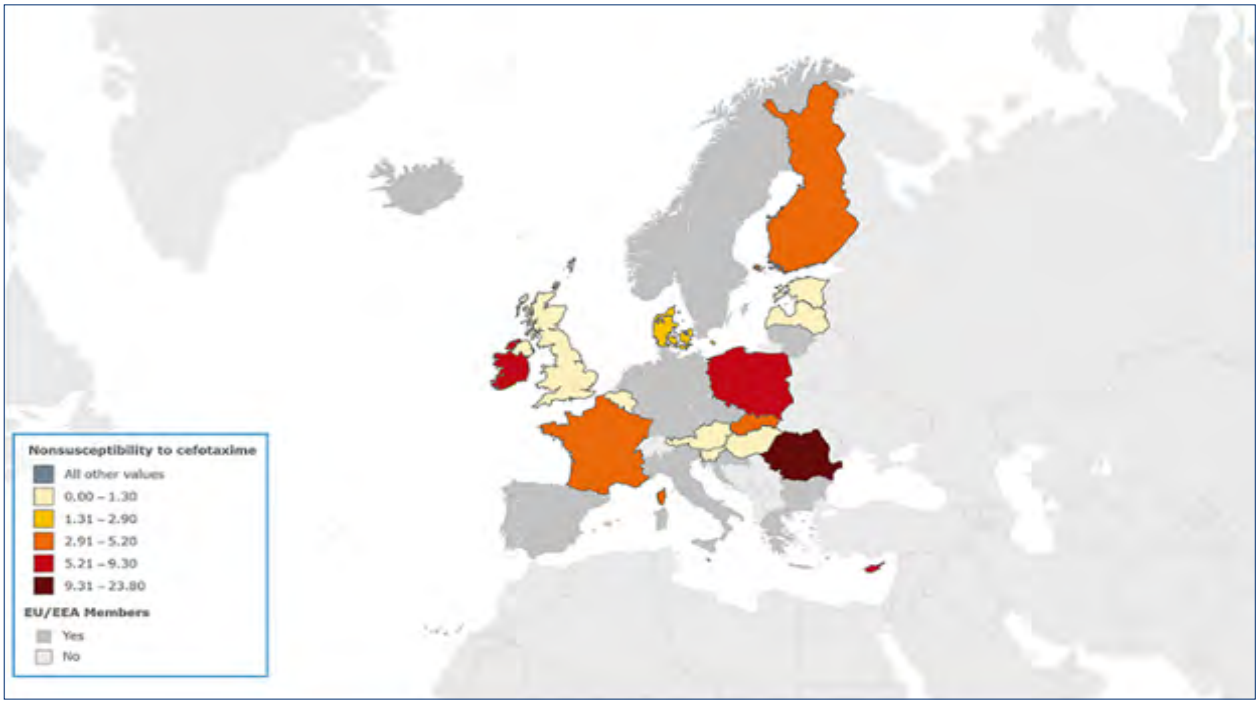
Cyprus (54.5%) and Romania (38.1%) reported the highest rates of non-susceptibility to erythromycin (Figure 13). (Denmark appears in the map with high non-susceptibility to erythromycin most likely due to a surveillance artefact because an incomplete reporting of denominators).

Figure 13. Non-susceptibility to erythromicyn (%) in EU/EEA countries, 2010



Romania (23.8%) and Ireland (9.3%) had the highest non-susceptibility rates to cefotaxime (Figure 14). (Finland reported susceptibility to ceftriaxone not to ceftaxime).

Figure 14. Non-susceptibility to cefotaxime (%) in EU/EEA countries, 2010



Overall penicillin MIC was ≤ 0.06 mg/L for 75.6% of isolates, 0.125 ≤ MIC ≤ 2mg/L for 23.3% and > 2mg/L for 1.1% of isolates tested. The erythromycin MIC was ≤0.25 mg/L for 70.9% of the isolates, 0.25 < MIC ≤ 0.5 mg/L for 5.4% of isolates and > 0.5 mg/L for 23.7 % of the isolates with this information. For cefotaxime, 91.3% of the isolates had MIC ≤ 0.5mg/L, 8.4% had 0.5 mg/L < MIC ≤ 2 mg/L and 0.3% had MIC > 2 mg/L.

Countries in Southern and Eastern Europe reported the highest proportion of non-susceptibility of *S. pneumoniae* to penicillin and/or erythromycin. However, Finland was an exception within the Northern countries with a non-susceptibility proportion of 23.3% for penicillin and with a non-susceptibility proportion of 28.2% for erythromycin. The overall percentage of non-susceptibility was 17.6% for erythromycin, 8.9% for penicillin and 2.7% for cefotaxime.

Simultaneous resistance to penicillin, erythromycin and cefotaxime (multidrug-resistance) was observed for serotypes 19A, 14, 19F, and 23F. Dual resistance to penicillin and erythromycin was reported in serotypes 19F, 19A, 14, 15A, 6A, 6B, 9V, 23A, 23F, and 24A. Non-susceptibility to penicillin was 6.9% for PCV7 and PCV10 serotypes whereas PCV13 serotypes non-susceptibility was 12.7%. For erythromycin, PCV7 serotypes non-susceptibility was 7.2%, PCV10 was 9.4% and PCV13 serotypes non-susceptibility was 17.2%.

Overall, non-susceptibility to the three antibiotics varied with age and children below five years presented the highest rates of non-susceptibility compared to 5-64 years and ≥65 years group (Table 9). Table 9 shows the three most frequent serotypes by age group and associated non-susceptibility to penicillin, erythromycin and cefotaxime. Percentages of resistance are higher for children under five years than for the other two age groups.

Table 9. Distribution of non-susceptible serotypes (3 most frequent) by age group

	Penicillin		Erythromycin		Cefotaxime	
	N	%	N	%	N	%
Serotype	**< 5 years**					
19A	130	10,3	192	15,3	60	5,0
14	46	3,6	63	5,0	23	1,9
19F	35	2,8	25	2,0	10	0,8
Serotype	**5-64 years**					
19A	123	3,6	128	3,8	59	1,8
14	102	3,0	93	2,8	54	1,6
19F	40	1,2	51	1,5	21	0,6
Serotype	**≥65 years**					
19A	155	5,0	164	5,3	69	2,3
14	114	3,7	119	3,9	68	2,3
6B	24	0,8	39	1,3	8	0,3

4.2 Risk factors for death from invasive pneumococcal disease

Once the epidemiological characteristics of invasive pneumococcal disease in EU/EEA countries were established, I decided to deepen into the burden of IPD within the variables available in the surveillance. I tried to determine the risk factors for fatal outcome. I studied the potential association between patient age, clinical presentation, serotype, antimicrobial resistance and death. The study sample consisted of 2,921 patients for which serotype and outcome were known. Death was reported in 264 (9%) cases (Figure 1).

Cases characteristics

In 2010, the European countries reported 21,565 IPD cases. Out of these, 17,549 cases (Figure 1) had information on laboratory variables, from which 4,637 had a known outcome. The study sample consisted of 2,921 cases with information on both serotype and outcome.

The study sample denoted (Table 10) that 56.8% of cases were men and 38.2% of cases were ≥65 years. Meningitis occurred in 18.5% of cases (Table 10). A total of 56.8% of cases (Table 10) occurred in men, and 38.2% of cases were among adults >65 years of age. Children <5 years of age accounted for 19.7% of cases. A total of 264 (9.0%) persons died. PCV13-specific serotypes (1, 3, 5, 6A, 7F, 19A) accounted for 42.7% of cases. Non-susceptibility (intermediate + resistant) to penicillin was reported in 122 (5.9%) of 2,071 cases; non-susceptibility to erythromycin was reported in 486 (23.6%) of 2,059 cases (Table 10).

Table 10. Characteristics of patients with invasive pneumococcal disease, EU/EEA countries, 2010*

Characteristic	No. cases (% total) (N = 17,549)	Sample size†, no. (%) (N = 2,921)	p-value§
Gender			
Women	7915 (45.3)	1257 (43.2)	0.039
Men	9565 (54.7)	1651 (56.8)	
Age group			
< 5	1980 (11.3)	570 (19.7)	<0.001
5-64	7819 (44.7)	1222 (42.1)	
≥ 65	7684 (44.0)	1108 (38.2)	
Outcome			
Non-fatal	4146 (89.4)	2657 (91.0)	0.029
Fatal	491 (10.6)	264 (9.0)	
Clinical presentation			
Non-meningitis	6047 (79.4)	1722 (81.5)	0.031
Meningitis	1572 (20.6)	391 (18.5)	
Serotype			
PCV13-specific‡	4185 (42.1)	1235 (42.3)	0.733
PCV7	1772 (17.8)	517 (17.7)	
Non-PCV	3989 (40.1)	1169 (40.0)	
Antimicrobial susceptibility testing			
Penicillin			
Susceptible	8420 (91.1)	1949 (94.1)	<0.001
Non-susceptible¶	827 (8.9)	122 (5.9)	
Erythromycin			
Susceptible	6911 (82.5)	1573 (76.4)	<0.001
Non-susceptible	1471 (17.5)	486 (23.6)	

* Numbers do not add to the total in each category because of missing data
† Defined patients for whom information was available about serotype and outcome
‡ Serotypes contained in PCV13 but not in PCV7
¶ Either resistant or intermediate resistance
§ Pearson $\chi 2$ test

The Pearson χ2 analysis (Table 11) demonstrated a lack of statistical association between gender and fatal outcome (p=0.631). Mortality was highest in cases ≥65 years (13.7%, p<0.001)) and 2.3% of children below five years of age, died.

Clinical presentation was related to death. The CFR for meningitis cases accounted for 15.9% of cases (p<0.001) whereas the CFR for non-meningitis cases was 8.8% (p<0.001). PCV7 serotypes were most associated with death (14.1%) when compared with the other two serotype categories (<0.001).

A fatal outcome was associated with non-susceptibility to penicillin: 13.1% of cases non-susceptible to penicillin had a fatal outcome (p=0.010) (Table 11). Non-susceptibility to erythromycin was not significantly associated with death (p=0.837).

Table 11. Associations between invasive pneumococcal disease study variables and death, Europe 2010

Variable	Outcome				p-value*
	Non-fatal		Fatal		
	N	%	N	%	
Sex					
Women	1147	91.3	110	8.8	0.631
Men	1498	90.7	153	9.3	
Age group					
< 5	557	97.7	13	2.3	<0.001
5-64	1123	91.9	99	8.1	
≥ 65	956	86.3	152	13.7	
Clinical presentation					
Non-meningitis	1571	91.2	151	8.8	<0.001
Meningitis	329	84.1	62	15.9	
Serotype					
PCV13-specific†	1155	93.5	80	6.5	<0.001
PCV7	444	85.9	73	14.1	
Non-PCV	1058	90.5	111	9.5	
Antimicrobial susceptibility testing					
Penicillin					
Susceptible	1815	93.1	134	6.9	0.010
Non-susceptible‡	106	86.9	16	13.1	
Erythromycin					
Susceptible	1464	93.1	109	6.9	0.837
Non-susceptible	451	92.8	35	7.2	

* Pearson χ2 test
† PCV13 specific serotypes: those contained in PCV13 but not in PCV7
‡ Non-susceptible: includes either resistant or intermediate resistance

Table 12 shows individual serotype association to death. Serotype 35B (RR=4.98, 95%CI 2.49-9.95), serotype 4 (RR=2.03, 95%CI 1.04-3.95) and serotype 11A (RR=1.97, 95%CI 1.33-2.94) presented the highest association to death. Serotype 3 (RR=1.39, 95%CI 0.88-2.21) accounted for the highest number and the highest pecentage (13.3%) of serotype-specific deaths, but the association with death was not statistically significant (p=0.161). In contrast, serotype 1 (RR=0.25, 95%CI 0.13-0.48) and serotype 5 (RR=0.15, 95%CI 0.09-0.26) were not associated with death.

A sub-analysis of the association between susceptibility to penicillin and outcome for serotype 35B cases revealed that there were not significant differences in risk for death between susceptible and non-susceptible cases.

Table 12. *Streptococcus pneumoniae* serotype association with death

Serotype	PCV*	Fatal (%)	non-Fatal (%)	RR	CI 95%	p-value†
3	PCV13-specific‡	13.3	9.6	1.39	(0.88-2.21)	0.161
4	**PCV7**	**6.1**	**2.8**	**2.03**	**(1.04-3.95)**	**0.038**
19A	PCV13-specific	6.1	7.6	0.80	(0.41-1.57)	0.515
14	PCV7	5.7	4.6	1.23	(0.78-1.85)	0.369
7F	PCV13-specific	4.9	8.3	0.59	(0.35-1.01)	0.053
6B	PCV7	3.8	1.7	2.01	(0.79-5.16)	0.144
19F	PCV7	3.8	1.9	1.85	(0.93-3.65)	0.078
22F	non-PCV	3.8	2.8	1.35	(0.89-2.03)	0.157
9V	PCV7	3.4	2.2	1.50	(0.95-2.38)	0.081
23F	PCV7	3.4	2.3	1.42	(0.60-3.32)	0.423
1	**PCV13-specific**	**3.4**	**13.1**	**0.25**	**(0.13-0.48)**	**<0.001**
11A	**non-PCV**	**2.3**	**1.1**	**1.97**	**(1.33-2.94)**	**0.001**
10A	non-PCV	2.3	1.4	1.52	(0.86-2.68)	0.147
6A	PCV13-specific	2.3	2.3	1.01	(0.39-2.57)	0.990
6C	non-PCV	1.9	0.7	2.33	(0.93-5.86)	0.072
9N	non-PCV	1.9	1.5	1.21	(0.52-2.82)	0.664
12F	non-PCV	1.9	1.8	1.07	(0.51-2.23)	0.867
35B	**non-PCV**	**1.5**	**0.2**	**4.98**	**(2.49-9.95)**	**<0.001**
33F	non-PCV	1.5	0.9	1.53	(0.55-4.28)	0.414
18C	PCV7	1.5	1.2	1.23	(0.40-3.76)	0.713
8	non-PCV	1.5	3.1	0.59	(0.25-1.06)	0.073
23A	non-PCV	1.1	0.7	1.51	(0.66-3.45)	0.323
15A	non-PCV	0.8	0.7	1.05	(0.46-2.43)	0.909
15B	non-PCV	0.8	1.0	0.79	(0.26-2.41)	0.677
24F	non-PCV	0.4	0.6	0.69	(0.12-4.09)	0.683
5	**PCV13-specific**	**0.4**	**2.6**	**0.15**	**(0.09-0.26)**	**<0.001**

* Classification of serotypes according to the study group
† Generalised linear model with log link function
‡ PCV13 specific serotypes: those contained in PCV13 but not in PCV7

Risk factors for IPD-associated death

Univariable analysis showed differences between non-fatal and fatal cases (Table 13). Compared to children <5 years, those 5-64 years (RR=3.55, 95%CI 1.66-7.61) and cases ≥65 years (RR=4.79, 95%CI 3.08-11.76) had a higher risk of death. In the univariable analysis significant associations with fatal outcome were also found for cases presenting with meningitis (RR=1.81, 95%CI 1.25-2.61) compared to non-meningitis cases. PCV7 serotypes were significantly associated with a fatal outcome (RR=2.18, 95%CI 1.06-4.48). Conversely, non-PCV serotypes were not related to fatal outcome (RR=1.47, 95%CI 0.94-2.28).

Non-susceptibility to penicillin was found to be associated with an increased risk of death (RR=1.91, 95%CI 1.16-3.13) while non-susceptibility to erythromycin was not significantly associated with death (RR=1.04, 95%CI 0.84-1.29).

Table 13. Association between study variables and death

Variable	RR* (95% CI)
Gender	
Women	Reference
Men	1.06 (0.88-1.28)
Age group	
< 5	Reference
5-64	3.55 (1.66-7.61)
≥ 65	4.79 (3.08-11.76)
Clinical presentation	
Non-meningitis	Reference
Meningitis	1.81 (1.25-2.61)
Serotype	
PCV13-specific‡	Reference
PCV7	2.18 (1.06-4.48)
Non-PCV	1.47 (0.94-2.28)
Antimicrobial susceptibility testing	
Penicillin	
Susceptible	Reference
Non-susceptible§	1.91 (1.16-3.13)
Erythromycin	
Susceptible	Reference
Non-susceptible	1.04 (0.84-1.29)

* Generalised linear model with log link function
‡ PCV13 specific serotypes: those contained in PCV13 but not in PCV7
§ Non-susceptible: includes either resistant or intermediate resistance

The study of susceptibility to penicillin versus outcome for clinical presentation showed that the association with the outcome only remained statistically significant for meningitis cases (RR = 1.82, 95%CI 1.27-2.62) and not for non-meningitis cases (RR = 1.31, 95%CI 0.28-6.01).

The analysis of the variable age as either confounder of effect modifier showed that age acted as an effect modifier. Therefore, the analysis was conducted stratifying by age group. After stratification, we found that in the age group below five years, there was an increased risk of death due to non-PCV serotypes (RR=3.68, 95%CI 1.27-10.69) (Table 14), whereas in the age group 5-64 years PCV7 serotypes presented the highest risk for fatal outcome (RR=2.68, 95%CI 1.37-5.23). In cases aged ≥65 years there were not significant differences among the serotype categories.

Table 14. Stratified analysis of *Streptococcus pneumoniae* serotype distribution

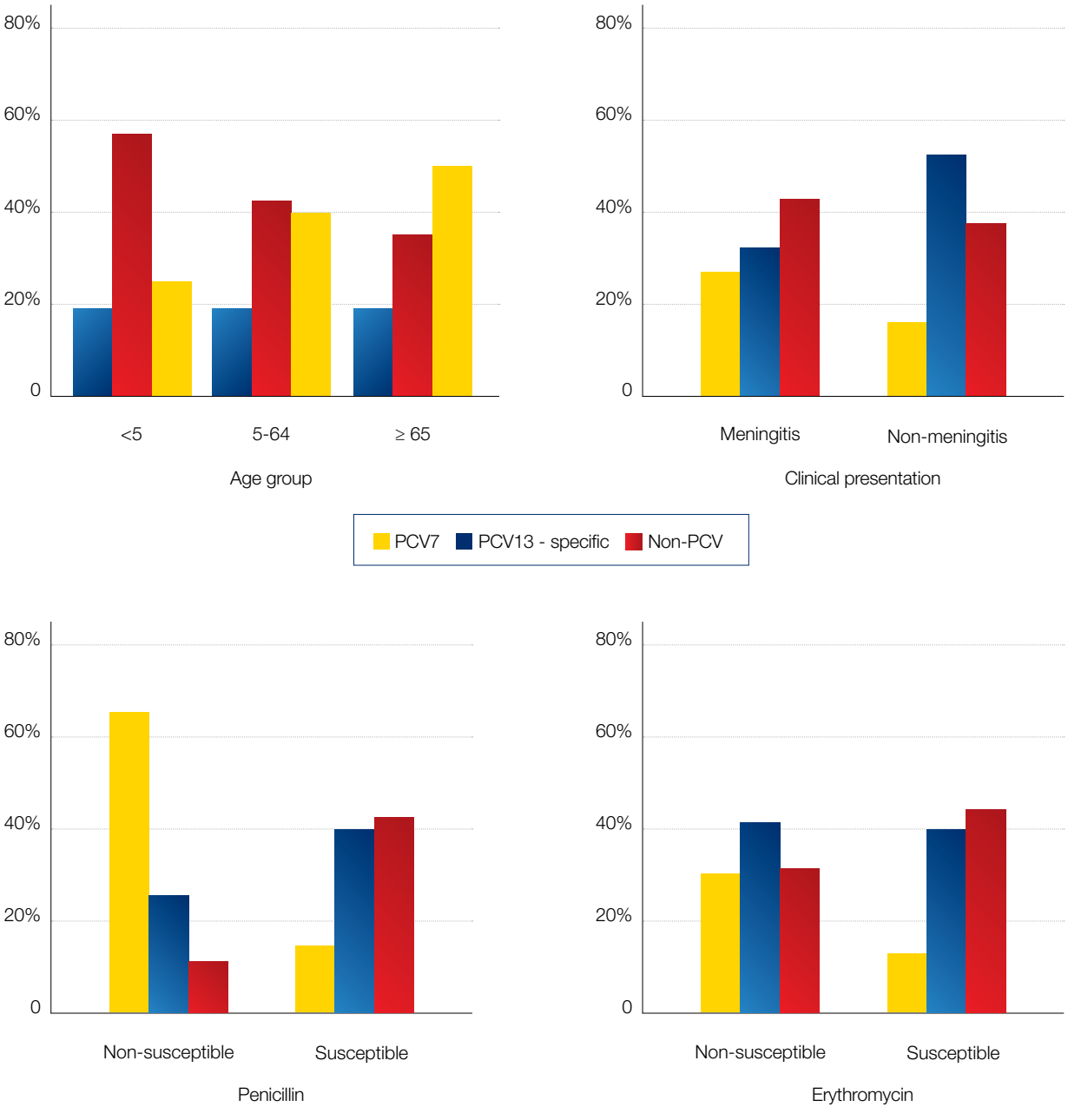
Age group		Survived (%)	Died (%)	RR (95% CI)	p-value
<5	PCV13 specific	325 (98.8)	4 (1.2)	1	
	PCV7	104 (97.2)	3 (2.8)	2.31 (0.35-15.02)	0.382
	Non-PCV	128 (95.5)	6 (4.5)	3.68 (1.27-10.69)	0.017
5-64	PCV13 specific	486 (94.4)	29 (5.6)	1	
	PCV7	186 (84.9)	33 (15.1)	2.68 (1.37-5.23)	0.004
	Non-PCV	451 (92.4)	37 (7.6)	1.35 (0.64-2.82)	0.429
≥65	PCV13 specific	338 (87.8)	47 (12.2)	1	
	PCV7	154 (80.6)	37 (19.4)	1.59 (0.90-2.79)	0.108
	Non-PCV	464 (87.2)	68 (12.8)	1.05 (0.64-1.72)	0.856

