REVIEW

Determinants of invasiveness and ability to cause invasive pneumococcal disease, pneumonia and acute otitis media of different serotypes of *Streptococcus pneumoniae*

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Key words

Streptococcus pneumoniae • Invasive pneumococcal disease • Pneumococcal virulence factors

Introduction

Streptococcus pneumoniae is one of the leading bacterial cause of community-acquired pneumonia, acute otitis media (AOM), bloodstream infection (bacteraemia), sinusitis and meningitis, particularly affecting young children and elderly people. In particular, pneumococcal pneumonia represents one of the major causes of childhood mortality in developing countries and of adult mortality worldwide, accounting for 30-50% of pneumonia cases [1].
The best preventive strategy is vaccination, that is made complicated by T-indipendent nature of polysaccharide antigen, and by high heterogeneity of pneumococcus. In fact, more than 90 immunologically different serotypes of pneumococci have been described but less than 30 types account for the majority (> 90%) of invasive disease [2]. Individual serotypes may vary in their distribution over time and geography [3] and differ in their prevalence, age distribution and degree of antimicrobial resistance [4]. To overcome the inability of bacterial polysaccharides to induce T-cell response, a second-generation conjugate vaccines were designed, developed and extensively evaluated during the 1990s.
The introduction in the USA in 2000 of the 7-valent conjugated vaccine (PCV7), and the rapid implementation of the coverage in children has determined a dramatic decrease of incidence of invasive (IPD) and non-invasive pneumococcal disease (non-IPD), not only among vaccinated children but even among unvaccinated children and adults as a result of a striking herd immunity effect [5]. Selective pressure applied by vaccination has lead to an increased role of non-vaccine serotypes (serotype replacement) and may favour the emergence of successful strains, originally of vaccine serotype, that have acquired a non vaccine serotype through capsular switching [6]. In particular, the emergence of non-vaccine serotypes such as 19A, 15, 1, 7F, involved in IPD and non-IPD, has been reported elsewhere [7, 8].
The earlier introduction in late 2008 to early 2009 of PCV10 (PCV7 plus serotypes 1, 5, 7F) and then of PCV13 vaccine, added with serotypes 3, 6A and 19A, has offered a significant benefit, with a reached coverage of 59% of IPD in children younger than 5 years, whereas PCV7 covered 36% of cases [9].
Serotypes included in both the 10- and 13-valent PCVs accounted in fact for 10 million cases and 600,000 deaths worldwide [10].
The ability of PCV7, PCV10 and PCV13 to maintain reduction of IPD and non-IPD rates has been questioned. Nowadays, the global effect of conjugate vaccines is not the reduction of the overall prevalence of nasopharyngeal colonization in the target population, i.e. children in the first years of life, but, by reducing vaccine serotypes, better suited to causing invasive disease, a decrease of IPD. Therefore, several factors are both critical and informative to understand the changing population biology of this pathogen and the future effectiveness of conjugate vaccine [11].
A central role is played by pathogenecity and virulence determinants of *S. pneumoniae* and by their distribution among vaccine and non-vaccine serotypes.
Although host and bacterial factors both seem to contribute to IPD pathogenicity [12], pneumococcal virulence factors, above all the capsular polysaccharide, are thought to play an important role on pneumococcal disease outcome and have been considered as potential vaccine candidates. Nevertheless, one study have suggested that even clonal types and properties may influence invasive capacity and render pneumococcal strains more efficient pathogens, pointing out the importance of the genotype [13].
Purpose of this review is to deepen the recent data emerged on determinants able to influence invasive ability of pneumococcus and its propensity to determine non-IPD, and to correlate invasiveness with bacteria serotype.

Pneumococcal virulence factors

In the recent years a great number of pneumococcal virulence factors have been described, but only certain proteins are probably the best candidates for use as vaccine antigens [14, 15]. Hereinafter the individual contributions of each known virulence factors to the pathogenesis of *S. pneumoniae* infections and their impact are reported.

