Analysing pneumococcal invasiveness using Bayesian models of pathogen progression rates

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Short title: Bayesian analyses of microbial progression rates

Abstract

The disease burden attributable to opportunistic pathogens depends on their prevalence in asymptomatic colonisation and the rate at which they progress to cause symptomatic disease. Increases in infections caused by commensals can result from the emergence of “hyperinvasive” strains. Such pathogens can be identified through quantifying progression rates using matched samples of typed microbes from disease cases and healthy carriers. This study describes Bayesian models for analysing such datasets, implemented in an RStan package (<https://github.com/nickjcroucher/progressionEstimation>). The models converged on stable fits that accurately reproduced observations from meta-analyses of *Streptococcus pneumoniae* datasets. The estimates of invasiveness, the progression rate from carriage to invasive disease, in cases per carrier per year correlated strongly with the dimensionless values from meta-analysis of odds ratios when sample sizes were large. At smaller sample sizes, the Bayesian models produced more informative estimates. This identified historically rare but high-risk *S. pneumoniae* serotypes that could be problematic following vaccine-associated disruption of the bacterial population. The package allows for hypothesis testing through model comparisons with Bayes factors. Application to datasets in which strain and serotype information were available for *S. pneumoniae* found significant evidence for within-strain and within-serotype variation in invasiveness. The heterogeneous geographical distribution of these genotypes is therefore likely to contribute to differences in the impact of vaccination in between locations. Hence genomic surveillance of opportunistic pathogens is crucial for quantifying the effectiveness of public health interventions, and enabling ongoing meta-analyses that can identify new, highly invasive variants.

Author summary

Opportunistic pathogens are microbes that are commonly carried by healthy hosts, but can occasionally cause severe disease. The progression rate quantifies the risk of such a pathogen transitioning from a harmless commensal to causing a symptomatic infection. The incidence of infections caused by opportunistic pathogens can rise with the emergence of “hyperinvasive” strains, which have high progression rates. Therefore methods for calculating progression rates of different pathogen strains using surveillance data are crucial for rapidly identifying emerging infectious disease threats. Existing methods typically measure progression rates relative to the overall mix of microbes in the population, but these populations can vary substantially between locations and times, making the outputs challenging to combine across studies. This work presents a new method for estimating progression rates from surveillance data that generates values useful for modelling pathogen populations, even from relatively small sample sizes.

Introduction

Opportunistic pathogens are commonly found in the environment, or microbiota of healthy hosts, but have the capacity to cause disease in some host organisms [1]. The frequency with which they transition from asymptomatic colonisation to symptomatic infection can be quantified as a progression rate [2]. Many opportunistic pathogens are diverse, and can be typed by a variety of phenotypic methods [3], or divided into strains using genotyping or whole genome sequencing [4,5]. Understanding the variation in progression rates across such species is crucial for understanding changes in the incidence of diseases caused by opportunistic pathogens. Recently, strains with high progression rates, sometimes referred to as “hypervirulent” [6] or “hyperinvasive” [7], have been ascribed as the primary cause of rising case numbers of disease caused by *Neisseria meningitidis* [8], *Streptococcus pyogenes* [9] and *Klebsiella pneumoniae* [10], among others. However, understanding whether such strains drive elevated disease burdens through more rapid transmission, or heightened progression rates, typically requires additional information on the carried population of the opportunistic pathogen.

Progression rates have been extensively studied in the nasopharyngeal commensal and respiratory pathogen *Streptococcus pneumoniae* (the pneumococcus). Over one hundred serotypes have been identified in this species, each corresponding to a structurally-distinct polysaccharide capsule [11,12]. These are clustered into 48 serogroups, based on serological cross-reactivity [13]. This capsule inhibits pneumococci being cleared by the immune system, and is crucial to their ability to cause invasive pneumococcal disease (IPD) [14,15], infections of normally sterile anatomical sites. Hence the rate of progression from carriage to IPD is referred to as

invasiveness [16]. It has long been assumed the capsule is an important determinant of pneumococcal invasiveness, based on differences in serotypes' ability to cause disease in animal experiments [17]. Epidemiological differences are also apparent in human disease, with paediatric serotypes (including serotypes within serogroups 6, 9, 19, 23, and serotype 14) common in sporadic infant disease, and 'epidemic' serotypes (such as 1, 2, 5 and 12F) capable of causing IPD outbreaks in adults [18,19].

Understanding these differences between serotypes has become crucial for *S. pneumoniae* epidemiology, owing to the introduction of polysaccharide conjugate vaccines (PCVs) [20]. A seven valent PCV (PCV7) was introduced in the USA in 2000, which has been supplanted by ten- and thirteen-valent formulations (PCV10 and PCV13, respectively). These induce immune responses against a specific subset of serotypes, resulting in their elimination from carriage [21]. However, the overall frequency of *S. pneumoniae* colonisation typically remains stable after any PCV's introduction, owing to the replacement of vaccine serotypes by the plethora of non-vaccine serotypes [22,23]. Nevertheless, PCVs have usually proved highly effective at reducing IPD through facilitating the replacement of the pre-PCV carried population with pneumococcal serotypes associated with lower invasiveness [24,25]. Hence optimal PCVs are those which minimise the overall invasiveness of the carried pneumococcal population [26,27]. Therefore estimation of invasiveness across vaccine-targeted serotypes, and those which may replace them post-PCV, is vital for reducing the incidence of IPD. Such quantification of invasiveness is typically achieved through paired studies of serotypes' prevalence in geographically and temporally matched surveys of pneumococcal carriage, and surveillance of IPD.

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105 Multiple methods have been used to estimate invasiveness using such paired case
 106 and carrier data. The most common approaches use odds ratios: the ratio of isolates
 107 of a given serotype recovered from disease against those recovered from carriage,
 108 divided by the same ratio calculated across all other serotypes in the population
 109 [28,29]. However, this statistic is intrinsically imperfect, because even if a serotype
 110 causes disease at a consistent rate across populations, the mix of serotypes against
 111 which it is compared will differ between locations [30,31]. This geographical
 112 heterogeneity has been exacerbated by post-PCV serotype replacement, which has
 113 driven increasing divergence between countries' serotype compositions [32]. The
 114 interpretability of the odds ratios may be improved by standardising them relative to
 115 the geometric mean across all odds ratios [33], but this does not resolve the
 116 underlying problem, which likely contributes to the substantial heterogeneity
 117 observed for some serotypes when odds ratios are combined in meta-analyses [34].
 118 This means carefully-conducted odds ratio analyses can be hampered by having to
 119 subsample data, and employ both fixed and random effects analyses, within a single
 120 analysis [35]. One solution has been to estimate invasiveness relative to a standard
 121 serotype that is common in both carriage and IPD across studied locations [34,36].
 122 However, these properties mean such serotypes are likely to be targeted by PCVs.
 123 Correspondingly, the original standard, serotype 14 [29], has been eliminated by
 124 PCV7 in many settings. Similarly, PCV13 has removed the replacement standard
 125 serotype used in post-PCV7 studies, 19A [34].

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127 Invasiveness has been more directly characterised as the ratio of disease cases
 128 relative to the estimated prevalence in carriage [25]. However, this approach did not

account for the stochasticity of disease cases being observed, and assumed the uncertainty associated with the estimated case-to-carrier ratio derived only from the colonisation survey data. More comprehensive quantification of the uncertainty in invasiveness can be achieved with Bayesian frameworks, which have been applied to individual pneumococcal populations [2,37]. Invasiveness has been modelled by jointly fitting distributions to carriage and disease data, then using random effects models to estimate invasiveness in different age groups [37]. An alternative approach, using Poisson regression, was used to estimate serotypes' progression rates for causing otitis media [2].

In this work, we apply Bayesian modelling to estimate progression rates from meta-analyses of multiple studies. The ability to synthesise data across populations is important for increasing the sample size of each of the many serotypes observed in species such as *S. pneumoniae*, particularly as many genotypes that have emerged post-PCV were previously rare, and vary geographically [32,38]. The model enables the estimation of progression rates as an opportunistic pathogen's hazard of progressing from carriage to disease in a specified population. Such absolute values avoid a measure that is relative either to the rest of the local pneumococcal population, or to one standard serotype. These properties are crucial for quantifying and modelling changes in disease incidence, such as following vaccine introduction, or the emergence of a hyperinvasive strain. They can be particularly valuable in settings where disease surveillance is not comprehensive, and only carriage data are available [37,39].

Using a Bayesian approach also enables hypothesis testing through statistical comparison of alternative model structures [2,37]. This is crucial for understanding the host and pathogen factors that affect progression rates. For instance, the correlation between pneumococcal serotypes and invasiveness has not been indisputably established as a causal link. In *Haemophilus influenzae*, changes to an isolate's serotype altered its virulence in an animal model of disease in such a manner that reflected the epidemiology of human disease [40]. While some equivalent experiments in *S. pneumoniae* have replicated observations from human IPD [41,42], others have found changes in an isolate's serotype did not change its invasiveness in an animal model [43,44], suggesting serotype-independent factors may contribute to this phenotype. Studies of isolates from disease and carriage using comparative genomic hybridisation [45,46] and whole genome sequencing [47,48] have both supported links between non-capsular loci and invasiveness. An unambiguous association cannot even be made between the distinctive phenotypes of the epidemic serotypes and their capsules, as these pneumococci tend to have low genetic diversity [35,49], and therefore their capsule polysaccharide synthesis locus is in linkage disequilibrium with many other variable genomic loci [50,51].