PCV: pneumococcal conjugate vaccine; PCV7, 7-valent PCV;PCV13, 13-valent PCV; RR, relative risk

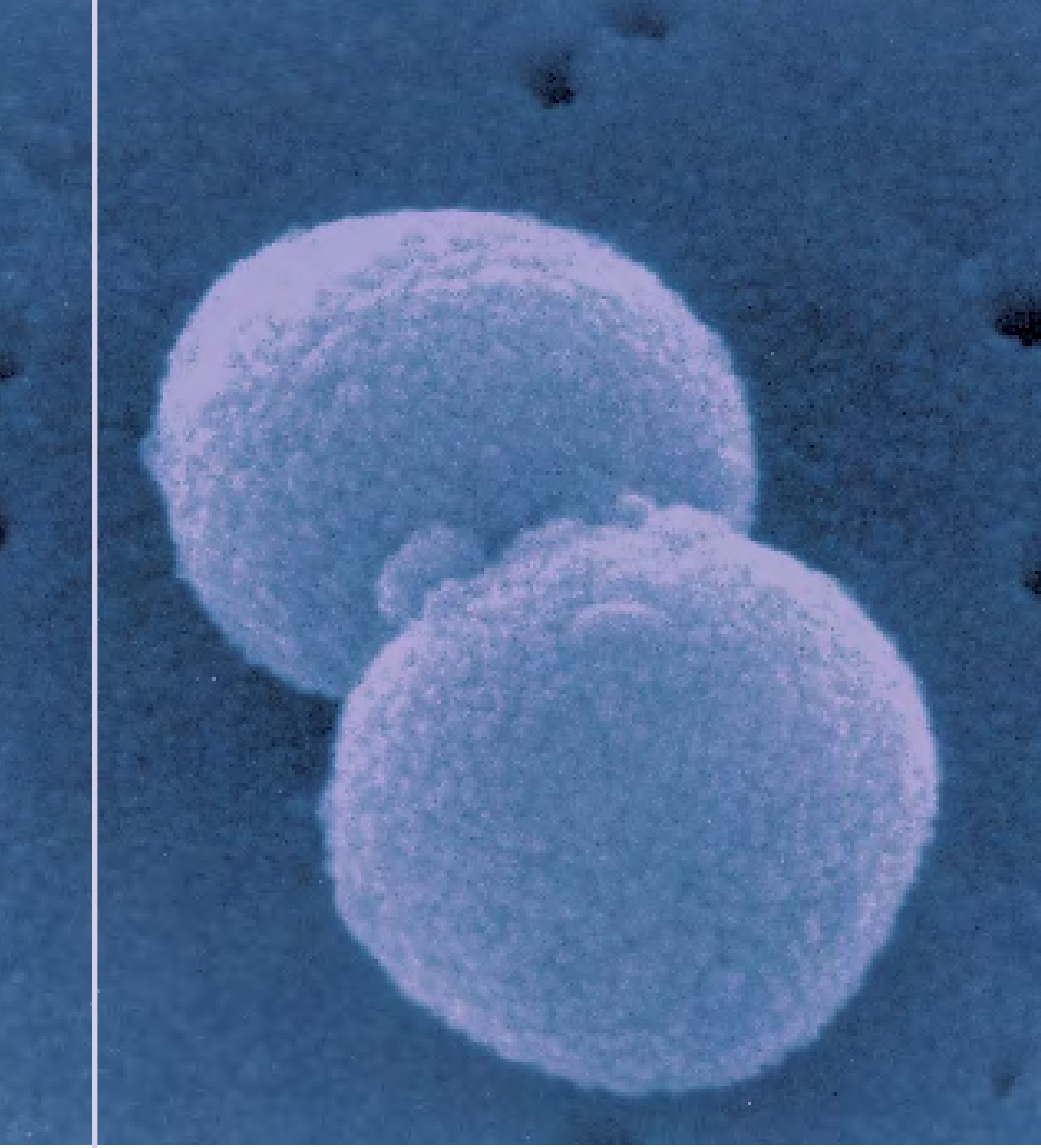
The analysis of the association between susceptibility to penicillin and outcome for the non-PCV serotypes and <5 years subgroup revealed that there were not differences between susceptible and non-susceptible cases for this group.

PCV13-specific serotypes caused 57.7% (p<0.001) of cases among children <5 years of age (Figure 15). Non-PCV serotypes accounted for 48.0% of cases among adults >65 years of age. Meningitis cases were predominantly caused by non-PCV serotypes (41.4%, p<0.001) (Figure 15). Non-susceptibility to penicillin was highest among PCV7 serotypes (64.8%, p<0.001) (Figure 15).

Figure 15. Invasive pneumococcal disease study variables and PCV coverage of *S. pneumoniae* serotypes, 2010



p<0.001



5. DISCUSSION

5. DISCUSSION

In 2010, 26 European countries reported 21,565 confirmed cases of IPD to The European Surveillance System (TESSy) (77). Notification rates varied markedly between these countries. This variation may be due not only to demographic divergence but also to differences in the surveillance systems across Europe: the case definition applied, diagnostic methods, medical practices (mainly blood culturing), and reporting methodologies (78). At the time of this surveillance, most of the European countries (27 countries, Annex 1) had already implemented the PCV7 vaccine in their immunisation programmes, albeit with different schedules, policies and at different introduction dates, which may have also impacted the results obtained by this surveillance.

There are geographic variations in the distribution as seen in other studies (79). Notification rates ranged from 0.3 per 100,000 inhabitants in Lithuania to 17.4 in Denmark and 17.1 in Belgium. Nordic countries and Belgium had the highest notification rates, most likely due to better ascertainment of cases, as these countries seem to have more robust surveillance systems for IPD. Isaacman et al. (79) also encountered great variability in notification rates in a study of the burden of invasive pneumococcal disease in young children in Europe. In addition to the above-mentioned reasons, the authors also attribute these differences to the insidious onset of IPD, the resistance to collect CSF in many settings, and certain under-reporting of cases.

The clonal spread of certain strains, probably due to antimicrobial pressure (80), may have also contributed to these disparities by selecting resistant strains that may cause more severe clinical presentations. The spread of a resistant strain can occur among children attending day-care centres and among adults living in nursing homes or other long-term-care facilities with an effect on geographical distribution. Resistant strains represent a greater challenge than susceptible strains in terms of clinical management and may result in invasive disease, a more severe presentation with a worse prognosis. European countries present different levels of resistance and this fact may also have affected the number of notification rates.

The highest notification rates were identified among children under one year of age (18.6 per 100,000) and adults of 65 years and over (15.6 per 100,000). The reason for this U-shaped age distribution might be multifaceted. With regard to the pathogen, *S. pneumoniae* has adherence and survival mechanisms such as a polysaccharidic capsule that allows it to attach and colonise the nasopharynx, enabling this pathogen to evade immune mechanisms. On the other hand, young children present an immature immune system that renders this age group more susceptible to pneumococcal colonisation and subsequent disease. Immunity in elderly people is usually impaired (immunosenescence) and thus this age group is more prone to severe pneumococcal infections.

The age distribution pattern of IPD has been consistent within European data since 2006 and has also been described in other parts of the world (5,81-83). This age distribution constituted the paradigm for targeting vaccination.

The most frequent clinical presentation was non-meningitis (mainly bacteraemic pneumonia or pneumonia/septicaemia as the variable was called in the surveillance). These findings are aligned with other reports since *S. pneumoniae* is the leading cause of pneumonia in the developed world (84). Meningitis is another severe presentation of IPD and often results in sequelae and a high CFR (85).

In 2010, IPD in Europe displayed a seasonal pattern with a greater number of cases occurring during the winter months, particularly evident amongst the elderly. Individual serotypes (the ten most frequent) followed a similar pattern. A number of studies have pointed to different possible causes, namely co-infection with respiratory viruses (i.e. influenza, syncytial respiratory virus, metapneumovirus), temperature and environmental factors (38,86-89).

If all age groups are taken into account, the sequence of the most frequent serotypes was 19A (10.0%, n=991), 1 (9.8%, n=978), 7F (9.7%, n=966), 3 (9.3%, n=928), and 14 (5.1%, n=503).

PCV13 could have potentially prevented more than 60% of the cases occurring in children under one year. Overall, the potential coverage of PCV13 is higher than 50% in all age groups except for 5-14 years. This age group accounts for the lowest notification rates and the smallest total number of cases. PCV13-specific serotypes (1, 5, 7F, 3, 6A, and 19A) would have covered 42.2% (n=4,166) of all cases with a reported serotype.

Low coverage of circulating serotypes included in PCV7 was most likely due to vaccination and replacement by non-vaccine serotypes (51, 90). Pneumococcal vaccination was introduced in all EU/EEA countries with differences in date of implementation, vaccine type, vaccination schedules and policies, with diverse scenarios combining whether it is mandatory or recommended, universal or restricted to risk groups, free of charge, reimbursed or with costs covered by the patients (Annex 1). In 2010, most of the EU/EEA countries had already implemented PCV7 in their national immunisation schedules on a universal basis with an acceptable coverage in many of them (Annex 1). Nevertheless, despite the scarce information of vaccination status and vaccine type of cases in this surveillance, the predominance of non-PCV serotypes could be attributed to the impact of the vaccine (91).

A considerable number (n=1,051, 10.6%) of the serotypes 4, 14, and 19F, included in all three PCVs, have been reported across Europe, especially in Finland and Spain. In both countries, PCV7 was introduced to the private market in 2001 but it was never incorporated into the routine childhood immunisation programme in either country, except in the autonomous region of Madrid in Spain; hence the estimated PCV7 vaccination coverage has been low (less than 1% in Finnish children). Recent publications (92) have shown that serotype 14 is still circulating in certain settings after immunisation with pneumococcal conjugate vaccine. Continuous monitoring of circulating serotypes after vaccination is therefore warranted to identify strains with particular virulence.

Serotypes 3 (9.3%, n=928; included in PCV13 and PPV23) and 8 (3.4%, n=343; included in PPV23), considered to have low invasive potential as described elsewhere (93, 94), were significantly represented and predominant in older adults, corroborating that serotypes causing disease in children and young adults differ from those causing IPD in the elderly, most likely due to concurrent conditions (75, 94). Among the non-PCV serotypes, serotype 22F (n=426) accounted for 4.28%, serotype 8 (n=343) 3.45%, serotype 12F (n=266) 2.67% and serotype 9N (n=193) accounted for 1.94%. These serotypes were predominant in adults and are only covered by PPV23. These findings support the recommendation of adult vaccination. At present, expert committees are evaluating different alternatives for the recommendation of PCV13 and PPV23 to prevent IPD in adults (95). Results from the long-awaited CAPITA clinical trial have revealed that among older adults, PCV13 was effective in preventing vaccine-type pneumococcal, bacteraemic and non-bacteraemic community-acquired pneumonia, and vaccine-type invasive pneumococcal disease, but not in preventing community-acquired pneumonia from any cause (96).

Serotype 6C was reported in 2.27% of cases for which information on serotype was available, mainly in adults aged 15 years and over. The increased prevalence in nasopharyngeal carriage of serotype 6C in certain settings after vaccination has been discussed elsewhere (97). Currently, serotype 6C is not covered by any of the licensed vaccines. However, there is evidence that PCV13 has the potential to confer cross-protection against serotypes not directly covered by the vaccine, namely serotypes 6C and 7A (98, 99). Nevertheless, in a recent population-based surveillance following the introduction of PCV13, Moore et al. were unable to identify any reductions in serotype 6C among adults and could not model it in children (60). Furthermore, PCV7 did not confer cross-protection to serotype 6C in a retrospective study in AOM (100).

Serotype 6C has been reported as being resistant to macrolides in this surveillance exercise.

The highest rate of antimicrobial non-susceptibility was reported for erythromycin (17.6%) followed by penicillin (8.9%). Pneumococcal non-susceptibility prevalence to penicillin and erythromycin varies across Europe (40) and predominance of non-susceptibility to erythromycin has been published elsewhere (101). Co-resistance to penicillin, erythromycin, and cefotaxime (multidrug-resistance) was observed amongst serotypes 19A, 14, 19F, and 23F, in accordance with other publications (40,102).

In the main, non-susceptibility was highest in children below five years, most likely due to repeated exposure of strains to antibiotics as respiratory infections; in particular, those caused by *S. pneumoniae* are the main clinical entities for the prescription of antimicrobial agents in young children.

Countries in Southern and Eastern Europe reported the highest proportion of non-susceptibility of *S. pneumoniae* to penicillin and/or erythromycin. This North-South gradient is more likely due to the overuse of antibiotics and less strict antibiotic-use policies in those countries, whereas in Northern countries (Nordic countries and the Netherlands) stringent measures to reduce antibiotic consumption and overuse apply.

Serotype 1 usually remains susceptible to penicillin (103) although resistance to erythromycin (macrolides) has been published. Simultaneous resistance to penicillin, erythromycin and cefotaxime (multidrug resistance) was observed in serotypes 19A, 14, 19F, and 23F. Serotypes 19A, 14, 19F, and 23F are considered to be the most antimicrobial resistant (40,102). High-level resistance to penicillin, erythromycin and cefotaxime was found in serotypes 14, 19A, and 19F.

Pneumococcal immunisation has decreased the number of antimicrobial-resistant infections. Nevertheless, some of the PCV10- and PCV13-specific serotypes exhibited antimicrobial resistance or multidrug resistance. Therefore, the judicious use of antimicrobials remains pivotal in curtailing the emergence and spread of antimicrobial resistance within pneumococcal strains (40, 41).

The CFR varied largely between countries in this surveillance. Nevertheless, the figures should be taken cautiously due to the limited clinical data available for this variable (overall, 79.5% missing data for the variable 'outcome', Table 5), i.e. information on the point at which the fatal outcome is defined and concurrent conditions is lacking. Additionally, capsular and clonal differences of pneumococcal strains predict their behaviour in relation to invasive potential and outcome (93, 94,105,106) as observed in this surveillance where the distribution of serotypes with the highest CFR varies with age.

As seen in this surveillance, *S. pneumoniae* causes a considerable burden in Europe in terms of morbidity and mortality, particularly affecting boths ends of life. Therefore, the study of the risk factors for death in IPD was planned. The possible association between patient age and sex, clinical presentation, pneumococcal serotype, antimicrobial resistance and death in invasive pneumococcal disease was analysed (107). This

study unveiled a significant association between death and older age, meningitis, serotypes contained in PCV7, and non-susceptibility to penicillin. In accordance with other studies (108-111) the analysis showed an association between increased age and death. However, the lack of information about a patient's clinical characteristics impedes an accurate assessment of these differences. Elderly persons have been postulated to have an increased susceptibility to – in addition to co-occurring conditions – pneumococcal disease because of reduced splenic function (24), age-related changes in respiratory tract, immunosenescence, and cellular senescence related to age-associated inflammation (24).

Sex was not significantly associated with death in this surveillance. Nevertheless, other studies showed association either with men (106) or with women (24,112).

The study showed that the presence of meningitis was significantly associated with death. Harboe et al. obtained similar results in a large population-based cohort study (105). Another Danish study concluded that patients with pneumococcal meningitis had increased death rates, but these rates derived from severe underlying conditions (113).

CFRs for pneumococcal meningitis are usually higher than for non-meningitis (114). More recently, Ladhani et al. found that the CFR was higher for children with meningitis in England and Wales (53). This study showed that the infecting serotype was not associated with death (53), whereas meningitis and co-occurring conditions were significantly associated with death. In our analysis, meningitis was predominantly caused by non-PCV serotypes; this finding could be an effect of PCV introduction, as observed in other studies (115). A sub-analysis of susceptibility to penicillin by clinical presentation showed a higher risk of death among persons with non-susceptible IPD than for those with susceptible IPD who had meningitis. Therefore, in the absence of information about the clinical management of cases and existing co-occurring conditions, the association between meningitis and non-susceptibility to penicillin might be an explanation.

Capsular differences between serotypes affect clinical presentation and outcome (93,106,116). These differences are in accordance with our study, which found that PCV7 serotypes were associated with death in the univariable analysis. Among children <5 years of age, PCV13-specific serotypes were the most frequent category, compared with PCV7 and non-PCV serotypes. In 2010, PCV13 was already licensed, and many European countries began moving from PCV7 towards the higher-valent vaccine, although with different schemes, policies, and dates of introduction. Nevertheless, these changes are unlikely to have affected our study findings because we analysed data from 2010.

After stratification, the highest risk of death among children <5 years of age corresponded to non-PCV serotypes. This finding could be attributed to serotype replacement after pneumococcal vaccination (53,115). Our analysis found no differences between penicillin-susceptible and non-susceptible cases among children <5 years of age and the non-PCV serotype subgroup with respect to death. However, the overall percentage of meningitis cases was high (18.5% of the study sample), and meningitis was predominantly caused by non-PCV serotypes ($p<0.001$). Hence, vaccines with enhanced serotype coverage (higher valency) might be needed to prevent IPD in this age group in the near future.

Among persons 5-64 years of age, the risk of death was highest for PCV7 serotypes, which were predominantly non-susceptible to penicillin ($p<0.001$). Reductions in IPD caused by PCV7 serotypes in non-vaccine-eligible age groups in countries with universal use of PCV7 might indicate the indirect effect of PCV7 (117). However, because vaccine policies differed among European countries at the time of the study, this indirect effect might not be reflected in the pooled data (Annex 1).

Serotypes 1, 5, and 7F have been described as having high potential for invasiveness (these serotypes are carried for a short time) but are associated with a milder disease and lower CFRs (93,108,118,119). As in those studies we found that serotypes 1 and 5 caused IPD and were not associated with death.

Serotype 35B has been reported as non-susceptible to penicillin (120). The sub-analysis on susceptibility to penicillin for serotype 35B showed that penicillin non-susceptibility did not affect the risk of death for serotype 35B. Nevertheless, the increased risk of death from non-PCV serotypes 11A and 35B merits further monitoring.

Penicillin non-susceptibility was significantly associated with death, as described by others (110,121). Nevertheless, in other large studies, this association was not found (93,112,122), and the effect of multidrug-resistant strains remains to be determined. Conversely, erythromycin non-susceptibility did not significantly affect death, as described by Song et al. (123) and Martens et al. (109). A plausible explanation might be the additional benefits of macrolides (i.e. their immunomodulatory/anti-inflammatory properties), which might be important when these drugs are used in combination with other therapeutic agents (124).

Antimicrobial resistance to *S. pneumoniae* is increasing in many countries in Europe (76), and the prudent use of antibacterial drugs, apart from immunisation, is pivotal in preventing and controlling IPD. Furthermore, these findings underpin the importance of antimicrobial susceptibility testing in order to assist with the clinical management of cases and to provide data on prevalence of antimicrobial resistance.

In conclusion, older age, meningitis, non-PCV serotypes among children <5 years of age and PCV7 serotypes among persons 5-64 years of age, and penicillin non-susceptibility were risk factors for death from IPD in Europe. The stratified analysis highlighted differences in risk of death according to the *S. pneumoniae* serotype and age group. This knowledge may assist in decision-making when implementing vaccination strategies as new immunisation strategies are needed to tackle the considerable IPDs and associated deaths in adults (125) and in designing new extended valency vaccines or protein-based pneumococcal vaccines that may confer serotype-independent immunity (23,125).

This work has shown that continued surveillance across Europe is important since serotype distributions and age group related incidences of IPD vary from country to country and the use of new vaccines is expected to have an impact on serotype distribution.

Notwithstanding its strengths, this study is affected by some limitations. The data analysis unveiled that there are limitations in the capacity of some countries to facilitate the provision of comprehensive data, e.g. some isolates were not characterised to the serotype level but only up to serogroup. The heterogeneity of laboratory methods for *S. pneumoniae* detection, characterisation and AST was highlighted in previous studies (73). Therefore, further efforts in the harmonisation and standardisation of laboratory methods for characterisation and AST of *S. pneumoniae* within European national reference laboratories are needed.

Surveillance of IPD varies markedly in Europe, including differences in laboratory methods for the confirmation of cases, in reporting, and in medical practices. Therefore there is probably a certain degree of under-diagnosis and under-reporting in this dataset. Moreover, surveillance systems for IPD differ in sensitivity, representativeness and specificity across European countries and these variations may have influenced the results as some countries were major contributors and ascertainment bias may have also occurred. Information on concurrent conditions or clinical management of cases that may have had an impact on outcome was also missing.

