# Pneumococcal surface protein C group 4 of Streptococcus pneumoniae is a significant factor to human invasive pneumococcal diseases

**Haruko Miyazaki** ( hmiya@tokyo-med.ac.jp )
Tokyo Medical University
**Bin Chang**
National Institute of Infectious Diseases
**Hiroyuki Kikuchi**
Tokyo Medical University
**Noritoshi Fukushima**
Tokyo Medical University
**Rie Shibuya**
Saiseikai Yokohamashi Tobu Hospital
**Yoshitusgu Miyazaki**
National Institute of Infectious Diseases
**Tetsuya Matsumoto**
International University of Health and Welfare

**Research article**

**Keywords:** Streptococcus pneumoniae, virulence factor, pneumococcal surface protein C, serotype, pathogenicity

**Posted Date:** June 5th, 2019

**DOI:** https://doi.org/10.21203/rs.2.10103/v1

# Abstract

Background Streptococcus pneumoniae is a major causative pathogen of non-invasive and invasive disease worldwide. Although many virulence factors of S. pneumoniae have been reported, the knowledge of the relationship between these factors and clinical features of pneumococcal infection is limited. To clarify factors leading from non-invasive to invasive disease, we analyzed virulence factor genes of S. pneumoniae isolates from patients and evaluated relationship between presence or absence of the genes and clinical features. Methods Pneumococcal surface protein (Psp)A families (pspA F1 and pspA F2), PspC group 4 (pspC.4), Pilus-1 (rrgC), and Pilus-2 (sipA) virulence factor genes were measured by PCR using 13 isolates from patients with invasive pneumococcal disease (IPD) and 111 from patients with non-invasive pneumococcal diseases (NIPD) in a community hospital in Japan during 2016. We also tested serotype of isolates. Statistical analysis was performed using multivariable logistic regression analysis for calculating adjusted odds ratio (AOR). Results Isolates from IPD carried pspC.4 at significantly higher rate (69.2%) than from NIPD (AOR 10.58 [95% CI, 2.67-41.87]). Proportions of pspC.4 positive isolates were varied depending on serotype. There found 13 serotypes (including non-typeable) that had pspC.4, and 7 of the 13 pspC.4-carrying serotypes caused invasive diseases while 2 of 12 non-pspC.4 serotypes did. Relationships between IPD and pspA F1, pspA F2, rrgC, or sipA were not significant. Conclusions It is suggested that positive pspC.4 gene in S. pneumoniae is related with invasive pneumococcal diseases in this study. Further studies of virulence factors are required to elucidate S. pneumoniae pathogenicity.


# Background

*Streptococcus pneumoniae*, one of the most prevalent human pathogens worldwide, constitutes a major causative agent of both non-invasive (e.g., otitis media and pneumonia) and invasive disease (e.g., bacteremia and meningitis), leading to the death of a large number of young children and the elderly in particular [ 1-3]. In Japan, pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23) have been approved for routine vaccination of children and the elderly, respectively.

*S. pneumoniae* colonises the nasopharynx of many healthy young children asymptomatically [ 4]. However, despite *S. pneumoniae* being one of the most extensively studied microorganisms and the identification of many virulence factors, little is known regarding the factors that contribute to cause of disease and invasion. The pneumococcal surface protein A (PspA), a cell-wall-associated protein, interferes with complement deposition on the bacterial surface [ 5] and binds human lactoferrin [ 6]. PspA is variable at the DNA level, and can be classified into three families by polymerase chain reaction (PCR) [ 7], with PspA family 1 and 2 as the major alleles. PspC, also termed choline binding protein A (CbpA), constitutes a major pneumococcal adhesin. PspC binds to the polymeric immunoglobulin receptor (pIgR) in human nasopharyngeal cells and promotes translocation across a mucosal barrier. PspC also binds secretory IgA (sIgA), platelet activating factor receptor and more specifically complement proteins such as C3 [ 8]. PspC can inhibit C3b deposition but also binds the host complement inhibitor factor H, leading to inhibition of alternative pathway activation. Dieudonné-Vatran et al. demonstrated that the