Epidemiological studies using genotyped and serotyped isolates have also found examples of within-serotype differences in invasiveness [33,52,53], although other studies found evidence of serotype being the primary determinant of invasiveness [29]. Genomic analysis by the Global Pneumococcal Sequencing project also identified differences in the invasiveness of strains of the same serotype [31]. However, given the multiple pairwise comparisons conducted in such analyses, it is not clear whether such observations might be expected, even if the capsule is the

primary determinant of invasiveness. Such questions can be addressed through comparison of different model fits to the data, thereby improving our understanding of the determinants of progression rates. This is essential for deciding on the most effective methods for ongoing pathogen surveillance, and to enable the rapid identification of emerging highly invasive types. Therefore the methods, models and data described in this study are made available as an R package (<https://github.com/nickjcroucher/progressionEstimation>), to enable the ongoing aggregation of case and carrier studies for any opportunistic pathogens,.

Methods

Model definitions and assumptions

All models were designed to be applied to a meta-population of a multi-strain opportunistic pathogen. Each population i corresponded to a matched sample of microbes from asymptomatic carriers, or the environment, and a sample from disease. It was assumed all cases of disease emerged independently from the carried or environmental population. Across the meta-population, the microbes were classified according to their serotype j , strain k , or the combination of both, j,k . If x denotes any one of these classifications, then the carriage rate in population i , $\rho_{i,x}$, was estimated based on the total number of samples (including negative results), η_i , and the number of samples positive for isolates of type x , $c_{i,x}$:

$$c_{i,x} \sim \text{Binom}(\rho_{i,x}, \eta_i)$$

Modelling the number of positive samples using a Binomial distribution follows the precedent of Weinberger *et al* [37], and assumes all carriage samples are independently drawn from the circulating population.

A set of related statistical models were used to estimate the progression rate [2], v , the hazard of developing a disease given carriage of a microbe per unit time. As v was assumed to be constant for each type x in each study i , the number of observed cases of disease caused by x in i , $d_{i,x}$, was modelled as the product of the number of potential hosts in i , N_i ; the proportion carrying x in i , $\rho_{i,x}$; the per unit time probability of x causing disease, v_x ; and the duration of the study, t_i . Hence, the expected number of isolates from disease, $\mathbb{E}[d_{i,x}]$, in all models was proportional to all these quantities:

$$\mathbb{E}[d_{i,x}] \propto \rho_{i,x} N_i t_i v_x$$

Some models also adjusted the expected $d_{i,x}$ between i through a scaling parameter, γ_i , that was relative to a reference population. This corrects for differences between studies both in the actual hazard of developing a disease, due to host population variation, and differences in the comprehensiveness of surveillance across the study populations. Hence, the expected number of isolates from disease, $\mathbb{E}[d_{i,x}]$, in such models was proportional the quantities:

$$\mathbb{E}[d_{i,x}] \propto \rho_{i,x} N_i t_i v_x \gamma_i$$

All these parameters were assumed to be constant within a study over t_i . All disease cases were assumed to develop independently, and therefore were modelled as a Poisson process. However, relevant unmodelled variation, or oversampling of local transmission chains in either carriage or disease sampling [54], could cause heterogeneity in the observed isolate counts in either component of the paired samples. Hence $d_{i,x}$ was additionally modelled as following a negative binomial distribution, in which overdispersion was quantified by the precision parameter, ϕ , relative to the mean, μ . Lower ϕ values indicate greater overdispersion, as the variance of the distribution was calculated as:

$$Var[d_{i,x}] = \mu + \frac{\mu^2}{\phi}$$

Models of disease prevalence

Four different structures were used to model $\mathbb{E}[d_{i,x}]$. For each, two different versions were fitted to the data. In the simpler version, the observed disease case counts were assumed to follow a Poisson distribution:

$$d_{i,x} \sim \text{Poisson}(\mathbb{E}[d_{i,x}])$$

The more complex version assumed a negative binomial distribution of the observed disease case counts:

$$d_{i,x} \sim \text{NegBin}(\mathbb{E}[d_{i,x}], \phi)$$

Null model: In this simplest model, the progression rate was independent of the pathogen's type ($v_x = v$ for all x) and host population ($\gamma_i = 1$ for all i). Therefore $d_{i,x}$ is expected to be correlated with $c_{i,x}$:

$$\mathbb{E}[d_{i,x}] = \rho_{i,x} N_i t_i v$$

Type-specific model: This model, similar to that Weinberger *et al* applied to a single population [37], allowed for variation in progression rate between types, but still assumed host populations to be homogeneous ($\gamma_i = 1$ for all i). Therefore $d_{i,x}$ depends on both the carriage prevalence and progression rate of x :

$$\mathbb{E}[d_{i,x}] = \rho_{i,x} N_i t_i v_x$$

Study-adjusted model: In this model, variation in disease prevalence between studies reflected differences in host population and surveillance, rather than differences between types. Hence the progression rate was independent of the pathogen's type ($v_x = v$ for all x), but varied between i due to γ_i :

$$\mathbb{E}[d_{i,x}] = \rho_{i,x} N_i t_i v \gamma_i$$

Study-adjusted type-specific model: This model allowed for both variation in the progression rate between types x and study populations i :

$$\mathbb{E}[d_{i,x}] = \rho_{i,x} N_i t_i v_x \gamma_i$$

Joint modelling of strain and type progression rates

For datasets in which information was available on both type (for *S. pneumoniae*, serotype) j and strain k , v_x was modelled in five different ways:

Serotype-determined progression rate: The progression rate was entirely determined by an isolate's type, and independent of its strain background ($v_x = v_j$).

Strain-determined progression rate: The progression rate was entirely determined by the strain to which an isolate belonged, and independent of its type ($v_x = v_k$).

Strain-modified type-determined progression rate: Two approaches were taken to modelling progression rates as being independently affected by type and genetic background. The first modelled the progression rate as being primarily determined by an isolate's serotype, which was modified by its strain background. This was calculated as $v_x = v_j v_k$, where the model priors meant v_j was less constrained than v_k . Hence most variation in v_x should be attributed to the type j .

Type-modified strain-determined progression rate: The second approach to analysing the independent effects of type and genetic background modelled progression rate as being primarily determined by the strain to which an isolate belonged, which was modified by the serotype it expressed. This was calculated as $v_x = v_j v_k$, where the model priors meant v_k was less constrained than v_j . most variation in v_x should therefore be attributed to the strain k .

Type and strain-determined progression rate: Each combination of serotype j and strain k was modelled as having a unique progression rate ($v_x = v_{j,k}$), suggesting non-multiplicative interactions between strain background and type on an isolate's progression rate.

Model priors and implementation

The characteristics of each study population (η_i , N_i , t_i) and observed counts of type x in carriage ($c_{i,x}$) and disease ($d_{i,x}$) were assumed to be accurate and without error. As each recovery of a type x isolate from carriage contributes to the estimation of $\rho_{i,x}$, even if an individual carries multiple types, the prior distribution was:

$$\rho_{i,x} \sim \text{Beta}(1,1)$$

This means each type's carriage was independently estimated, and the sum of carriage prevalences ($\sum_x \rho_{i,x}$) may be greater than one, if there is high level of multiple carriage in a population. The beta distribution was used, as it is the conjugate prior of the binomial distribution, although setting the scale and shape parameters to one make it equivalent to a uniform distribution bounded by zero and one.

Invasiveness has been estimated to vary over orders of magnitude in *S. pneumoniae* [27], and therefore a uniform prior was placed on the logarithm of v_x in all models. The lower bound was set at one IPD case per million carriers per year, as the largest studies used surveillance of host populations consisting of tens of millions of individuals (Table S1). The upper bound was ten cases per carrier per year, which

would effectively correspond to an obligate pathogen for a bacterium with the carriage duration of *S. pneumoniae* [55]:

$$\log_{10}(v_x) \sim U(-6, 1)$$

As the scaling factors were relative measures of disease prevalence between populations, γ_i for the population with the largest sample size was fixed at one. The scaling factor for each other population i was allowed to vary higher or lower. The Cauchy distribution was assumed as the prior for the logarithm of γ_i , as this describes the ratio of two normally-distributed random variables. However, the Hamiltonian Monte Carlo algorithm used for model fitting does not efficiently sample heavy-tailed distributions, and therefore the prior was reparameterised to use a random variable Γ_i with a unit-sized uniform prior [56]:

$$\Gamma_i \sim U\left(-\frac{\pi}{2}, \frac{\pi}{2}\right)$$

The Γ_i values were then transformed to generate γ_i values with a Cauchy distribution using the location ($\mu_{study} = 0$) and scale ($\tau_{study} = 2$) parameters:

$$\log_{10}(\gamma_i) = \mu_{study} + \tau_{study} \tan(\Gamma_i)$$

The τ_{study} value allowed for variation in observed incidence over multiple orders of magnitude between datasets, ensuring the model makes allowance for substantial heterogeneity in observed disease incidence [57].