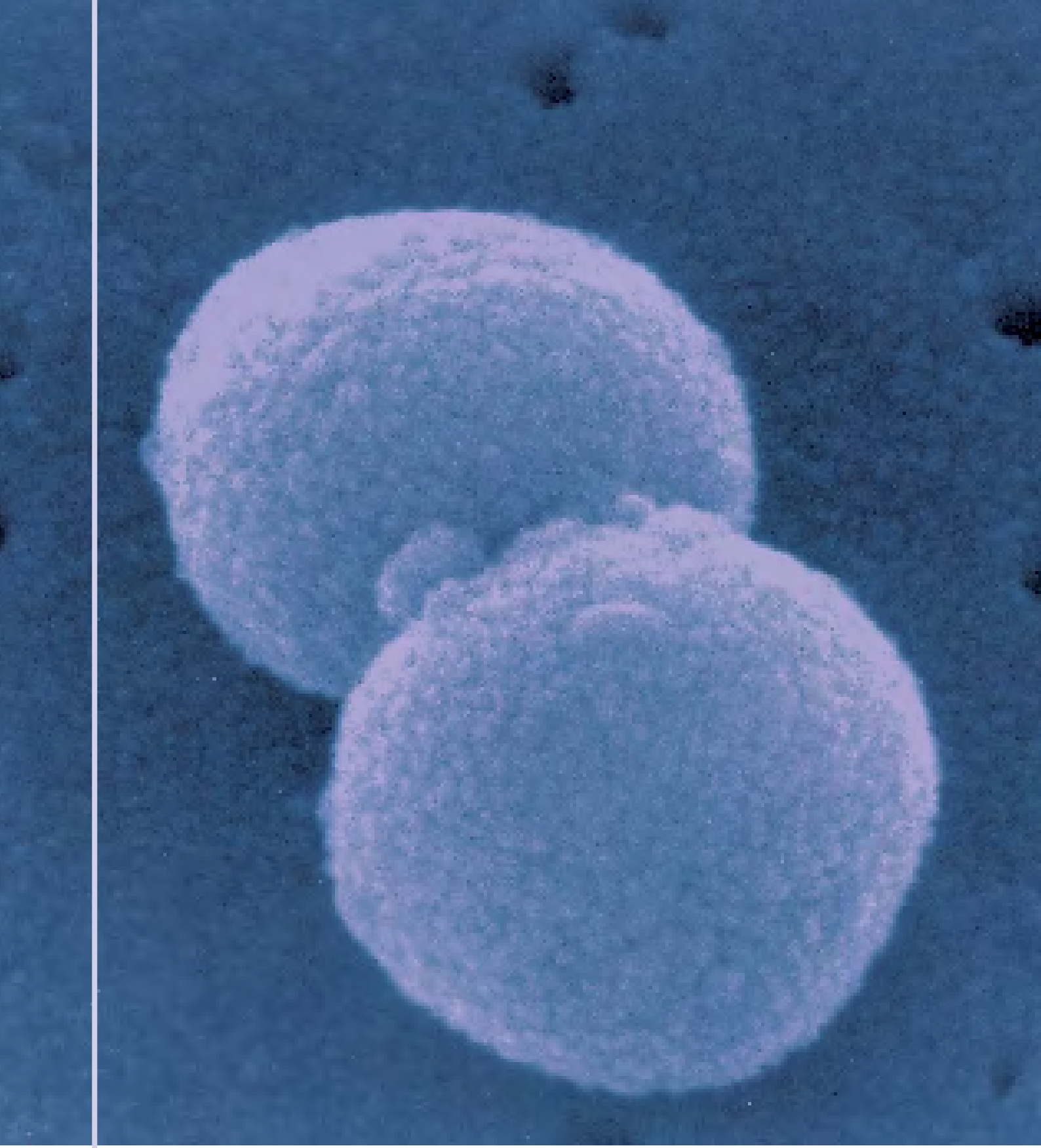
Pneumococcal vaccination was introduced in European countries at different time and with different policies and this may have affected the serotype distribution throughout Europe. Furthermore, the incomplete information on the vaccination status of cases makes it difficult the interpretation of results.

These limitations emphasise the need for continued and improved surveillance of IPD throughout European countries.

The major strength of this study is its large sample size as data came from national surveillance systems across Europe. To our knowledge, this was the first study in Europe that analysed IPD data at individual level in all the population, using data to characterise IPD of the entire population of a large geographical area. In 2010, European IPD surveillance collected data corresponding to approximately 82% of the total population of EU/EEA countries. This enhanced surveillance for IPD data pooled together at supranational level allows for comparisons with other parts of the world.

European IPD pooled-data analysis is relevant to assess differences across the world and to help formulate public health policies at a European level. However, differing national surveillance systems in terms of coverage and vaccination schedules make it difficult to compare data throughout Europe.

Despite these caveats, the establishment of the IPD enhanced surveillance at European level has provided baseline information on the epidemiology of IPD and has allowed an estimate of the burden of the disease across Europe in the post-heptavalent conjugate vaccine era. This baseline study will allow for comparisons after the implementation of PCV10/PCV13 immunisation in European countries, to assess the impact of the second generation conjugate vaccines. Finally, it provides information to the European countries to call for the prudent use of antibiotics to prevent the emergence and spread of antimicrobial resistance.



6. CONCLUSIONS

6. CONCLUSIONS

1. Despite the introduction of the hepta-valent pneumococcal conjugate vaccine, most European countries report a significant number of cases of invasive pneumococcal disease. Notification rates vary markedly between countries (from 17.4 per 100,000 in Denmark to 0.28 in Lithuania), most likely due to differences in surveillance systems, medical practice, diverging vaccination schemes and policies, and probably due to temporal trends in geographical distribution of the different serotypes.

2. The highest notification rates were among children under one year (18.6 per 100,000) and adults of 65 years and over (15.6 per 100,000), which constitutes the paradigm for pneumococcal vaccination.

3. Bacteraemic pneumonia was the predominant clinical presentation (48% of cases with known clinical presentation).

4. Quellung is the preferred technique for serotyping in Europe (62% of all cases for which serotype was reported), followed by slide agglutination and Pneumotest®.

5. Antimicrobial gradient is the preferred method (60% of cases with reported MIC) for determining MIC among the countries reporting this variable.

6. In 2010, the distribution of IPD cases displayed a seasonal pattern with a clear increase during the winter months, peaking in December. This sequence was observed for both the total number of cases and the 'top ten' serotypes. This distribution was slightly more pronounced for adults (age groups 15-64 and ≥ 65 years).

7. Overall, the most frequent serotypes were 19A, 1, 7F, 3, and 14. Among children below 15 years of age, the sequence was serotype 19A, 1, 7F, and 14. Since the most predominant serotypes are not included in PCV7 we can postulate that this is most probably due to the impact of the vaccine (serotype replacement).

8. Serotype 1 was the most frequent serotype reported among cases with pneumonia/septicaemia, whereas serotype 19A was predominant among cases presenting with meningitis.

9. PCV13-specific serotypes (1, 5, 7F, 3, 6A, and 19A) would have covered 42.2% (n=4,166) of all cases with reported serotype. PCV13 could have potentially prevented more than 60% of the cases occurring in children below one year.

10. The highest rate of antimicrobial non-susceptibility was reported for erythromycin (17.6%) followed by penicillin (8.9%).

11. Southern and Eastern European countries showed higher rates of antimicrobial resistance to penicillin and/or erythromycin in IPD compared to Nordic countries.

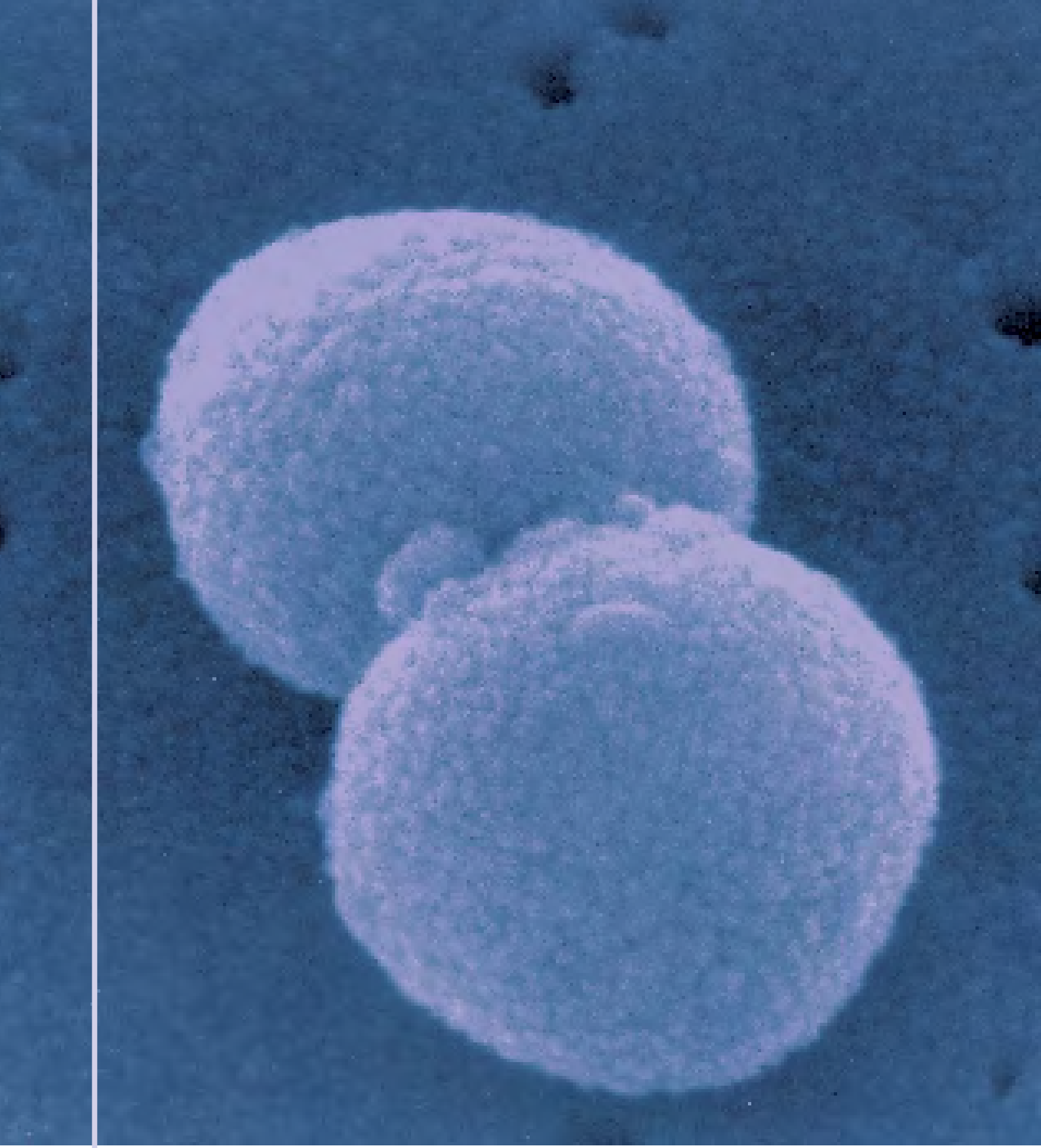
12. Co-resistance to penicillin, erythromycin, and cefotaxime (multidrug resistance) was observed amongst serotypes 19A, 14, 19F, and 23F.

13. Non-susceptibility was highest in children below five years.

14. Older age, meningitis, non-PCV serotypes among children <5 years of age and PCV7 serotypes among persons 5-64 years of age, and penicillin non-susceptibility were risk factors for death from IPD in Europe.

15. Among cases with a fatal outcome and known serotype, meningitis was predominantly caused by non-PCV serotypes (those not contained in any pneumococcal conjugate vaccine). Therefore, new extended-valency or serotype-independent vaccines are needed.

16. Serotypes 11A and 35B were significantly associated with death and are not covered by any pneumococcal conjugate vaccine. There is a need for close monitoring of emerging serotypes, particularly if they are associated with antimicrobial resistance, as in serotype 35B.



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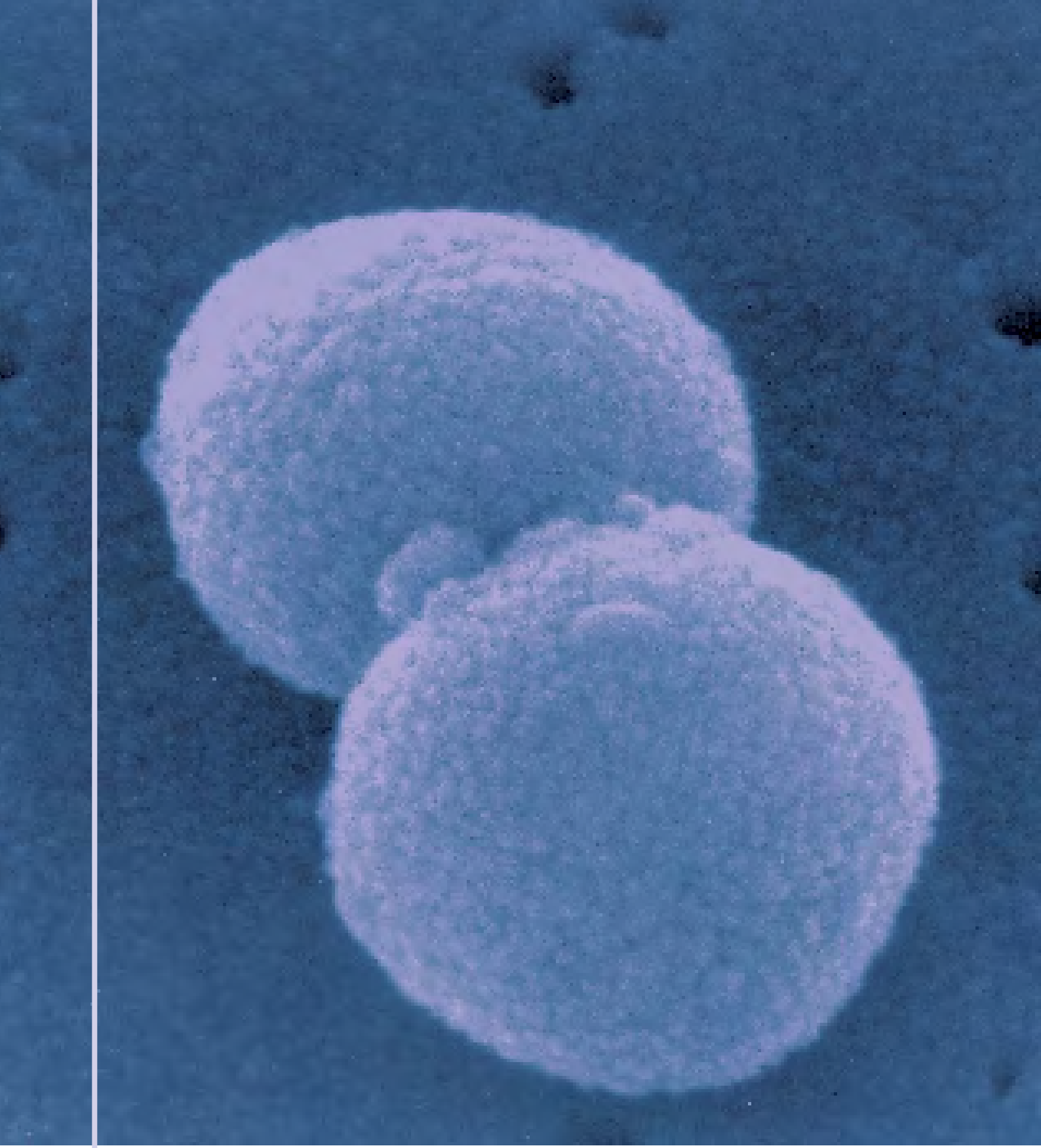

8. ANNEXES

8. ANNEXES

8.1. Annex 1

Characteristics of national pneumococcal vaccination programmes in EU/EEA countries in 2010

Country	Date PCV7 first introduction	Scope of PCV vaccination programme	Immunisation schedule	1st d (m)	2nd d (m)	3rd d (m)	4th d (m)	Vaccine coverage[d]	Year of measure-ment
Austria	July 2004	Universal	3+1 dose	3	5	7	12-24	-	-
Belgium	January 2005	Universal	2+1 dose	2	4	12		97	2010
Bulgaria	April 2010	Universal	3+1 dose /2+1 dose	2	3	4	12	-	-
Cyprus	August 2008	Universal	3+1 dose	2	4	6	12-15	-	-
Czech Republic	January 2010	Risk-based	3+1 dose	2	4	6	18	86.3	2010
Denmark	October 2007	Universal	2+1 dose	3	5	12		85	2010
Estonia	-	-	not decided	-	-	-	-	-	-
Finland	January 2009	Risk-based	2+1 dose	3	5	12		-	-
France	June 2006	Universal	2+1 dose	2	4	12		81	2008
Germany	July 2006	Universal	3+1 dose	2	3	4	11-14	52.9	2010
Greece	January 2006	Universal	3+1 dose	2	4	6	12-15	-	-
Hungary	October 2008	Universal	2+1 dose	2	4	15		81.1	2009
Iceland	December 2006	Risk-based	2+1 dose	3	5	12		-	-
Ireland	October 2002	Universal	2+1 dose	2	6	12		89	2009
Italy	May 2005	Universal/Risk Based	2+1 dose	3	5	11		55	2008
Latvia	January 2010	Universal	3+1 dose	2	4	6	12-15	51	2010
Lithuania	-	-	3+1 dose	2	4	6	24	-	-
Luxembourg	February 2003	Universal	3+1 dose	2	3	4	12-15	86	2010
Malta	January 2007	Risk-based	3+1 dose	2	4	13	none	-	-
Netherlands	June 2006	Universal	3+1 dose	2	3	4	11	94	2009
Norway	July 2006	Universal	2+1 dose	3	5	12		90	2009
Poland	May 2008	Risk-based	3+1 dose/2+1 dose	N/A	N/A	N/A	N/A	1.70	2008
Portugal	June 2010	Risk-based	2+1 dose	2	4	12-15		52	2009
Romania[a]			3+1 dose	2	4	6	15-18		
Slovakia[b]	January 2006	Risk-based	2+1 dose	2	4	10		99.2	2009
Slovenia	September 2005	Risk-based	3+1 dose	2-3	4	6	24	-	-
Spain[c]	June 2001	Risk-based	3+1 dose	2	4	6	15	-	-
Sweden	January 2009	Universal	2+1 dose	3	5	12		-	-
United Kingdom	September 2006	Universal	2+1 dose	2	4	13		90	2010

[a] PCV7 was registered in September 2007 for voluntary use on a private basis.
[b] Universal as of April 2008.
[c] Universal introduction in the autonomous region of Madrid in November 2006.
[d] Sources: VENICE II and WHO estimates of PCV7 coverage.
N/A: not applicable; -: not available

8.2. Annex 2

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Austria	PCV13/PPV23	National (2014)	At risk and high risk (≥6)	**High risk** Asplenia (anatomical, functional) Chronic renal insufficiency Cochlear implant Complement and properdin deficiency Haematopoietic organ disorder HIV Hypogammaglobulinemia Immunodeficiency (congenital, acquired) Liquor fistula Nephritic syndrome Nephrotic syndrome prior to immunosuppressive therapy Neurological disorder (in children) Sickle-cell anaemia Transplantation (organ, subsequent to stem cell transplantation) **At risk** Body weight below third percentile (in infants and children) Chronic cardiovascular disease (except hypertension) Chronic respiratory disease Cirrhosis Diabetes Metabolic disease Neoplastic disease	Private	**Naïve** PCV13 followed by PPV23 after ≥8 weeks **Pre-vaccinated with PCV** After interval of ≥8 weeks 1xPPV23 **Pre-vaccinated with PPV23** After interval of ≥8 weeks 1xPCV13 and after another interval of ≥8 weeks 1xPPV23 again (second PPV23 dose recommended ≥5 years after first PPV23 dose) **Investigations ongoing into necessity of further vaccinations**
Austria	PCV13/PPV23	National (2014)	All (≥50)	N/A	Private	**Naïve** PCV13 followed by PPV23 after 1 year **Pre-vaccinated with PCV13** After interval of ≥1 year 1xPPV23 **Pre-vaccinated with PPV23** After interval of ≥2 years 1xPCV13 **Investigations ongoing into necessity of further vaccinations**

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Belgium	PCV13/PPV23	National (2013)	High risk (≤17)	Asplenia Chronic disease (heart, lung, renal) Cochlear implant CSF leak Diabetes (non-stable) Immunodeficiency (congenital, immunosuppressant induced) Metabolic disease	Private	PCV13 (schedule depending on age) followed by PPV23 (revaccination every 5 years for asplenia)
Belgium	PCV13/PPV23		High risk (≥18)	Autoimmune disease/immune-mediated inflammatory disease Asplenia Cancer (haematological) Cochlear implant HIV Immunodeficiency Transplantation (organ)	Private	**High-risk populations** PCV13 followed by PPV23 after at least 8 weeks and revaccination with PPV23 every 5 years **Adults aged ≥50 years with certain comorbidities and all ≥65 years** Either PPV23 with 1 revaccination after 5 years or PCV13 followed by PPV23 after 8 weeks with 1 revaccination after 5 years (except >75 years who do not require revaccination)
			At risk (≥50)	Alcoholism Chronic disease (heart, kidney, liver, respiratory) Smoking		
			All (≥65)	N/A		
Denmark	PCV13	National (2012)	At risk (any age)	Asplenia (functional) Cochlear implant CSF leak History of IPD HIV Lymphoma Splenectomy (completed/planned) Transplantation (organ)	Limited subsidy (to cover vaccination of at-risk groups and some age groups)	For individuals at risk aged ≥6 years vaccination with PCV13 should be followed by 1 dose of PPV23 after ≥8 weeks
Denmark	PCV13		At risk (<18)	Chronic lung disease Cyanotic heart disease Heart failure/insufficiency Hypodynamic respiratory insufficiency Immunodeficiency (excluding agammaglobulinemia and SCID) Nephrotic syndrome Palliative surgery for heart disease		
			At risk (18-65)	Chronic disease (heart, kidney, liver, lung) Diabetes	Private	For individuals at risk vaccination with PCV13 should be followed by 1 dose of PPV23 ≥8 weeks after PCV13 vaccination
			All (≥65)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Finland	PCV13	National (2013)	High risk (≥ 5)	Asplenia (anatomical, functional) Cochlear implant HIV Immunodeficiency (congenital, acquired) Liquor fistula Lymphoma Multiple myeloma Nephrotic syndrome Patients treated with systemic corticosteroids or other immunosuppressants Transplantation (organ and tissue)	Private (except stem cell trans-plantation patients)	PCV13 preferred in high-risk individuals (e.g. immunocompromised) and may be followed by PPV23. However, physicians can choose whether to give PCV13 or PPV23 PCV13 is funded for stem cell transplantation patients of all ages. PCV13 may also be considered in healthy individuals of all ages
Finland	PPV23		At risk or in permanent institutional care (≥5) All (≥65)	Chronic disease (cardiac, pulmonary) Diabetes (type 1) Hepatic insufficiency Patients treated with systemic corticosteroids or other immunosuppressants Renal insufficiency Transplantation (organ, tissue) N/A		
France	PCV13	National (2013)	At risk (≥2)	Asplenia or hyposplenia Cancer treated by chemotherapy (solid tumour, haematological) Cochlear implant or planned cochlear implant HIV Immunodeficiency (congenital) Immunosuppressive therapy, biotherapy, or corticotherapy for autoimmune disease or chronic inflammation Meningeal fistula Nephrotic syndrome Transplantation or waiting for transplantation (organ, haematopoietic stem cell)	Public	For all at-risk individuals aged ≥2 years, PCV13 followed by PPV23 after ≥8 weeks In some cases the vaccination schedule may differ and there are slight differences for specific populations (for asplenic and immunosuppressed patients PCV is preferred), but PCV13 should be administered first in all cases For high-risk individuals aged ≥6 years to <50 years funding procedure ongoing