pneumococcus exploits host C4b-binding protein (C4BP) for complement evasion in a PspC group 4-dependent manner [ 9]. Pilli have been identified on several gram-positive bacteria; two different pilus islets have been described in *S. pneumoniae* that encode for two different types of pilli, Pilus-1 and Pilus-2 [ 10, 11]. Pilus-1 is encoded by a pathogenicity islet including genes for three structural proteins, *rrgABC*, three sortases, *srtBCD*, and a regulator, *rlrA*. Pilus-1 was reported to mediate host-bacterial interactions as an adhesin, and a proinflammatory stimulus [ 10, 12]. In particular, an isolate is defined as pilus-1 positive if a PCR for the *rrgC* gene is positive [ 13]. In turn, Pilus-2, detected via primers for the signal peptidase-like protein (SipA) gene [ 14], mediates adhesion of *S. pneumoniae* to eukaryotic cells [ 11].

To clarify bacterial factors which foresee invasive diseases, we analysed five virulence factor genes, *pspA F1*, *pspA F2*, *pspC group 4* (*pspC.4*), *rrgC*, and *sipA*, of clinical isolates and evaluated the relationship between gene possession and clinical features in this study.

## Methods

# Clinical isolates

A total of 511 clinical *S. pneumoniae* isolates were collected at Saiseikai Yokohamashi Tobu Hospital, a regional core hospital in Kanagawa prefecture, Japan, during January–December 2016. Those patients from whom multiple pneumococcal isolates were obtained within a 30-day period, they were regarded as a single episode. In the 331 episodes (187 males and 144 females), 124 episodes were confirmed pneumococcal diseases based on both bacteriological isolation and consistent clinical symptom, signs and laboratory findings with *S. pneumoniae* infection.

Table 1 shows the distribution of patient's age, type of specimen, clinical diagnosis, and serotype. Thirteen episodes were diagnosed as invasive pneumococcal diseases (IPD) according to that *S. pneumoniae* had been isolated from normally sterile body sites in patients. Patients who had been diagnosed with pneumonia as well as bacteremia were designated as having IPD. Thirteen isolates from normally sterile body sites were defined as IPD isolates; 11 isolates from blood, and 2 from cerebrospinal fluid. We defined the other 111 episodes as non-invasive pneumococcal diseases (NIPD), although NIPD might have contained IPD because all episodes did not have blood culture test. The first isolate per NIPD episode subjected to genetic analysis; 97 isolates were from sputum, 10 from bronchial wash, 3 from nasopharyngeal swab, and 1 from otorrhea.

# DNA extraction and serotyping

Isolates were cultured on 5% blood agar plates at 37 °C with 5% $CO_2$. DNA was extracted using the Cica Geneus DNA Extraction Reagent (Kanto Chemical Co, Tokyo, Japan) according to manufacturer instruction. Capsular serotypes were determined via sequential multiplex PCR analysis [ 15] using QIAGEN Multiplex PCR Kits (QIAGEN, Hilden, Germany) or pneumococcal capsule-specific antisera

(Statens Serum Institut, Copenhagen, Denmark) per manufacturer instruction. Strains whose serotypes could not be determined by PCR or the Quellung reaction were defined as non-typeable.

# Analysis of PspA family 1 and 2

PspA family classification was performed by PCR using DNA extracted from isolates with primers reported by Hollingshead, et al. [ 16] as follows: LSM12 and SKH63 for PspA family 1 (*pspA F1*), and LSM12 and SKH52 for PspA family 2 (*pspA F2*) (Table 2). PCR reactions were carried out using Quick Taq HS DyeMix (TOYOBO, Osaka, Japan). The PCR conditions were 95 ℃ for 3 min; then 30 cycles of 95 ℃ for 1 min, 62 ℃ for 1 min, 72 ℃ for 3 min, and finally 72 ℃ for 10 min. PCR products were loaded onto 1% agarose gels, electrophoresed at 100 V for 30 min, and stained with 0.5 μg/ml ethidium bromide. The isolates that were not initially amplified were further processed with the same cycling pattern at an annealing temperature of 58 ℃, or, if that also failed, of 55 ℃.