A similar reparameterization of the Cauchy distribution was used for the models in which type and strain background independently contributed to a genotype's progression rate (the strain-modified type-determined, and type-modified strain-determined progression rate models). The categorisation modelled as determining the progression rate (v_x) had the same prior as when a single factor determined the progression rate. This was multiplied by a second value, v_y , determined by the classification modifying the progression rate. The prior distribution for the logarithm of this value was a truncated Cauchy, symmetrical around zero. This represented the expectation that the modification of the progression rate would be small (approximately one) in most cases, but may be substantial in rare instances. The truncation was found to be necessary for efficient sampling with the Hamiltonian Monte Carlo algorithm. This was parameterised with a random variable Y_y , sampled from a uniform distribution with narrower boundaries than those for the analogous Γ_i variable:

$$Y_y \sim U(-1.25, 1.25)$$

The Y_y values were then transformed to generate v_y values using the location ($\mu_{mod} = 0$) and scale ($\tau_{mod} = 0.5$) parameters:

$$\log_{10}(v_y) = \mu_{mod} + \tau_{mod} \tan(Y_y)$$

This meant v_y values could vary over three orders of magnitude (between 0.0313 and 32.0), while enabling models to converge with reasonable MCMC lengths.

The precision parameter of the negative binomial distribution, ϕ , was sampled from a uniform distribution. Where there is little evidence of overdispersion, ϕ will be large relative to $\mathbb{E}[d_{i,x}]$, whereas highly overdispersed data can be modelled with a smaller, albeit non-zero, ϕ value. Therefore, the prior distribution was chosen to be broad, but always positive:

$$\log_{10}(\phi) \sim U(-3, 3)$$

Model parameters are summarised in Table 1. All models were implemented using R [58] and Rstan [59]. Each model was fitted using two Hamiltonian Markov chain Monte Carlo (MCMC) evaluations for 25,000 iterations. Convergence was assessed through analysing the MCMC traces, using the criterion of all $\hat{R}$ values below 1.05, and no reports of divergent transitions in the sampling [56]. This was achieved using default MCMC sampling parameters for the serotype analyses, but the strain analyses required a smaller step size (0.01), higher acceptance probability (0.99) and larger tree depth (20). The data and code used for the analyses are available as an R package from <https://github.com/nickjcroucher/progressionEstimation/>.

Model evaluation and comparison

Models were compared using likelihoods, independent of priors, through approximate leave-one-out cross-validation with the loo package [60]. The marginal likelihoods of different models, given the data, were compared with Bayes factors calculated using the bridgesampling package [61]. The same number of iterations were used to calculate the logarithmic marginal likelihoods as were used to fit the

models. Model outputs were analysed and plotted with tidyverse and ggpubr packages [62,63].

***S. pneumoniae* serotype data**

The matched carriage and invasive pneumococcal disease serotype datasets were combined from two meta-analyses [27,34]. Twelve of the reported studies were omitted due to lack of publicly-available data [29,64–69]; difficulty in defining independent cross-sectional carriage samples [70,71]; small sample sizes once stratified by age [72], or study design biased towards particular serotypes [73]. If a PCV were introduced during a study, then samples were divided into pre-PCV and post-PCV datasets. This generated 21 paired case and carrier datasets, each with substantial matched systematically-sampled and comprehensively serotyped asymptomatic carriage and disease samples (Table S1). Of these studies, 20 included data on child carriage and child IPD, and five included data on child carriage and adult IPD.

Data that were only resolved to serogroup level, based on the currently-known serotypes, were omitted from the analysis, although the overall number of samples taken (n_i) was not adjusted. However, data on serotypes in historical studies that are now known to correspond to multiple serotypes (e.g. serotype 6A now being resolved into 6A and 6C [74]) were included unaltered. Additionally, isolates of serotypes 15B and 15C were combined into the single 15B/C category.

***S. pneumoniae* strain data**

Analysis of variation of invasiveness between *S. pneumoniae* strains in children used population genomic data from South Africa [35], a mixture of population genomic and genotyped data from the USA [35], and genotyped data from Finland [71], Oxford [29] and Stockholm [75]. The equivalent analysis of strain invasiveness in adults used data from Portugal [52]. Some of the genotyped datasets were not as thoroughly documented as the serotyped datasets, and therefore it was not appropriate to fit models that lacked a study-specific scale factor that reduced their reliance upon accurate carriage sample information (Text S1).

Results

Bayesian meta-analysis of *S. pneumoniae* serotype invasiveness

Twenty matched disease case and nasopharyngeal carrier datasets were used to quantify *S. pneumoniae* serotype invasiveness in children (Table S1). From these, 7,340 carriage isolates and 2,851 disease isolates were extracted, across 72 serotypes (Fig. S1). Multiple models of invasiveness were proposed to analyse these data (see Methods). The null model assumed there was no systematic difference between serotypes' invasiveness across datasets. The type-specific model assumed each serotype had a characteristic invasiveness that was consistent across locations. The study-adjusted null model assumed invasiveness did not vary across serotypes, but could differ between studies. Finally, the study-adjusted type-specific model allowed invasiveness to vary between both types and studies. All four approaches to estimating the expected numbers of disease cases and carriage isolates were fitted assuming the number of disease cases would follow a Poisson

distribution, and allowing for overdispersion by fitting a negative binomial distribution to these values, resulting in eight total models.

Datapoints at which both the count of both carriage and disease isolates were low for a given type in a study offered limited power for distinguishing the different model structures. Therefore only paired observations from an individual study in which either the number of carriage or disease isolates was at least five were used for the model comparison. This reduced the dataset to 7,048 carriage isolates and 2,617 disease isolates across 45 serotypes (Fig. S2). The eight models were each fitted to the data using two Hamiltonian Markov chain Monte Carlo samplings, both run for 25,000 iterations. All chains appeared to have converged, based on the traces of the posterior probabilities (Fig. S3) and all $\hat{R}$ values being below 1.001 (Fig. S4).

Comparisons of the observed and predicted isolate counts showed all models could accurately reproduce the observations from carriage studies, with the exception of the simplest null Poisson model (Fig. 1). However, only the most complex models, adjusting for both *S. pneumoniae* serotype and study, were able to accurately reproduce the disease case counts.

These deviations can be quantified as root mean squared error (RMSE) values (Fig. S5). These show the negative binomial models replicate the carriage values closely, relative to the equivalent Poisson models, with correspondingly greater deviation from the disease counts enabled by the overdispersion permitted by the negative binomial distribution's precision parameter, ϕ . The low ϕ values for the three simplest negative binomial models suggest there is substantial unmodelled variation (Fig. S6). However, for the study-adjusted type-specific models, the high ϕ value suggests little

evidence of overdispersion, and the similar RMSE values for the Poisson and negative binomial versions of this model suggested adjusting for study and serotype enabled disease cases to be modelled using a Poisson distribution (Fig. S5).

The success of the study-adjusted type-specific models in reproducing the observations may represent overfitting by the most complex models. Therefore formal model comparisons were undertaken using leave-one-out cross validation [60]. Although this suggested the study-adjusted type-specific models were the most appropriate, the large number of model parameters meant the Pareto k statistic diagnostic values were too high for these comparisons to be reliable (Table S2) [76]. This was still true if the models were compared using only the likelihoods calculated from the disease counts, which were more constrained than those calculated from the carriage data (Table S3). Hence models were instead compared using Bayes factors calculated from bridge sampling [61]. This found the null models to be the poorest fitting, and identified the study-adjusted type-specific Poisson model to be the most strongly favoured by the data (Table S4).

Identification of highly invasive non-vaccine serotypes

The study-adjusted type-specific model was therefore applied to the full dataset. Both the MCMC traces and $\hat{R}$ statistics indicated the model fit converged after 25,000 iterations (Fig. S7). This enabled the estimation of invasiveness for 72 serotypes (Fig. 2; Dataset S1), including all 24 included in formulations that are currently licensed or under development [20,77,78]. The analysis shows the serotypes associated with high invasiveness and narrow credibility intervals are the three serotypes added to PCV7 to generate PCV10 (1, 5 and 7F), which are also

present in higher-valency PCVs, and serotype 12F, included in PCV20. Other serotypes not included in current vaccine designs, but likely to be highly invasive, include 9L, 19C, 28A and 46. However, the credibility intervals of these estimates were substantial, resulting from small overall sample sizes.

The serotypes associated with low invasiveness and narrow credible intervals were 11A (included in PCV20), 6C, 21, 23A, 23B, 34, 35B and 35F. Many other serotypes (e.g. 10F, 11F, 19B, 33A, 35A, 42 and 45) are likely to have low invasiveness, but these estimates have wide credibility intervals. The higher uncertainty generally corresponded with lower sample sizes, although some of these serotypes had a total sample size above 50 (e.g. 10F, 35A, 42), but were nevertheless rare in IPD. The reference population for the model fit was the post-PCV7 sample from the Navajo nation [37], as this study contributed the largest number of isolates (Fig. S1). The model fits found the maximal progression rates were above 10^{-2} IPD cases per carrier per year, with the minimum point estimates approximately 10^{-4} IPD cases per carrier per year. However, the incidence of IPD in the Navajo population is high, with excellent disease surveillance [79], and therefore other studies were expected to report lower disease cases per carriage episode. Correspondingly, the study-adjustment scale factors were below one for most other datasets (Fig. S8), suggesting most locations would expect to detect around half the number of IPD cases per carriage episode for the same carriage serotype composition.