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
France	PPV23		At risk (≥5)	Asthma (severe with continuous treatment) Chronic liver disease (alcoholic or non-alcoholic origin) Chronic respiratory failure COPD Cyanotic congenital heart disease Diabetes (not balanced by diet) Emphysema Heart failure Kidney failure	Public	-
Germany	PCV	Saxony (updated January 2014)	At risk (>2)	Asplenia Autoimmune disease Bone marrow transplantation Chronic disease (heart, kidney, respiratory) CSF leaks, cochlea implant HIV Haematological diseases Immunodeficiency (primary) Metabolic disease Neurological diseases in children Occupational risk (laboratory personnel at risk of infection, medical personnel in contact with patients) Sickle-cell anaemia Transplantation (organ)	Public	All infants from the age of 2 months to 5 years should receive PCV (vaccination should be started in the third month of life, according to schedule of vaccine manufacturer) PCV may be PCV10 or PCV13 for those aged 2-<5 years; PCV will be PCV13 for those aged ≥5 years. Children with persisting risk of pneumococcal infection should be vaccinated in the third year of life with PPV23 in addition to PCV (interval of at least 2 months after last vaccination with PCV) Non-vaccinated infants (aged ≥5 years), adolescents and adults should receive one dose of PCV or PPV23 (according to approval) PCV can be supplemented with PPV23 if protection against further serotypes is required (interval at least 4 years). In those pre-vaccinated with PPV23, catch-up vaccination with PCV is useful (interval at least 5 years). In at-risk individuals and those aged ≥60 years revaccination with PPV23 is possible (≥5 years for adults, ≥3 years for children aged<10 years)
			All (≥60)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Germany	PCV	National (PCV funding 2013; PCV recommendations 2014)	At risk (2-<5)	Chronic disease (e.g. heart, kidney, liver, respiratory diseases, metabolic disorders [e.g. diabetes], neurological diseases [e.g. cerebral pareses, seizure disorders]) Immunodeficiency (congenital, acquired, e.g. T-cell, B-cell or antibody deficiency, deficiency or functional disorders of myeloic cells [e.g. neutropenia, chronic granulomatosis, leukocyte adhesion or signal transduction defects], complement or properdin deficiency, functional hypersplenism or splenectomy, neoplastic diseases, HIV, infection, bone marrow transplantation, immunosuppressive therapy [e.g. due to organ transplantation, autoimmune disease]) Anatomic risks, risks associated with foreign bodies for pneumococcal meningitis (e.g. liquor fistula, cochlea implant)		For this age group, PCV may be PCV10 or PCV13 For congenital or acquired immunodeficiencies, chronic renal diseases/nephrotic syndrome, revaccination can be considered every 5 years (for those aged >10 years) or every 3 years (for those aged <10 years)
Germany	PCV13/PPV23	National (PCV funding 2013; PCV recommendations 2014; PPV 1982)	At risk (≥5)	Chronic disease (e.g. heart, kidney, liver, respiratory diseases, metabolic disorders [e.g. diabetes], neurological diseases [e.g. cerebral pareses, seizure disorders]) Immunodeficiency (congenital, acquired, e.g. T-cell, B-cell or antibody deficiency, deficiency or functional disorders of myeloic cells [e.g. neutropenia, chronic granulomatosis, leukocyte adhesion or signal transduction defects], complement or properdin deficiency, functional hypersplenism or splenectomy, neoplastic diseases, HIV infection, bone marrow transplantation, immunosuppressive therapy [e.g. due to organ transplantation, autoimmune disease]) Anatomic risks, risks associated with foreign bodies (e.g. liquor fistula, cochlea implant)		For this age group, PCV may be PCV10 or PCV13 For congenital or acquired immunodeficiencies, chronic renal diseases/nephrotic syndrome, revaccination can be considered every 5 years (for those aged >10 years) or every 3 years (for those aged <10 years)
	PPV23	National (1998)	All (≥60)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Greece	PCV13	National (2011)	All (>50)	N/A	Public	-
Ireland	PCV13/PPV23	National (2013)	Medium risk and high risk (2-<5)	**Medium risk** Children <5 years of age following IPD Chronic heart, lung, or liver disease Chronic renal disease or nephrotic syndrome Diabetes mellitus requiring insulin or oral hypoglycemic drugs Down syndrome **High risk** Asplenia, hyposplenia (including splenectomy, sickle-cell disease, haemoglobinopathies, and celiac disease) Candidates for, or recipients of, a cochlear implant Complement deficiency (particularly C1–C4) CSF leaks (congenital or complicating skull fracture or neurosurgery) Immunosuppressive conditions (e.g. some B- and T-cell disorders, HIV infection, leukaemia, lymphoma) and those receiving immunosuppressive therapies Intracranial shunt Post-haematopoietic stem cell transplant Solid organ transplant	PCV13 supplied free of charge to all those in risk groups; individuals pay an administration fee	2-5 years: 1 or 2 doses of PCV13 at 2-month intervals followed by 1 dose of PPV23 ≥2 months after final PCV dose

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Ireland	PCV13/PPV23		High risk (5-<18; 18-64)	Asplenia, hyposplenia (including splenectomy, sickle-cell disease, haemoglobinopathies, and celiac disease) Candidates for, or recipients of, a cochlear implant Complement deficiency (particularly C1-C4) CSF leaks (congenital or complicating skull fracture or neurosurgery) Immunosuppressive conditions (e.g. some B- and T-cell disorders, HIV infection, leukaemia, lymphoma) and those receiving immunosuppressive therapies Intracranial shunt Post-haematopoietic stem-cell transplant Solid organ transplant	PCV13 supplied free of charge to all those aged <18 years in risk groups; individuals pay an administration fee PCV13 is not free of charge to those aged ≥18 years PPV23 supplied free of charge to all those in risk groups; individuals pay an administration fee unless they have a medical or doctor-only card	>5-<18 years: 0, 1 or 2 doses of PCV13 followed by 1 dose of PPV23 ≥2 months after PCV
Ireland	PPV23		Medium risk (5-<18)	Children <5 years of age following IPD Chronic heart, lung, or liver disease Chronic renal disease or nephrotic syndrome Diabetes mellitus requiring insulin or oral hypoglycemic drugs Down syndrome	Vaccine supplied free of charge to all those in risk groups; individuals pay an administration fee unless they have a medical or doctor-only card	1 dose of PPV23

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Ireland	PPV23		Medium risk (18-64)	Chronic heart, lung, or liver disease Chronic renal disease or nephrotic syndrome Diabetes mellitus requiring insulin or oral hypoglycemic drugs Smokers and alcoholics Individuals with occupational exposure to metal fumes (e.g. welders)		
Ireland	PPV23		All (≥65)	N/A		
Italy	PCV13/PPV23	Basilicata (2012)	At risk (any age) All (≥65)	Chronic disease (heart, liver [hepatic cirrhosis], respiratory) Metabolic disease N/A	Public	For at-risk adults aged <50 years, PCV13 is recommended in addition to PPV23. PPV23 should be administered after >8 weeks
Italy	PCV13/PPV23	Bolzano (2013)	At risk (any age) All (>65)	Alcoholism Asplenia Chronic disease (cardiac, liver, pulmonary) Cirrhosis Cochlear implant Diabetes HIV Immunodeficiency Immunosuppression (clinically significant) Leukaemia Liquor fistula Lymphoma Multiple myeloma Neoplastic spread Nephrotic syndrome SCID Thalassemia Transplantation (organ, bone marrow) N/A	Public	

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Italy	PCV13/PPV23	Cagliari (LHU Cagliari 8) (2011)	At risk (≥50)	Asplenia Chronic disease (heart, kidney, liver, respiratory) Cochlear implant, CSF leak HIV Immunodeficiency Metabolic disease Other pathologies predisposed to high IPD risk Transplantation (organ)	Public	PCV13 recommended in addition to PPV23 PPV23 to be administered 8 weeks following PCV13
Italy	PCV13/PPV23	Emilia Romagna (2014)	At risk and high risk (any age)	High risk Asplenia Chronic disease (kidney [renal failure]) Cochlear implant CSF leak Haemoglobinopathy HIV Immunodeficiency (acquired) Immunosuppression (iatrogenic) Leukaemia Lymphoma Multiple myeloma Neoplastic spread Nephrotic syndrome Transplantation (organ, bone marrow) At risk Alcoholism Chronic disease (heart, liver [hepatic cirrhosis], respiratory) Diabetes Residents in an institution (e.g. nursing home) aged >65 years	Public	**High-risk individuals** PCV13 recommended in addition to PPV23 PPV23 should be administered >8 weeks after PCV13; for bone marrow transplantation, 3 doses of PCV13 (interval 2 months); a fourth dose is recommended in case of chronic graft versus host disease
			In permanent institutional care (≥65)	N/A		
Italy	PCV13/PPV23	Friuili-Venezia Giulia (2012)	At risk (≥18)	Asplenia Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Metabolic disease Others	Public	PCV13 recommended in addition to PPV23 At-risk individuals: PCV13, 2 doses 8 weeks apart (3 doses for bone marrow transplantation)
			All (≥65)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Italy	PCV13/PPV23	Lazio (2012)	At risk (any age)	Asplenia Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Metabolic disease	Public	-
		Liguria (2013)	At risk (any age)	Asplenia Cancer (haematological) Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CSF leak Diabetes Immunodeficiency (congenital, acquired) Neoplastic spread Transplantation (organ, bone marrow)	Public	
		All (>70)	N/A			
Italy	PCV13/PPV23	Lombardia (LHU Milan) (2012)	At risk and high risk (>18)	High risk Asplenia Chronic disease (renal) Cochlear implant CSF leak Haemoglobinopathy HIV Immunodeficiency (congenital, acquired) Leukaemia Lymphoma Multiple myeloma Neoplastic spread Previous IPD Transplantation (organ, bone marrow) At risk Chronic disease (heart, liver, pulmonary) Diabetes	Public (PCV13 is available on medical prescription)	PCV13 recommended in addition to PPV23 PPV23 should be administered 8 weeks after PCV13 Individuals already vaccinated with PPV23 should be vaccinated with PCV13 1 year after PPV23 For adults aged<50 years PPV23 is recommended For adults aged ≥50 years PCV13 is recommended
			All (≥65)	N/A		PCV13 if not previously vaccinated

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Italy	PCV13/PPV23	Marche (2013)	At risk (any age)	Asplenia Chronic disease (heart, kidney disease [renal failure], respiratory) Cochlear implant CSF leak Diabetes Hepatic cirrhosis and chronic liver disease due to alcoholism HIV Immunodeficiency (congenital, acquired) Immunosuppression (iatrogenic) Leukaemia Lymphoma Multiple myeloma Neoplasia Thalassemia Transplantation (organ, bone marrow)	Public	-
		Piemonte (2012)	At risk (>5)	Asplenia Chronic disease (heart [excluding hypertension], kidney [renal failure], liver, respiratory) Cochlear implant Complement deficiency CSF leak Diabetes (type 1) Haemoglobinopathy Immunodeficiency (congenital, acquired)	Private	PCV13 + PPV23 6 months apart
Italy	PCV13/PPV23	Puglia (2012)	At risk (≥50)	Asplenia Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) Cochlear implant CSF leak Diabetes Haemoglobinopathy HIV Immunodeficiency (congenital, acquired) Leukaemia Lymphoma Multiple myeloma Neoplasia	Public	-
			Cohort (65, 70, 75)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Italy	PCV13/PPV23	Sicilia (2012)	At risk (50-64)	Asplenia Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Metabolic disease	Public	-
			Cohort (65, 75)	N/A		
		Trento (2012)	At risk or nursing home residents (any age)	Asplenia Chronic cardiac disease Chronic renal failure Cochlear implant COPD Diabetes HIV Immunodeficiency (congenital) Immunosuppression Liquor leakage Nephrotic syndrome SCID	Public	-
			All (>65)	N/A		
Italy	PCV13/PPV23	Tuscany LHU (Local Directive to GPs – April 2012) (2012)	At risk or in permanent institutional care (≥6)	Asplenia Cancer (haematological, solid) Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Immunodeficiency (primary) Metabolic disease Transplantation (organ)	Public	-
			All (≥50)			
Italy	PCV13/PPV23	Umbria (2012)	At risk or in permanent institutional care (≥50)	Asplenia Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Metabolic disease	Public	-

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Italy	PCV13/PPV23	Veneto (2012)	At risk (any age)	Asplenia Cancer (haematological, solid) Chronic disease (heart, kidney [renal failure], liver [hepatic cirrhosis], respiratory) CNS disease Immunodeficiency (primary) Metabolic disease Transplantation (organ)	Public	-
Italy	PPV23	National (2005)	At risk (any age)	Agammaglobulinemia Asplenia Asthma Autoimmune disease Cancer (haematological, solid tumour) Chronic disease (heart, kidney, liver, respiratory) Cyanotic heart disease Immunodeficiency (primary) Metabolic disease SCID Transplantation (organ)	Public	-
Luxembourg	PCV13	National (2011)	At risk (<5)	Asplenia Chronic disease (heart, liver, renal, respiratory [excluding asthma]) Cochlear implant CSF leak Diabetes HIV Immunocompromised Premature birth		
	PPV23	National (2008)	At risk or in permanent institutional care (≥18)	Alcoholism Asplenia Chronic disease (cardiovascular, renal, respiratory) Cirrhosis Cochlear implant CSF leak Diabetes HIV Liquor fistula Lymphoma Multiple myeloma Nephrotic syndrome Sickle-cell disease Transplantation (organ)	Private	-
			All (>60)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Netherlands	PCV13/PPV23	National (2012)	At risk (any age)	Asplenia	Private	1 Dose of PCV13 followed by 1 dose of PPV23 after ≥8 weeks PPV23 should be repeated once after 5 years
Norway	PCV13	National (2013)	At risk (any age)	Asplenia HIV Stem cell transplantation Considered for following groups after collective evaluation of risk: B-cell deficiency Cancer (haematological) Cochlear implant CSF leak Transplantation (organ, bone marrow)	Public (for asplenia, HIV, and stem cell transplantation only)	PCV13 recommended only in addition to PPV23 Administer PCV13 ≥8 weeks prior to PPV23 For asplenia and HIV administer PPV23 in addition to PCV13 Repeat PPV23 every 5 years for asplenia and every 10 years for other risk groups
	PPV23		At risk (any age)	Asplenia B-cell deficiency Cancer (haematological) Cochlear implant CSF leak HIV Transplantation (organ, bone marrow)		
			All (≥65)	N/A		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Portugal	PCV13	National (2010)	At risk and high risk (<5, 59 months)	**High risk** Asplenia (anatomical, functional) Cochlear implant or cochlear implant placement planned Down syndrome HIV infection Premature birth (≤28 weeks) Sickle-cell disease and other haemoglobinopathies **Presumable high risk** Acquired immunodeficiency Immunosuppressive therapy, prolonged corticosteroid therapy, chemotherapy, or radiotherapy Haematological cancer, mainly lymphocytic leukaemia (acute and chronic), Hodgkin disease and multiple myeloma **Bone marrow donor** **Chronic disease** (cardiac [cyanotic congenital cardiopathy, heart failure], liver, pulmonary [excluding asthma, except patients on high doses of corticosteroids]) **Chronic renal failure** **Congenital immunodeficiency** **Diabetes** **CSF fistula** (congenital malformation, cranial fracture, or neurosurgery procedure) **Nephrotic syndrome** **Organ or bone marrow transplantation**	Public	

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Portugal	PPV23		At risk and high risk (2-17)	**High risk** Asplenia (anatomical, functional) Cochlear implant or cochlear implant placement planned Down syndrome HIV infection Premature birth (≤28 weeks) Sickle-cell disease and other haemoglobinopathies **Presumable high risk** Acquired immunodeficiency Immunosuppressive therapy, prolonged corticosteroid therapy, chemotherapy, or radiotherapy Haematological cancer, mainly lymphocytic leukaemia (acute and chronic), Hodgkin disease and multiple myeloma **Bone marrow donor** **Chronic disease** (cardiac [cyanotic congenital cardiopathy, heart failure], liver, pulmonary [excluding asthma, except patients on high doses of corticosteroids]) **Chronic renal failure** **Congenital immunodeficiency** **Diabetes** **CSF fistula** (congenital malformation, cranial fracture, or neurosurgery procedure) **Nephrotic syndrome** **Organ or bone marrow transplantation**		
Spain	PCV13	National (2012)	At risk (≥50)	Cancer (haematological) Chemotherapy or immunosuppressive treatment HIV Nephrotic syndrome Renal insufficiency Transplantation (organ, haematopoietic cell)	Public	

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Spain	PCV13	Cataluña (2014)	At risk (≥5)	Asplenia or asplenic dysfunction Cancer (haematological) Cochlear implant CSF leak HIV Immunodeficiency (congenital, acquired) Immunosuppressive treatment, including systemic steroids and radiotherapy Nephrotic syndrome Renal insufficiency Sickle-cell disease Transplantation		
		Galicia (2012)	At risk (≥50)	Asplenia Cancer (haematological) Chemotherapy or immunosuppressive treatment Chronic renal disease (stage C3) Cochlear implant CSF leak HIV Nephrotic syndrome Transplantation (organ, haematopoietic cell)		
		Murcia (2014)	At risk (≥6)	Asplenia or asplenic dysfunction B- or T-cell deficiency Cancer (haematological) Chemotherapy or radiotherapy Chronic liver disease (including cirrhosis) Chronic renal insufficiency (advanced) Complement deficiency Haemodialysis History of IPD HIV Phagocytosis dysfunction Transplantation (organ, haematopoietic cell)		
			At risk (6-50)	Cochlear implant CSF leak		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Spain	PCV13	Basque Country (2013)	At risk (≥50)	Asplenia Cancer (haematological) Chemotherapy or immunosuppressive treatment Chronic renal insufficiency (advanced) Cochlear implant CSF leak Haemodialysis History of IPD HIV Immunodeficiency (congenital, acquired) Transplantation (organ, haematopoietic cell)		
		Valencia (2013)	At risk (≥18)	Asplenia or asplenic dysfunction B- or T-cell deficiency Cancer (haematological) Chemotherapy or radiotherapy Chronic renal disease (stage C3) Complement deficiency Cochlear implant CSF leak Haemodialysis HIV Nephrotic syndrome Phagocytosis dysfunction Transplantation (organ, haematopoietic cell)		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Spain	PCV13	Madrid (2013)	At risk (≥50)	Asplenia (including elective splenectomy and late complement component deficiency) Cancer (haematological) Chemotherapy or immunosuppressive treatment Chronic alcoholism Chronic liver disease Cirrhosis Coagulation factor concentrate recipients Cochlear implant CSF leak Haemodialysis HIV Nephrotic syndrome Renal disease (end-stage) Renal insufficiency Sickle-cell disease Transplantation (organ, haematopoietic cell)		
		Navarra (2013)	At risk (≥18)	Asplenia Cancer (haematological) Chemotherapy or immunosuppressive treatment HIV Nephrotic syndrome Renal insufficiency (severe) Transplantation (organ, haematopoietic cell)		
		Extremadura (2013)	At risk (≥50)	Cancer (haematological) Chemotherapy or immunosuppressive treatment HIV Nephrotic syndrome Renal insufficiency Transplantation (organ, haematopoietic cell)		

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Spain	PPV23	All Spanish autonomous regions (varies)	At risk or older adults in permanent institutional care (≥2 to ≤60/65)	Alcoholism Asplenia Cancer (haematological) Chronic disease (cardiovascular, respiratory) Cirrhosis Cochlear implant Diabetes HIV Nephrotic syndrome Renal insufficiency Sickle-cell disease Transplantation (organ)		Funded by Public Health of the different Spanish Regions Date of implementation differs between the 19 different autonomous regions
		Most Spanish autonomous regions (varies)	All (≥60/≥65)	N/A		Recommended vaccination by age at time of influenza vaccination campaign Date of implementation varies between different regions
Sweden	PCV13/PPV23	Stockholm (2013)	At risk (≥2)	Asplenia Cochlear implant Cystic fibrosis Immunosuppression (e.g. transplantation, receiving cytostatics or other medication severely affecting the immune system) Liquor fistula Nephrotic syndrome Transplantation (organ)	Public (for high-risk individuals)	Regional recommendations for high-risk individuals in Stockholm, PCV13 recommended followed by PPV23 after ≥8 weeks

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
Sweden	PPV23	National (1994)	At risk (≥2)	Agammaglobulinemia Alcoholism Asplenia Asthma Autoimmune disease Cancer (haematological, solid tumour) Chronic disease (heart, kidney, liver, respiratory) Cyanotic heart disease CNS disease CSF leak Haemodynamically significant residual lesion after surgery Haemodynamic respiratory insufficiency History of IPD HIV Immunodeficiency (primary) Intracranial shunt Metabolic disease SCID Sickle-cell disease and other haemoglobinopathies Transplantation (organ)	Varies	Funding is decided by the local county council, in some areas vaccination of individuals aged ≥65 years is free of charge, in other areas it is partially subsidised, and in the remainder the full cost is paid by the individual
			All (≥65)	N/A		
United Kingdom	PCV13	National (2013)	At risk (<5)	Asplenia Asthma (only if high-dose systemic steroids) Cancer (haematological, solid tumour) Chronic disease (heart, kidney, liver, respiratory) Cochlear implant CSF leak Diabetes (excludes diet controlled) HIV Immunosuppression Sickle-cell disease Transplantation (organ)	Via the National Health Service	-