# Analysis of pspC.4, rrgC, and sipA genes

The presence of *pspC.4* was investigated by PCR using primers LU9 and LU10 (Table 2), which were designed to specifically amplify the *pspC group 4* locus [ 9]. The PCR conditions were 96 ℃ for 5 min; then 30 cycles of 96 ℃ for 45 sec, 50 ℃ for 45 sec, 72 ℃ for 3 min, and finally 72 ℃ for 10 min. PCR products were electrophoresed at 100 V for 30 min, then stained with ethidium bromide.

For detection of the presence of the Pilus-1 operon, an isolate was defined as Pilus-1 positive if PCR for the *rrgC* gene was positive. Primers to evaluate the presence or absence of *rrgC* were c5 and c3 (Table 2) [13]. The PCR conditions were 94 ℃ for 3 min; then 35 cycles of 94 ℃ for 15 sec, 60 ℃ for 15 sec, 72℃ for 1 min, and finally 72℃ for 5 min. PCR products were electrophoresed in 2% agarose gels and stained.

To determine whether Pilus-2 was present, primers *sipA*-up and *sipA*-dn (Table 2), designed against the Pilus-2-specific gene *sipA*, were used [ 17]. The PCR conditions were 95 ℃ for 15 min; then 35 cycles of 95 ℃ for 20 sec, 55 ℃ for 30 sec, 68 ℃ for 1 min, and finally 68 ℃ for 10 min.

# Statistical analysis

Statistical analysis was performed using multivariable logistic regression model to calculate adjusted odds ratios (AORs) of developing IPD by existence of virulence factors. In the model IPD status was used as dependent variables, and five virulence factor genes (i.e. *pspA F1*, *pspA F2*, *pspC.4*, *rrgC*, and *sipA*) were used simultaneously as independent variables. Findings of $p$ <0.01 were considered significant.

## Results

# Serotype distribution of isolates

Serotype distribution of the isolates showed that serotype 11A/11E was the most common serotype, followed by 15A, among isolates from total pneumococcal diseases including IPD (Table 1). IPD distribution did not follow the total serotype distribution. Among IPD isolates, serotype 7F was the most common, and serotype 24B and 38 were the only one isolates in total studied (Table 1). It was noted that three of five 7F isolates caused IPD (bacteremia), and two of the three 7F isolates that caused bacteremia were isolated from patients with no underlying diseases in age group of 5–64 years.

# Virulence factor gene distribution in clinical pneumococcal isolates

Figure 1 shows the proportions of the *pspA F1*, *pspA F2*, *pspC.4*, *rrgC*, and *sipA* presence in IPD and NIPD. Among the 124 strains tested, 96.0% had *pspA F1* or *pspA F2*; 46.8% were identified as belonging to PspA family 1, whereas 50.8% belonged to family 2. Two isolates of serotype 22 belonged to both families 1 and 2. All IPDs were included in PspA family 1 or family 2 groups. However, the relationship between IPD and *pspA F1* (adjusted odds ratio [AOR] 4.09 [95% CI 0.13-125.70]) or *pspA F2* (AOR 1.77 [95% CI 0.06-55.25]) were not statistically significant. Isolates from the IPD group showed significant relation with *pspC.4* carriage than the NIPD groups (AOR 10.58 [95% CI 2.67-41.87], p <0.001). *rrgC* did not show difference between IPD isolates and NIPD isolates (AOR 0.25 [95%CI 0.02-2.62]). Although *sipA* was more frequently detected in the IPD group (23.1%) than in the NIPD group (8.1%), the differences were not significant statistically (AOR 2.65 [95% CI 0.41-17.00]).

Proportions of *pspC.4* presence varied depending on serotype (Figure 2). For example, all isolates of 7F, 19F, 24B, 33F, and more than half of 6C, 20 were *pspC.4* positive, whereas, no isolate had *pspC.4* in serotype 11A/11E which was the most common serotype in this study. Neither of the second common serotype 15A had *pspC.4* even in 2 IPD isolates. Some serotypes such as 24B and 33F had *pspC.4* and caused IPD although they were not frequent serotypes. There found 13 serotypes (including non-typeable) that had *pspC.4*, and 7 of the 13 *pspC.4*-carrying serotypes caused invasive diseases while 2 of 12 non-*pspC.4* serotypes did (Figure 2).

