The relative invasive disease potential of *Streptococcus pneumoniae* among children after PCV introduction: a systematic review and meta-analysis

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Summary

Objectives: Burden of pneumococcal disease depends on the prevalence and invasive disease potential of serotypes. We aimed to estimate the invasive disease potential of serotypes in children under 5 years of age by combining data from different settings with routine immunisation with pneumococcal conjugate vaccines (PCV).

Methods: We conducted a systematic review, supplemented by unpublished data, to identify data on the frequency of pneumococcal serotypes in carriage and invasive pneumococcal disease (IPD). We estimated the invasive disease potential of serotypes as the ratio of IPD in relation to carriage (odds ratio and 95%CI) compared with 19A (reference serotype) by meta-analysis. We report results based on a random effects model for children aged 0–23, 24–29, and 0–59 months and by invasive clinical syndromes.

Results: In comparison with 19A, serotypes 1, 7F, and 12F had a significantly higher invasive disease potential in children aged 0–23 and 0–59 months for all IPD and clinical syndromes (OR>5). Several non-vaccine types (NVTs) (6C, 15A, 15BC, 16F, 23B, in these two age groups) had a lower invasive disease potential than 19A (OR 0·1−0·3). NVTs 8, 12F, 24F, and 33F were at the upper end of the invasiveness spectrum.

Conclusions: There is substantial variation among pneumococcal serotypes in their potential to cause IPD and disease presentation, which is influenced by age and time after PCV introduction. Surveillance of IPD and carriage is critical to understand the expected effectiveness of current PCVs (in the longer term) and guide the development of future vaccines.

Keywords

Streptococcus pneumoniae; serotype; invasive disease potential; pneumococcal conjugate vaccine; meta-analysis

Introduction

Current pneumococcal conjugate vaccines (PCVs) protect against 10 to 13 serotypes (VT) (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F (PCV10), plus 3, 6A, 19A (additional in PCV13) of the 97 different serotypes identified to date.[1] Widespread and routine use of PCVs among children has resulted in alterations in carriage and disease due to shift in the distribution of pneumococcal serotypes. An analysis of 21 large surveillance systems in populations after the introduction of PCV7 in routine immunisation programmes demonstrated that while there was an overall and sustained decrease in childhood invasive pneumococcal disease (IPD), disease due to non-vaccine serotypes (NVT) had increased in all age groups.[2] Additionally, replacement of VT with NVT in nasopharyngeal colonisation in both vaccinated children and unvaccinated populations has resulted in a small or no change of overall *S. pneumoniae* carriage prevalence in different settings globally.[3] The effect of increases in prevalence of NVT nasopharyngeal carriage on childhood IPD and the contribution of specific serotypes to invasive disease in the long run remains uncertain. Since nasopharyngeal colonisation is a key prerequisite for pneumococcal disease, the extent of serotype replacement in IPD is likely to be influenced by colonisation with NVT with low or high invasive disease potential.[3]

S. pneumoniae serotypes differ in their potential to cause IPD. In a meta-analysis of 7 datasets from the pre-PCV era,[4] serogroups 1, 5 and 7 were associated with a higher invasive disease potential among children in relation to 14, the most frequent disease-causing type during this period. Those associated with a lower invasive disease potential included 3, 6A, and 15. Several studies have also found varying levels of invasive disease potential among serotypes.[5] After routine use of PCV7, serotype 19A emerged as the most frequent serotype in childhood IPD across industrialised settings.[3] No predominant serotypes have emerged as yet post-PCV10 or PCV13 implementation, though this may occur in the future. Assessing the invasive disease potential of VT and NVTs in relation to predominant serotypes subsequent to the introduction of higher valent PCVs in diverse geographical settings with routine immunisation programmes can assist in understanding the future effectiveness of higher valent PCVs against childhood IPD. Thus, we aimed to estimate the invasive disease potential of *S. pneumoniae* serotypes in young children, by age and syndrome, by pooling data from different countries with routine use of PCV.

Methods

Sources of data

We identified published studies by systematic searches of electronic databases: Medline, Embase, and Global Health (Ovid), Global Health Library (WPRO, EMRO, and SEA), Web of Science, and LILACs. Searches were conducted between October and November 2015 by two reviewers (EB, SD). Search strategies are available in appendix pp 2–4. Eligibility criteria are available in Box 1.

We requested re-analysed or an extension of previously published serotype-specific IPD/carriage data for the years when PCV was available in each setting up to the year 2015 from investigators in 20 locations (3 in North America, 12 in Europe, 4 in Africa and 1 in Latin America) who were invited to collaborate. If sites evaluated multiple serotypes for morphologically distinct colonies, investigators were asked to report each serotype for which individual children tested positive separately. Data were collected between October 2015 and May 2016. A data collection template was developed and piloted before its final use. EB maintained files and communication with collaborators. Datathief (http://www.datathief.org/) was used to extract data from figures in published studies. Serotype data for IPD, meningitis, bacteraemia/sepsis, pneumonia, or other syndromes were extracted or requested for three age groups (0–<12, 12–23, and 24–59 months). We did not re-distribute serotypes 6A and 6C and analysed 15BC as a single serotype.[6,7]

Definitions

We defined IPD as the identification of *S. pneumoniae* from a normally sterile site and carriage from nasopharyngeal specimens. PCV coverage was defined as the percentage of children from carriage studies who received their age-specific PCV recommended dose. Other definitions from settings with routine use of PCV were available and accepted, e.g. percentage of children who received their primary immunisation series by 12 months of age. Annual data on IPD, but not carriage, were available for all years in all datasets. In each setting, we considered the year following the introduction of PCV for which data on isolates in both IPD and carriage were available as the first year for analysis. Since the annual number of IPD isolates was low in some settings, we included data from all eligible years after the initial year.

Data analysis

Our primary objective was to develop overall estimates of the invasive disease potential of individual serotypes compared to the reference serotype among children. Since not all settings had IPD and carriage data

for two key age groups (0–23 and 0–59 months), we developed two sets of data with strict criteria by age for our main analyses. The invasive disease potential for narrower age groups (0–23 and 24–59 months) and across 3 clinical syndromes were estimated as secondary outcomes. For these analyses we only included datasets that reported data for all categories. The individual contribution of each serotype to IPD or carriage in each combined dataset was estimated (box 1). We restricted our meta-analyses to serotypes representing at least 1% of IPD in our combined dataset for 0–59 months. The reference serotype was selected based on criteria used in the pre-PCV era:[4] a) serotype identified in both IPD and carriage studies for all datasets, b) serotype was among the largest overall proportion in both IPD and carriage datasets, c) serotype was among top 5 in individual datasets.