The fitted study-adjustment scale factors were all associated with similarly narrow credibility intervals, demonstrating there were enough shared types between samples to robustly infer these parameters. For four locations, both pre-PCV and

post-PCV samples were included in the meta-analysis, which might be expected to have similar scale factors, if the host population and disease surveillance were consistent across these eras. However, this was not the case for three locations (Atlanta, Bogota and the Netherlands). The model's assumption that serotype invasiveness is constant across studies may be violated by a reduction in vaccine-type invasiveness following PCV introduction, if vaccine-induced immunity inhibited progression from carriage to IPD. Therefore the study-adjusted type-specific Poisson model was refitted, treating vaccine-type serotypes as different types pre- and post-PCV (including 6A as a PCV7 type, and 6C as a PCV13 type [80]; Fig. S9). The point estimates of invasiveness for most vaccine serotypes dropped following PCV introduction, with the evidence strongest for serotypes 14 and 7F falling after the introduction of PCV7 and PCV10, respectively (Fig. 2). This fit meant the scale factor credibility intervals for the Netherlands' pre-PCV7, post-PCV7 and post-PCV10 studies overlapped (Fig. S10). These data suggest PCVs can reduce the invasiveness of vaccine types before they are eliminated by herd immunity, and therefore distinguishing between pre- and post-PCV invasiveness may help standardisation across meta-analyses.

Comparison of Bayesian modelling with odds ratios

The outputs of the adjusted type-specific Poisson model were compared to those from estimating pneumococcal invasiveness from the same dataset using odds ratios (Fig. 3). Odds ratios were combined across studies using either random (Fig. 3A) or fixed effects (Fig. 3B) models, and the results disaggregated based on the total number of isolates, summed across carriage and disease, for each serotype. Across all sample sizes, there was a significant positive correlation between the two

measures of invasiveness. The range of invasiveness values extended over two orders of magnitude for both methods. Hence the study-adjusted type-specific Poisson model produced similar relative estimates of invasiveness to odds ratio analyses, while having the advantage of providing both overall, and location-specific, absolute estimates of these progression rates that can be used in quantitative analyses.

Fixed effects analyses assume there is a single invasiveness value across all locations, whereas random effects analyses allow for variation in the underlying invasiveness estimate between studies. The identification of between-location heterogeneity typically suggests random effects models are more appropriate for analyses of pneumococcal invasiveness [27,34,35]. This variation may either reflect genuine differences in serotype behaviour, or arise from standardisation relative to a variable mix of serotypes between locations. By accounting for this variation, random effects models often calculate wider confidence intervals than fixed effect models (Fig. 3). Additionally, random effects models are typically not recommended where there are fewer than five studies in which a serotype features [81], and therefore meta-analyses of odds ratios may be a mixture of fixed- and random-effects models [35]. By contrast, the Bayesian framework rejected the over-dispersed negative binomial model in favour of the Poisson model (Fig. 1). This may reflect reduced heterogeneity in invasiveness estimates, because standardisation does not use differing mixtures of serotypes, as for odds ratios. This single, consistent model can be applied across serotypes, regardless of their frequency.

Deviations between the methods in the rank order of invasiveness point estimates were most evident at small sample sizes. This was most notable for serotypes 33A and 36, both of which were associated with relatively high log odds ratios (above zero) and relatively low Bayesian progression rate estimates (below 10^{-3} cases per carrier per year). Both were observed only in carriage: five isolates across four studies for serotype 33A, and one isolate in each of three studies for serotype 36. Whereas logarithmic odds ratio analyses necessarily generate symmetrical confidence intervals, those from the Bayesian analysis were asymmetric, with long tails spanning lower values representing the possibility that the invasiveness of these serotypes may be negligible, as they were not observed to cause IPD. Hence the Bayesian model estimates provide a more informative representation of the limited available data.

At the upper end of the scale, the Bayesian model found the most highly invasive serotype to be 46, with credibility intervals indicating this serotype is unlikely to be of intermediate invasiveness [28]. By contrast, the confidence intervals for the odds ratios estimated using random effects spanned the full range of invasiveness point estimates across the species. This serotype was observed in two studies, but never isolated from carriage, and there is further circumstantial evidence that serotype 46 is likely to be highly invasive (see Discussion). Hence this Bayesian framework can use case-carrier studies to provide informative estimates of invasiveness even from small sample sizes.

Odds ratios were previously found to correlate with another measure of invasiveness, the “attack rate”, which in turn negatively correlated with carriage

duration [18]. This could result from IPD being most common shortly after the acquisition of a novel serotype in the nasopharynx, which occurs more frequently for serotypes carried for only a short period in each host. The invasiveness values from child IPD data were compared with recent estimates of carriage duration from multi-state modelling of longitudinally-sampled carriage studies in Maela, Thailand [55,82]. Although the highest invasiveness values were associated with short carriage durations, likely as longer carriage duration is expected to result in higher cross-sectional carriage prevalences, there was no strong overall relationship (Fig. S11).

Differences in invasiveness between host age demographics

Although *S. pneumoniae* primarily circulate between children, they frequently cause disease in adults. Five datasets were identified in which adult disease cases could be matched with child nasopharyngeal carrier datasets, to estimate serotype invasiveness in adults. Overall, 3,756 carriage isolates and 3,041 disease isolates were extracted across 53 serotypes (Fig. S12). As with the child IPD samples, this dataset was filtered for observations where either the number of carriage or disease isolates was at least five for the model comparison, to improve the power for identifying the best fitting model. This filtering left in 3,704 carriage isolates and 2,969 disease isolates across 40 serotypes (Fig. S13). As with the child dataset, two Hamiltonian MCMC samplings for 25,000 iterations were used to fit the eight models, which converged based on the traces of the posterior probability (Fig. S14) and $\hat{R}$ values being below 1.001 (Fig. S15). Similar to the analysis of invasiveness in children, comparisons of the observed and predicted isolate counts again showed that only the models adjusting for both *S. pneumoniae* serotype and study were able to accurately reproduce the adult disease case counts (Fig. S16). Bayes factors

concluded that the study-adjusted type-specific Poisson model was the most likely, given the data (Table S5).

Thus, this model was applied to the complete adult disease dataset. MCMC traces and $\hat{R}$ statistics again indicated the model fit converged after 25,000 iterations (Fig. S17). Like the child analysis, the reference population for the model fit was the post-PCV7 sample from the Navajo nation [37]. The high incidence of IPD in this population is again evident in the adult population, and therefore the other studies report lower study-adjustment scale factors (Fig. S18), with most locations expecting to detect around a third or fewer the number of IPD cases per carriage episode for the same carriage serotype composition.

This analysis therefore enabled the estimation of invasiveness of 53 serotypes in adults (Fig. 4A; Dataset S2), including the 24 included in PCV formulations currently licensed or under development. The analysis shows the non-vaccine serotypes associated with high invasiveness and narrow credibility intervals were serotypes 8 and 12F, included in the PCV20 formulation, and the non-vaccine type serotype 13. Other serotypes not included in current vaccine designs, but likely to be highly invasive, include serotypes 9L, 12B and 29. However, the credibility intervals of these estimates were substantial, resulting from small overall sample sizes. The non-PCV serotypes associated with low invasiveness (mean estimates $<10^{-3}$ disease cases per carrier per year) and narrow credible intervals were serotypes 7C, 15A, 16F, 23A, 31, 34, 35A, 35B and 35F.

Comparisons with the corresponding child invasiveness estimates (Fig. 4B) showed many PCV serotypes had similar invasiveness estimates in both children and adults [37], with PCV7 and PCV10 types having mid-range and high invasiveness estimates for both children and adults, respectively. Serotype 6C, which is affected by PCV13-induced immunity against the 6A component, was low in both children and adults. The non-PCV serotype 9L appears to be highly invasive in both populations, although this value was associated with considerable uncertainty. However, other non-PCV types showed a discrepancy between the two age groups. For example, serotypes 12B and 29 were highly invasive in adults but not children, though the credible interval ranges were large for both.

Serotypes included in the PCV15 and PCV20 formulations appeared to have mid-range invasiveness in both age groups, except for the consistently highly invasive serotypes 8 and 12F. This suggests the removal of these two serotypes through herd immunity from higher-valency PCV infant immunisation programmes would likely be beneficial to the adult population [83,84]. However, this would concomitantly reduce the effectiveness of the current PPV23 adult vaccine, due to its overlap in serotype coverage with PCVs meaning it would protect against a lower proportion of the post-vaccine *S. pneumoniae* population [27,85]. Its residual effect would be determined by the prevalence and invasiveness of the non-PCV PPV23 serotypes. Both serotypes 2 and 20 were estimated to be highly invasive for both age groups, albeit with broad credible intervals for adults for the former, due to it only being included in one adult dataset. Other PPV23 serotypes had elevated invasiveness compared to many non-vaccine types, suggesting that PPV23 may offer protection against a

limited number of higher-risk serotypes following the implementation of PCV15 or PCV20 infant vaccination programmes.