## Discussion

Pneumococci express multiple virulence factors, which include, for example, the polysaccharide capsule, pneumolysin, pneumococcal surface proteins, and pilli. It is considered that they can cause diseases because they possess efficient complement evasion strategies and resist opsonophagocytosis. Complement resistance constitutes a major contributor to pneumococcal virulence and pathogenesis. In particular, the capsule reduces the amount of bound C3b and restricts the access of phagocytes to cell-

bound C3b, which hampers opsonophagocytosis [ 18]. Pneumolysin quenches complement away from the pneumococcal surface [ 19].

PspA is present on almost all strains of *S. pneumoniae* [ 16] and PspA families 1 and 2 (fusion PspA) are considered as promising candidate antigens for pneumococcal vaccines [ 7, 20, 21]. Our data showed that all isolates from patients with IPD and 96.0% of total isolates in this study contained PspA families 1 or 2 and that relationship between clinical invasiveness and each of them were not significant in this study.

PspC proteins contribute to virulence in colonisation and systemic mouse models [ 22, 23]. PspC binds to the secretory component of pIgR to promote the adherence and invasion of epithelial cells [ 24] and also binds soluble host factors such as sIgA and IgM as well as C3 and complement inhibitor factor H ([ 8, 25]). PspC exhibit high variability at the sequence level, with allelic variants of PspC being divided into 11 groups. The presence of PspC group *4* was found to be correlated with the ability to bind C4BP [ 9]. C4BP retains its inhibitory function when bound to the bacteria, although the pneumococcus binds via the same site as C4b. Our study showed that isolates from IPD exhibited significantly higher rate of *pspC.4*, suggesting that *pspC.4* is related to invasiveness. It is also suggested that the serotype distribution of IPD isolates did not follow that of total isolates because proportions of *pspC.4* positive isolates were serotype dependent. Seven of isolates from IPD were serotypes, over half of which shows *pspC.4* positive regardless of serotype frequency. Whereas, the serotype 3 from a patient with pneumococcal bacteremia was the only *pspC.4*-positive isolate among the 22 serotype 3 isolates. However, no serotype 15A isolate including two from IPD carried *pspC.4*, further supporting that *pspC.4* constitutes one of the factors related to invasiveness but not related with invasiveness in certain serotypes.

The presence of *rrgC* had no appreciable difference in frequency between IPD and NIPD, as reported previously [ 26]. In comparison, *rrgC* was significantly associated with penicillin susceptibility (data not shown).

All *sipA* positive isolates in IPD were serotype 7F. Notably, 7F was the most frequent serotype in IPD in this study and all of them carried both *pspC.4* and *sipA*. Although we studied just five among many potential virulence factors, the results may relate to the observation that 7F is among the serotypes with highest invasive disease potential [ 27].

The pathogenicity of *S. pneumoniae* has been reported to differ between clones and even isolates of the same clone [ 28]. Our study suggested, although factors responsible for pathogenicity vary depending on serotypes and isolates, bacterial virulence factors are associated with clinical invasive aspects.

A limitation of this study is that it was performed in a single center for one year and sample number is limited. There are concerns regarding geographical and time variation, and it is not known whether our data are representative of that in the world.

# Conclusions

Our results suggested that the presence of genes for PspC group 4 is associated with clinical invasiveness and the results will promote the understanding of virulence factors in pneumococcal diseases. Further studies including more virulence factors are required to elucidate the pathogenic mechanisms of *S. pneumoniae*.

## List Of Abbreviations

PspA: pneumococcal surface protein A, PspC: pneumococcal surface protein C, PCR: polymerase chain reaction, IPD: invasive pneumococcal disease, NIPD: non-invasive pneumococcal disease, AOR: adjusted odds ratio, pIgR: polymeric immunoglobulin receptor, sIgA: secretory immunoglobulin A, PCV: pneumococcal conjugate vaccine, PPSV: pneumococcal polysaccharide vaccine, C4BP: C4b-binding protein

## Declarations

# Ethics approval and consent to participate

This study was approved by the Saiseikai Yokohamashi Tobu Hospital ethics committee (approval number 2016002) and by the Tokyo Medical University ethics committee (approval number 2016-218). Patients' data were anonymised for analysis. The committees permitted to omit informed consents by providing a means to opt out, according to Ethical Guideline for Medical and Health Research Involving Human Subjects.