The *metan* command in Stata Version 13 (College Station, TX: StataCorp LP) was used to estimate serotype-specific invasive disease potential odds ratio (ORs) and 95% confidence intervals (CI) by comparing with the reference serotype[4] (box 1). Serotype-specific meta-estimates are reported if carriage or IPD data for a specific serotype were reported in at least 3 datasets. We decided to use a random effects model (DerSimonian & Laird method) for meta-analysis as we anticipated substantial heterogeneity in the included studies.[8] We report the heterogeneity for each serotype included in the meta-analyses using the I^2 where values of less than 25% indicate low heterogeneity, of 25% to 50% as moderate and above 50% as considerable heterogeneity.[29] We applied a continuity correction of 0·005[10] when there were zero cases in either of the outcomes to estimate the invasive disease potential for serotypes detected among carriers but not causing IPD (or vice-versa). We report 95% CIs estimates and for the main analyses we use a p-value <0.002 to denote statistical significance when assessing differences between OR for individual serotypes and the reference serotype using the Bonferroni correction to address issues of multiple comparisons. Sensitivity analyses were conducted to explore the effect of differences across datasets on overall meta-estimates by restricting analysis to datasets with the following characteristics: a) ≥70% PCV coverage, b) low prevalence of HIV, c) industrialised country settings, d) case counts from years subsequent to introduction of a higher valent PCV (10/13), e) implementation of PCV10 or PCV13.

Results

The PRISMA flowchart depicts the process to identify datasets eligible for analysis (figure 1). We included 13 datasets (9 included data provided by collaborators and 4 from published studies).[11-14] Datasets were from settings with routine use of PCVs from Europe, North America, Latin America, and Africa. The characteristics of IPD and carriage studies are shown in Table 1. While age groups were similar for the IPD and carriage data in our meta-analyses, there are differences across sites as well as within individual settings. Across sites, carriage studies included cross-sectional surveys among children in the community or sampled at different type of health facilities (Table 1). Within individual sites, the geographical/racial overlay of carriage data and IPD data are not exact (e.g. individual cities and nationwide data, respectively). In these cases, we aimed to obtain the carriage and IPD data that best correlated in each site and assumed that the carriage data are representative of the entire country.

Serotype distribution in IPD and carriage

Table 2 shows the overall distribution of serotypes in the different datasets included in the meta-analyses. The combined dataset for the post-PCV introduction period for children 0–59 months included 2,648 IPD isolates and 15,931 pneumococci isolates from carriers. The leading IPD-causing serotypes in our combined datasets included PCV10/13 serotypes, except for serotypes 4 and 9V. Serotypes included in meta-analysis accounted for 85·3% of all IPD cases in the combined dataset (of which 48·6% were PCV13 and 36·8% non-PCV13) and 69·6% of carriers (21·7% PCV13 and 48·1% non-PCV13). Among children 0–23 months, 2,677 IPD and 10,930 carriage isolates were examined. Serotypes analysed were associated with 86·8% (46·0% PCV13 and 40·7% non-PCV13) of IPD and 70·2% (23·2% PCV13 and 47·1% non-PCV13) of carriers. PCV13-type 19A was selected as the reference serotype. The distribution of serotypes in IPD cases and carriers in the not included in meta-analysis is provided in appendix p 6.

Invasive disease potential by age group

Nine settings were included in the analyses for children aged 0−59 months. Figure 2 shows results from meta-analyses of the invasive disease potential (OR) as a continuum of invasive disease potential. Overall, significant differences in the meta-estimates of the invasive disease potential of serotypes were found. Among VTs, 1 and 7F were significantly more invasive than 19A (OR between 5−15). Conversely, the invasive disease potential for 6A, 6B, 19F, and 23F was significantly lower (OR between 0·3−0·4). The invasive disease

potential of other VTs (3, 5, 14, and 18C), at the upper end of the spectrum, was not significantly different from 19A. The invasive disease potential of NVT 12F was higher than 19A, 5·8 times higher in relation to 19A while for other NVTs (6C, 15A, 15BC, 16F, 22F, 23B), the invasive disease potential was significantly lower than 19A (ORs ranged between 0·1−0·6). Estimates for the remaining NVTs (8, 10B, 24F, 33F, 35B, 38) were not significantly different from that of 19A. Figure 2 shows that the invasive disease potential relative to 19A of NVTs 12F, 8, 33F, 24F, 22F, and 38 ranked higher than other NVTs in this age group.

In sensitivity analyses, the point-estimates from the overall analysis for children 0−59 months remained similar for serotypes with high or low invasive disease potential in relation to 19A (appendix p 7). Heterogeneity was negligible to moderate for serotypes with a higher or lower invasive disease potential than 19A, except for 12F, 15A, 15BC. Sensitivity analyses did not influence the heterogeneity for these meta-estimates. The invasive disease potential of serotype 5 was significantly higher than 19A when analysis was restricted to data from settings with PCV coverage >70% or when considering data from years subsequent to the introduction of the higher PCV, for which, the heterogeneity in the meta-analysis was low or negligible. There was low heterogeneity in the estimate of invasive disease potential for 35B when analyses were restricted to settings with low HIV prevalence of industrialised settings. Restricting analysis to data for the period with current higher valent PCV did not change the point estimate for serotypes with lower invasive disease potential (6A, 6B, 22F, and 23F), but results were no longer significantly different to 19A.

The analyses of data for 0−23 months olds (11 settings) showed similar results as for the 0−59 months old children (figure 3). VTs 1 and 7F were more invasive (by 5 to 7 fold) compared to 19A, while 6A, 6B, 19F, and 23F were significantly less invasive than 19A (OR ranged between 0·3−0·4). The invasive disease potential of other VTs (3, 5, 14, and 18C) was not significantly different from 19A. For NVTs, the invasive disease potential relative to 19A of 12F was higher and ranked higher than other NVTs; while estimates for 15A, 15BC, 16F, 35B, 6C, and 23B were lower compared to 19A and ranked lower than other NVTs. The sensitivity analyses in this age group demonstrated similar patterns as in the 0−59 months. There was low to moderate heterogeneity for serotypes with a higher or lower invasive disease potential than 19A, except for serotypes 6A, 12F, 15BC, and 35B. Less heterogeneity was noted in the meta-estimate for serotypes 5 and 35B when analysis was restricted to data from settings with PCV coverage >70% and with low HIV prevalence or industrialised,

respectively. Inclusion of a PCV10 dataset in this age group did not impact the overall conclusion as results were similar to those when all datasets were considered (appendix pp 8–9).