Invasiveness varies between pneumococcal strains and serotypes

S. pneumoniae populations are genetically diverse, and can be divided into discrete strains [50,86]. Isolates of a particular serotype are often found across multiple strain backgrounds, and a single strain may be associated with multiple serotypes through switching [51]. To test how each of these characteristics affected pneumococcal progression rates, five different models of isolates' invasiveness were fitted using the study-adjusted type-specific Poisson framework (see Methods). These corresponded to the hypotheses that an isolate's invasiveness was determined by its serotype; by its strain background; primarily by its serotype, but modified by strain background; primarily by its strain background, but modified by its serotype; and determined by the combination of its serotype and strain background. All five models were fitted using Poisson and negative binomial distributions.

These ten models were used to conduct a meta-analysis of six studies in which both serotype and strain background could be determined, for at least some isolates (Fig. S19). Three of the studies (post-PCV7 and post-PCV13 South Africa; post-PCV7 USA) were modified versions of a comparison primarily conducted using genomic data [35]. These were combined with pre-PCV genotyped studies from Oxford, UK [29]; Stockholm, Sweden [75], and Finland [71] (see Text S1 and Table S6). For each dataset i , isolates were grouped by both their serotype j and strain k . Models were fitted to a subset of these samples that excluded rare strain-serotypes (with a maximum $c_{i,j,k}$ or $d_{i,j,k}$ below five; Fig. S20), leaving 38 serotypes, 74 strains, and 99

serotype-strain combinations. All models converged on stable set of parameter estimates, as inferred from the traces of logarithmic posterior probability values (Fig. S21) and distribution of $\hat{R}$ values (Fig. S22). All models were able to generate reasonably accurate predictions (Fig. S23). There was little evidence of negative binomial distributions improving the fit of these models, and the precision parameters were correspondingly high, albeit with evidence of greater overdispersion when serotype information was omitted from the model fit (Fig. S24). The lowest RMSEs were associated with the models that accounted for both strain background and serotype, either through modification, or when considered in combination (Fig. S25).

Model comparison with LOO-CV found the best-performing models were those that combined information on isolates' serotype and strain background (Tables S7 and S8), although the Pareto k statistic diagnostic values were again too high for this comparison to be reliable. Comparisons using Bayes factors agreed that the most likely model was that in which invasiveness was determined individually for each combination of strain and serotype (Table S9). Some carriage sample sizes had to be approximated in this analysis (for the Oxford and Finland studies in particular; see Text S1), but the study adjustment factors were robustly estimated and significantly differed across studies, suggesting the model was able to compensate for this aspect of the data (Fig. S26). To test whether these problems could have affected relative model likelihoods, the model comparison was repeated with a 100-fold change in the sample size values for those studies in which the precise number was not reported. The study-adjustment factor meant the comparisons were relatively insensitive to these changes, and the same model was identified as the most likely,

given the data (Table S10). Hence model comparisons demonstrated that neither serotype nor strain background alone determines a genotype's invasiveness.

Identification of invasive pneumococcal genotypes

The best-fitting model converged on a stable solution when applied to the full dataset (Fig. S27). The invasiveness estimates can be plotted to analyse variation by serotype within strains (Fig. 5; Dataset S3). Some strains were uniformly low invasiveness (serotypes 11A, 15A and 20A within GPSC22), whereas others had consistently higher invasiveness estimates (serotypes 14, 9V, 19F and 19A within GPSC6). By contrast, 19A had an elevated invasiveness relative to 19F within GPSC1, and relative to serotype 15B/C in GPSC4. Similarly, serotypes 8 and 33F were substantially more invasive than 11A within GPSC3. Hence there was considerable variation in estimated invasiveness both between, and within, strains.

The same invasiveness estimates were plotted grouped by serotype (Fig. 6). The meta-analysis identified significant evidence to support many of the observations of within-serotype invasiveness variation noted in the individual analyses (Table S11). Heterogeneity was most strongly evident within some of the traditional paediatric types [19] (including 6A, 6B, 14, 19F), as well as 16F and 19A. This may represent these common serotypes being found across a wider diversity of strain backgrounds. However, the estimates for other low invasiveness serotypes disseminated across multiple backgrounds, such as 15B/C, 23F and 35B, were more consistent. Furthermore, some serotypes (such as 1 and 12F) were similarly highly invasive in multiple strains. This implies some serotypes may have a strong effect on an isolate's invasiveness.

The adjusted type-specific model was applied to the full dataset, including low frequency types, to identify high-risk genotypes that might emerge as causes of IPD post-PCV (Fig. S28). The most invasive genotypes not targeted by current PCVs were mainly of serotypes typically considered invasive, such as 8 and 12F. Yet there were also examples of genotypes expressing serotypes regarded as less invasive (e.g. 10A, 16F, 23B and 35B) with high invasiveness point estimates, albeit with wide credibility intervals reflecting small sample sizes. Most of these are likely to remain rare, or regress to the population-wide mean as more data emerge, but ongoing surveillance to rapidly identify whether any represent a potentially problematic type post-PCV will be valuable.

An additional genotyped dataset from Portugal (Fig. S29), in which the majority of disease cases were isolated from adults [52], was analysed with the type-specific Poisson model in which invasiveness was specific to each combination of serotype and strain background (Fig. S30). There were few instances of multiple serotypes being isolated from the same strain, but several serotypes were distributed across multiple genetic backgrounds (Fig. S31). This found evidence of heterogeneity in invasiveness within serotypes 3, 14 and 23F. The elevated invasiveness of serotype 3 was associated with the GPSC6 strain, found to have high invasiveness when expressing multiple serotypes in children (Fig. 5). For serotypes 14 and 23F, this involved strains that did not substantially differ in the child dataset (Fig. 6), suggesting further within-serotype variation in invasiveness may emerge as studies include a greater diversity of host age groups.

Discussion

This study describes novel models for using data from cases and carriers to estimate the hazard of an asymptotically carried pathogen causing a defined disease over a time period, previously defined as a progression rate [2]. This quantity is crucial for determining the efficacy of partial coverage vaccines, such as the PCVs [26,27], and the flexible framework developed here should be applicable to any diverse microbial population. These models calculate progression rates as absolute quantities, rather than dimensionless ratios as with previous meta-analyses [27,34,36]. These estimates can enable the translation of alterations in microbial population structures to changes in disease incidence, which is crucial for understanding the consequences of strain- or type-specific interventions [27]. Furthermore, given suitable datasets for multiple pathogens, these models could also be used to estimate changes in disease burdens for alterations across microbiota.

When applied to *S. pneumoniae* serotype data, the best-performing model structures were those in which type-specific progression rates were adjusted by dataset-specific scale factors. In principle, these scale factors should correlate with the burden of disease in a given host population, representing a combination of socio-economic factors and co-morbidities that affect IPD incidence [87,88]. Yet in practice, they will also represent the probability of an isolate causing IPD being included in a study dataset. This is affected by multiple factors, including the probability of attending a hospital participating in the study, the efficiency of retrieving bacteria from blood cultures, and the consistency with which cultured isolates are collected by research centres [87].

These model structures facilitate comparisons across populations by standardising progression rate estimates between datasets using all common serotypes or strains shared between them. Existing methods for meta-analysis typically standardise data relative to a single type, required to be present in all datasets [34,36], or combine estimates derived from ratios calculated relative to variable mixes of types in different locations. The dataset-adjusted type-specific progression rate models are better suited to the extensive international variation in *S. pneumoniae* population structures [35,38], particularly given their ongoing diversification following vaccine introduction [32]. Comparison with odds ratios showed that estimates were correlated at large sample sizes, demonstrating both methods converged on similar values when data are available on many isolates. However, this comes with the caveat that 16 of the 21 studies in the meta-analysis originated from North America or Europe, with most collected prior to PCV introduction. Hence there is a pressing need for paired case and carrier studies from post-PCV settings, particularly from Asia and Africa. As more diverse populations are combined, the improved standardisation across studies from these Bayesian analyses will become more important.

At smaller sample sizes, the Bayesian models were able to produce more informative estimates of invasiveness than odds ratios. Information on types that are rare in individual datasets is particularly important for an opportunistic pathogen as diverse as *S. pneumoniae*, in which serotypes can emerge and expand rapidly post-PCV [23,32,83]. A recent meta-analysis using odds ratios highlighted the conclusion that 12F was the only non-PCV13 serotype to have an invasiveness higher than 19A [34], the serotype responsible for much post-PCV7 IPD [32,89]. However, this

previous study limited its results to 25 serotypes, omitting less common examples that could rise in frequency when serotype replacement is driven by higher-valency vaccines [32,89]. The Bayesian analysis described in this work identified rare serotypes that may be highly invasive in adults (e.g. 9L, 12B and 29) and children (e.g. 19C, 28A and 46), based on small sample sizes. Serotype 46 has already been observed causing child IPD post-PCV13 within the strain GPSC26, which is more commonly associated with the highly-invasive serotype 12F [31]. These two capsule types are structurally similar [90], which is common for serotypes expressed by isolates of the same strain [51]. Future post-PCV13 surveillance will likely show whether 12F and 46 are truly similarly invasive. As higher-valency PCVs become available, with the potential to remove commonly-carried serotypes such as 11A and 15B/C [20,91], reducing the uncertainty in invasiveness estimates for remaining non-vaccine types will be of great importance in understanding the vaccine-associated changes in the incidence of IPD.