Capsule

The pneumococcal capsular polysaccharide represents an indispensable virulence factor due to its strongly anti-phagocytic activity, in fact spontaneous non-encapsulated strains are almost completely avirulent [14].
The capsule is covalently linked to the surface of the cell-wall peptidoglycan, which itself represents a virulence determinant, and protects *S.pneumoniae* against phagocytic clearance by blocking the deposition and function of opsonins such as C3b factor and the interaction with the Fc region of IgG.
Interestingly, the presence of a heavy capsule could contribute to disease severity. More heavily encapsulated serotypes tend to be more prevalent among paediatric carriage isolates even if they appear to be less invasive [16]. Nevertheless, they are more frequently associated to severe disease when they do invade. It has been demonstrated that pneumococcal isolates can be distinguished by their opaque or transparent colony morphology and that the organisms spontaneously alternate between the two phases. During the initial stages of colonization, transparent variants with a thinner capsule predominate since their greater capacity for adherence. However, the presence of a thick capsule could promote the persistence of pneumococcal strains in the nasopharynx, lungs and blood by protecting bacteria against host immune response [15, 17] such as neutrophil-mediated killing [18].
Capsular composition in different serotypes may influence their invasive capacity as a reflection of their ability to resist phagocytosis together with their different ability to trigger inflammatory responses [19].
Finally, the presence of a heavy capsule could contribute to antibiotic resistance [20].

Pilus

Pilus-like structures have been identified beyond the polysaccharide capsule in *S. pneumoniae* and a possible role in enhancing adhesion to host cells was supposed.
These adhesive pili-like appendages are encoded by the pneumococcal *rlrA* islet, present in some, but not all, clinical isolates. Introduction of the *rlrA* islet into an encapsulated *rlrA*-negative isolate allowed pilus expression, enhanced adherence to lung epithelial cells, and provided a competitive advantage upon mixed intranasal challenge of mice [21].
Basset et al. [22] have suggested that the pilus does not appear to be associated with increased virulence in humans, despite the data obtained from a mouse study [21] and that the presence of pilus may be associated only with certain serotypes, especially vaccine serotypes. Therefore, the reduction observed in invasive disease caused by vaccine serotypes after the introduction of conjugate vaccines have also meant a reduction in piliated strains.

Cell-wall components

Pneumococcal cell-wall is composed by several molecules involved in the adhesion to the host and able to induce strong inflammatory responces.
In particular, phosphorylcoline linked to teichoic and lipoteichoic acids acts as an adhesion, binding platelet-activating factor receptor (rPAF) on host tissues and promoting airway colonization.
Associated to the cell-wall have been described three major complexes of cell surface proteins: choline-binding proteins (PspA, PspC or CbpA, LytA) covalently anchored to phosphorylcoline, lipoprotein and proteins covalently associated to the cell-surface anchorage domain LPXTG [15].

Choline-binding proteins

CBPs are cell-surface proteins characterized by a specific choline binding motif consisting of several repeat sequences of approximately 20 amino acids which appears to bind to the pneumococcal cell surface through phosphorylcoline associated to teichoic and lipoteichoic acids [23].
PspA, known as the protective antigen, is an 84-kDa protein that inhibits complement mediated opsonization, probably through its electronegative properties [15]. Moreover, the capacity of PspA to prevent the fixation of complement seemed to be correlated to the capsular type [24].
CbpA is considered the most important pneumococcal adhesin, essential for pneumococcal carriage. CbpA is also characterized by its ability to bind C3 and secretory IgA thereby favouring translocation of pneumococcus across the respiratory epithelium [25].
LytA, a N-acetylmuramoyl-L-alanine amidase, represents the major enzyme responsible for cellular autolysis and plays a role in cell-wall growth and turnover [26].

Lipoproteins

More than 40 pneumococcal cell surface lipoproteins have been described [27]. Pneumococcal surface antigen A (PsaA), Pneumococcal iron acquisition A (PiaA) and pneumococcal iron uptake A (PiuA) are reported to be essential for pneumococcal virulence [17]. PsaA plays an important role in pneumococcal adherence as is a component of an ATP-binding cassette transport system, essential for manganese uptake.
The importance of PiaA and PiuA resides in their immunogenicity and, consequently, in their possible use in pneumococcal vaccine development [28].