# Consent for publication

Not applicable

# Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

# Competing interests

HM received a grant for education from Pfizer (grant no. 30049557). Other authors declare no conflicts of interest associated with this study.

# Funding

This study was supported by Pfizer (grant no. 30049557). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

# Authors' contributions

HM designed the study, collected data, performed the experiments, analyzed results and wrote the manuscript. BC performed the laboratory examinations. HK and NF carried out the statistical analysis. RS participated in the data collection and laboratory examinations. YM and TM involved in assessment and reviewed the manuscript. All authors approved the manuscript.

# Acknowledgements

We thank Ms. Hisa Goke for her assistance in conducting the experiments.

## References

1. Mortality GBD, Causes of Death C: Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016; 388(10053):1459-1544.

2. Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, Damaske B, Stefonek K, Barnes B, Patterson J et al: Epidemiology of invasive Streptococcus pneumoniae infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. JAMA. 2001; 285(13):1729-1735.

3. Morimoto K, Suzuki M, Ishifuji T, Yaegashi M, Asoh N, Hamashige N, Abe M, Aoshima M, Ariyoshi K, Adult Pneumonia Study G-J: The burden and etiology of community-onset pneumonia in the aging Japanese population: a multicenter prospective study. PLoS One. 2015; 10(3):e0122247.

4. Bogaert D, De Groot R, Hermans PW: Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004; 4(3):144-154.

5. Ren B, Szalai AJ, Hollingshead SK, Briles DE: Effects of PspA and antibodies to PspA on activation and deposition of complement on the pneumococcal surface. Infect Immun. 2004; 72(1):114-122.

6. Shaper M, Hollingshead SK, Benjamin WH, Jr., Briles DE: PspA protects Streptococcus pneumoniae from killing by apolactoferrin, and antibody to PspA enhances killing of pneumococci by apolactoferrin [corrected]. Infect Immun. 2004; 72(9):5031-5040.

7. Hotomi M, Togawa A, Kono M, Ikeda Y, Takei S, Hollingshead SK, Briles DE, Suzuki K, Yamanaka N: PspA family distribution, antimicrobial resistance and serotype of Streptococcus pneumoniae isolated from upper respiratory tract infections in Japan. PLoS One. 2013; 8(3):e58124.

8. Jarva H, Janulczyk R, Hellwage J, Zipfel PF, Bjorck L, Meri S: Streptococcus pneumoniae evades complement attack and opsonophagocytosis by expressing the pspC locus-encoded Hic protein that binds to short consensus repeats 8-11 of factor H. J Immunol. 2002; 168(4):1886-1894.

9. Dieudonne-Vatran A, Krentz S, Blom AM, Meri S, Henriques-Normark B, Riesbeck K, Albiger B: Clinical isolates of Streptococcus pneumoniae bind the complement inhibitor C4b-binding protein in a PspC allele-dependent fashion. J Immunol. 2009; 182(12):7865-7877.

10. Barocchi MA, Ries J, Zogaj X, Hemsley C, Albiger B, Kanth A, Dahlberg S, Fernebro J, Moschioni M, Masignani V et al: A pneumococcal pilus influences virulence and host inflammatory responses. Proc Natl Acad Sci USA. 2006; 103(8):2857-2862.

11. Bagnoli F, Moschioni M, Donati C, Dimitrovska V, Ferlenghi I, Facciotti C, Muzzi A, Giusti F, Emolo C, Sinisi A et al: A second pilus type in Streptococcus pneumoniae is prevalent in emerging serotypes and mediates adhesion to host cells. J Bacteriol. 2008; 190(15):5480-5492.

12. LeMieux J, Hava DL, Basset A, Camilli A: RrgA and RrgB are components of a multisubunit pilus encoded by the Streptococcus pneumoniae rlrA pathogenicity islet. Infect Immun. 2006; 74(4):2453-2456.