The need for meta-analysis of multiple datasets is exacerbated by the model comparison in this study that concluded that invasiveness is most accurately quantified at the level of strain-serotype combination. This conclusion is consistent with the results of some individual studies [35,52,53], although not all [29]. Such a result raises the spectre of separately estimating invasiveness for multiple serotypes within the hundreds of known *S. pneumoniae* strains, many of which are rare in individual populations [35,86]. However, that such models fit better than those using only strain or serotype as a predictor only implies that neither factor alone determines an isolate's invasiveness. The improved fit might simply represent the benefit of more precise classification of bacterial genotypes, and therefore remains

possible that some other component of the *S. pneumoniae* genome that varies over short, within-strain timescales may additionally affect, or entirely determine, isolates' invasiveness [50,92,93]. This could explain the heterogeneity of invasiveness within strain GPSC3, where the serotypes are separated into distinct clades (<https://microreact.org/project/gpsGPSC3>), in contrast to the comparative homogeneity of invasiveness within GPSC6 (containing the PMEN3, or Spain^{9V}-3, lineage), where a single lineage dominates the strain's structure [94].

However, it seems unlikely that serotypes and invasiveness are not causally linked. Some serotypes (e.g. 1, 12F) exhibited high invasiveness across multiple strain backgrounds, whereas others were consistently low (e.g. 15B/C, 35F). This is consistent with the model fitting to serotype data, in which there was not a substantial improvement of model fitting when using negative binomial versions of the models. Additionally, the associated precision parameters were large when dataset-adjusted models were fitted (Fig. S6). If other loci were strongly affecting invasiveness, then a greater degree of overdispersion in the observed incidence of IPD might be expected, unless they were in linkage disequilibrium with the locus determining serotype.

By contrast, some paediatric serotypes exhibited a more heterogeneous distribution of invasiveness values. This may reflect these capsules having little effect on invasiveness, or their greater prevalence in the pre-PCV pneumococcal population providing greater power to detect variation between strains [30]. Heterogeneity has previously been noted in serotype 14, with both GPSC6 [52,71] and GPSC18 [29,35] identified as highly invasive clones (Table S11). In this analysis, both were found to

be more invasive than GPSC9 within the same serotype. This analysis estimated high invasiveness values for all serotypes within GPSC6, including an elevated invasiveness within serotype 3 in the Portuguese dataset. Similarly, invasiveness was consistently low for serotypes 14 and 15A for GPSC9. This could result in heterogeneous vaccine impacts between regions, as elimination of serotype 14 by PCVs will likely have a greater benefit across Europe and South America, where the highly-invasive GPSC6 is mainly distributed (<https://microreact.org/project/gpsGPSC6>), rather than Africa and India, where the less invasive GPSC9 is common (<https://microreact.org/project/gpsGPSC9>).

Whether these models are useful long-term, or will ultimately be superseded by alternatives based on genome-wide association studies, will depend on the extent to which progression rates are affected by interactions between genetic loci [95]. It may prove possible to explain a high proportion of the variation in invasiveness between microbes through a tractable number of polymorphic loci that each independently contribute to a microbe's invasiveness using genome-wide association studies [45–48]. If so, then the individual estimates for each of the multitude of subtype categorisations can be replaced with a simpler genome-based approach, which would be capable of predicting the invasiveness of previously unseen genotypes. However, if invasiveness depends on combinations of loci [95], it may only be possible to estimate progression rates for extant common types from epidemiological studies. Other alternative model structures include random effects models and multivariate regression analyses, which can incorporate information on the host population characteristics [2,37,96]. However, these models typically require comprehensive surveillance of disease and detailed information on host populations,

which limits the range of studies that can be combined in meta-analyses of diverse bacterial populations.

Even using more conventional approaches to estimating invasiveness, whole genome sequencing clearly represents an important tool in future case-carrier studies of opportunistic pathogen invasiveness. In addition to assigning isolates to strains, genomic data is a reliable and cost-effective means of classifying microbes according to their capsular structures [97,98]. Furthermore, it also enables the extraction of additional clinically-relevant information, such as antibiotic resistance loci [94,99], that may be incorporated into future extensions of these models. However, the use of genomic data exacerbates an underlying problem with these models, as high-quality genome assemblies are not necessarily generated from all isolates recovered from carriage. The model assumes there are no false negative swabs in carriage studies, meaning there is an expectation that all carriage samples will be present in the genomic data. Hence the omission of genomic data adds to a false negative rate that, even with serotype data, already reflects the imperfect detection of colonisation by nasopharyngeal swabbing. This can be the consequence of all resident bacteria being missed, or the difficulty of detecting lower prevalence types in instances of multiple carriage [100]. Unless there is variation in the ability to detect carriage of different types, the underestimation of carriage means all progression rates will be uniformly overestimated. However, if only a subset of datasets in a meta-analysis has a lower sensitivity for detecting carriage, the model will adjust for this by increasing the associated scale factors. Hence the model structure is sufficiently flexible to account for such differences. This is necessary, as even with standard protocols for detecting colonisation, the population-wide

estimates for carriage rates for a location will depend on the exact demographics sampled.

Some of these problems with detecting colonisation can be addressed using new techniques with improved sensitivity for detecting multiple serotypes in carriage [101]. However, to simultaneously obtain the genotyping information needed to most precisely characterise an isolate's invasiveness, deep genome sequencing, or similar high-sensitivity molecular genotyping techniques, will be necessary. Such data will not only improve the input to epidemiological models, such as those outlined here, but also potential future models based on combinations of genetic loci. Yet meta-analyses of case-carrier studies can also be improved by simple changes to reporting, without any alterations to current procedures. The data and models described in this work are made available for re-use, modification, and application to other multi-strain microbes (see Methods). The fitting of the model to new datasets will require studies to report their raw data in a standard format, and include the total number of swabs included in the carriage study, as well as an informed estimate of the size of the population under surveillance for IPD. To improve comparability between future studies, it would also be ideal for studies to stratify these data by age category, to enable specific subsets to be employed in different meta-analyses studying specific demographics. Therefore, as the repertoire of vaccines against diverse pathogens expands, we can hope to monitor and understand their impact on population-wide microbial invasiveness using improved models, advances in sequencing technology, and greater transparency of reporting.

Acknowledgements

We thank Raquel Sá-Leão, Mário Ramirez, Bill Hanage and Birgitta Henriques-Normark for providing further information on case-carrier datasets, and Daniel Weinberger for helpful discussions on model structure and fitting. AL was funded by an investigator-initiated grant from GSK to NJC. NJC was supported by the UK Medical Research Council and Department for International Development (grants MR/R015600/1 and MR/T016434/1) and a Sir Henry Dale Fellowship, jointly funded by Wellcome and the Royal Society (grant no. 104169/Z/14/A). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests

We have read the journal's policy and the authors of this manuscript have the following competing interests: this work was partially funded by GSK, who manufacture the PCV10 vaccine, although they had no role in study design, data collection or analysis.

Data Availability

All data and code used for the described analyses are available in a GitHub repository at <https://github.com/nickjcroucher/progressionEstimation/>. This repository has been assigned the DOI 10.5281/zenodo.5154066.

References

1. Brown SP, Cornforth DM, Mideo N. Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. *Trends Microbiol.* 2012;20: 336–342. doi:10.1016/j.tim.2012.04.005
2. Lewnard JA, Givon-Lavi N, Weinberger DM, Lipsitch M, Dagan R. Pan-serotype reduction in progression of *Streptococcus pneumoniae* to otitis media after rollout of pneumococcal conjugate vaccines. *Clin Infect Dis.* 2017;65: 1853–1861. doi:10.1093/cid/cix673
3. Didelot X, Bowden R, Wilson DJ, Peto TEA, Crook DW. Transforming clinical microbiology with bacterial genome sequencing. *Nat Rev Genet.* 2012;13: 601–612. doi:10.1038/nrg3226
4. Feil EJ, Enright MC. Analyses of clonality and the evolution of bacterial pathogens. *Curr Opin Microbiol.* 2004;7: 308–313. doi:10.1016/j.mib.2004.04.002
5. Lees JA, Harris SR, Tonkin-Hill G, Gladstone RA, Lo S, Weiser JN, et al. Fast and flexible bacterial genomic epidemiology with PopPUNK. *Genome Res.* 2019;29: 304–316. doi:10.1101/360917
6. Cartman ST, Heap JT, Kuehne SA, Cockayne A, Minton NP. The emergence of “hypervirulence” in *Clostridium difficile*. *Int J Med Microbiol.* 2010;300: 387–395. doi:10.1016/j.ijmm.2010.04.008
7. Fearnley C, Manning G, Bagnall M, Javed MA, Wassenaar TM, Newell DG. Identification of hyperinvasive *Campylobacter jejuni* strains isolated from poultry and human clinical sources. *J Med Microbiol.* 2008;57: 570–580. doi:10.1099/jmm.0.47803-0