LPXTG-anchored proteins

LPXTG is an amino acids sequence motif which is recognized by a specific transpeptidase enzyme responsible for the attachment of several surface-located proteins. StrA, a sortase transpeptidase, has been reported to have a role in pneumococcal adhesion and invasion [29].

Esoenzymes

Beyond cell-wall related virulence factors, other important determinants of *S. pneumoniae* are involved in

bacterial pathogenicity, in particular pneumolysin and neuraminidase enzymes.

PNEUMOLYSIN

This enzyme is a powerful cytoplasmic toxin of 52kDa, belonging to the family of thiol-activated cytolysins, that oligomerizes in the membrane of the target cell to form a large transmembrane pore. Pneumolysin is released by autolysis of the cell and binds to cholesterol present on the host cell surfaces. This enzyme shows its cytolitic activity on ciliated bronchial epithelial cells, alveolar epithelial cells and pulmonary endothelium. This activity includes inhibition of ciliary beating and of respiratory burst, separation of tight junctions of alveolar epithelial cells, induction of cytokine synthesis and CD4+ T-cell activation and chemotaxis [30]. Further, pneumolysin is essential for bacterial spread to the bloodstream and is involved in brain damaging through direct neurotoxicity [31].
Interestingly, strains producing a non-cytolitic pneumolysin was shown to be more virulent and it was suggested that this non-haemolytic pneumolysin could activate TLR4-dependent responces and stimulate the production of interferon-γ [32].

NEURAMINIDASE

This sialidasis cleave sialic acid residues from a wide range of soluble proteins, such as lactoferrin, IgA2 and other secretory components facilitating colonization and invasion by increasing the number of adhesins available for pneumococcal binding [33]. There are three pneumococcal enzymes with neuraminidase activity: NanA, NanB, NanC. It has been suggested that NanA and NanB promote pneumococcal survival in the respiratory tract and bloodstream [34], whereas NanC is frequently found in isolates from cerebrospinal fluid and tissue-specific role has been proposed [35].