Comparison of the recommendations and funding for pneumococcal immunisation outside routine vaccination programmes for children in Western European countries

Country	Recommended vaccine	Region (date of recommendation)	Population (age, years)	Definition of risk	Funding	Additional information
United Kingdom	PCV13		At risk – severely immuno-compro-mised (≥5)	Genetic disorders severely affecting the immune system (e.g. IRAK-4, NEMO, complement deficiency) Leukaemia (acute, chronic) Multiple myeloma Transplantation (bone marrow)		
United Kingdom	PPV23	National (1992)	At risk (≥2)	Asplenia Asthma (only if high-dose systemic steroids) Cancer (haematological, solid tumour) Chronic disease (heart, kidney, liver, respiratory) Cochlear implant CSF leak Diabetes (excludes diet controlled) HIV Immunosuppression Sickle-cell disease Transplantation (organ)		
		National (2003)	All (≥65)	N/A		

CNS Central nervous system, COPD chronic obstructive pulmonary disease, CSF cerebrospinal fluid, GPs general practitioners, HIV human immunodeficiency virus, IPD invasive pneumococcal disease, LHU local health unit, N/A not applicable, PCV pneumococcal conjugate vaccine, PPV pneumococcal polysaccharide vaccine, SCID severe combined immunodeficiency disease

Source: Castiglia P. Adv. Ther.2014; 31:1011-1044 (with permission)

8.3 Annex 3

Definition of the study variables

Variable	Definition
Technical fields	
1. RecordID	Identification of the record in the database
2. RecordType	Internal identification of the metadataset
3. RecordTypeVersion	Version of the metadataset
4. Subject	Disease of interest
5. Status	Status
6. DataSource	Data source of origin (in each country)
7. DateUsedForStatistics	Date preferred for statistics (as selected by each country)
8. ReportingCountry	Reporting country
9. NRLData	Data from National Reference Laboratory (yes or no)
Epidemiological variables	
10. DateOfNotification	Date of notification
11. PlaceOfNotification	Place of notification
12. PlaceOfResidence	Place of residence
13. Age	Age in `years´
14. AgeMonth	Age in `months´ (up to 12 months)
15. Gender	Gender
16. DateOfDiagnosis	Date of diagnosis
17. Outcome	Outcome (`dead´ or `alive´)
18. Classification	Case classification according to the case definition (possible, probable, confirmed)
19. ClinicalPresentation	Clinical presentation of the case
20. VaccStatus	Vaccination status of the case
21. VaccType	Vaccine type (if vaccinated)

Variable	Definition
Laboratory variables	
22. DateOfSpecimen	Date of collection of the specimen
23. Specimen	Type of specimen
24. Serotype	Serotype
25. TestMethodTyping	Method used for serotyping
26. ResultMICValuePEN	MIC value for penicillin
27. ResultMICValueERY	MIC value for erythromycin
28. ResultMICValueCTX	MIC value for cefotaxime
29. ResultMICSign_PEN	MIC sign (>, <, =, ≤, ≥) for penicillin
30. ResultMICSign_ERY	MIC sign (>, <, =, ≤, ≥) for erythromycin
31. ResultMICSign_CTX	MIC sign (>, <, =, ≤, ≥) for cefotaxime
32. TestMethodMIC	Method used for antimicrobial susceptibility testing
33. SIR_PEN	Sensitive, intermediate of resistant to penicillin
34. SIR_ERY	Sensitive, intermediate of resistant to erythromycin
35. SIR_CTX	Sensitive, intermediate of resistant to cefotaxime

Distribution of reported IPD cases by serotype and country, EU/EEA countries, 2010 (n=9946*)

Country	Serotype 19A**		Serotype 1@		Serotype 7F~		Serotype 3#		Serotype 14&		Serotype 22F^		Serotype 8^		Serotype 4$		Serotype 12F^		Serotype 19F£		Other	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Austria	22	8	14	5	19	7	44	15	25	9	9	3	5	2	13	5	3	1	14	5	120	42
Belgium	124	7	279	15	108	6	124	7	35	2	32	2	56	3	28	2	50	3	4	0	1011	55
Cyprus	0	0	4	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	60
Czech Republic	8	3	42	14	18	6	51	17	16	5	5	2	5	2	16	5	3	1	9	3	121	41
Denmark	72	8	170	18	89	9	73	8	22	2	56	6	57	6	48	5	39	4	15	2	319	33
Finland	31	4	1	0	49	6	77	10	147	18	46	6	5	1	73	9	2	0	49	6	317	40
France	168	15	93	8	163	14	79	7	25	2	36	3	21	2	6	1	80	7	30	3	426	38
Greece	2	8	1	4	0	0	4	17	0	0	0	0	0	0	0	0	0	0	0	0	17	71
Hungary	9	8	1	1	3	3	36	34	1	1	1	1	1	1	4	4	1	1	2	2	48	45
Ireland	18	7	7	3	21	9	12	5	13	5	19	8	23	9	9	4	7	3	8	3	109	44
Italy	26	9	42	15	35	13	40	14	13	5	9	3	7	3	8	3	3	1	8	3	85	31
Lithuania	0	0	0	0	0	0	0	0	1	33	0	0	0	0	0	0	0	0	0	0	2	67
Malta	0	0	0	0	0	0	1	14	1	14	0	0	2	29	0	0	0	0	0	0	3	43
Netherlands	11	24	6	13	4	9	2	4	0	0	0	0	3	7	0	0	0	0	0	0	19	42
Poland	9	4	11	5	5	2	21	10	28	14	3	1	5	2	10	5	6	3	22	11	85	41
Romania	0	0	5	24	2	10	4	19	0	0	0	0	0	0	0	0	0	0	4	19	6	29
Slovenia	18	8	16	7	14	6	35	16	32	14	3	1	3	1	12	5	0	0	5	2	86	38
Spain	321	15	169	8	244	11	238	11	119	5	82	4	86	4	49	2	60	3	49	2	795	36
UK	71	14	63	13	88	18	36	7	5	1	36	7	45	9	1	0	10	2	5	1	137	28
Iceland	2	6	1	3	1	3	2	6	4	13	0	0	0	0	2	6	0	0	4	13	16	50
Norway	79	11	53	7	103	14	49	7	16	2	89	12	19	3	30	4	2	0	11	2	268	37
Total	**991**		**978**		**966**		**928**		**503**		**426**		**343**		**309**		**266**		**239**		**3996**	

** Serotype 19A protected against by the 13-valent vaccine and PPV23
£ Serotype 19F protected against by the 7, 10 and 13-valent vaccines & PPV23
$ Serotype 4 protected against by the 7, 10 and 13-valent vaccines & PPV23
@ Serotype 1 protected against by 10 and 13-valent vaccines and PPV23
# Serotype 3 protected against by the 13-valent vaccine and PPV23
^ Serotype protected against by the PPV23
Serotype 7F protected against by 10 and 13-valent vaccines and PPV23
& Serotype 14 protected against by the 7, 10 and 13-valent vaccines & PPV23
* 9 946 cases with reported serotype

8.5. Annex 5

Publications

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RESPIRATORY INFECTIONS (F ARNOLD, SECTION EDITOR)

Epidemiologic and Clinical Implications of Second-Generation Pneumococcal Conjugate Vaccines

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Abstract This review is based on published literature about some of the potential advantages and challenges of the second generation of pneumococcal conjugate vaccines, with special reference to 13-valent vaccine in children and adults.

Keywords Epidemiologic · Clinical implications · Second-generation pneumococcal conjugate vaccines

Introduction

Pneumococcal disease remains a major public health problem across the world in the XXI century. The etiological agent *Streptococcus pneumoniae* (pneumococcus) is still the most common cause of bacterial community-acquired pneumonia (CAP) and meningitis in developed countries, often causing sequelae and death [1•]. More than 800,000 children die of pneumococcal disease every year, the majority by pneumococcal pneumonia in developing countries [2•]. In addition, *S. pneumoniae* is by far the leading cause of pneumonia and acute otitis media in children in developed countries [3, 4]. Among elderly patients, pneumococcus is the most common microorganism isolated from patients with CAP [5].

The differences in the composition of the capsule of pneumococcus permit serological differentiation among 94 capsular types, of which only 30–40 of the serotypes are associated with pneumococcal disease. Capsular characteristics have been related to differences in properties, such as carriage prevalence, propensity to cause invasive disease, and lethality [6]. Differences in pathogenicity and clinical impact of different serotypes are relevant from the public health viewpoint, since available vaccines are serotype specific and target a reduced number of serotypes.

The first vaccines to be licensed against a limited number of *S. pneumoniae* serotypes were pneumococcal polysaccharide vaccines (PPSVs). The 14-valent vaccine was first introduced in 1977, being replaced in 1983 by the 23-valent PPSV (PPSV23) [7]. PPSV23 induces a T-cell independent response and does not elicit immunological memory, so there is no anamnestic or booster response to revaccination. Moreover, reimmunization has been associated with decreased response [8]. These immunological characteristics explain that PPSV23 does not induce an effective immune response in younger children.

In 2000, a conjugate vaccine (PCV7) against seven of the most frequent serotypes (4, 6B, 9V, 14,18C, 19F, and 23F) associated with invasive disease was licensed for use in children less than 5 years of age in the U.S. A large clinical trial demonstrated significant protection against invasive pneumococcal disease, especially bacteraemia, but also

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against pneumonia and otitis media [9–11]. Over the subsequent 10 years, PCV7 was introduced in many other countries, mostly in the developed world. In 2007, the World Health Organization (WHO) recommended the inclusion of PCV7 in national immunization programs of developing countries with high rates of childhood mortality. Postlicensure surveillance across countries has documented significant reductions in PCV7-type invasive pneumococcal disease (IPD) and carriage, especially in the age group targeted for vaccination (direct effects), but also in non-vaccinated groups (indirect effects) [12–14]. Simultaneously, while rates of PCV7-type IPD have declined, rates of IPD caused by non-PCV7 serotypes have increased in many settings [15–17]. The increase in non-PCV7 serotypes may be a multifactorial phenomenon, and it is unclear to what extent it could be attributed to vaccination. Nevertheless, this increase has partially eroded the recognized benefits of PCV7. One of the emergent serotypes has been serotype 19A. Multidrug-resistant strains expressing serotype 19A (i.e., clonal type ST320) are of special concern because some of these strains have developed resistance to many oral antimicrobial agents, except for linezolid and levofloxacin [18].

The association of different serotypes with different clinical manifestations is well known [19]. In the vaccine era, the change of serotypes causing IPD has been related to a change in clinical types of IPD [20]. While the proportions of pneumococcal bacteremia and meningitis have declined in the majority of countries, an increase of pneumonia and empyema has been reported worldwide; this increase has mainly been associated with serotypes 1,19A, and 3 [21–24].

Vaccination is the most effective tool for preventing pneumococcal disease. Due to the limitations of PCV7 in preventing these emergent serotypes, two new pneumococcal conjugate vaccines have been licensed: a 10-valent pneumococcal conjugate vaccine (PCV10, which includes the seven serotypes of PCV7 and serotypes 1, 5, and 7F) and a 13-valent pneumococcal conjugate vaccine (PCV13 contains PCV10 serotypes and additional serotypes 3, 6A, and 19A). In 2012, WHO issued a position paper on the use of PCV7 that replaced the former one published in 2007. WHO recommended the inclusion of new pneumococcal conjugate vaccines in childhood immunization programs worldwide, because the inclusion of these additional serotypes is an important progress against pneumococcal disease [25].

In adults, PPSV23 has been available for more than 25 years, but the impact of this vaccine in the target population has been limited [12] . In the U.S., this vaccine has been recommended for all adults ≥65 years of age since 1983, and in the last 10 years, the vaccination rate has been around 60 % [26]. Despite this fact, the burden of disease in the vaccine target population remains high, with more than 30,000 IPD episodes, including a high proportion caused by vaccine serotypes [27].

PCV13 offers protection against the major serotypes causing IPD. The Centers for Disease Control and Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP) recommend routine vaccination with PCV13 for infants and young children [28]. Other committees around the world have also made some recommendations to introduce PCV13 for the prevention of pneumococcal infections in children [29, 30].

Recently, ACIP extended the recommendation of PCV13 to adults ≥19 years of age with immunocompromising conditions, functional or anatomic asplenia, cerebrospinal fluid leaks, or cochlear implants. If the patient has not previously been vaccinated with any pneumococcal vaccine, ACIP recommends a first dose of PCV13, followed by a dose of PPSV23 at least 8 weeks later. For those previously vaccinated with PPSV23, they should be given a PCV13 dose ≥1 year after the last PPSV23 dose has been received. With regard to the additional doses of PPSV23, the first such dose should be given no sooner than 8 weeks after PCV13 and at least 5 years after the most recent dose of PPSV23 [31].

What Is Expected About PCV13 Vaccine?

A Safe, Immunogenic, and Tolerable Vaccine

Different clinical trials have proved PCV13 to be at least as safe, immunogenic, and tolerable as PCV7 [32–35]. These and other studies [36] show that the majority of unwanted reactions to vaccines were mild fever, temporary loss of appetite, or redness at the injection site.

Postlicensure surveillance might be deemed necessary with regard to immunogenicity for serotype 3 because clinical trials showed lower immunogenicity than for other serotypes, which may have an effect on long-term efficacy of the vaccine against serotype 3-caused disease [36]. Other PCV13 serotypes that probably need specific postlicensure surveillance are serotypes 1 and 5 because these serotypes are not commonly detected in nasopharyngeal carriage in children (the main target of the vaccine), and thus other patterns of transmission not preventable by vaccination could be expected.

PCV13 is the first and only pneumococcal conjugate vaccine licensed for use in adults 50 years of age or older. The extension of therapeutic indications for PCV13 in the prevention of IPD was based on noninferiority to PPSV23 . In adults previously vaccinated with PPSV23, a certain hyporesponsiveness to PCV has been reported, when compared with those vaccinated with PCV without prior vaccination with PPSV23 [8]. Nevertheless, this does not seem to have important clinical consequences.

Better Coverage than PCV7, but with Dramatic Differences Between Countries

A high coverage with PCV13 vaccine is expected. A number of developed countries have included the new conjugate vaccines in their vaccination programs [25].

In addition, a better introduction of the new vaccine is expected than was obtained with PCV7 in developing countries according to GAVI Alliance expectations. GAVI is a public–private partnership (including WHO, UNICEF, and the Bill and Melinda Gates Foundation) focused on protecting people's health by increasing access to immunization in poor countries. In May 2011, a total of 46 GAVI-eligible countries were approved for GAVI support to introduce the new pneumococcal conjugate vaccine in their national immunization programs. Presumably, 90 million children will be immunized by 2015 [37]. Of note, important differences about coverage of PCV13 are observed in developed countries greatly depending on national policies to provide immunization services. Nonetheless, there is concern about a potential reduction in pneumococcal vaccine coverage due to the economic crisis, particularly in those countries where pneumococcal vaccination is neither universal nor reimbursable by their governments.

A progressive coverage of PCV13 in adults ≥50 years of age is expected because the immunologic properties of conjugate vaccine suggest that PCV might offer advantages over PPSV23 in adults. Additionally, serotypes included in PCV13 are causing a high percentage of IPD in adults. A recent document of the Vaccines and Related Biological Products Advisory Committee (VRBPAC) about the indication of PCV13 in adults concludes that there is a need to improve the current pneumococcal vaccine strategies in the adult population and that PCV13 could help achieve this objective [38].

Impact on the Global Burden of Disease

Due to the fact that PCV13 is designed against the main serotypes causing disease, and the high introduction of the vaccine in numerous countries, a positive impact on reducing the global burden of disease is expected. A recent review article on serotype distribution worldwide found that serotypes included in PCV13 cause more than 70 % of all IPD episodes worldwide, with a range of 74 % in Asia to 88 % in Europe [39]. A study performed in Alaskan native children less than 5 years showed a significant decrease of global IPD incidence after PCV13 introduction. Intriguingly, the decrease was observed in both PCV13 serotypes and non-PCV13 serotypes. A transitory decrease of PCV7 and non-PCV7 serotypes was also reported in the same population after introduction of PCV7 in 2001 [40•]. Another study by the CDC has demonstrated a decline in the incidence of IPD after the introduction of PCV13 in children younger than 2 years [41]. Mathematic models have predicted that the administration of a catch-up dose for all children 14–59 months of age who had previously been fully vaccinated with PCV7 would significantly reduce cases of pneumococcal disease, reduce vaccine-preventable deaths, and increase quality-adjusted life-years within the U.S. population [42, 43]. In the same direction, Miller et al. [44•] recently presented the efficacy of PCV13-specific serotypes against IPD in the U.K.

Regarding adult vaccination with PCV13, there are not controlled clinical trials in adults to date showing evidence of decrease of IPD or nonbacteremic pneumococcal pneumonia following vaccination with PCV13. However, the efficacy of PCV13 in preventing CAP is being assessed by the Community-Acquired Pneumonia Immunization Trial in Adults (CAPITA) [45]. Despite the lack of knowledge of the public health and economic impact of PCV13 on this age group, a recent modeling analysis conducted in the U.S. predicted that using PCV13 instead of PPSV23 would result in a significant reduction of IPD and nonbacteremic pneumococcal pneumonia and savings in health-care and societal costs, on the basis of the assumption that the effectiveness of PCV13 in adults is similar to that of PCV7 in children [46•]. Furthermore, a German health economic study has estimated that the implementation of PCV13 in adults, as compared with PPSV23, would avoid a greater number of cases and deaths due to IPD and CAP and would be cost effective, as compared with PPSV23 vaccination and no-vaccination [47].

Impact on Clinical Manifestations

The impact of PCV7 was different according to clinical manifestations. While a decrease of bacteremia was observed quite consistently in different countries [48, 49], heterogeneous data were available for meningitis [50, 51] and, especially, for pneumonia (including complicated pneumonia with empyema) and otitis. The significant increase of empyema due to epidemic serotypes not included in PCV7 was of special concern in some geographical areas. These epidemic serotypes, such as serotypes 1 and 5 and other serotypes (e.g., serotype 19A) also related to an increase of empyema, are included in PCV13. So, the new vaccine should have a positive impact in preventing this clinical manifestation. A preliminary study performed in Northern England reported a decrease of pediatric empyema after introduction of PCV13 [52].

The impact of pneumococcal conjugate vaccination on otitis remains unclear [53], and this also applies to the new conjugate vaccines. The human nasopharynx is an ecologic reservoir of multiple pathogens, and interactions between them occur. A recent study showed a competitive

association between *Haemophilus influenzae* and *S. pneumoniae*; thus, the authors argued that *H. influenzae* might become a more common cause of acute otitis media among children who receive pneumococcal conjugate vaccine [54].

Apart from increases in nontypable *H. influenzae* [55], *S. aureus* might also be filling the niche left by the suppression of the vaccine serotypes. Van Gils et al. [56•], in a randomised controlled trial, found that PCV7 vaccination induced an increase in *S. aureus* colonisation. Another recent Dutch study [57] concluded that *S. aureus* carriage was inversely associated with non-vaccine-type *S. pneumoniae* carriage. Serotype 19A showed higher *S. aureus* co-carriage rates, as compared with other serotypes. Higher valent vaccines are predicted to have an effect on overall pneumococcal carriage, and the clinical significance of other bacterial species replacement remains unknown. Nonetheless, close monitoring and surveillance are needed, particularly now in the era of multidrug-resistant strains.

On the other hand, a decrease of the major serotypes causing pneumococcal disease may modify pneumococcal–respiratory viral interactions because a synergism or association between some pneumococcal serotypes and respiratory virus has been observed by different authors [58, 59].

Impact on Antimicrobial Resistance

Studies performed just before the introduction of PCV13 show that serotypes covered by this vaccine cause the majority of penicillin nonsusceptible IPDs. A study carried out in the Active Bacterial Core surveillance areas of the U.S. during 2007–2008 found that serotypes in PCV13, but not in PCV7, caused 78 %–97 % of penicillin nonsusceptible IPD, depending on age [60]. The main serotype related to antimicrobial resistance was serotype 19A, as reported by other authors [61, 62]. Furthermore, PCV13 has demonstrated an ability to induce opsonophagocytic activity (OPA) to serotype 19A that translates to a potentiality to prevent serotype 19A invasive disease, which has been described as multidrug resistant [63]. In adults, for serotypes common to both vaccines, PCV13 elicits greater OPA responses than PPSV23 in the majority of serotypes and noninferior in the remainder [38].

PCV13 could be a notable progress in reducing rates of disease and antimicrobial resistance, but the use of vaccine must be accompanied by a judicious use of antibiotics and the availability of screening diagnostic tests [64]. Reductions in disease prevalence and appropriate use of antibiotics may decrease carriage of resistant pneumococcal strains in children [65]. However, continuous surveillance is needed

Table 1 Potential advantages and challenges of the new pneumococcal conjugate vaccines

	Vaccine	Evidence
Advantages		
• Reduction of pneumococcal burden of disease by targeting additional serotypes (1, 5, 7 F in PCV10, plus 3, 6A, 19A in PCV13)	PCV10, PCV13	Esposito et al., 2010 Vesikari et al., 2012 Martín-Arias et al., 2012 Singleton et al., 2012 Moore et al., 2012 Miller et al., 2011
• Potential cross-protection against vaccine-related serotypes (6C, 6D, 7 F)	PCV13	Nahm et al., 2009 Cooper et al., 2011 McEllistrem et al., 2012
• Use in adults ≥50 years and younger adults with comorbidities	PCV13	Elek et al., 2000 Weycker et al., 2012 Kobayasi et al., 2012 De Roux et al., 2008
• Decrease in antibiotic usage and resistance	PCV10, PCV13	Hampton et al., 2012 Gladstone et al., 2011
Challenges		
• Serotype replacement	PCV10, PCV13	van Hoek et al., 2012
• Nonpneumococcal bacterial replacement	PCV10, PCV13	Xu et al., 2012 De Wals et al., 2009 Dunne-Meijnen et al., 2012 van Gils et al., 2011
• Potential selection of highly resistant serotypes	PCV10, PCV13	Wiley et al., 2012

to monitor how vaccination strategies affect the prevalence of resistant pathogens and to anticipate the potential selection of highly resistant strains.