Invasive disease potential by narrow age groups and clinical IPD syndromes

For six settings, the serotype-specific invasive disease potential could be estimated for children 0–23 and 24–59 months (table 1). The distribution of the meta-estimates for the invasive disease potential of individual serotypes is shown in appendix p 1. There was overlap of 95%CI for estimates of invasive disease potential with the reference type for most serotypes. Considering all meta-estimates of invasive disease, there was considerable heterogeneity for potential in 11 serotypes in the 0–23 months age groups. However, heterogeneity was negligible to moderate for most serotypes in the 24–59 months age group (except for 4 serotypes: 5, 6A, 33F, NT) (appendix p 10). Though there was overlap of the wide 95% CIs, point estimates of individual serotypes for both age groups were largely in agreement in terms of magnitude as well as in direction of the OR in relation to 19A, with a few exceptions. The point estimate of serotypes' invasive disease potential was 3−4 fold higher for serotypes 1 and 5 in the 24−59 months age group. The point estimates for serotypes 14 and 18C suggested a higher invasive disease potential than 19A in the 24−59 months age group, despite a lower potential in children 0−23 months of age (appendix pp 1, 10).

Five datasets provided serotype data from isolates for meningitis, bacteraemia/sepsis and pneumonia for children 0–59 months (table 1). Confidence intervals of meta-estimates of invasive disease potential for individual serotypes were wide and, for most of the serotypes these overlapped with the reference serotype. Considering all serotypes, heterogeneity of meta-estimates was generally low to moderate for most serotypes in bacteraemia/sepsis and meningitis cases, while considerable heterogeneity was noted when pooling invasive disease potential for pneumonia (appendix 11). Overall, point estimates of invasive disease potential for individual serotypes showed consistency in the direction of invasive disease potential in relation to 19A across syndromes, with a few exceptions (figure 4, appendix p 11). Meta-analyses of invasive disease potential and differences with the reference type by narrow age groups and by syndromes should be interpreted with caution due to a reduced number of datasets and small sample sizes per serotype.

Discussion

Our study shows that estimates of invasive disease potential of non-PCV13 serotypes differ and were usually lower than that of 19A. Serotypes with an invasive disease potential similar to 19A were also identified. This comprehensive assessment of serotype-specific disease potential across different geographic locations informs our understanding of the invasive disease potential of NVTs and thereby the potential of current PCVs for the prevention of IPD in the longer term.

In agreement with pre-PCV findings, we found that serotypes differ in their ability to cause IPD[4]. In our dataset, VTs 1 and 7F that are included in PCV10 and PCV13 were significantly more invasive than 19A in children aged 0−59 and 0−23 months. Additionally, we observed that 12F, a serotype currently not included in PCVs, had a high invasive disease potential (compared with 19A), which is consistent with other findings.[15] In 2015, 12F was identified as the lead cause of IPD due to NVT (19%) in children 0−23 months in Belgium.[16] Increases in incidence of IPD associated with 12F have also been noted among adults and in association with antibiotic resistance in South Africa.[17] Considering the observed ability of 19A to rapidly fill in the vacant niche after eradication of PCV7-types[18] and non-significant differences in IPD potential of other types like 22F, 24F, and 33F, the possibility of an emerging role in IPD for these serotypes post-introduction of PCV10 and PCV13 cannot be excluded. Our results thus re-emphasise the need for ongoing surveillance of circulating pneumococcal strains, despite the fact that available PCVs cover the majority of currently identified highly invasive serotypes.

Overall incidence of childhood IPD in settings with mature PCV programmes has decreased, even though replacement disease has been noted across settings.[2] Our meta-estimates provide further insights into the phenomenon of limited serotype replacement in childhood IPD post-higher valent PCVs. Highly invasive disease strains in relation to 19A in this study, such as 1 and 12F are rarely detected in the nasopharynx by conventional culture and serotyping methods[13,19] or are known to have cyclical fluctuations.[20,21] Since serotype 1 is covered by PCV10 and PCV13, we need to await whether serotype 12F will become dominant in the future in childhood IPD. While our results indicate that the relative invasiveness of serotypes is higher or lower than 19A, our meta-estimates should be considered in light of the level of heterogeneity. The heterogeneity identified for some NVTs in our meta-analysis can also be reflective of a fluctuating, by time and locality, invasive disease potential across settings included in the meta-analyses. Several factors may contribute to this heterogeneity, including factors assessed in our sensitivity analyses but also differences in blood culture rates and antibiotic

susceptibility patterns. It is also important to note that the true heterogeneity across studies is also influenced by differences in study designs, populations, etc. (Table1) and the true uncertainty in estimates of invasive disease potential is wider than those reported by confidence intervals. It is as yet unpredictable whether replacement by a particular NVT will reach a similar level as with 19A replacement disease post-PCV7; e.g. 35% of all IPD cases in young children in 2005 in the USA.[22] Increasing trends of disease and drug resistance due to 15A, 23B, and 35B have recently been reported in Europe and the USA.[23-25] Our meta-analyses indicate that the invasive disease potential of these serotypes in the settings represented in our study is at the lower end of spectrum of invasiveness, and that we need to await developments of these serotypes, that may also depend on setting, antibiotic resistance and co-morbidities, like HIV exposure.

Our review also shows that there is a clear gap in the evidence base as the invasive disease potential of serotypes in low-income countries in Asia and Africa in the post-PCV era remains poorly described. In these regions, serotypes' proportional contribution to childhood IPD differed from industrialised settings before the introduction of PCV.[26-28] Following PCV10 introduction, strains with serotypes such as 2, 8, 10F, 12A, 12F, 18A, 38, and 45 have recently been found to be highly invasive in South Asia.[29] As serotype replacement in carriage continues to take place after PCV implementation, evidence suggests that circulation of a greater number of serotypes, some with high invasive disease potential, may be found in low-income countries.

The risk of invasive disease by specific serotypes in different childhood age groups has not been clearly determined. From our meta-estimates, though with overlapping confidence intervals, serotype 1 and 5 were likely to be about 3−4 times more invasive in children 24−59 months than in those less than 2 years, while 14 and 18C appeared to be more invasive in the younger age group compared than the older. In another study, a higher invasive disease capacity was observed for 13 out of 15 serotypes in children 0−23 months, compared with those aged 24−84 months,[30] which suggested that the varying propensity of strains to cause IPD may contribute to decline in incidence with increasing age. Direct comparisons between our study and this study cannot be made, as methodologies and serotypes analysed differed (e.g. methods to estimate invasiveness and geographic/temporal representation). Nonetheless, agreements in findings that serotypes vary in their capacity to cause IPD events by age groups is important for public health purposes. If replacement in carriage results in more carriage of serotypes with lower invasive disease potential, these serotypes may nevertheless act like opportunistic serotypes in individuals at high risk of IPD (e.g. elderly or with co-morbidities) and severe IPD

outcomes. These groups may constitute a large part of the remaining burden of *S. pneumoniae* in the future, even though, the overall IPD burden in the whole population would be lower. Data from ongoing studies in South Africa indicate that the invasiveness of serotypes are likely to differ by immune status (e.g. by HIV status). Further research in other settings is needed to explore differences in invasive disease potential by different populations.