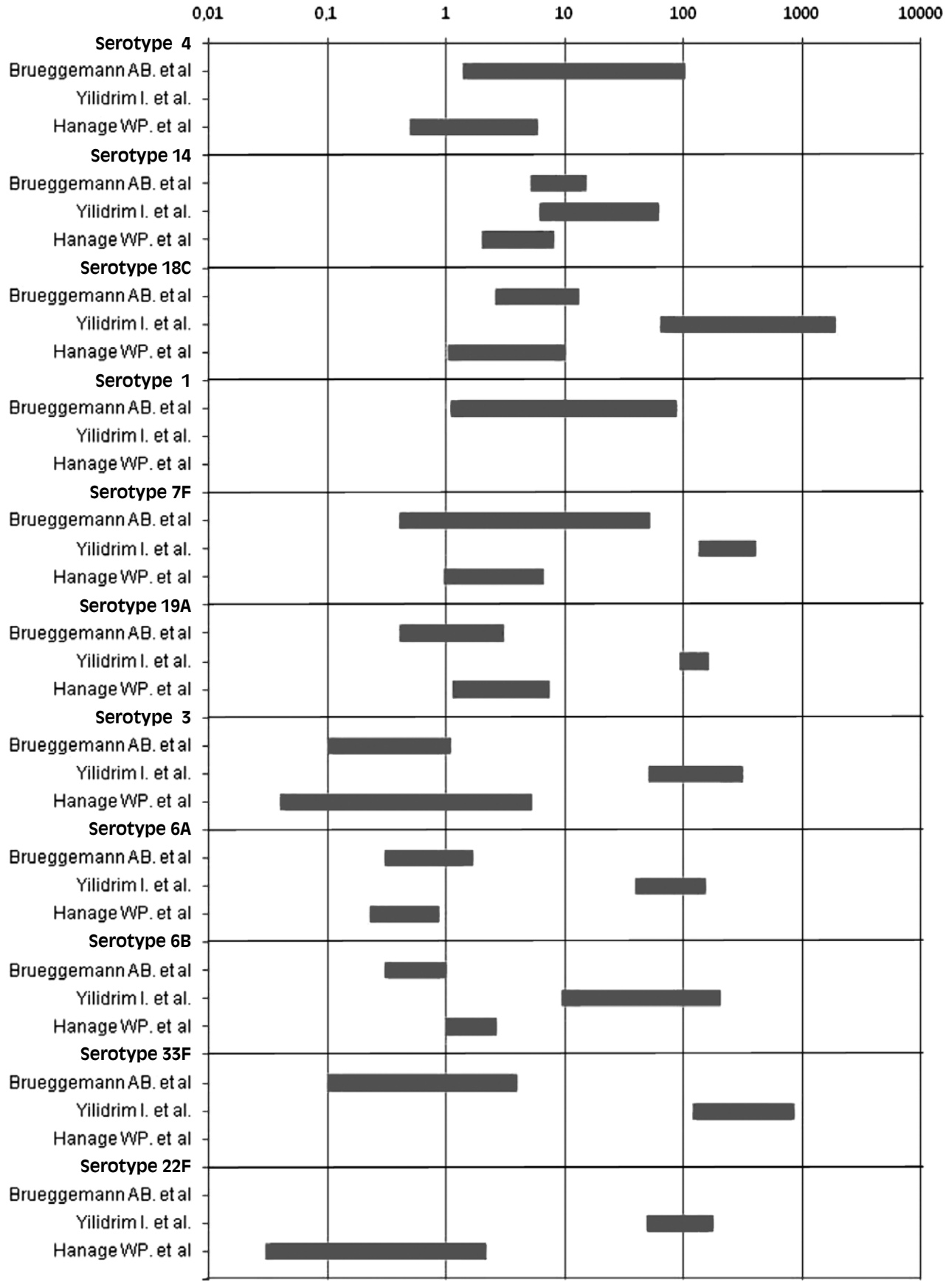
HYALURONIDASE

The importance of this enzyme is connected to its degrading activity of connective tissue and its involvement in the pathogenesis of human pneumococcal meningitis. Strains with higher hyaluronidase activity have been demonstrated to cross the blood-brain barrier and disseminate more effectively [36].

Ability of *S. pneumoniae* serotypes to cause invasive disease

Invasive pneumococcal disease represents one of the leading causes of morbidity and mortality worldwide, with a 5-35% of mortality depending on site of infection, age and comorbidity [37].
Changes in serotype distribution of IPD are inextricably connected to the serotype distribution of pneumococcal colonization among children, the main reservoir of the pathogen.
Many studies have described the serotypes of isolates involved in invasive disease and in carriage [38,39] but only few have investigated, in the same community and in the same time period, the relationship between the isolates involved in invasive disease and those carried by the same population.
Brueggemann et al. [40] have suggested and demonstrated the importance of the capsular serotype in defining pneumococcal invasiveness, quantified and compared the disease potential of individual serotypes or clones thus demonstrating their different ability to cause invasive disease (Fig. 1). Moreover, the study, conducted among children < 5 years in Oxford, confirmed that invasive strains own significantly less genetic diversity than the carriage isolates and that in both the populations a strong association between serotype and ST exists. Noteworthy, serotypes most commonly found among invasive disease in children were the ones included in the 7-valent conjugate vaccine, but they differed in their disease potential. In particular, vaccine serotypes 4, 14 and 18C were significantly associated with invasive disease. Of particular concern resulted non vaccine serotypes 1, 7F and 19A (Tab. I).
In another study, a meta-analysis was conducted on 7 data sets of invasive and carriage pneumococcal isolates from young children coming from the same region during roughly the same time period. Odds ratios of pneumococcal serogroups were calculated for each study and it was demonstrated that serogroup invasive disease potential did not differ temporally or geographically [41]. Finally, the meta-analysis results showed an inverse correlation between invasive disease and carriage prevalence: serotypes with the highest odds ratios were the least commonly circulating whereas serotypes more commonly carried were the least invasive. This could be partly explained by differences in serotype and serogroup-specific carriage duration but serotype or serogroup remains one of the most important determinants of invasiveness.
Although some limitations, these 2 studies provide important information and instruments for the evaluation of the disease incidence of the different serotypes circulating in a community, which largely depends on their disease potential and could be altered after vaccination.
A similar study was conducted in Finland [42] and the odds ratios for invasive disease of different serotypes and clones were obtained. Data found in Oxford resulted to be comparable with finnish results, indeed, all serotypes with an ORs > 1 in the Oxford study also had ORs > 1 in Finland, with the exception of serotype 1 (Tab. I, Fig. 1).
Furthermore, both host and bacterial factors seem to contribute to IPD pathogenicity. A nationwide population-based study conducted in Denmark, demonstrated the association between specific invasive pneumococcal serotypes and mortality related to IPD and also showed the influence of several risk factors, such as: age, ethnicity, meningitis, comorbid conditions etc, in mortality after IPD [38] (Tab. I). Serotype 1 represented 15% of all IPD cases in the study population, followed by serotypes 14, 4, 7F, 9V, 3, 8, 12F, 23F and 6B. Serotypes 14, 6B, 18C, 7F, 19F, 1, 6A, 23F, 4 and 9V accounted for 80% of IPD cases in children < 5 years of age, with serotypes 6B, 14, 7F, 18C, 19F, 6A and 23F involved in 70% of meningitis cases. In patients older than 5 the most fre-