Emergence of Serotypes not Included in PCV13

At present, serotypes included in PCV13 are those that are causing the majority of the burden of the disease. However, as occurred with PCV7, the use of PCV13 might be accompanied by an increase in non-PCV13 serotypes in healthy carriers and, subsequently, cause of disease (serotype replacement). Nevertheless, the magnitude of the predictable serotype replacement is uncertain. Among carriers, the impact of PCV13 remains unclear. A preliminary study performed among PCV13-vaccinated children with otitis showed a significant decrease in nasopharyngeal carriage of 19A, 7 F, and 6C serotypes without emergence of new serotypes [66]. However, other authors have reported an increase of non-PCV13 serotypes before the introduction of this vaccine, and probably these emergent serotypes continue to increase. For example, since the introduction of PCV7, nasopharyngeal carriage of serotype 15A has increased [67], and this serotype has also been reported as responsible for outbreaks [68]. Remarkably, serotype 22 F not covered by PCV13 has experienced an increase in the U.K. [69], where PCV13 was introduced in January 2010. Therefore, this particular non-PCV13 serotype and others merit a careful monitoring in the PCV13 era [70•].

Despite the probable serotype replacement, the benefit of PCV13 for reducing pneumococcal disease leaves no doubt that its use might represent a great contribution. Table 1 reports some of the potential advantages and challenges of the new pneumococcal conjugate vaccines. Notwithstanding, efforts to develop low-cost and broad serotype-independent vaccines are warranted. Recent data suggest that some vaccine candidates based on conserved pneumococcal proteins could be the future response in the prevention of pneumococcal disease [71].

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European enhanced surveillance of invasive pneumococcal disease in 2010: Data from 26 European countries in the post-heptavalent conjugate vaccine era

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ABSTRACT

Streptococcus pneumoniae is a leading cause of severe infectious diseases worldwide. This paper presents the results from the first European invasive pneumococcal disease (IPD) enhanced surveillance where additional and valuable data were reported and analysed. Following its authorisation in Europe in 2001 for use in children aged between two months and five years, the heptavalent pneumococcal conjugate vaccine (PCV7) was progressively introduced in the European Union (EU)/European Economic Area (EEA) countries, albeit with different schemes and policies. In mid-2010 European countries started to switch to a higher valency vaccine (PCV10/PCV13), still without a significant impact by the time of this surveillance. Therefore, this surveillance provides an overview of baseline data from the transition period between the introduction of PCV7 and the implementation of PCV10/PCV13.

In 2010, 26 EU/EEA countries reported 21 565 cases of IPD to The European Surveillance System (TESSy) applying the EU 2008 case definition. Serotype was determined in 9 946/21 565 (46,1%) cases. The most common serotypes were 19A, 1, 7F, 3, 14, 22F, 8, 4, 12F and 19F, accounting for 5 949/9 946 (59,8%) of the serotyped isolates. Data on antimicrobial susceptibility testing (AST) in the form of minimum inhibitory concentration (MIC) were submitted for penicillin 5 384/21 565 (25,0%), erythromycin 4 033/21 565 (18,7%) and cefotaxime 5 252/21 565 (24,4%). Non-susceptibility to erythromycin was highest at 17,4% followed by penicillin at 8,9%.

PCV7 serotype coverage among children <5 years in Europe, was 19,2%; for the same age group, the serotype coverage for PCV10 and PCV13 were 46,1% and 73,1%, respectively.

In the era of pneumococcal conjugate vaccines, the monitoring of changing trends in antimicrobial resistance and serotype distribution are essential in assessing the impact of vaccines and antibiotic use control programmes across European countries.

1. Introduction

Streptococcus pneumoniae infections are a major public health threat and cause high morbidity and mortality worldwide especially among children under 5 years and amongst the elderly [1,2]. It is the leading cause of bloodstream infection (BSI), meningitis, upper respiratory tract infections and otitis media [2]. It is the most frequent causative agent of community acquired pneumonia (CAP), resulting in high case-fatality ratios (CFR) [3].

S. pneumoniae is surrounded by a polysaccharide capsule that protects the bacterium from phagocytosis and intracellular killing and therefore is an important virulence factor [4]. Based on differences in the capsule and recognition by different specific antibodies, 93 serotypes with different invasiveness and mortality potential have been identified [5,6].

Different medical practices [7] and country differences in reporting and surveillance systems of IPD may well explain the large variation of IPD notification rates from 0,4 to 20 cases per 100 000 population per year [8] between European countries that have been reported previously [9].

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[1] See Appendix A.

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The introduction of PCV7 targeting children less than 5 years of age has proven highly successful in reducing invasive and mucosal disease caused by the vaccine serotypes and in decreasing antibiotic resistance associated with vaccine serotypes [10]. An additional benefit of the PCV is the decrease in nasopharyngeal carriage of vaccine serotypes that confers a degree of herd immunity to the population [11]. Nevertheless, this success may be partially offset by an increase in non-vaccine serotypes [12,13]. Furthermore, antimicrobial resistance has emerged and spreads in these non-vaccine serotypes [14].

In response, new pneumococcal conjugate vaccines (PCV10, PCV13) that include additional serotypes have been licensed and EU/EEA countries started introducing them gradually since 2010. The impact of pneumococcal conjugated vaccines and the burden of pneumococcal infections should be closely monitored and better quality data should be analysed in order to assess vaccine strategies throughout Europe. Moreover, it may prove useful to indicate where new expanded valency vaccines should be developed in response to serotype replacement observed after the implementation of PCV7 and as expected for PCV13 [15]. Here we report on the results from an analysis of data from the first enhanced surveillance programme for IPD set up by the European Centre for Disease Prevention and Control (ECDC) in collaboration with the EU/EEA Member States in order to assess the burden of IPD and the prevalence of the different serotypes across Europe.

2. Materials and methods

2.1. Scope

Twenty-six European countries participated to the surveillance for IPD from 1st January to 31st December 2010 inclusive, namely Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, The Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. This corresponded to approximately 82% of the total population of EU/EEA countries in 2010. A case of IPD was defined, in accordance with the EU 2008 case definition [16], as the isolation of *S. pneumoniae* or detection of *S. pneumoniae* nucleic acid or antigen from a normally sterile site of a patient.

2.2. Surveillance systems and case definitions

Notification of IPD is mandatory in 19 European countries: Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, Hungary, Iceland, Ireland, Latvia, Lithuania, Malta, The Netherlands, Norway, Poland, Slovakia, Slovenia, Spain and Sweden. It is voluntary in 5 countries: Belgium, France, Germany, Italy, and the United Kingdom [17]. Cyprus and France have a sentinel surveillance system while all other countries operate a comprehensive surveillance system [17]. The official EU 2008 [16] case definition for IPD was applied in 18 countries, Bulgaria applied the EU 2002 case definition [18], whereas the United Kingdom and Denmark applied other non-specified case definitions. All countries reported case-based data with the exception of Bulgaria that submitted aggregated data. Thus, it is highly unlikely that the vaccine change impacted this surveillance.

2.3. Vaccination programmes

PCV7 (including serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) received marketing authorisation in the EU in 2001. Since then it was gradually introduced in almost all EU/EEA countries though under different schemes and policies (Table 1). PCV10 (PCV7 serotypes plus 1, 5, 7F) and PCV13 (PCV10 plus 3, 6A, 19A) were licensed and EU/EEA countries started their implementation in mid-2010.

2.4. Data sources

ECDC experts in collaboration with European national experts developed an additional set of variables. EU/EEA countries reported to TESSy[2] these enhanced data on IPD for the first time in 2010.

2.5. Laboratory methods

In Europe, serotyping of pneumococcal strains is performed by various laboratory methods; Quellung, Pneumotest-Latex®, slide agglutination, multiplex PCR, coagglutination and gel diffusion. Quellung (83.6%) was the preferred technique for serotyping in Europe, followed by slide agglutination (21%) and Pneumotest-Latex® (11.6%). Serotype data were reported by Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Hungary, Ireland, Italy, Malta, The Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, the United Kingdom and Norway.

Fifteen countries reported data on MIC; Austria, Belgium, Cyprus, Denmark, Finland, France, Hungary, Ireland, Italy, Latvia, Poland, Romania, Slovenia, Spain and the United Kingdom. As an indication to the European Antimicrobial Resistance Surveillance Network (EARS-Net) report[3] for 2010, 68% of reporting laboratories in Europe used Clinical and Laboratory Standards Institute (CLSI)[4] standards whereas 29% applied the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.[5] According to these guidelines we considered as non-susceptible to penicillin isolates with MIC ≥0.12 mg/L, which is considered the cut-off value for meningeal isolates and the most used for surveillance studies. Antimicrobial gradient was the preferred method for AST of *S. pneumoniae* in most reporting European countries (68%), followed by agar dilution.

2.6. Data quality

Data were uploaded, validated and approved in TESSy by the countries. Individual datasets were further manually checked, validated and cleaned for inconsistencies, double reporting and impossible values.

2.7. Data analysis

Data comparisons were performed using the Pearson χ^2 test as appropriate. The notification rate was defined as the number of laboratory confirmed cases of IPD per 100 000 inhabitants. Population data for denominators were retrieved from the European Statistics (EUROSTAT) website.[6] STATA® 11.0 software was used to perform statistical tests and analysis.

3. Results

3.1. Epidemiology

In 2010, 21 585 cases of IPD were reported by 26 EU/EEA countries. Notification rates ranged from 0.3 in Lithuania to 17.4

[2] For more on this platform go to: http://ecdc.europa.eu/en/activities/surveillance/TESSy/Pages/TESSy.aspx.
[3] EARS-Net report 2010 data, http://www.ecdc.europa.eu/en/publications/Publications/1111_SUR_EARS_data.pdf.
[4] CLSI standards, http://www.clsi.org/Standards/.
[5] EUCAST guidelines, http://www.eucast.org/.
[6] EUROSTAT, http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home/.

Table 1
Characteristics of national pneumococcal vaccination programmes in EU/EEA countries in 2010.

Country	Date PCV7 introduction	Scope of PCV7 vaccination programme	Immunisation schedule	1st d (m)	2nd d (m)	3rd d (m)	4th d (m)	Vaccine coverage[d]	Year of measurement
Austria	July 2004	Universal	3+1 dose	3	5	7	12–24	–	–
Belgium	January 2005	Universal	2+1 dose	2	4	12		87	2010
Bulgaria	April 2010	Universal	3+1 dose(2+1 dose	2	3	4	12	–	–
Cyprus	August 2008	Universal	3+1 dose	2	4	8	12–15	–	–
Czech Republic	January 2010	Risk-based	3+1 dose	2	4	8	18	88,3	2010
Denmark	October 2007	Universal	2+1 dose	3	5	12		85	2010
Estonia	–	–	not decided	–	–	–	–	–	–
Finland	January 2010	Risk-based	2+1 dose	3	5	12		–	–
France	June 2006	Universal	2+1 dose	2	4	12		81	2008
Germany	July 2006	Universal	3+1 dose	2	3	4	11–14	52,8	2010
Greece	January 2006	Universal	3+1 dose	2	4	8	12–15	–	–
Hungary	October 2008	Universal	2+1 dose	2	4	15		81,1	2008
Iceland	December 2006	Risk-based	2+1 dose	3	5	12		–	–
Ireland	October 2002	Universal	2+1 dose	2	8	12		88	2008
Italy	May 2005	Universal/risk-based	2+1 dose	3	5	11		55	2008
Latvia	January 2010	Universal	3+1 dose	2	4	8	12–15	51	2010
Lithuania	–	–	3+1 dose	2	4	8	24	–	–
Luxembourg	February 2003	Universal	3+1 dose	2	3	4	12–15	88	2010
Malta	January 2007	Risk-based	3+1 dose	2	4	13	None	–	–
Netherlands	June 2006	Universal	3+1 dose	2	3	4	11	84	2008
Norway	July 2006	Universal	2+1 dose	3	5	12		88	2008
Poland	May 2008	Risk-based	3+1 dose(2+1 dose	NA	NA	NA	NA	1,78	2008
Portugal	June 2008	Risk-based	2+1 dose	2	4	12–15		52	2008
Romania[a]			3+1 dose	2	4	8	15–18		
Slovakia[b]	January 2008	Risk-based	2+1 dose	2	4	10		88,2	2008
Slovenia	September 2005	Risk-based	3+1 dose	2–3	4	8	24	–	–
Spain[c]	June 2001	Risk-based	3+1 dose	2	4	8	15	–	–
Sweden	January 2006	Universal	2+1 dose	3	5	12		–	–
United Kingdom	September 2006	Universal	2+1 dose	2	4	13		88	2010

[a] PCV7 was registered in September 2007 for voluntary use on a private basis.
[b] Universal as of April 2008.
[c] Universal introduction in the autonomous region of Madrid in November 2008.
[d] Source: VENICE II and WHO estimates of PCV7 coverage.

per 100 000 in Denmark (Table 2). The Nordic countries (Denmark, Norway, Finland and Sweden) and Belgium had the highest notification rates.

Of the 21 473 reported cases for which age information was provided, 45,5% were 65 years of age or older, 42,1% 15–64 years of age and 12,5% 0–14 years of age. The highest notification rates were reported among children below 1 year (18,5 per 100 000) followed by adults aged 85 years or older (15,8 per 100 000) (Fig. 1). Of the 21 486 reported cases with gender information, 54,9% (n = 11 798) occurred in men and 45,1% (n = 9 688) in women, corresponding to a male to female ratio of 1,2:1 (Fig. 1), although this difference was not statistically significant.

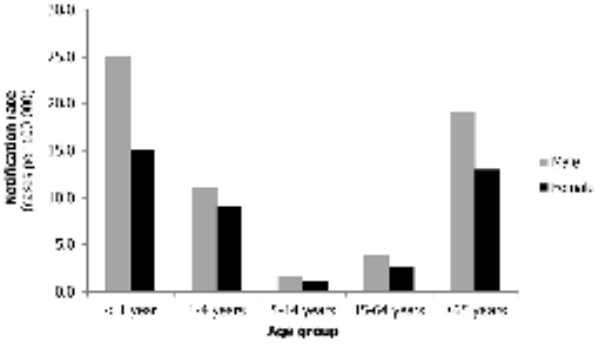


Fig. 1. Notification rate of IPD cases by age group and gender, EU/EEA countries, 2010 (n = 21 486).

The clinical presentation was available in 57,1% (n = 7 948) of cases. Non-meningitis was the most frequent clinical presentation for all age groups.

Overall the CFR was 9,8% and ranged from 0,0% for Cyprus, Lithuania and Malta to 28,9% for Hungary, but interpretation needs caution as the completeness for the variable outcome varied widely from country to country (data in Supplement Table S1). In children under 5 years of age, meningitis was the clinical presentation that accounted for the greatest number of deaths while non-meningitis was the major cause in the age group ≥65 years. The case fatality under 5 years was low (overall 2,4%). Among the age group 5–64 years the overall CFR was 8,1%. Cases aged 85 years and over presented the highest CFR (18,6%).

Vaccination status was known in 8,7% of the reported cases. Of the 1879 cases for which vaccination status was reported, 345 (17,4%) were fully vaccinated, 4,2% partially vaccinated and 78,5% not vaccinated, according to the national schedule (Table 1).

The distribution of IPD cases during 2010 followed a seasonal pattern with a clear increment in the winter months, peaking in December. This temporal distribution was observed both for the total number of cases and for the 10 most common serotypes.

3.2. IPD serotypes

Of the 21 585 reported confirmed cases of IPD, 9 840 (46,1%) included information on the serotype. Of these, the most common serotypes were 19A, 1, 7F, 3, 14, 22F, 8, 4, 12F and 19F, accounting for 5 949/9 840 (58,8%) of the typed isolates reported (Fig. 2).

Serotypes 19A and 7F were the most commonly reported in children <1 year of age, whereas serotypes 1 and 19A were the

Table 2
Number of reported cases and notification rates of invasive pneumococcal disease cases in EU/EEA countries, 2010 (N = 21 585).

Country	No. of reported cases (N)	Notification rate (cases per 100 000)
Austria	325	3,8
Belgium	1851	17,1
Bulgaria[a]	20	0,3
Cyprus	23	2,8
Czech Republic	308	2,8
Denmark	988	17,4
Estonia	14	1,1
Finland	830	15,8
France[b]	5117	10,8
Greece[c]	38	0,3
Hungary	107	1,1
Iceland	304	8,2
Italy	858	1,3
Latvia	18	0,7
Lithuania	8	0,3
Malta	11	2,7
Netherlands[d]	55	4,8
Poland	333	0,8
Romania	88	0,4
Slovakia	18	0,3
Slovenia	224	10,7
Spain[e]	2212	4,7
Sweden	1458	14,8
United Kingdom[f]	5618	0,8
EU Total	20785	5,1
Iceland	32	11,5
Norway	748	16,2
Total	21 585	5,2

[a] Aggregated reporting.
[b] France: no national coverage for invasive pneumococcal disease (see Section 2).
[c] National coverage only for meningitis.
[d] Netherlands reports data on IPD only on children up to 5 years. Notification rate was calculated accordingly.
[e] No national surveillance in Spain. The notification rate needs to be interpreted cautiously and may be much higher.
[f] There is not a single surveillance system in the UK covering the four health services.

most frequently reported in the group aged 1–4 years. Among those 15–64 years, serotypes 1, 7F and 3 were predominant while serotypes 19A, 3, 7F and 8 were most common among those aged ≥65 years.

Among the non-PCV serotypes, serotype 22F (436/9 048) accounted for 4,5%, serotype 8 (345/9 048) for 3,5%, 12F (268/9 048) for 2,5% and 8C (228/9 048) accounted for 2,5% of all serotyped isolates (*n* = 9 048) (Table 3).

Serotype 1 was the most frequent serotype reported among cases presenting with non-meningitis (413/3 588, 11,5%), followed

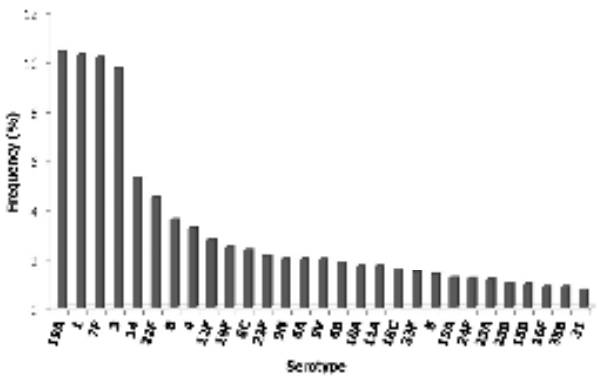
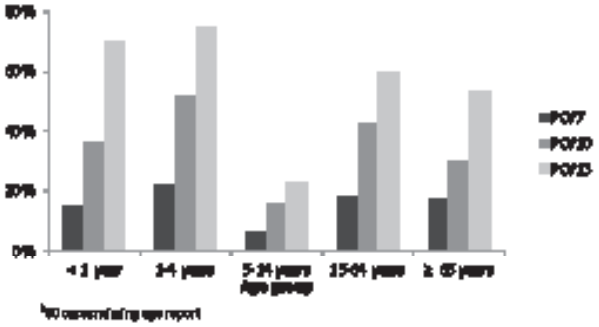


Fig. 2. Distribution of the most frequent serotypes of reported IPD cases, EU/EEA countries, 2010 (*N* = 8 604).



[a] No corresponding age report.

Fig. 3. Potential coverage of pneumococcal conjugate vaccines by age group, 2010 (*N* = 8 885)[a].

by serotypes 19A, 7F and 3. Similarly serotype 19A was the most frequent serotype reported among cases presenting with meningitis (112/1 075, 10,4%), followed by serotypes 3 and 7F.

In children below 5 years the serotype with the highest CFR was 10A (18,6%) although serotype 19A caused the highest number of deaths (*n* = 3, serotype specific CFR 2,8%). In age groups 5–64 years and 65 years and over, serotype 3 accounted for the highest number of deaths (*n* = 35, serotype specific CFR 11,2% and 14,1% respectively) but in both age groups serotype 4 accounted for the highest CFR (21,4% and 14,3% respectively).

3.3. Serotype coverage of pneumococcal conjugate vaccines

Overall, PCV7 serotype coverage among children <5 years in Europe, was 18,2%; for the same age group, the serotype coverage for PCV10 was 48,1% and for PCV13 was 73,1% (Fig. 3). Among adults, PCV13 serotype coverage was 60,1% for cases from 15 to 64 years, and 53,9% for the elderly (≥65 years).

3.4. Antibiotic susceptibility

Romania (42,2%), Cyprus (36,4%) and France (27,5%) reported the highest rates of non-susceptibility to penicillin, Cyprus (54,5%) and Romania (38,1%) reported the highest rates of non-susceptibility to erythromycin, Romania (25,8%) and Ireland (8,3%) had the highest non-susceptibility rates to cefotaxime. Overall penicillin MIC was ≤0,06 mg/L for 75,6% of isolates, 0,125 ≤ MIC ≤ 2 mg/L for 23,3% and >2 mg/L for 1,1% of isolates tested. The erythromycin MIC was ≤0,25 mg/L for 70,9% of the isolates, 0,25 < MIC ≤ 0,5 mg/L for 5,4% of isolates and >0,5 mg/L for 23,7% of the isolates with this information. For cefotaxime, 91,3% of the isolates had MIC ≤ 0,5 mg/L, 8,4% had 0,5 mg/L < MIC ≤ 2 mg/L and 0,3% had MIC > 2 mg/L.