8. Knol MJ, Hahné SJM, Lucidarme J, Campbell H, de Melker HE, Gray SJ, et al. Temporal associations between national outbreaks of meningococcal serogroup W and C disease in the Netherlands and England: an observational cohort study. *Lancet Public Heal.* 2017;2: e473–e482. doi:10.1016/S2468-2667(17)30157-3
9. Lynskey NN, Jauneikaite E, Li HK, Zhi X, Turner CE, Mosavie M, et al. Emergence of dominant toxigenic M1T1 *Streptococcus pyogenes* clone during increased scarlet fever activity in England: a population-based molecular epidemiological study. *Lancet Infect Dis.* 2019;19: 1209–1218. doi:10.1016/S1473-3099(19)30446-3
10. Du P, Zhang Y, Chen C. Emergence of carbapenem-resistant hypervirulent *Klebsiella pneumoniae*. *Lancet Infect Dis.* 2018;18: 23–24. doi:10.1016/S1473-3099(17)30625-4
11. Ganaie F, Saad JS, McGee L, van Tonder AJ, Bentley SD, Lo SW, et al. A new pneumococcal capsule type, 10D, is the 100th serotype and has a large *cps* fragment from an oral streptococcus. *MBio.* 2020;11: e00937-20. doi:10.1128/mBio.00937-20
12. Ganaie F, Maruhn K, Li C, Porambo RJ, Elverdal PL, Abeygunwardana C, et al. Structural, genetic, and serological elucidation of *Streptococcus pneumoniae* serogroup 24 serotypes: Discovery of a new serotype, 24C, with a variable capsule structure. *J Clin Microbiol.* 2021; JCM.00540-21. doi:10.1128/JCM.00540-21
13. Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal capsules and their types: Past, present, and future. *Clin Microbiol Rev.* 2015;28: 871–899. doi:10.1128/CMR.00024-15

- 1012 14. Nelson AL, Roche AM, Gould JM, Chim K, Ratner AJ, Weiser JN. Capsule
1013 enhances pneumococcal colonization by limiting mucus-mediated clearance.
1014 Infect Immun. 2007;75: 83–90. doi:10.1128/IAI.01475-06
- 1015 15. Hyams C, Camberlein E, Cohen JM, Bax K, Brown JS. The *Streptococcus*
1016 *pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis
1017 by multiple mechanisms. Infect Immun. 2009/12/02. 2010;78: 704–715.
1018 doi:IAI.00881-09 [pii]10.1128/IAI.00881-09 [doi]
- 1019 16. Barker J, Gratten M, Riley M, Lehmann D, Montgomery J, Kajoi M, et al.
1020 Pneumonia in children in the eastern highlands of Papua New Guinea: A
1021 bacteriologic study of patients selected by standard clinical criteria. J Infect
1022 Dis. 1989;159: 348–352. doi:10.1093/infdis/159.2.348
- 1023 17. Eyre JW, Washbourn JW. Varieties and virulence of the pneumococcus. Lancet.
1024 1899;153: 19–22. doi:10.1016/S0140-6736(01)78950-7
- 1025 18. Sleeman KL, Griffiths D, Shackley F, Diggle L, Gupta S, Maiden MC, et al.
1026 Capsular serotype-specific attack rates and duration of carriage of
1027 *Streptococcus pneumoniae* in a population of children. J Infect Dis. 2006;194:
1028 682–688. doi:10.1086/505710
- 1029 19. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among
1030 pneumococcal serotypes. Lancet Infect Dis. 2005;5: 83–93.
1031 doi:10.1016/S1473-3099(05)01280-6
- 1032 20. Croucher NJ, Løchen A, Bentley SD. Pneumococcal Vaccines: Host
1033 Interactions, Population Dynamics, and Design Principles. Annu Rev Microbiol.
1034 2018;72: 521–549. doi:10.1146/annurev-micro-090817-062338
- 1035 21. Miller E, Andrews NJ, Waight PA, Slack MPE, George RC. Herd immunity and
1036 serotype replacement 4 years after seven-valent pneumococcal conjugate

1037 vaccination in England and Wales: An observational cohort study. *Lancet*
 1038 *Infect Dis.* 2011;11: 760–68. doi:10.1016/S1473-3099(11)70090-1
 1039 22. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after
 1040 pneumococcal vaccination. *The Lancet.* 2011. pp. 1962–1973.
 1041 doi:10.1016/S0140-6736(10)62225-8
 1042 23. Lewnard JA, Hanage WP. Making sense of differences in pneumococcal
 1043 serotype replacement. *Lancet Infect Dis.* 2019. doi:10.1016/s1473-
 1044 3099(18)30660-1
 1045 24. Devine VT, Cleary DW, Jefferies JMC, Anderson R, Morris DE, Tuck AC, et al.
 1046 The rise and fall of pneumococcal serotypes carried in the PCV era. *Vaccine.*
 1047 2017;35: 1293–1298. doi:10.1016/j.vaccine.2017.01.035
 1048 25. Southern J, Andrews N, Sandu P, Sheppard CL, Waight PA, Fry NK, et al.
 1049 Pneumococcal carriage in children and their household contacts six years after
 1050 introduction of the 13-valent pneumococcal conjugate vaccine in England.
 1051 *PLoS One.* 2018;13: e0195799. doi:10.1371/journal.pone.0195799
 1052 26. Nurhonen M, Auranen K. Optimal Serotype Compositions for Pneumococcal
 1053 Conjugate Vaccination under Serotype Replacement. *PLoS Comput Biol.*
 1054 2014;10: e1003477. doi:10.1371/journal.pcbi.1003477
 1055 27. Colijn C, Corander J, Croucher NJ. Designing ecologically optimized
 1056 pneumococcal vaccines using population genomics. *Nat Microbiol.* 2020;5:
 1057 473–485. doi:10.1038/s41564-019-0651-y
 1058 28. Smith T, Lehmann D, Montgomery J, Gratten M, Riley ID, Alpers MP.
 1059 Acquisition and invasiveness of different serotypes of *Streptococcus*
 1060 *pneumoniae* in young children. *Epidemiol Infect.* 1993;111: 27–39.
 1061 doi:10.1017/S0950268800056648

29. Brueggemann AB, Griffiths DT, Peto T, Meats E, Crook DW, Spratt BG. Clonal Relationships between Invasive and Carriage *Streptococcus pneumoniae* and Serotype- and Clone-Specific Differences in Invasive Disease Potential. J Infect Dis. 2003;187: 1424–32. doi:10.1086/374624
30. Johnson HL, Deloria-Knoll M, Levine OS, Stoszek SK, Hance LF, Reithinger R, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: The pneumococcal global serotype project. PLoS Med. 2010;7: e1000348. doi:10.1371/journal.pmed.1000348
31. Lo SW, Gladstone RA, van Tonder AJ, Lees JA, du Plessis M, Benisty R, et al. Pneumococcal lineages associated with serotype replacement and antibiotic resistance in childhood invasive pneumococcal disease in the post-PCV13 era: an international whole-genome sequencing study. Lancet Infect Dis. 2019;19: 759–769. doi:10.1016/S1473-3099(19)30297-X
32. Løchen A, Croucher NJ, Anderson RM. Divergent serotype replacement trends and increasing diversity in pneumococcal disease in high income settings reduce the benefit of expanding vaccine valency. Sci Rep. 2020;10: 18977. doi:10.1038/s41598-020-75691-5
33. Hanage WP, Auranen K, Syrjanen R, Herva E, Makela PH, Kilpi T, et al. Ability of pneumococcal serotypes and clones to cause acute otitis media: implications for the prevention of otitis media by conjugate vaccines. Infect Immun. 2004;72: 76–81.
34. Balsells E, Dagan R, Yildirim I, Gounder PP, Steens A, Muñoz-Almagro C, et al. The relative invasive disease potential of *Streptococcus pneumoniae* among children after PCV introduction: A systematic review and meta-analysis. J Infect. 2018;77: 368–378. doi:10.1016/j.jinf.2018.06.004

- 1087 35. Gladstone RA, Lo SW, Lees JA, Croucher NJ, van Tonder AJ, Corander J, et
1088 al. International genomic definition of pneumococcal lineages, to contextualise
1089 disease, antibiotic resistance and vaccine impact. EBioMedicine. 2019;43:
1090 338–346. doi:10.1016/j.ebiom.2019.04.021
- 1091 36. Brueggemann AB, Peto TEA, Crook DW, Butler JC, Kristinsson KG, Spratt
1092 BG. Temporal and Geographic Stability of the Serogroup-Specific Invasive
1093 Disease Potential of *Streptococcus pneumoniae* in Children. J Infect Dis.
1094 2004/09/04. 2004;190: 1203–1211. doi:10.1086/423820
- 1095 37. Weinberger DM, Grant LR, Weatherholtz RC, Warren JL, O'Brien KL, Hammitt
1096 LL. Relating Pneumococcal Carriage among Children to Disease Rates among
1097 Adults before and after the Introduction of Conjugate Vaccines. Am J
1098 Epidemiol. 2016;183: 1055–62. doi:10.1093/aje/kwv283
- 1099 38. Corander J, Fraser C, Gutmann MU, Arnold B, Hanage WP, Bentley SD, et al.
1100 Frequency-dependent selection in vaccine-associated pneumococcal
1101 population dynamics. Nat Ecol Evol. 2017;1: 1950–1960. doi:10.1038/s41559-
1102 017-0337-x
- 1103 39. Weinberger DM, Bruden DT, Grant LR, Lipsitch M, O'Brien KL, Pelton SI, et al.
1104 Using pneumococcal carriage data to monitor postvaccination changes in
1105 invasive disease. Am J Epidemiol. 2013;178: 1488–1495.
1106 doi:10.1093/aje/kwt156
- 1107 40. Zwahlen A, Winkelstein JA, Moxon ER. Surface Determinants of *Haemophilus*
1108 *influenzae* Pathogenicity: Comparative Virulence of Capsular Transformants in
1109 Normal and Complement-Depleted Rats. J Infect Dis. 1983;148: 385–394.
1110 doi:10.1093/infdis/148.3.385
- 1111 41. Kelly T, Dillard JP, Yother J. Effect of genetic switching of capsular type on