13. Regev-Yochay G, Hanage WP, Trzcinski K, Rifas-Shiman SL, Lee G, Bessolo A, Huang SS, Pelton SI, McAdam AJ, Finkelstein JA et al: Re-emergence of the type 1 pilus among Streptococcus pneumoniae isolates in Massachusetts, USA. Vaccine. 2010; 28(30):4842-4846.

14. Zahner D, Gudlavalleti A, Stephens DS: Increase in pilus islet 2-encoded pili among Streptococcus pneumoniae isolates, Atlanta, Georgia, USA. Emerg Infect Dis. 2010; 16(6):955-962.

15. Pai R, Gertz RE, Beall B: Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. J Clin Microbiol. 2006; 44(1):124-131.

16. Hollingshead SK, Baril L, Ferro S, King J, Coan P, Briles DE, Pneumococcal Proteins Epi Study G: Pneumococcal surface protein A (PspA) family distribution among clinical isolates from adults over 50 years of age collected in seven countries. J Med Microbiol. 2006; 55(Pt 2):215-221.

17. Aguiar SI, Melo-Cristino J, Ramirez M: Use of the 13-valent conjugate vaccine has the potential to eliminate pilus carrying isolates as causes of invasive pneumococcal disease. Vaccine. 2012; 30(37):5487-5490.

18. Abeyta M, Hardy GG, Yother J: Genetic alteration of capsule type but not PspA type affects accessibility of surface-bound complement and surface antigens of Streptococcus pneumoniae. Infect Immun. 2003; 71(1):218-225.

19. Paton JC, Rowan-Kelly B, Ferrante A: Activation of human complement by the pneumococcal toxin pneumolysin. Infect Immun. 1984; 43(3):1085-1087.

20. Tart RC, McDaniel LS, Ralph BA, Briles DE: Truncated Streptococcus pneumoniae PspA molecules elicit cross-protective immunity against pneumococcal challenge in mice. J Infect Dis. 1996; 173(2):380-386.

21. Piao Z, Akeda Y, Takeuchi D, Ishii KJ, Ubukata K, Briles DE, Tomono K, Oishi K: Protective properties of a fusion pneumococcal surface protein A (PspA) vaccine against pneumococcal challenge by five different PspA clades in mice. Vaccine. 2014; 32(43):5607-5613.

22. Balachandran P, Brooks-Walter A, Virolainen-Julkunen A, Hollingshead SK, Briles DE: Role of pneumococcal surface protein C in nasopharyngeal carriage and pneumonia and its ability to elicit protection against carriage of Streptococcus pneumoniae. Infect Immun. 2002; 70(5):2526-2534.

23. Quin LR, Moore QC, 3rd, McDaniel LS: Pneumolysin, PspA, and PspC contribute to pneumococcal evasion of early innate immune responses during bacteremia in mice. Infect Immun. 2007; 75(4):2067-2070.

24. Zhang JR, Mostov KE, Lamm ME, Nanno M, Shimida S, Ohwaki M, Tuomanen E: The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell. 2000; 102(6):827-837.

25. Hammerschmidt S: Adherence molecules of pathogenic pneumococci. Curr Opin Microbiol. 2006; 9(1):12-20.

26. Basset A, Trzcinski K, Hermos C, O'Brien KL, Reid R, Santosham M, McAdam AJ, Lipsitch M, Malley R: Association of the pneumococcal pilus with certain capsular serotypes but not with increased virulence. J Clin Microbiol. 2007; 45(6):1684-1689.