Pneumococcal serotypes have also been shown to vary in their ability to cause particular clinical outcomes, such as case fatality or disease syndrome like empyema or meningitis.[31] We estimated the disease potential of strains by three IPD syndromes in children. Compared with 19A, among meningitis and pneumonia a higher invasive disease potential was estimated for 12F. There is a paucity of reliable data describing relationships between specific serotypes and individual clinical syndromes. Nevertheless, several studies have shown that serotype 12F is associated with meningitis and has been documented indirectly from outbreaks to be hyper-invasive.[5,32] Increases in cases of overall IPD and antibiotic non-susceptible serotype 12F following PCV introduction have been recently reported in Israel and France. This increase was caused by a single clone expansion and 89% of 12F IPD cases were penicillin non-susceptible in Israel, suggesting the need to monitor the invasiveness of 12F.[33,34] Similarly, although 24F was not significantly more invasive than 19A, it appeared to be prone to cause meningitis. Serotype 24F has emerged as the leading cause of pneumococcal meningitis in France after PCV13 introduction in children 0−23 months.[35] In Norway, 24F showed an increase in incidence and clinical severity.[36] Further studies are required to understand the epidemiology of individual serotypes on the burden of IPD from a clinical perspective to inform on new prevention strategies in the PCV era.

Our study has several limitations. Firstly, we chose 19A as the reference type even though it is not included in PCV10. This serotype is likely to be prone to selective advantages due to high genetic diversity, clonal shifts, and antibiotic resistance.[37] However, it was the only serotype present in all datasets and this enabled an estimation of ORs across multiple settings. The comparison with 19A represents 19A invasiveness mostly in population immunised. Our sensitivity analysis showed no impact on the study conclusion when PCV10 dataset was excluded. Secondly, some of our serotype-specific estimates are affected by low numbers of cases and heterogeneity was noted. As the number of childhood IPD cases has decreased upon PCV use, estimates of invasive disease potential based on incidence rates (which were not available for this analysis), will be required. Furthermore, sampling of carriage cases differed across settings, where antibiotic use is likely to

vary. As these limitations affect precision and the ability to detect significant differences, we conducted a wide range of sensitivity analyses and focused on describing estimates and their plausible range of values rather than conducting significance tests to avoid issues of multiple comparisons. However, we did not assess the impact of other factors on the estimates of invasiveness, such as role of rates of blood culturing or antibiotic use. As these factors are likely to vary across sites, their role on estimates of invasive disease potential and heterogeneity remains to be assessed. Biases leading to under or overestimation of invasive disease potential cannot be excluded. Our IPD data came from passive surveillance systems and carriage data usually from cross-sectional studies. These sources are vulnerable to reporting and ascertainment biases. However, it has been shown that cross-sectional data can be used reliably to examine invasive disease potential of capsular types.[15] Changes to clinical practices and blood culturing in the post-PCV era could also lead to underestimation of the role of *S. pneumoniae* in particular in ambulatory cases of pneumonia or bacteraemia.[3] Additionally, introduction of PCV would have likely changed the ratio of bacteraemic and non-bacteraemic pneumonia (the proportion of latter having increased substantially post-PCV).[38] The use of post-PCV data only a few years after introduction for settings that have transitioned to PCV10/13 is also a source of bias since development of new equilibria after PCV introduction may take time and up to 6-16 years.[39] PCV immunisation is effective on decreasing IPD and colonisation for targeted serotypes, but replacement by NVT takes time which could have led to underestimation of the role of NVTs.

Our study has several strengths including the wide geographical spread of the included settings and the supplementation of published literature with data from collaborators, which enabled serotype-specific analyses and minimised information biases. We also included long study periods to minimise risk of random error due to small sample sizes or outbreaks of serotypes causing IPD. We have presented analyses for a large number of serotypes, selected by their role in causing disease in various settings. Additionally, we provide results for various sensitivity analyses and report meta-estimates based on random effects model to address issues of heterogeneity across studies. Limitations withstanding, this paper provides a comprehensive view of the invasive disease potential of *S. pneumoniae* serotypes causing childhood IPD post-PCV.

Conclusion

There is substantial variation among pneumococcal serotypes in invasive potential to cause IPD and disease presentation which is influenced by age and time after PCV introduction. This poses challenges to the

design of the optimal composition of PCV in different settings. Because of the diversity of pneumococcal serotypes, surveillance of IPD and carriage is critical to understand the sustained effectiveness of current PCV products in the longer term and guide the development of future PCVs for use in specific settings.

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Contributions

MHK, HC and HN conceptualised the study. EB led the literature review with contributions from SD. EB analysed data. EB, MHK, and HN led data interpretation and wrote the first draft. All other named authors contributed to analysis of primary data, data interpretation, and critically reviewed drafts of the manuscript. All authors read and approved the final draft of the manuscript. EB and MHK are accountable for accuracy and integrity of contents in this manuscript.

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Competing interests

EB, HC and HN are employees of the University of Edinburgh and funding for this study was provided via an agreement between Sanofi Pasteur and the University. MHK is an employee of Sanofi Pasteur. AP reports grants from Pfizer for collection of primary data used in this meta-analysis, outside the submitted work; and that he is Chair of UK Department of Health's Joint Committee on Vaccination and Immunisation and a member of WHO's SAGE committee. AvG reports grants from Pfizer, other from Pfizer, Sanofi and Novartis, outside the submitted work. AvdE reports grants from Pfizer, grants from National Institute of Public Health and the Environment, during the conduct of the study; grants from Pfizer, other from GSK, other from Pfizer, outside the submitted work. CMA reports grants from Pfizer, personal fees from GSK, outside the submitted work. KT reports grants from Pfizer outside the submitted work. RK reports funding from a Pfizer Investigator Initiated Research grant and support from NIHR Oxford Biomedical Research Centre, during the conduct of the UK carriage study. ES reports research grants from Pfizer and GSK outside the submitted work. SP reports membership of the advisory boards for Merck, Pfizer, Sequiris, consultancy activities for Pfizer, including projects at PAI, Brookline, MA, grants from Pfizer, payment for lectures by J&J and Pfizer, and royalties, all outside the submitted work. AV, AS, DFV, DF, PG, LB, LD, CM, NG, RD, SD, SM, IY, ZV have nothing to disclose. No financial support in any form from Sanofi Pasteur was given to AP, AvG, AvdE, KT, RK, KR, SP, AV, AS, CMA, DFV, DF, ES, PG, LB, LD, CM, NG, RD, SD, SAM, SM, SN, IY, GZ for this work.