Fig. 1. Odds ratio association (95%CI) of pneumococcal sertypes with invasive disease [40, 48, 42].



quent serotypes resulted to be 1, 4, 14, 7F, 3, 9V, 12F, 8 and 23F, accounting for 67% of all cases. Furthermore, danish results, according to previous studies [38] demonstrated that highly invasive serotypes, e.g. serotypes 1, 7F and 14, were not related to high mortality rates and resulted more invasive in children with low rates of comorbid conditions (Tab. I). No statistically significant serotype-mortality association could be demonstrated for children because of low childhood mortality (< 3%), whereas in developing countries mortality ranges from 10%-40%, probably due to poorer access to health care, higher prevalence of comorbid conditions, malnutrition and HIV. Serotypes 3 and 19A were associated with high- mortality in older children and adults, together with serotypes 31, 11A, 35F, 17F, 16F, 19F, 15B and 10A as compared with serotype 1. In addition, comorbidity resulted to influence IPD related-mortality rate, supporting data of a Swedish study which suggested that comorbidity may influence the invasiveness of specific serotypes in susceptible populations, indicating that serotypes with low invasive disease potential may behave as opportunistic pathogens in patients with unapparent comorbidity [43].

Several other studies already suggested relationships between serotype and outcome of disease [44,45] and demonstrated that host related factors are relevant as well [46]. In contrast, some other authors suggested that there is no association between serotype and outcome of disease, and that host factors are better predictors of IPD and associated morbidity and mortality than are microbial factors [47]. However, in this work information on serotype appeared to be dichotomized thus altering the real risk associated to specific serotypes.

Recently Yildirim et al. [48] suggested that the most invasive serotypes were 18C, 33F, 7F, 19A, 3 and 22F both among vaccine and non-vaccine serotypes (Tab. I, Fig. 1).

Association of *S. pneumoniae* serotype and pneumococcal pneumonia

S. pneumoniae is the most common cause of community-acquired pneumonia (CAP) in children and the elderly [49].

Pneumonia is considered one of the major causes of childhood mortality in developing countries, and of adult mortality worldwide [50].

In Europe and the United States, pneumococcal pneumonia is the most common community-acquired bacterial pneumonia, estimated to affect approximately 100 per 100 000 adults each year [51]. For adults with pneumococcal pneumonia the mortality rate averages 10-20%.

No more less is known about the strains and serotypes from pneumonia, although several studies have suggested a relationship between some serotypes and certain disease presentations. In particular, a high proportion of severe pneumonia cases were reported for serotypes 1 and 3 [52-53] (Tab. I).

A report on the association of nasopharyngeal *S. pneumoniae* serotypes with CAP suggested that carriage of serotypes 1 and 5 may be highly associated with radiographycally and clinically more severe childhood pneumonias [54] (Tab. I).