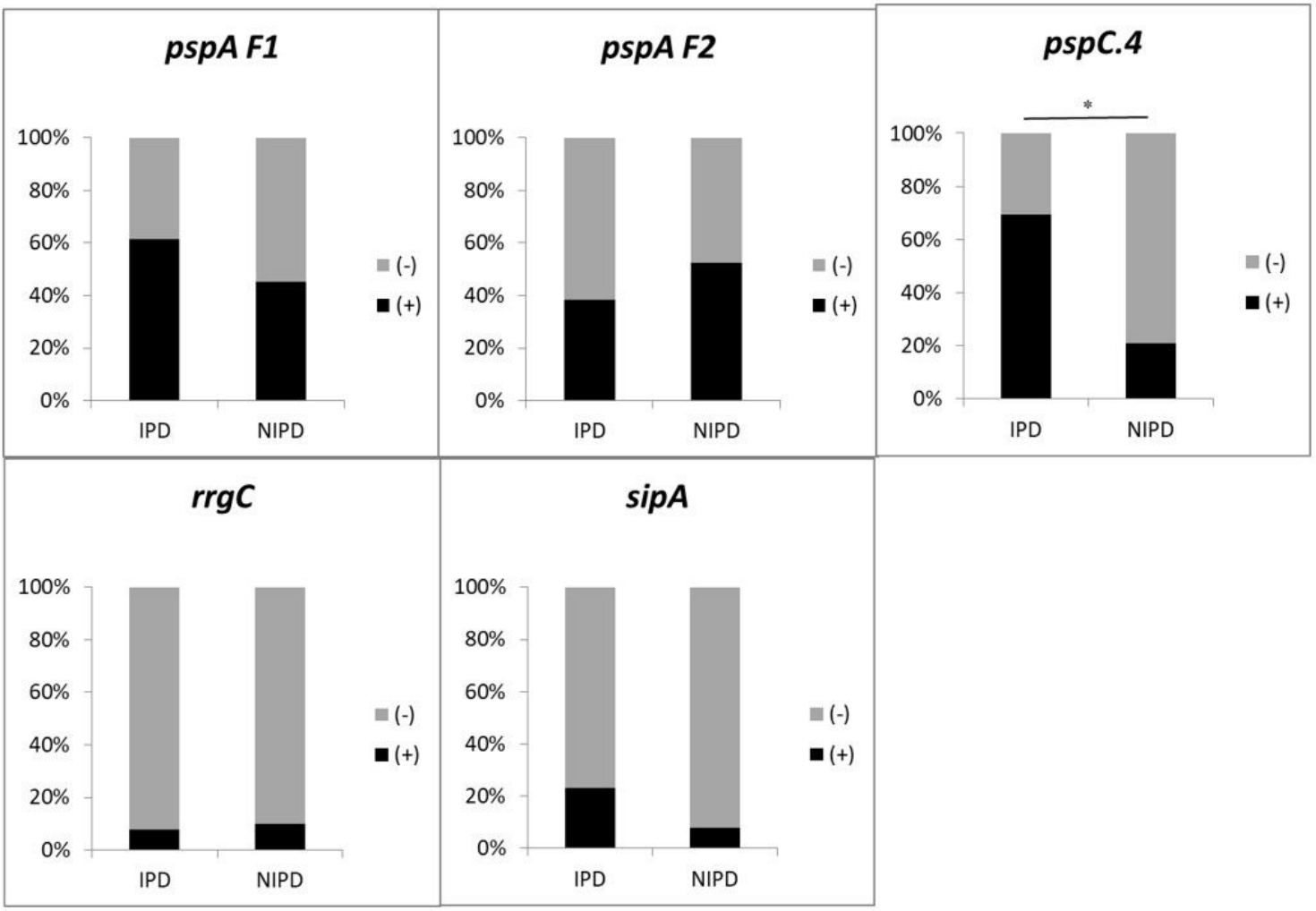
27. Klugman KP, Bentley SD, McGee L: Determinants of invasiveness beneath the capsule of the pneumococcus. J Infect Dis. 2014; 209(3):321-322.

28. Blomberg C, Dagerhamn J, Dahlberg S, Browall S, Fernebro J, Albiger B, Morfeldt E, Normark S, Henriques-Normark B: Pattern of accessory regions and invasive disease potential in Streptococcus pneumoniae. J Infect Dis. 2009; 199(7):1032-1042.

# Tables

Due to technical limitations, all Tables are only available as a download in the supplemental files section.

# Figures




## Figure 1

Proportions of the presence of the pspA F1, pspA F2, pspC.4, rrgC, and sipA, in isolates from each diagnosis group. IPD: isolates from invasive pneumococcal diseases; NIPD: isolates from non-invasive pneumococcal diseases. *: statistically significant difference ($p < 0.001$). Black: gene positive isolates; grey: gene negative isolates.