Box 1: Eligibility criteria for databases with *S. pneumoniae* carriage and/or IPD serotype data

Inclusion criteria

- Observational studies (prospective, retrospective) published between 2000−2015
- *S. pneumoniae* serotypes' data are available from carriage *and* invasive disease studies among children 0−59 months from similar population during similar periods.
- Study population included children vaccinated with PCVs or from settings with wide-spread routine use of PCVs. For carriage, data had to be from healthy or not exclusively from severely sick children.
- IPD was defined as the identification of a pneumococcus isolate from a normally sterile site (e.g. blood, cerebrospinal, pleural effusions, or joint fluid)

Exclusion criteria:

- Study does not report data on *S. pneumoniae* serotypes or serotype-specific data are not reported for all carriage or IPD cases
- Serotype data for either IPD or carriage are not available specifically for a period post−PCV introduction
- Serotype data are from study populations exclusively of immunocompromised populations or data include adults
- If data overlap with other publications exists, studies with the longest study period or larger sample size are to be included
- Isolates were recovered to address a specific question and high risk of bias (e.g. rates of antimicrobial resistance, severe cases)
- IPD and carriage serotype data are not from similar paediatric populations
- Pneumococcus recovered from nasopharynx with a diagnosis of invasive disease used as a surrogate from a normally sterile site (IPD)

Serotypes' overall contributions to IPD or carriage in the combined dataset were estimated as described in the following equation using the 0−59 months as an example: $IPD_i = \frac{\sum_{i=1}^{j=9} x_{ij}}{N} \times \mathbf{100}(\%)$

Where x_{ij} is the number of isolates in serotype *i* in study *j*, *j* is the index of settings, *N* is total number of isolates serotyped in the combined dataset

Invasive disease potential (OR) was estimated using the following formula:

$$\boldsymbol{OR} = \left(\frac{\boldsymbol{a} \times \boldsymbol{d}}{\boldsymbol{b} \times \boldsymbol{c}}\right) = \frac{\boldsymbol{number\ of\ invasive\ serotype\ X\ isolates} \times \boldsymbol{number\ of\ carriage\ reference\ isolates}}{\boldsymbol{number\ of\ carriage\ serotype\ X\ isolates} \times \boldsymbol{number\ of\ invasive\ reference\ isolates}}$$

385 **Table 1: Characteristics of datasets included in meta-analysis**

	Carriage			IPD			
Country, year PCV7 and PCV10-13 introduction	**Study design**	**Study population**	**Study period in meta-analysis**	**Study design**	**Case ascertainment**	**Study period in meta-analysis**	**Analyses dataset included**
USA Alaska†, PCV7 2001, PCV13 2009/10	Cross-sectional annual surveys	Children at urban pediatric clinics and at households in rural Alaska villages. ≥80% vaccination coverage in carriage studies.	2002, 2003, 2004, 2008, 2009, 2010, 2011, 2012, 2013, 2014	Statewide surveillance by clinical laboratories. IPD is a reportable condition in Alaska	Positive culture from a normally sterile site from Alaska residents. IPD cases from south east Alaska were also excluded so the IPD data better correlates with the carriage data	2002 to 2014, inclusive	0–59 months, 0-23 months, narrow age groups, syndromes
USA Atlanta ABCs PCV7 2001, PCV13 2010‡	Cross-sectional survey	Children 6–59 months of age residents of the study area and who sought medical care, regardless of presenting symptom at the emergency department	2009 Jan/Aug	Continuous active population-based surveillance	Positive culture from a normally sterile site from the residents of the study area	2008Jun/2009 May	0–59 months,
USA Massachusetts† PCV7 2000, PCV13 2010	Cross-sectional surveys	Children attending well-child or sick visits at primary care practices in 16 (2003/04) and 8 (other study periods) communities during respiratory virus season	2003Nov/04Apr, 2006Oct/07Apr, 2008OCt/09Apr, 2010OCt/11Apr, 2013Oct/14Apr	Passive, prospective surveillance	Positive culture from a normally sterile site in Massachusetts	Oct-Sep in years 2003/04, 2006/07, 2008/09, 2010/11, 2013/14	0-23 months
USA Navajo PCV7 2000, PCV13 2010‡	Prospective longitudinal observational cohort	Representative selection of nasopharyngeal samples from the 861 first acquisition isolates from a prospective longitudinal observational cohort study of children <5 years	2006Mar-2008Mar	Active surveillance of clinical microbiology laboratories	Children <5 years of age who resided in the carriage cohort study communities, and who had an incident episode of IPD identified through active surveillance	2006March to 2008March	0–59 months
Colombia PCV7: 2009; PCV13 ‡	Cross-sectional survey	Nasopharyngeal samples recovered at six urban areas of Bogotá from healthy children of 12 to 18 months of age, which were vaccinated with PCV7.	2011Jun/Nov	Passive, prospective surveillance	Children ≤2 years of age diagnosed with IPD who were living in Bogotá through the National Surveillance Program*	2010-201	0-23 months
France PCV7: 2006; PCV13 2010	Cross-sectional surveys	300 healthy children aged 6–24 months for well-baby visits among 90 paediatricians.	2008/09 and 2012/13	Prospective surveillance	Cases reported from 400 laboratories located in the 22 regions of France	2008/09 and 2012/13	0-23 months
Israel† PCV7: 2009; PCV13 2010	Prospective health-facility based surveillance.	Collection of NP among healthy children visiting the paediatric emergency or maternal and child health centres for vaccination or regular check-up in Southern Israel	2010, 2011, 2012, 2013, 2014, 2015	Prospective surveillance	Positive culture from a normally blood or cerebrospinal fluid from the entire country	2010, 2011, 2012, 2013, 2014, 2015	0–59 months, 0-23 months, narrow age groups, syndromes
Italy† PCV7: 2006; PCV13 2010	Prospective, cross-sectional surveys	PCV13-vaccinated healthy children in Milan, Lombardy	Sep/Dec 2011, Jun 2011, Sep12/Dec12	Prospective surveillance system	Positive culture from blood and/or cerebrospinal fluid Lombardy	2011 to 2015, inclusive	0–59 months, 0-23 months