A recent meta-analysis of serotype-specific outcomes for adults and paediatric patients with pneumococcal pneumonia and meningitis reported that clinical outcome in bac-

Tab. I. Recent studies noting associations between serotypes and clinical manifestations among different age groups.

Reference and Location	Study period	Age group	Number of isolates	Serotypes
Invasive pneumococcal diseases				
Martens P, et al., Holland [37]	1990-2001	> 16 years old	464	1, 3
Brueggemann AB, et al., UK [40]	1995-2001	> 5 years old	150	4, 14, 18C, 1, 7F, 19A
Hanage WP, et al., Finland [42]	1995-1999	< 2 years old	224	14, 18C, 19A, 6B
Harboe ZB, et al., Denmark [38]	1977-2007	All age gropus	18858	1, 14, 4, 7F, 9V, 3, 8, 12F, 23F, 6B
Ruckinger S, et al., Germany [46]	1997-2003	> 16 years old	494	7F
Yildirim I, et al., USA [48]	2003/2004,2006/2007 2008/2009	≤ 7 years old	206	18C, 33F, 7F, 19A, 3, 22F
Pneumonia				
Camou, et al., Uruguay [53]	1994-2001	> 5 years old	506	1, 3
Dagan R, et al., Israel [54]	2001-2004	> 5 years old	435	1, 5
Yu J, et al., USA [57]	2007-2009	pediatric patients	49	1, 3, 7F/A, 19A
Resti M, et al., Italy [56]	2007-2009	0-16 years old	753	1
Acute otitis media				
Hanage WP, et al., Finland [64]	1994-1997	< 2 years old	149	19F, 23F
Kilpi T, et al., Finland [58]	1994-1997	< 2 years old	329	19F, 23F, 6A, 6B, 14
Shouval DS, et al., Israel [65]	200-2004	< 3 years old	3200	1, 3, 5, 12F, 19A, 19F

teriemic pneumonia, such as carriage prevalence and invasiveness, seemed to be a stable serotype-associated property. In particular, an increased risk of death was associated with serotypes 3, 6A, 6B, 9N and 19F among pneumonia patients, and a higher risk was observed for serotypes 19A and 23F, although not statistically significant [55].
Several studies have reported the distribution of specific serotypes as cause of IPD by age groups. In particular, serotype 1 was classified as leading cause of bacteriemic pneumonia and paediatric parapneumonic empyema, in children older than 2 years [56].
A recent study conducted in USA reported a strong association between serotypes 1, 3, 7F/A and 19A and pneumonia with pleural effusion in paediatric patients [57] (Tab. I).
An observational study conducted from April 2007 to June 2009 on children aged 0-16 years admitted to 83 paediatric hospital in Italy with a diagnosis of community-acquired pneumonia, reported that serotype 1 was the most frequently found and was significantly associated with complicated cases and older age according to previous finding [56] (Tab. I).
Highly invasive disease potential serotypes such as 1, 5 and 7F were correlated to complicated parapneumonic effusion or empyema in younger adults [1].