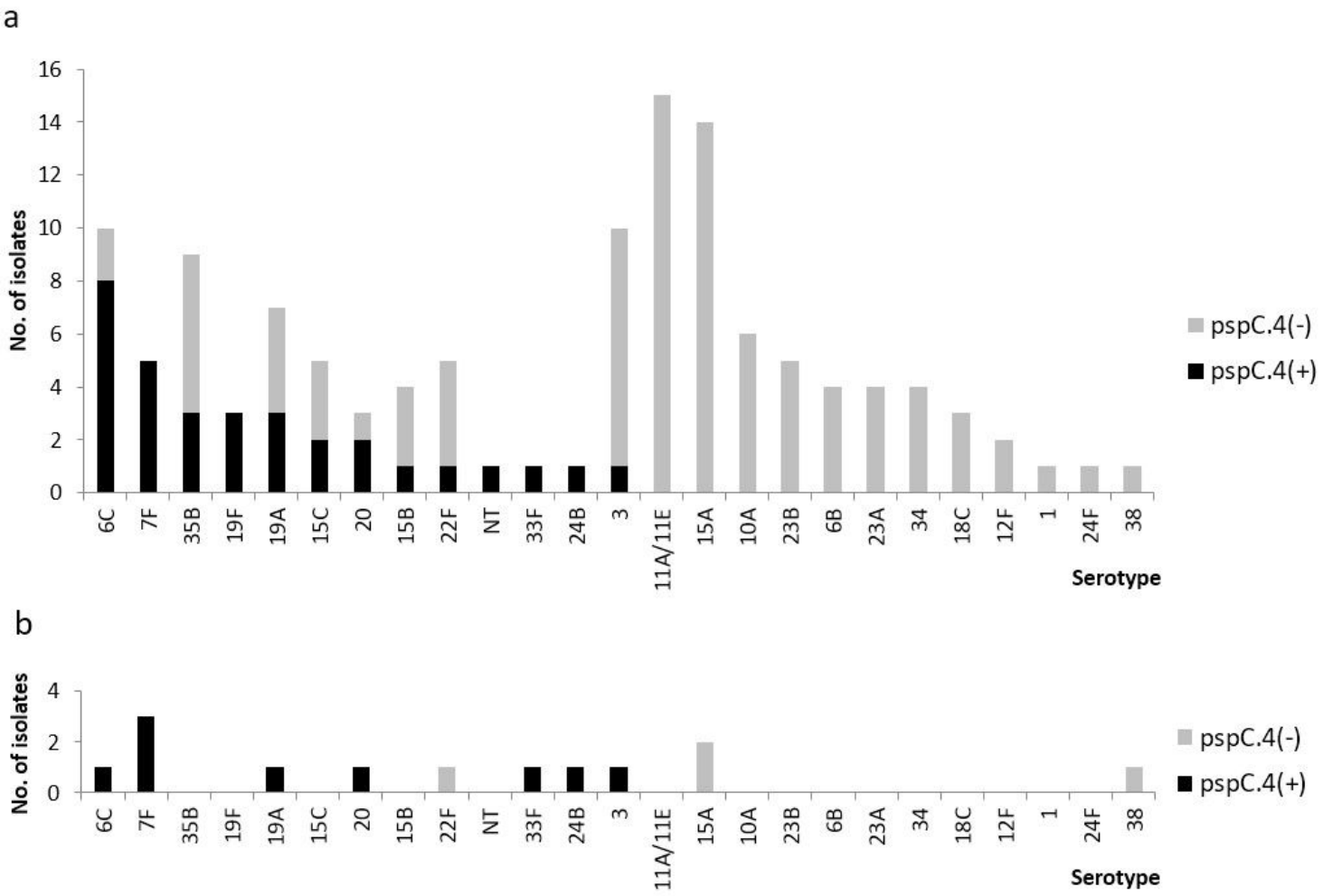



**Figure 2**

Serotype distributions and pspC.4 gene presence of isolates from patients with pneumococcal diseases and invasive pneumococcal disease (IPD). a. Serotype distribution and pspC.4 gene presence of total isolates (n=124). b. Serotype distribution and pspC.4 gene presence of isolates from patients with IPD (n=13). Black: pspC.4 gene positive; grey: pspC.4 gene negative.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.xlsx
- supplement2.xlsx