Countries in Southern and Eastern Europe reported the highest proportion of non-susceptibility of *S. pneumoniae* to penicillin and/or erythromycin. However, Finland was an exception within the Northern countries with a non-susceptibility proportion of 23,3% for penicillin and with a non-susceptibility proportion of 28,1% for erythromycin. The overall percentage of non-susceptibility was 17,6% for erythromycin, 8,6% for penicillin and 3,7% for cefotaxime.

Simultaneous resistance to penicillin, erythromycin and cefotaxime (multidrug-resistance) was observed for serotypes 19A, 14, 19F, and 23F. Dual resistance to penicillin and erythromycin was reported in serotypes 19F, 19A, 14, 15A, 6A, 6B, 9V, 23A, 23F, and 24A. Non-susceptibility to penicillin was 0,9% for PCV7 and PCV10 serotypes whereas PCV13 serotypes non-susceptibility was 12,7%. For erythromycin, PCV7 serotypes non-susceptibility was 7,2%, PCV10 was 8,4% and PCV13 serotypes non-susceptibility was 17,2%,

Table 3
Distribution of reported serotyped cases of invasive pneumococcal disease by vaccine type and by country, EU/EEA countries, 2010 (N = 8 848).

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a] AT, Austria; BE, Belgium; CY, Cyprus; CZ, Czech Republic; DK, Denmark; ES, Spain; FI, Finland; GR, Greece; HU, Hungary; IE, Ireland; IS, Iceland; IT, Italy; LT, Lithuania; MT, Malta; NL, Netherlands; NO, Norway; PL, Poland; RO, Romania; SI, Slovenia; UK, United Kingdom.
[b] Percentage referred to the total number of serotyped cases per country.
[c] This table corresponds to 79% (7 [illegible]/8 848) of serotyped isolates. Serotypes not covered by any of the pneumococcal vaccines accounted for 21% ([illegible]/8 848).

Table 4
Distribution of non-susceptible serotypes (3 most frequent) by age group.

Serotype	<5 years					
	Penicillin		Erythromycin		Cefotaxime	
	n	%	n	%	n	%
19A	130	18,3	102	15,3	38	5,8
14	48	3,8	63	5,0	23	1,8
19F	35	2,8	25	2,0	10	0,8
	5–64 years					
	Penicillin		Erythromycin		Cefotaxime	
	n	%	n	%	n	%
19A	123	3,6	128	3,8	58	1,8
14	102	3,0	83	2,8	54	1,6
19F	40	1,2	51	1,5	21	0,6
	≥65 years					
	Penicillin		Erythromycin		Cefotaxime	
	n	%	n	%	n	%
19A	155	5,0	184	5,3	88	2,3
14	114	3,7	119	3,8	68	2,3
6B	24	0,8	30	1,3	3	0,3

Overall, non-susceptibility to the three antibiotics varied with age and children below 5 years presented the highest rates of non-susceptibility compared to 5–64 years and ≥65 years groups (Table 4).

4. Discussion

The 21 565 confirmed cases of IPD reported in the EU/EEA in 2010 showed a wide variation in notification rates. The variation in the notification rate of IPD in Europe may well be due to differences in case definitions of IPD, surveillance methods, medical practices (mainly blood culturing) and clinical presentation of IPD cases [17]. Therefore, a certain degree of under-diagnosis and under-reporting is suspected. Geographic variations in the distribution have been described elsewhere [18] and it is also seen in this surveillance where Nordic countries and Belgium had the highest notification rates, probably due to better ascertainment. In addition to differences in clinical practice and population characteristics, overuse of antibiotics may have caused clonal spread of certain serotypes that are more prone to produce more severe presentations [20].

The age distribution pattern of IPD is consistent within European data since 2006 and is seen in other parts of the world [21–24]. This age distribution of the disease has been the paradigm for targeting vaccination. In our analysis non-meningitis (mainly bacteraemic pneumonia) was the most frequent clinical presentation. These findings are similar to those from other reports since S. pneumoniae is by far the leading cause of pneumonia in the developed world [25] and also causes meningitis, resulting in sequelae and a high CFR [26]. The CFR differs between countries but these figures should be interpreted cautiously due to the limited clinical data available for this variable, e.g. information on the time-point at which the fatal outcome is defined and concurrent conditions is lacking. In addition, capsular and clonal differences of pneumococcal strains predict their behaviour in relation to invasive disease potential and outcome [8,27–29] as observed in this surveillance where distribution of serotypes with highest CFR varies with age. The overall CFR increases with age but data mandate prudence in interpretation due to overall low childhood-related mortality.

The four most frequent serotypes, 19A, 1, 7F and 3 were most prevalent in children under 15 years and none of these serotypes are covered by PCV7. Low coverage of circulating serotypes included in PCV7 is most likely due to vaccination and replacement by non-vaccine serotypes [12,13]. Pneumococcal vaccination

was introduced in all EU/EEA countries with differences in date of implementation, vaccine type, vaccination schedules and policies, with diverse scenarios combining whether it is mandatory or recommended, universal or restricted to risk groups, free of charge, reimbursed or with costs covered by the patient (Table 1). In 2010 the majority of EU/EEA countries had already implemented PCV7 in their national immunisation programmes after several years on a universal basis with acceptable vaccine coverage in many of them (Table 1). A number of EU/EEA countries switched to higher valency vaccines (PCV10/PCV13) late in 2010, thus presumably without significant effect on our data. Therefore, despite the scarce information on vaccination status and vaccine type of cases in this surveillance, the predominance of non-PCV7 serotypes could be attributed to the impact of the vaccine.

Serotypes 3 (included in PCV13 and the polysaccharide pneumococcal vaccine PPV23) and 8 (included in PPV23) combined with low invasive potential, predominantly occurred in older adults underpinning that serotypes causing disease in children and younger adults differ from those causing IPD in the elderly most likely due concurrent conditions [27,28]. Serotypes 22F, 8, 12F and 9N occurred principally in adults and are only covered by PPV23. At present, expert committees are evaluating different alternatives for the recommendation of PCV13 and PPV23 to prevent IPD in adults for which there is some evidence [30]. However, compelling evidence to support the routine use of PPV23 to prevent all-cause pneumonia or mortality is lacking [30]. There are studies that indicate that immunological properties of PCV13 are higher than PPV23 in adults while safety and tolerability are comparable [31] but data of the efficacy of PCV13 to adults is still unavailable. Nevertheless, new immunisation strategies are needed to tackle the considerable burden of morbidity and mortality that pneumococcal infections represent for adults [32].

IPD displayed a seasonal pattern with greater numbers of cases occurring during the winter months, and this was even more evident within the older age groups. Individual serotypes including the ten most frequent follow the same pattern. Several studies have pointed to different possible causes: co-infection with respiratory viruses (e.g. influenza, syncytial respiratory virus, metapneumovirus), temperature and environmental factors [33,34]. From the public health perspective this might constitute an opportunity for strengthening delivery of pneumococcal vaccines (PCV13, PPV23) within older age groups by administering them at the annual visit for influenza vaccination.

The highest rate of antimicrobial non-susceptibility was reported for erythromycin followed by penicillin. Co-resistance to penicillin, erythromycin, and cefotaxime (multidrug-resistance) was observed amongst serotypes 19A, 14, 19F and 23F, in accordance with other publications [14,35]. Non-susceptibility was highest in children below 5 years most likely due to repeated exposure of strains to antibiotics as respiratory infections and in particular those caused by *S. pneumoniae* are the main clinical entities for prescription of antimicrobial agents in young children. Pneumococcal immunisation has decreased the number of antimicrobial resistant infections. Nevertheless, some of the PCV10 and PCV13-specific serotypes exhibited antimicrobial resistance or multidrug-resistance. Therefore, the judicious use of antimicrobials remains pivotal in curtailing the emergence and spread of antimicrobial resistance within pneumococcal strains [36,37].

Continued surveillance across Europe is important since serotype distributions and age group related incidences of IPD vary from country to country and the use of the new vaccines (PCV10/PCV13) is expected to have an impact on serotype distribution.

The heterogeneity of laboratory methods for *S. pneumoniae* detection, characterisation and AST was highlighted in previous studies [9]. A limitation of this surveillance is the current coded values for the variable 'clinical presentation'. In future editions, bacteraemia/septicaemia cases should be grouped as a unique clinical entity. Although ECDC works for the harmonisation and standardisation of those laboratory methods within European national reference laboratories [38], further efforts in this direction are needed.

5. Conclusions

European IPD pooled-data analysis is relevant to assess differences across the world and to help formulate policies at a European level. However, differing national surveillance systems and differing vaccination schedules make it difficult to compare data throughout Europe.

Despite these caveats, the establishment of the IPD enhanced surveillance at a European level has provided baseline information on the epidemiology of IPD and has allowed an estimate of the burden of the disease across Europe after the introduction of PCV7 immunisation in most EU/EEA countries and before PCV10/PCV13 implementation. Furthermore, this information is useful in prioritisation policies, in evaluating the impact of vaccination and in informing the development of future vaccines.

Authors' contributions

Adauctión Navarro Torné coordinated the collection of data, performed the data analysis and wrote the manuscript.

Jesus Gomes Dias, Chantal Quinten, and Maria Cecilia Ramos contributed to the data analysis.

Pramila Ladhani, Pier Luigi Lopalco, Andrew J. Amato Gauci and Lucia Pastore-Celentano reviewed the manuscript.

The ECDC country experts for pneumococcal disease contributed to the data collection and reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

Appendix A.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.04.088.

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Risk Factors for Death from Invasive Pneumococcal Disease, Europe, 2010

Adoración Navarro-Torné, Joana Gomes Dias, Frantiska Hruba, Pier Luigi Lopalco, Lucia Pastore-Celentano, Andrew J. Amato Gauci, Invasive Pneumococcal Disease Study Group[1]

We studied the possible association between patient age and sex, clinical presentation, *Streptococcus pneumoniae* serotype, antimicrobial resistance, and death in invasive pneumococcal disease cases reported by 17 European countries during 2010. The study sample comprised 2,921 patients, of whom 56.8% were men and 38.2% were ≥65 years of age. Meningitis occurred in 18.5% of cases. Death was reported in 284 (9.8%) cases. Older age, meningitis, and nonsusceptibility to penicillin were significantly associated with death. Non–pneumococcal conjugate vaccine (PCV) serotypes among children <5 years of age and 7-valent PCV serotypes among persons 5–64 years of age were associated with increased risk for death; among adults ≥65 years of age, risk did not differ by serotype. These findings highlight differences in case-fatality rates between serotypes and age; thus, continued epidemiologic surveillance across all ages is crucial to monitor the long-term effects of PCVs.

Streptococcus pneumoniae causes severe invasive disease that results in considerable illness and death. The incidence of invasive pneumococcal disease (IPD) is higher during the early years of life and among elderly persons (*1*). Geographic and ethnic differences also exist (*1*,*2*). Environmental factors (i.e., ambient temperature, humidity, and air pollution) affect IPD incidence (*3*,*4*). IPD has also been related to recent respiratory viral infection (*4*).

The ability of the different *S. pneumoniae* serotypes to cause disease has been related to serotype-specific characteristics and the molecular size of the capsular polysaccharide and chemical composition, among other factors (*5*). Therefore, it seems plausible that different serotypes exhibit different virulence and propensity to cause certain clinical presentations (*5*).

Brueggemann et al. studied the invasive disease potential of different *S. pneumoniae* serotypes (*6*). They concluded that so-called "highly invasive" serotypes (including 4, 1, 14, 18C, and 7F), convey a higher risk for invasive disease than do the "low invasive" serotypes (including 3, 15B/C, and 6B), which are more frequently isolated as colonizers (*7*). Furthermore, serotype distribution varies with patient age, both in disease and in nasopharyngeal colonization (*2*,*8*–*10*). However, evidence exists that pneumococcal invasiveness does not necessarily mean lethality (*7*). Low invasive serotypes usually account for higher case-fatality rates (CFRs).

The availability of 7-valent, 10-valent, and 13-valent pneumococcal conjugate vaccines (PCV7, PCV10, and PCV13, respectively) and their introduction as part of national immunization schedules have contributed to reducing illnesses and death from IPD (*10*–*12*). Nevertheless, the subsequent replacement of vaccine serotypes by nonvaccine serotypes is an accepted and global phenomenon (*13*,*14*).

The incidence of drug- and multidrug-resistant *S. pneumoniae* strains is increasing worldwide (*15*). Antimicrobial use and abuse is a main driver for the emergence of antimicrobial resistance in respiratory pathogens. Persons who carry (nasopharyngeal colonization), and hence share the potential to transmit resistant pneumococci, also are more susceptible to IPD caused by resistant strains (*16*).

Monitoring antimicrobial resistance trends and serotype distribution is paramount because this information is essential in helping to determine risk factors and optimizing the appropriate clinical management of cases and public health interventions. We studied the possible association between age, sex, serotype, clinical presentation, antimicrobial resistance, and death among persons reported to have IPD in European countries during 2010.

Materials and Methods

Data

IPD data derived from passive national surveillance case notification systems were collected during 2010 by 26 European Union (EU)/European Economic Area countries

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(Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, and United Kingdom); data were submitted to The European Surveillance System. The platform of The European Surveillance System is a metadata-driven system for the collection, validation, cleaning, and analysis of data hosted by the European Centre for Disease Prevention and Control. Surveillance systems differ across Europe, and data were reported with varying levels of completeness. Countries reported only laboratory-confirmed cases based on the EU 2008 case definition.

Study Sample

The study sample was the subsample of cases for which information was available about both serotype and outcome (Figure 1). The sample represents data from 17 European countries (Table 1).

Study Variables

An episode of IPD was defined as the isolation of a strain or detection of nucleic acid or antigen of *S. pneumoniae* from a normally sterile site. Countries reported IPD outcome according to their national surveillance and guidelines. The

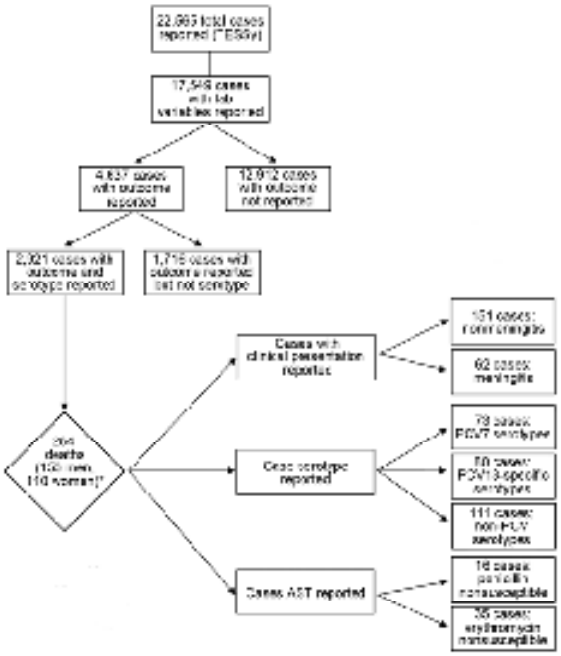

Figure 1. Flow of invasive pneumococcal disease cases through the study, Europe, 2010. *Sex was unknown for 1 patient. AST, antimicrobial susceptibility testing; PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV; TESSy, The European Surveillance System.

Table 1. Characteristics of patients with invasive pneumococcal disease, Europe, 2010*

Characteristic	No. cases (% of total), N = 17,549	Sample size, no. (%), n = 2,921†
Sex		
F	7,915 (45.3)	1,257 (43.2)
M	9,565 (54.7)	1,651 (56.8)
Age group, y		
<5	1,980 (11.3)	570 (19.7)
5–64	7,819 (44.7)	1,222 (42.1)
≥65	7,684 (44.0)	1,108 (38.2)
Outcome		
Nonfatal	4,146 (89.4)	2,657 (91.0)
Fatal	491 (10.6)	264 (9.0)
Clinical presentation		
Nonmeningitis	6,047 (79.4)	1,722 (81.5)
Meningitis	1,572 (20.6)	391 (18.5)
Serotype		
PCV13-specific‡	4,185 (42.1)	1,235 (42.7)
PCV7	1,772 (17.8)	517 (17.9)
Non-PCV	3,989 (40.1)	1,137 (39.4)
Antimicrobial susceptibility		
Penicillin		
Susceptible	8,420 (91.1)	1,949 (94.1)
Nonsusceptible§	827 (8.9)	122 (5.9)
Erythromycin		
Susceptible	6,911 (82.5)	1,573 (76.4)
Nonsusceptible	1,471 (17.5)	486 (23.6)

*Numbers do not add to the total in each category because of missing data. †See Figure 1. PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV.
†Defined as patients for whom information was available about serotype and outcome.
‡Serotypes contained in PCV13 but not in PCV7.
§Either resistant or intermediate resistance.

following age groups were defined for the study: <5 years, 5–64 years, and ≥65 years. For purpose of this analysis, clinical presentation was recoded as "meningitis" and "non-meningitis." Clinical presentation was grouped on the basis of a literature review (*3*), which suggested that meningitis and nonmeningitis had different degrees of severity and conveyed different rates of death.

Serotypes were grouped into 3 categories: PCV7 serotypes (serotypes in PCV7: 4, 6B, 9V, 14, 18C, 19F, and 23F), PCV13-specific serotypes (serotypes in PCV13 but not in PCV7: 1, 3, 5, 6A, 7F, and 19A), and non-PCV serotypes (serotypes not in any PCV). Results of antimicrobial susceptibility testing to penicillin and erythromycin were reported as "susceptible," "intermediate," or "resistant" by the countries according to their national standards and protocols. Therefore, information was not available about the breakpoints and guidelines used for antimicrobial susceptibility testing in each country. For example, in the European Antimicrobial Resistance Surveillance Network report for 2010 (*17*), 68% of reporting laboratories in Europe used Clinical and Laboratory Standards Institute standards, whereas 29% applied the European Committee on Antimicrobial Susceptibility Testing guidelines.

For this study, we redefined the variable to include just 2 categories: "susceptible" (cases reported as susceptible

by the countries) and "nonsusceptible" (intermediate and resistant), both for penicillin and erythromycin. Methods for the characterization of isolates and for antimicrobial susceptibility testing are provided in detail in the 2010 IPD enhanced surveillance report by the European Centre for Disease Prevention and Control (*18*).

Statistical Analysis

Categorical variables are presented as number of cases and percentages. We used the Pearson χ^2 test to compare the proportion of deaths by PCV7, PCV13-specific, and non-PCV serotypes; the proportion of deaths by the defined age groups and by sex; the proportion of deaths by clinical presentation; and the proportion of deaths in antimicrobial-susceptible and -nonsusceptible cases, according to antimicrobial drug type. We used the Fisher exact test to analyze the association between penicillin-susceptible/penicillin-nonsusceptible IPD and outcome for patients <5 years of age and non-PCV serotypes and to assess differences between penicillin-susceptible/penicillin-nonsusceptible cases and outcome for serotype 35B. In addition, we assessed the associations between each serotype and death using a generalized linear model with log-link function. This analysis was performed for the 28 serotypes that accounted for up to 80% of cases with fatal outcomes; each individual serotype was also compared with all the others.

Univariable analysis was performed for the 264 fatal cases to identify factors associated with a fatal outcome. To test the association between age, serotype, clinical presentation, and death, a generalized linear model with robust SEs accounting for the country effect was fitted because data came from different national surveillance systems and vaccination policies and practices differ widely across Europe. We studied the role of variables as potential confounders/modifiers, but only age was statistically significant. Age was an effect modifier of the association between serotype and risk for death, and thus the analysis was stratified by age group.

We also conducted regression analysis. The regression model comprised factors that were significant by univariable analysis and that had previously been hypothesized to affect IPD CFRs.

All p values were 2 tailed, and statistical significance was defined as $p<0.05$. We conducted statistical analyses by using STATA 12.0 (StataCorp, College Station, TX, USA).

Results

Case Characteristics

In 2010, the European countries reported 22,585 IPD cases. Of these, information was available about laboratory variables for 17,549 cases (Figure 1); outcome was known for 4,837 of these. The study sample comprised 2,921 cases for which information was available about serotype and outcome.

A total of 56.8% of cases (Table 1) occurred in men, and 38.2% of cases were among adults ≥65 years of age. Children <5 years of age accounted for 19.7% of cases. A total of 264 (9.0%) persons died. Meningitis occurred in 18.5% of cases. PCV13-specific serotypes (1, 3, 5, 6A, 7F, 19A) accounted for 42.7% of cases. Nonsusceptibility (intermediate + resistant) to penicillin was reported in 122 (5.9%) of 2,071 cases; nonsusceptibility to erythromycin was reported in 488 (23.6%) of 2,059 cases (Table 1).

PCV13-specific serotypes caused 57.7% ($p<0.001$) of cases among children <5 years of age (Figure 2). Non-PCV serotypes accounted for 48.0% of cases among adults ≥65 years of age. Meningitis cases were predominantly caused by non-PCV serotypes (41.4%, $p<0.001$) (Figure 2). Nonsusceptibility to penicillin was highest among PCV7 serotypes (64.8%, $p<0.001$) (Figure 2).

The Pearson χ^2 analysis (Table 2) demonstrated a lack of statistical association between sex and death ($p=0.831$). The CFR was highest for adults ≥65 years of age (13.7%, $p<0.001$); 2.3% of children <5 years of age died.

Clinical presentation was associated with death. The CFR for persons with meningitis was 15.9% compared with 8.8% for those without meningitis ($p<0.001$).

Death was also associated with nonsusceptibility to penicillin. Death occurred in 13.1% of cases in which *S. pneumoniae* was not susceptible to penicillin ($p = 0.010$) (Table 2). Nonsusceptibility to erythromycin was not significantly associated with death ($p = 0.837$).