Association of *S. pneumoniae* serotype and acute otitis media

S. pneumoniae is the most frequent pathogen isolated from middle ear fluid (MEF) in patients with Acute Otitis Media (AOM), being detected in 26-48% of cases by bacterial culture methods [58, 59].
The nasopharyngeal carriage of *S. pneumoniae* is important in the pathogenesis of AOM. The carriage rate of *S. pneumoniae* varies from 9% to 52% between populations in healthy children, but increases during a respiratory infection without AOM and is at its highest, 45% to 91%, during AOM episodes. During pneumococcal AOM the nasopharyngeal carriage rate of *S. pneumoniae* reaches 97 to 100% [60]. The occurrence of AOM is affected by both host factors and environmental factors. Predisposing host factors include young age, male gender, racial factors, allergic rhinitis, immune deficiency, cleft palate, craniofacial abnormalities and genetic predisposition [61]. Further, factors that increase nasopharyngeal colonization in children include young age, day-care attendance, parental smoking, the winter season and antibiotics [62].
Several studies have suggested a relationship between specific serotypes of *S. pneumoniae* and their ability to cause invasive disease [63], though, for mucosal infections like AOM it seems that there is much less variation in the ability of different pneumococcal serotypes and clones to cause AOM, thus suggesting that all serotypes are equally able to cause AOM [64].
Moreover, association of the common childhood serotypes 6B, 9V, 14 and 19F with AOM, not necessarily implies a propensity of these serotypes to cause AOM, as they are the most commonly carried in the nasopharynx of children [65, 66].
In a recent Finnish study the most frequent serotypes in MEF isolates from children with AOM were 19F, 23F, 6A, 6B and 14, which accounted for 73% of all cases [42] (Tab. I).
Hanage et al. [64] investigated the abilities of pneumococcal serotypes and clones to cause AOM, by comparing the distribution of pneumococcal serotypes and clones causing AOM in children < 2 years of age with those carried in the nasopharynx of children of the same age, in the same region and time period (Tab. I).
No significant association with AOM of individual serotypes was demonstrated, suggesting little variation in their ability to cause disease, with the exception of serotypes 19F and 23F, and no significant difference in distribution of AOM and NP isolates was observed. Furthermore, genotype appeared to be an important marker of the ability of a strain to cause AOM, thus suggesting that serotype may play a lesser role than that commonly associated to IPD. Consequently, serotype replacement potentially may reduce the long-term efficacy of the conjugate vaccines against AOM because it would lead to AOM caused by non vaccine serotypes instead of vaccine serotypes, without reducing the overall incidence of disease.
Another study demonstrated that specific serotypes causing IPD, AOM or AC possess different disease potential in relation with the site of infection. In particular, serotypes 1, 3, 5, 12F, 19A and 19F were associated with AOM, providing important information regarding specific disease potential of non-vaccine serotypes [65] (Tab. I).

Discussion

Pneumococcal serotypes and clones widely differ in their ability to cause disease, as a result not only of their differences at the capsular locus and genetic background, but also of bacteria-host interactions which play a significant role in determining IPD.
The introduction of the heptavalent pneumococcal conjugate vaccine formulations into the US infant-immunization programme in 2000 has determined a significantly reduction in IPD incidence both in young children and in old age groups as a consequence of herd immunity [66].
However, a statistically significant rise in disease caused by non vaccine serotypes has been observed, in particular by serotypes 15B/15C/15F and 19A, but now also by serotypes 1, 3, 5, 6A, 7F. These serotypes and clones may be better adapted to colonize the nasopharynx, evade the human immune response, and cause disease. Further, a recent study showed an increase in prevalence of serotype 6C in children and a corresponding decrease in serotype 6A after introduction of PCV7 [67].
Serotypes included in the recently licensed 10-valent pneumococcal *Haemophilus influenzae* protein D conjugate vaccine (PCV10) and 13-valent pneumococcal conjugate vaccine (PCV13) account for pneumococcal disease burdens in most developed countries of 65–85% and 80–90%, respectively [68].

The increasing diffusion of non vaccine serotypes may not necessarily reflect an increase in disease, infact, these serotypes are thought to have a relatively low disease potential. Nonetheless, the real potential of a serotype to cause disease also resides on the different exposures of children to each serotype. Therefore, serotypes with low pathogenicity, but to which children are often exposed, may be more virulent than serotypes highly invasive but to which children are rarely exposed to [40].

Moreover, the risk of vaccine-to-non vaccine serotype switch contributes to serotype replacement and may lead to vaccine escape events, which have the potential to reduce vaccine effectiveness in the longer term [69].

Such considerations underline the importance of a continued epidemiological surveillance for the monitoring of changes in the incidence of IPD and non IPD, for detecting genetic events such as transformation and recombination, which may result in vaccine escape, and for determining eventual increase in non vaccine serotypes virulence. Furthermore, the importance of such a surveillance resides on the consideration that the resulting knowledge of serotype-specific properties will represent a useful tool for the design and introduction of future pneumococcal vaccines into national programmes.

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