	Carriage			IPD			
Country, year PCV7 and PCV10-13 introduction	**Study design**	**Study population**	**Study period in meta-analysis**	**Study design**	**Case ascertainment**	**Study period in meta-analysis**	**Analyses dataset included**
	evaluated by home visits						
Netherlands† PCV7: 2006; PCV13 2011	Prospective, cross-sectional surveys in two age-cohorts of healthy children vaccinated evaluated by home visits	Child had to be vaccinated according to the national immunization schedule, the parents have to be willing and able to participate in the trial according to procedure. The child is either 11 or 24 months old (+/- 1-4 weeks) in the Western region	2009 Feb-Jul, 2010/11 Sep-March2012/13 Sep-March	Prospective surveillance	IPD cases (isolates from CSF and/or blood) provided by the reference laboratory, nationwide	2009−14, inclusive	0-23 months**
Norway† PCV7: 2006; PCV13 2011	Cross-sectional surveys	Children in daycare centres in and around Oslo.	2006 Autumn, 2008 Autumn, 2013 Autumn, 20015 Autumn	Prospective surveillance	Positive culture from a normally sterile site Reference Laboratory from the entire country	2008 to 2015(Nov), inclusive	0–59 months, 0-23 months, narrow age groups, syndromes
Spain† PCV7: 2001; PCV13 2011	Prospective surveillance	Healthy Children who attended University Hospital in Barcelona for minor surgical procedures in our hospital (i.e phimosis or dermatologic surgery)	2004, 2005, 2006, 2007, 2008, 2009, 2010, 2014, 2015	Prospective surveillance	Presence of clinical findings of infection, together with the isolation by culture and/or DNA detection by real-time polymerase chain reaction (PCR) of *S. pneumoniae* in any usually sterile fluid at a University Hospital in Barcelona, Spain.	2004 to 2015, inclusive	0–59 months, 0-23 months, narrow age groups, syndromes
UK† PCV7: 2006; PCV13 2010	Cross-sectional surveys	Children recruited via the child health computer department and/or day-care facility. Children that had received PCV13, incomplete PCV7 schedule, or with an acute respiratory infection were excluded.	2010Nov/2011Sep2014Feb/2015Aug	Prospective surveillance	IPD cases identified through 10 laboratories sending isolates to Oxfordshire surveillance program	2010–15 inclusive	0–59 months, 0-23 months, narrow age groups
South Africa PCV7: 2009; PCV13 2011	Cross-sectional surveys	Children recruited from well-baby clinics and ART clinics as part of a mother-infant pair study with concordant HIV status. Excluded from study: Underlying illness that contraindicated an nasopharyngeal swab or discordant HIV status with mother	2010May/2011Feb, 2012May/2013Apr	Passive, Population-based surveillance	IPD cases were identified through the laboratory at Chris Hani Baragwanath Hospital, Soweto	2010 to 2013, inclusive	0–59 months, 0-23 months, narrow age groups, syndromes

Notes: ABCs: Active Bacterial Core Surveillance, * Surveillance Networks System of Bacterial Agents Causing of Pneumonias and Meningitis † Includes data obtained from collaborators. ‡Data included in analyses are for post-PCV7, but PCV13 has now been introduced in this setting. PCV coverage in the datasets ranged between 50% to >90%. **The variation within any group at the time of sampling was within +/- 1 month. Strains cultured from these children were likely to be acquired before they reached 24 months.

Table 2: Serotype distribution and number of isolates included in meta-analyses

Analysis	Overall				By age		By clinical syndromes		
IPD definition	Any IPD				Any IPD		Meningitis	Bacteraemia Sepsis	Pneumonia
Age (months)	0−23		0−59		0−23	24−59	0−59	0−59	0−59
Number of settings	11		9		6	6	5	5	5
	Number of (n) IPD/Carriage Isolates (%)								
Total	2677/10930		2648/15931		1552/8214	862/6947	323/14408	719/14408	1133/14408
PCV10	599/1158	(22·4/10·6)	767/1756	(29·0/11·0)	(21·5/11·8)	(42·6/10·8)	(25.1/11.7)	(23·4/11.7)	(37·2/11.7)
PCV13 not 10	657/1443	(24·5/13·5)	545/1825	(20·6/11·7)	(21·4/11.7)	(17·7/11·3)	(13·3/11·6)	(17·5/11·6)	(23·9/11·6)
NVT	1421/8329	(53·1/75·9)	1336/12350	(50·5/76·9)	(57·1/76·4)	(39·7/77·9)	(61·7/78·4)	(59·1/76·4)	(38·8/76·4)
1*	111/23	(4.1/0.2)	255/41	(9.6/0.3)	65/15	165/25	7/39	27/39	186/39
3†	134/129	(5/1.2)	146/299	(5.5/1.9)	79/90	56/195	9/258	25/258	89/258
5*	77/15	(2.9/0.1)	160/26	(6/0.2)	68/14	86/11	19/25	30/25	103/25
6A†	48/358	(1.8/3.3)	52/434	(2/2.7)	31/267	21/159	9/426	18/426	26/426
6B*	29/201	(1.1/1.8)	33/294	(1.2/1.8)	18/162	14/129	6/289	12/289	14/289
6C	20/397	(0.7/3.6)	20/559	(0.8/3.5)	10/224	7/272	4/468	5/468	7/468
7F*	189/45	(7.1/0.4)	124/83	(4.7/0.5)	70/25	40/57	12/74	32/74	53/74
8	40/52	(1.5/0.5)	33/75	(1.2/0.5)	23/41	6/30	13/70	5/70	6/70
10A	105/278	(3.9/2.5)	51/377	(1.9/2.4)	45/170	5/177	10/310	22/310	5/310
10B	20/115	(0.7/1.1)	25/166	(0.9/1)	19/114	6/52	2/166	10/166	7/166
12F	202/55	(7.5/0.5)	239/94	(9/0.6)	169/52	54/37	32/80	101/80	65/80
14*	57/130	(2.1/1.2)	69/180	(2.6/1.1)	33/115	26/59	4/174	19/174	35/174
15A	55/482	(2.1/4.4)	43/681	(1.6/4.3)	28/374	10/245	9/587	7/587	16/587
15BC	154/1107	(5.7/10.1)	131/1503	(4.9/9.4)	93/844	31/613	22/1372	55/1372	25/1372
16F	31/509	(1.2/4.7)	32/813	(1.2/5.1)	20/446	11/353	5/779	12/779	12/779
18C*	21/41	(0.8/0.4)	19/66	(0.7/0.4)	7/34	11/32	4/66	11/66	3/66
19A†	475/956	(17.7/8.7)	346/1092	(13.1/6.9)	222/607	76/429	25/1000	83/1000	156/1000
19F*	63/404	(2.4/3.7)	47/602	(1.8/3.8)	38/340	4/248	13/586	19/586	9/586
22F	87/238	(3.2/2.2)	76/401	(2.9/2.5)	46/174	17/207	8/350	19/350	17/350
23B	34/562	(1.3/5.1)	42/873	(1.6/5.5)	21/411	15/421	9/756	13/756	5/756
23F*	29/229	(1.1/2.1)	36/350	(1.4/2.2)	20/205	12/141	12/345	12/345	8/345
24F	107/98	(4/0.9)	58/173	(2.2/1.1)	34/73	14/93	9/148	14/148	14/148
33F	113/197	(4.2/1.8)	94/305	(3.5/1.9)	72/157	11/137	13/278	31/278	34/278
35B	39/520	(1.5/4.8)	35/711	(1.3/4.5)	30/383	2/304	5/660	13/660	9/660
38	36/103	(1.3/0.9)	39/202	(1.5/1.3)	28/86	7/109	1/181	18/181	8/181
NT	48/431	(1.8/3.9)	56/727	(2.1/4.6)	42/356	14/328	9/663	6/663	37/663