We determined the association between individual serotype and death (Table 3). Serotype 35B (relative risk [RR] 4.98, 95% CI 2.49–9.95), serotype 4 (RR 2.03, 95% CI 1.04–3.95), and serotype 11A (RR 1.97, 95% CI 1.33–2.94) were most associated with death. Serotype 3 (RR 1.39, 95% CI 0.88–2.21) accounted for the highest number and the highest percentage (13.3%) of serotype-specific deaths, but the association with death was not statistically significant ($p = 0.161$). In contrast, for serotype 1 (RR 0.25, 95% CI 0.13–0.48) and serotype 5 (RR 0.15, 95% CI 0.09–0.26), the association with death was significant. Subanalysis of the association between susceptibility to penicillin and outcome for serotype 35B found no significant differences in risk for death between susceptible and nonsusceptible cases.

Risk Factors for IPD-Associated Death

Univariable analysis showed differences between non-fatal and fatal cases (Table 4). Persons 5–64 years of age (RR 3.55, 95% CI 1.66–7.61) and ≥65 years of age (RR 4.79, 95% CI 3.08–11.76) had a higher risk for death than did children <5 years of age. In the univariable

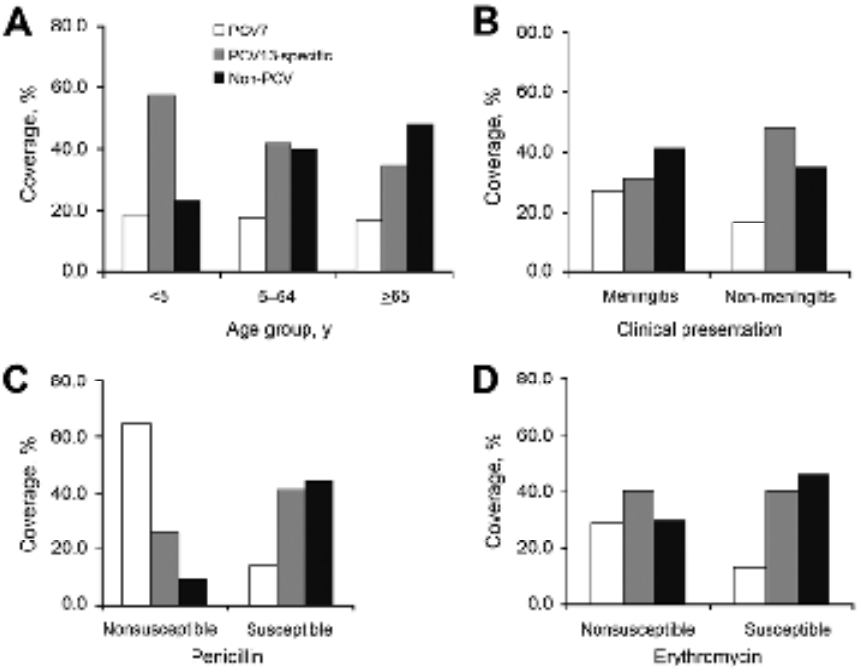

Figure 2. Invasive pneumococcal disease study variables and PCV coverage of *Streptococcus pneumoniae* serotypes, Europe, 2010. A) Age group. B) Clinical presentation. C) Penicillin susceptible. D) Erythromycin susceptible. For all 4 variables, p<0.001. White bars, PCV7 serotypes; gray bars, PCV13 serotypes; black bars, non-PCV serotypes. PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV.

analysis, meningitis (RR 1.81, 95% CI 1.25–2.61, p = 0.002) was significantly associated with death. PCV7 serotypes were also significantly associated with death (RR 2.18, 95% CI 1.06–4.48, p = 0.034). Conversely, non-PCV serotypes were not associated with death (RR 1.47, 95% CI 0.94–2.28).

Nonsusceptibility to penicillin was associated with increased risk for death (RR 1.91, 95% CI 1.16–3.13). Nonsusceptibility to erythromycin was not significantly associated with death (RR 1.04, 95% CI 0.84–1.29).

Our comparison of susceptibility to penicillin and outcome for clinical presentation showed that the association with the outcome remained statistically significant only for meningitis cases (RR 1.82, 95% CI 1.27–2.62, p = 0.001). These factors were not associated with nonmeningitis cases (RR 1.31, 95% CI 0.28–6.01).

Age was an effect modifier. In the stratified analysis, we found that among children <5 years of age, risk for death from non-PCV serotypes increased (RR 3.68, 95% CI 1.27–10.69) (Table 5), whereas among persons 5–64 years of age, PCV7 serotypes conveyed the highest risk for death (RR 2.68, 95% CI 1.37–5.23). Among adults ≥65 years of age, risk for death among the serotypes did not differ significantly.

We analyzed the association between susceptibility to penicillin and outcome for non-PCV serotypes. Children <5 years of age showed no differences between susceptible and nonsusceptible cases.

Table 2. Associations between invasive pneumococcal disease study variables and death, Europe, 2010*

Variable	Outcome: Nonfatal, no. (%)	Outcome: Fatal, no. (%)	p value†
Sex			
F	1,147 (91.3)	110 (8.8)	0.631
M	1,498 (90.7)	153 (9.3)	
Age group, y			
<5	567 (97.7)	13 (2.3)	
5–64	1,123 (91.9)	99 (8.1)	<0.001
≥65	956 (86.3)	152 (13.7)	
Clinical presentation			
Nonmeningitis	1,571 (91.2)	151 (8.8)	<0.001
Meningitis	329 (84.1)	62 (15.9)	
Serotype			
PCV13-specific‡	1,155 (93.5)	80 (6.5)	<0.001
PCV7	444 (85.9)	73 (14.1)	
Non-PCV	1,028 (90.5)	111 (9.5)	
Antibiotic susceptibility			
Penicillin			
Susceptible	1,815 (93.1)	134 (6.9)	
Nonsusceptible§	106 (86.9)	16 (13.1)	
Erythromycin			0.010
Susceptible	1,464 (93.1)	109 (6.9)	
Nonsusceptible	451 (92.8)	35 (7.2)	

*PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV.
†Pearson χ^2 test.
‡Serotypes contained in PCV13 but not in PCV7.
§Either resistant or intermediate resistance.

Discussion

Our analysis of IPD surveillance data from Europe in 2010 unveiled a significant association between death and older age, meningitis, serotypes contained in PCV7, and non-

Table 3. *Streptococcus pneumoniae* serotype in invasive pneumococcal disease and association with death, Europe, 2010*

Serotype	PCV†	Fatal, %	Nonfatal, %	RR (95% CI)	p value‡
3	PCV13-specific§	13.3	9.6	1.39 (0.88–2.21)	0.161
4	PCV7	6.1	2.8	2.03 (1.04–3.95)	0.038
19A	PCV13-specific	6.1	7.6	0.80 (0.41–1.57)	0.515
14	PCV7	5.7	4.6	1.23 (0.76–1.85)	0.369
7F	PCV13-specific	4.9	8.3	0.59 (0.35–1.01)	0.053
6B	PCV7	3.8	1.7	2.01 (0.79–5.16)	0.144
19F	PCV7	3.8	1.9	1.85 (0.93–3.65)	0.078
22F	Non-PCV	3.8	2.8	1.35 (0.89–2.03)	0.157
9V	PCV7	3.4	2.2	1.50 (0.95–2.38)	0.081
23F	PCV7	3.4	2.3	1.42 (0.80–3.32)	0.423
1	PCV13-specific	3.4	13.1	0.25 (0.13–0.48)	<0.001
11A	Non-PCV	2.3	1.1	1.97 (1.33–2.94)	0.001
10A	Non-PCV	2.3	1.4	1.52 (0.86–2.68)	0.147
6A	PCV13-specific	2.3	2.3	1.01 (0.39–2.57)	0.990
6C	Non-PCV	1.9	0.7	2.33 (0.93–5.86)	0.072
9N	Non-PCV	1.9	1.5	1.21 (0.52–2.82)	0.664
12F	Non-PCV	1.9	1.8	1.07 (0.51–2.23)	0.867
35B	Non-PCV	1.5	0.2	4.58 (2.43–8.65)	<0.001
33F	Non-PCV	1.5	0.9	1.53 (0.55–4.28)	0.414
18C	PCV7	1.5	1.2	1.23 (0.40–3.76)	0.713
8	Non-PCV	1.5	3.1	0.59 (0.25–1.06)	0.073
23A	Non-PCV	1.1	0.7	1.51 (0.65–3.45)	0.323
15A	Non-PCV	0.8	0.7	1.05 (0.45–2.43)	0.909
15B	Non-PCV	0.8	1.0	0.79 (0.26–2.41)	0.677
24F	Non-PCV	0.4	0.6	0.69 (0.12–4.09)	0.683
5	PCV13-specific	0.4	2.6	0.15 (0.03–0.26)	<0.001

*PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV; RR, relative risk. Boldface indicates statistical significance.
†Classification of serotypes according to study group.
‡Generalized linear model with log-link function.
§Serotypes contained in PCV13 but not in PCV7.

susceptibility to penicillin. As have many other studies, we found an association between increased age and death (*19*–*22*). The risk was higher for adults ≥65 years of age (RR 4.79, 95% CI 3.08–11.76) than for persons 5–64 years of age (RR 3.55, 95% CI 1.86–7.61). However, the lack of information about patients' clinical characteristics impedes accurate assessments of these differences.

Elderly persons have been postulated to have an increased susceptibility to—in addition to co-occurring conditions—pneumococcal disease because of reduced splenic function (*23*), age-related changes in respiratory tract, immunosenescence, and cellular senescence related to age-associated inflammation (*23*). The higher incidence and death rates for IPD in this age group is remarkable and highlights the need to direct vaccination toward the elderly. These findings may present an opportune moment to revisit adult vaccination recommendations and programs in European countries (*24*).

We did not find sex to be significantly associated with death. However, other studies have shown association either with men (*23*) or women (*21*,*24*).

In our study, presence of meningitis was significantly associated with death. Harboe et al. obtained similar results in a large population-based cohort study (*25*). In Denmark, another study concluded that patients with pneumococcal meningitis had increased death rates, but these rates derived from severe underlying conditions (*27*). CFRs for pneumococcal meningitis are usually higher than for nonmeningitis (*26*). More recently, Ladhani et al. found that the CFR was higher for children with meningitis in England and Wales (*29*). This study showed that infecting serotype was not associated with death (*29*), whereas meningitis and co-occurring conditions were significantly associated with death. In our analysis, meningitis was predominantly caused by non-PCV serotypes; this finding could be an effect of PCV introduction, as observed in other studies (*30*). Another analysis of susceptibility to penicillin by clinical presentation showed a higher risk for death among persons with nonsusceptible IPD than for those with susceptible IPD who had meningitis. Therefore, in the absence of information about clinical management of cases and existing co-occurring conditions, the association between meningitis and nonsusceptibility to penicillin might be an explanation.

Capsular differences between serotypes affect clinical presentation and outcome (*18*,*31*,*32*). These differences are in accordance with our study, which found PCV7 serotypes were associated with death in the univariable analysis. Among children <5 years of age, PCV13-specific serotypes were most frequently identified, compared with PCV7 and non-PCV serotypes, as defined in our study. In 2010, PCV13 was already licensed, and many European countries began moving from PCV7 to the higher-valent vaccine, although with different schemes, policies, and dates

Table 4. Association between invasive pneumococcal disease study variables and death, Europe, 2010*

Variable	Relative risk† (95% CI)
Sex	
F	Reference
M	1.06 (0.88–1.28)
Age group, y	
<5	Reference
5–64	3.55 (1.66–7.61)
≥65	4.79 (3.08–11.76)
Clinical presentation	
Nonmeningitis	Reference
Meningitis	1.81 (1.25–2.61)
Serotype	
PCV13-specific‡	Reference
PCV7	2.18 (1.06–4.48)
Non-PCV	1.47 (0.94–2.28)
Antimicrobial susceptibility	
Penicillin	
Susceptible	Reference
Nonsusceptible§	1.91 (1.16–3.13)
Erythromycin	
Susceptible	Reference
Nonsusceptible	1.04 (0.84–1.29)

*PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV.
†Generalized linear model with log-link function.
‡Serotypes contained in PCV13 but not in PCV7.
§Either resistant or intermediate resistance.

of introduction. Nevertheless, these changes are unlikely to have affected our study findings because we analyzed data from 2010.

After stratification, the highest risk for death among children <5 years of age corresponded to non-PCV serotypes. This finding could be attributed to serotype replacement after pneumococcal vaccination (*29*,*30*). Our analysis found no differences between penicillin-susceptible and -nonsusceptible cases among children <5 years of age and non-PCV serotypes subgroup with respect to death. However, the overall percentage of meningitis cases was high (18.5% of the study sample), and meningitis was predominantly caused by non-PCV serotypes (p<0.001) (Figure 2). Hence, vaccines with enhanced serotype coverage (higher valency) might be needed to prevent IPD in this age group in the near future.

Among persons 5–64 years of age, the risk for death was highest for PCV7 serotypes, which were predominantly nonsusceptible to penicillin (p<0.001) (Figure 2). Reductions in IPD caused by PCV7 serotypes in non–vaccine-eligible age groups in countries with universal use of PCV7 might indicate the indirect effect of PCV7 (*33*). However, because vaccine policies differed among European countries at the time of the study, this indirect effect might not be reflected in the pooled data (Table 6).

Serotypes 1, 5, and 7F have been described as having high potential for invasiveness (these serotypes are carried for a short time) but are associated with milder disease and lower CFRs (*7*,*9*,*19*,*34*). As in those studies, we found that serotypes 1 and 5 caused IPD but were not associated with death.

Serotype 35B has been reported as nonsusceptible to penicillin (*35*). The subanalysis on susceptibility to penicillin for serotype 35B showed that penicillin nonsusceptibility did not affect the risk for death for serotype 35B. Nevertheless, the increased risk for death of non-PCV serotypes 11A and 35B merits further monitoring.

We found penicillin nonsusceptibility to be significantly associated with death, as described by others (*20*,*36*). Nevertheless, in other huge studies, this association was not found (*21*,*26*,*34*,*37*), and the effect of multidrug-resistant strains remains to be determined. Conversely, we found that erythromycin nonsusceptibility did not significantly affect death, as described by Song et al. (*37*) and Montero et al. (*20*). A plausible explanation might be the additional benefits of macrolides (i.e., their immunomodulatory/antiinflammatory properties), which might be important when these drugs are used in combination with other therapeutic agents (*38*).

Antimicrobial resistance to *S. pneumoniae* is increasing in many countries in Europe (*17*), and the prudent use of antibacterial drugs, apart from immunization, is pivotal in preventing and controlling IPD. Furthermore, these findings underpin the importance of antimicrobial susceptibility testing to assist with the clinical management of cases and to provide data on prevalence of antimicrobial resistance.

Table 5. Stratified analysis of *Streptococcus pneumoniae* serotype distribution in a study of invasive pneumococcal disease, Europe, 2010*

Age group, y	Survived, no. (%)	Died (%)	RR (95% CI)	p value
<5				
PCV13-specific	325 (98.8)	4 (1.2)	1	
PCV7	104 (97.2)	3 (2.8)	2.31 (0.35–15.02)	0.382
Non-PCV	128 (95.5)	6 (4.5)	3.68 (1.27–10.68)	0.017
5–64				
PCV13-specific	486 (94.4)	29 (5.6)	1	
PCV7	186 (84.9)	33 (15.1)	2.68 (1.37–5.23)	0.004
Non-PCV	451 (92.4)	37 (7.6)	1.35 (0.64–2.82)	0.429
≥65				
PCV13-specific	338 (87.8)	47 (12.2)	1	
PCV7	154 (80.6)	37 (19.4)	1.59 (0.90–2.79)	0.108
Non-PCV	464 (87.2)	68 (12.8)	1.05 (0.64–1.72)	0.856

*PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV; PCV13, 13-valent PCV; RR, relative risk.

Table 6. Characteristics of national pneumococcal vaccination programs in European Union/European Economic Area countries, 2010*

Country	Date of PCV7 introduction	Scope of PCV vaccination program	Immunization schedule	Dose				Vaccine coverage†	Year of measurement
				First, mo	Second, mo	Third, mo	Fourth, mo		
Austria	2004 Jul	Universal	3+1 dose	3	5	7	12–24	NA	NA
Belgium	2006 Jan	Universal	2+1 dose	2	4	12		97	2010
Bulgaria	2010 Apr	Universal	3+1 dose/2+1 dose	2	3	4	12	NA	NA
Cyprus	2008 Aug	Universal	3+1 dose	2	4	6	12–15	NA	NA
Czech Republic	2010 Jan	Risk-based	3+1 dose	2	4	6	18	86.3	2010
Denmark	2007 Oct	Universal	2+1 dose	3	5	12		85	2010
Estonia	NA	NA	not decided	NA	NA	NA	NA	NA	NA
Finland	2009 Jan	Risk-based	2+1 dose	3	5	12		NA	NA
France	2006 Jun	Universal	2+1 dose	2	4	12		81	2008
Germany	2006 Jul	Universal	3+1 dose	2	3	4	11–14	52.9	2010
Greece	2006 Jan	Universal	3+1 dose	2	4	6	12–15	NA	NA
Hungary	2008 Oct	Universal	2+1 dose	2	4	15		81.1	2009
Iceland	2006 Dec	Risk-based	2+1 dose	3	5	12		NA	NA
Ireland	2002 Oct	Universal	2+1 dose	2	6	12		89	2009
Italy	2005 May	Universal/Risk-based	2+1 dose	3	5	11		55	2008
Latvia	2010 Jan	Universal	3+1 dose	2	4	6	12–15	51	2010
Lithuania	NA	NA	3+1 dose	2	4	6	24	NA	NA
Luxembourg	2005 Feb	Universal	3+1 dose	2	3	4	12–15	86	2010
Malta	2007 Jan	Risk-based	3+1 dose	2	4	13	None	NA	NA
Netherlands	2006 Jun	Universal	3+1 dose	2	3	4	11	94	2009
Norway	2006 Jul	Universal	2+1 dose	3	5	12		90	2009
Poland	2008 May	Risk-based	3+1 dose/2+1 dose	NA	NA	NA	NA	1.70	2008
Portugal	2010 Jun	Risk-based	2+1 dose	2	4	12–15		52	2009
Romania‡			3+1 dose	2	4	6	15–18		
Slovakia§	2006 Jan	Risk-based	2+1 dose	2	4	10		99.2	2009
Slovenia	2005 Sep	Risk-based	3+1 dose	2-3	4	6	24	NA	NA
Spain¶	2001 Jun	Risk-based	3+1 dose	2	4	6	15	NA	NA
Sweden	2009 Jan	Universal	2+1 dose	3	5	12		NA	NA
United Kingdom	2006 Sep	Universal	2+1 dose	2	4	13		90	2010

*NA, not available; PCV, pneumococcal conjugate vaccine; PCV7, 7-valent PCV. Blank cells indicate not applicable.
†Source: Vaccine European New Integrated Collaboration Effort: 8 project and World Health Organization estimates of PCV7 coverage.
‡PCV7 was registered in September 2009 for voluntary use on a private basis.
§Universal as of April 2008.
¶Universal introduction in the autonomous region of Madrid in November 2006.

The major strength of our study is its large sample size; data were from national surveillance systems across Europe (i.e., we analyzed IPD individual-level data from populations in a large geographic area). In 2010, European IPD surveillance collected data corresponding to ≈82% of the total population of EU/European Economic Area countries. This enhanced surveillance for IPD data pooled together at supranational level enables comparisons with other parts of the world.

Despite its strengths, our study has some limitations. Surveillance of IPD varies markedly in Europe, including differences in laboratory methods to confirm cases, reporting, and medical practices. Therefore, a certain degree of underdiagnosis and underreporting are likely to exist in this dataset. Moreover, surveillance systems for IPD differ in sensitivity, representativeness, and specificity across Europe; these variations might have influenced the results because some countries were major contributors (Table 7) and ascertainment bias might have also occurred. Information about co-occurring conditions or clinical management of cases that might have affected outcome was also missing. European countries introduced pneumococcal vaccination at different times and with different policies, which might have affected the serotype distribution throughout Europe. Furthermore, the incomplete information about the vaccination status of cases makes difficult the interpretation of results. These limitations emphasize the need for continued and improved surveillance of IPD throughout Europe.

In conclusion, we found that older age, meningitis, non-PCV serotypes among children <5 years of age and PCV7 serotypes among persons 5–64 years of age, and penicillin nonsusceptibility were risk factors for death from IPD in Europe. The stratified analysis highlighted differences in risk for death according to *S. pneumoniae* serotype and age group. This knowledge may assist in decision making when implementing vaccination strategies as new immunization strategies are needed to tackle the considerable IPD and associated death in adults (*39*) and in designing new extended valency vaccines or protein-based pneumococcal vaccines that may confer serotype-independent immunity (*40*).

Table 7. Geographic distribution of cases and deaths of invasive pneumococcal disease for which *Streptococcus pneumoniae* serotype and disease outcome were known, Europe, 2010

Reporting country	No. (%) cases	No. (%) deaths
Austria	190 (6.5)	15 (7.9)
Belgium	1,255 (43.0)	67 (5.3)
Cyprus	3 (0.1)	0
Czech Republic	242 (8.3)	43 (17.8)
Denmark	35 (1.2)	0
Greece	20 (0.7)	1 (5.0)
Hungary	26 (0.9)	7 (26.9)
Ireland	78 (2.7)	4 (5.1)
Italy	209 (7.2)	31 (14.8)
Lithuania	3 (0.1)	0
Malta	7 (0.2)	0
Netherlands	45 (1.5)	4 (8.9)
Norway	357 (12.2)	41 (11.5)
Poland	205 (7.0)	43 (21.0)
Romania	21 (0.7)	2 (9.5)
Slovenia	224 (7.7)	6 (2.7)
Slovakia	1 (0)	0
Total	2,921 (100.0)	264 (9.04)

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