Notes: IPD: Invasive pneumococcal disease. n: number of cases, NVT: non-PCV13, *PCV10/13 and †PCV13 serotype. Data reported for “Other” clinical syndromes are not shown

Figures

Figure 1: PRIMSA flowchart
Process to identify dataset to estimate *S. pneumoniae* serotypes invasive disease potential

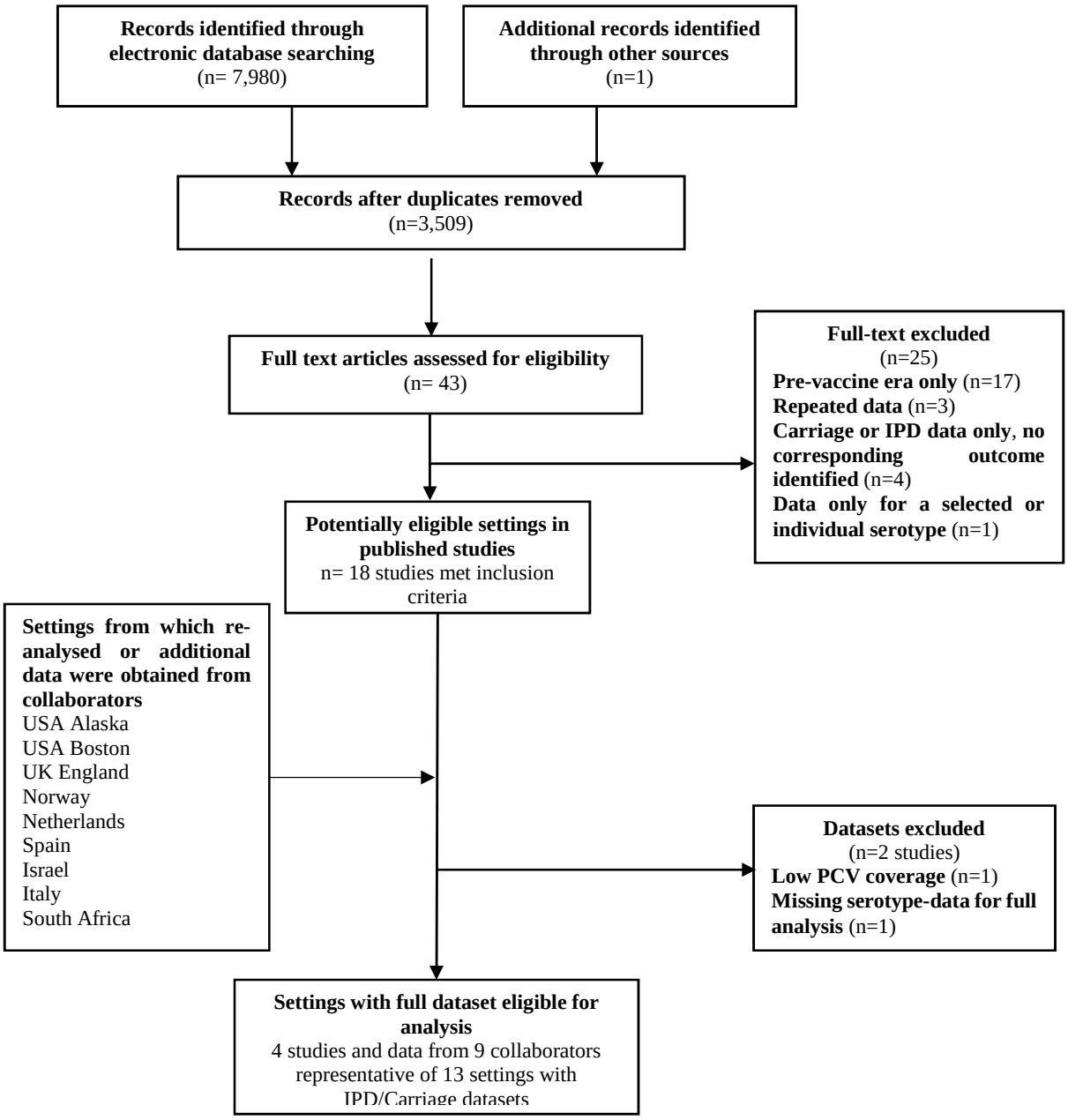


Figure 2: Serotype-specific contribution to IPD, carriage, and invasive disease potential in children 0–59 months

Serotypes are ranked by highest to lowest estimate of invasive disease potential. Bars depict overall contribution of each serotype to IPD and carriage in the combined dataset (%, left axis, N=9 settings). Dots show meta-estimates of serotype-specific invasive disease potential (OR 95%CI, right axis on a log-scale, point estimate shown in boxes). Dotted black line: Reference line for invasive disease potential (19A; right axis). *PCV10/13 and †PCV13 serotype

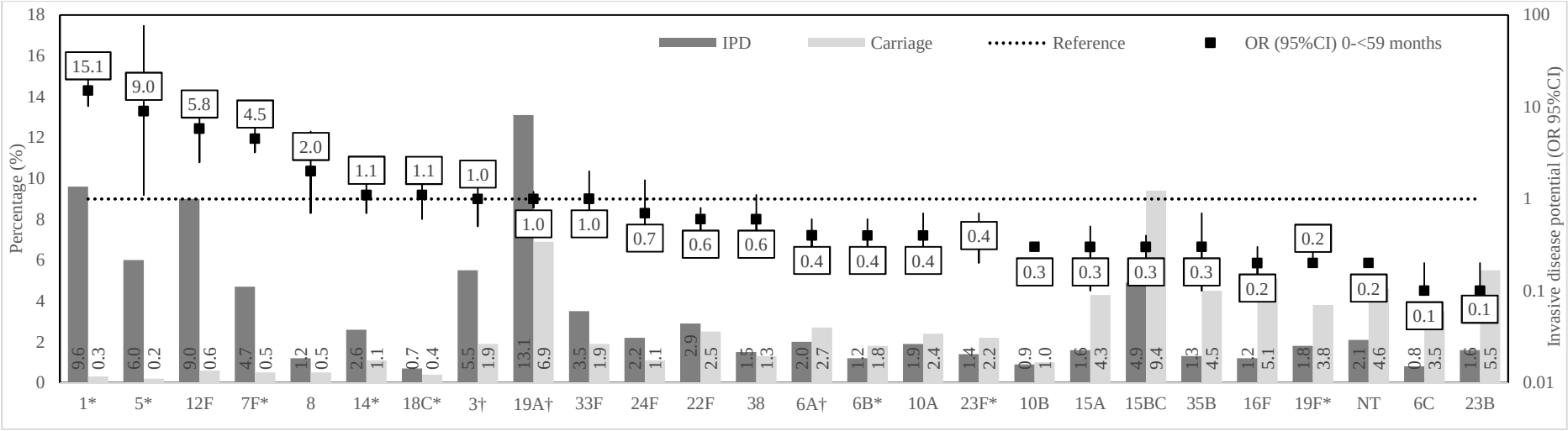


Figure 3: Serotype-specific contribution to IPD, carriage, and invasive disease potential in children 0–23 months
Serotypes are ranked by highest to lowest estimate of invasive disease potential. Bars depict overall contribution of each serotype to IPD and carriage in the combined dataset (%, left axis, N=11 settings). Squares depict meta-estimates of serotype-specific invasive disease potential (OR 95%CI, right axis on a log-scale, point estimate shown in boxes). Dotted black line: Reference line for invasive disease potential (19A; right axis). *PCV10/13 and †PCV13 serotype

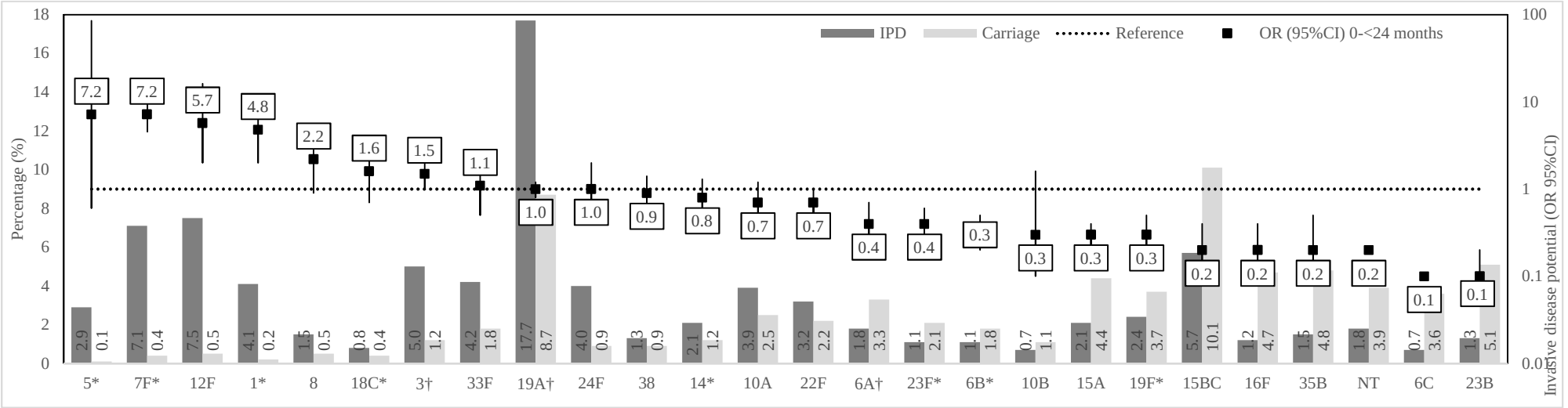
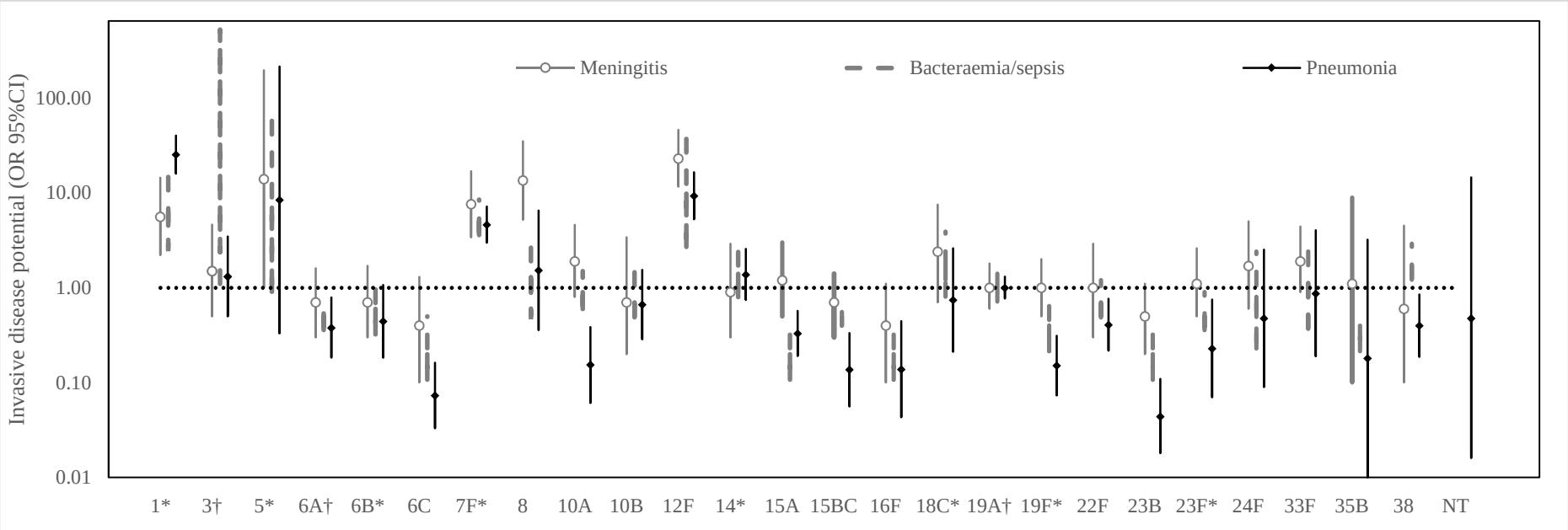


Figure 4: Serotype-specific invasive disease potential by syndromes in children 0−59 months

Meta-estimates of serotypes' invasive disease potential (OR 95%CI left axis on a log-scale) among cases of meningitis: grey solid line and circle, bacteraemia/sepsis: grey dotted line, and pneumonia: black solid line and dots. Dotted horizontal black line: Reference line for invasive disease potential (19A). *PCV10/13 and †PCV13 serotype



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