- 1112 virulence of *Streptococcus pneumoniae*. Infect Immun. 1994;62: 1813–1819.
- 1113 Available: <https://iai.asm.org/content/iai/62/5/1813.full.pdf>
- 1114 42. Hathaway LJ, Grandgirard D, Valente LG, Täuber MG, Leib SL. *Streptococcus*
- 1115 *pneumoniae* capsule determines disease severity in experimental
- 1116 pneumococcal meningitis. Open Biol. 2016;6: 150269.
- 1117 doi:10.1098/rsob.150269
- 1118 43. Hu FZ, Eutsey R, Ahmed A, Frazao N, Powell E, Hiller NL, et al. *In vivo*
- 1119 capsular switch in *Streptococcus pneumoniae* - Analysis by whole genome
- 1120 sequencing. PLoS One. 2012;7: e47983–e47983.
- 1121 doi:10.1371/journal.pone.0047983
- 1122 44. Mizrachi Nebenzahl Y, Porat N, Lifshitz S, Novick S, Levi A, Ling E, et al.
- 1123 Virulence of *Streptococcus pneumoniae* may be determined independently of
- 1124 capsular polysaccharide. FEMS Microbiol Lett. 2004;233: 147–152.
- 1125 doi:10.1016/j.femsle.2004.02.003
- 1126 45. Obert C, Sublett J, Kaushal D, Hinojosa E, Barton T, Tuomanen EI, et al.
- 1127 Identification of a candidate *Streptococcus pneumoniae* core genome and
- 1128 regions of diversity correlated with invasive pneumococcal disease. Infect
- 1129 Immun. 2006;74: 4766–4777. doi:10.1128/IAI.00316-06
- 1130 46. Blomberg C, Dagerhamn J, Dahlberg S, Browall S, Fernebro J, Albiger B, et al.
- 1131 Pattern of Accessory Regions and Invasive Disease Potential in *Streptococcus*
- 1132 *pneumoniae*. J Infect Dis. 2009;199: 1032–1042. doi:10.1086/597205
- 1133 47. Lees JA, Ferwerda B, Kremer PHC, Wheeler NE, Serón MV, Croucher NJ, et
- 1134 al. Joint sequencing of human and pathogen genomes reveals the genetics of
- 1135 pneumococcal meningitis. Nat Commun. 2019;10: 2176. doi:10.1038/s41467-
- 1136 019-09976-3

- 1137 48. Cremers AJ, Mobegi F, van der Gaast –de Jongh C, van Weert M, van
1138 Opzeeland F, Vehkala M, et al. The contribution of genetic variation of
1139 *Streptococcus pneumoniae* to the clinical manifestation of invasive
1140 pneumococcal disease. Clin Infect Dis. 2019;68: 61–69. doi:10.1101/169722
- 1141 49. Donati C, Hiller NL, Tettelin H, Muzzi A, Croucher NJ, Angiuoli S V, et al.
1142 Structure and dynamics of the pan-genome of *Streptococcus pneumoniae* and
1143 closely related species. Genome Biol. 2010;11: R107–R107. doi:10.1186/gb-
1144 2010-11-10-r107
- 1145 50. Croucher NJ, Coupland PG, Stevenson AE, Callendrello A, Bentley SD,
1146 Hanage WP. Diversification of bacterial genome content through distinct
1147 mechanisms over different timescales. Nat Commun. 2014;5: 5471.
- 1148 51. Croucher NJ, Kagedan L, Thompson CM, Parkhill J, Bentley SD, Finkelstein
1149 JA, et al. Selective and Genetic Constraints on Pneumococcal Serotype
1150 Switching. PLoS Genet. 2015;11: e1005095.
1151 doi:10.1371/journal.pgen.1005095
- 1152 52. Sá-Leao R, Pinto F, Aguiar S, Nunes S, Carriço JAJA, Frazao N, et al.
1153 Analysis of invasiveness of pneumococcal serotypes and clones circulating in
1154 Portugal before widespread use of conjugate vaccines reveals heterogeneous
1155 behavior of clones expressing the same serotype. J Clin Microbiol. 2011;49:
1156 1369–75. doi:10.1128/jcm.01763-10
- 1157 53. Browall S, Norman M, Tångrot J, Galanis I, Sjöström K, Dagerhamn J, et al.
1158 Intracolon Variations Among *Streptococcus pneumoniae* Isolates Influence the
1159 Likelihood of Invasive Disease in Children. J Infect Dis. 2014;209: 377–388.
1160 doi:10.1093/infdis/jit481
- 1161 54. Fraser C, Hanage WP, Spratt BG. Neutral microepidemic evolution of bacterial

- 1162 pathogens. Proc Natl Acad Sci U S A. 2005;102: 1968–1973.
- 1163 doi:10.1073/pnas.0406993102
- 1164 55. Lees JA, Croucher NJ, Goldblatt D, Nosten F, Parkhill J, Turner C, et al.
- 1165 Genome-wide identification of lineage and locus specific variation associated
- 1166 with pneumococcal carriage duration. Elife. 2017;6: e26255.
- 1167 doi:10.7554/eLife.26255
- 1168 56. Stan Development Team. Stan User’s Guide, Version 2.27. In: Interaction Flow
- 1169 Modeling Language [Internet]. 2020 p. 23.7. Available: [https://mc-](https://mc-stan.org/docs/2_27/stan-users-guide/reparameterization-section.html)
- 1170 [stan.org/docs/2_27/stan-users-guide/reparameterization-section.html](https://mc-stan.org/docs/2_27/stan-users-guide/reparameterization-section.html)
- 1171 57. Wahl B, O’Brien KL, Greenbaum A, Majumder A, Liu L, Chu Y, et al. Burden of
- 1172 *Streptococcus pneumoniae* and *Haemophilus influenzae* type b disease in
- 1173 children in the era of conjugate vaccines: global, regional, and national
- 1174 estimates for 2000–15. Lancet Glob Heal. 2018;6: E744–E757.
- 1175 doi:10.1016/S2214-109X(18)30247-X
- 1176 58. R Core Team. R: A Language and Environment for Statistical Computing. R
- 1177 Found Stat Comput. 2019. doi:10.1007/978-3-540-74686-7
- 1178 59. Stan Development Team. RStan: the R interface to Stan. 2020.
- 1179 60. Vehtari A, Gelman A, Gabry J. Practical Bayesian model evaluation using
- 1180 leave-one-out cross-validation and WAIC. Stat Comput. 2017;27: 1413–1432.
- 1181 doi:10.1007/s11222-016-9696-4
- 1182 61. Gronau QF, Singmann H, Wagenmakers EJ. Bridgesampling: An R package
- 1183 for estimating normalizing constants. J Stat Softw. 2020;92: 1–29.
- 1184 doi:10.18637/jss.v092.i10
- 1185 62. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al.
- 1186 Welcome to the Tidyverse. J Open Source Softw. 2019;4: 1686.

doi:10.21105/joss.01686

63. Kassambara A. ggpubr: “ggplot2” Based Publication Ready Plots. 2020.
Available: <https://cran.r-project.org/package=ggpubr>

64. Valles X, Roca A, Lozano F, Morais L, Suarez B, Casals F, et al. Serotype-specific pneumococcal disease may be influenced by mannose-binding lectin deficiency. Eur Respir J. 2010;36: 856–863. doi:10.1183/09031936.00171409

65. Principi N, Marchisio P. Epidemiology of *Streptococcus pneumoniae* in Italian children. Acta Paediatr Suppl. 2000;89: 40–43.

66. Smart LE, Platt DJ, Timbury MC. A comparison of the distribution of pneumococcal types in systemic disease and the upper respiratory tract in adults and children. Epidemiol Infect. 1987;98: 203–209.

67. Mogdasy MC, Camou T, Fajardo C, Hortal M. Colonizing and invasive strains of *Streptococcus pneumoniae* in Uruguayan children: type distribution and patterns of antibiotic resistance. Pediatr Infect Dis J. 1992;11: 648–652.

68. Scott J, Hall A, Hannington A, Edwards R, Mwarumba S, Lowe B, et al. Serotype distribution and prevalence of resistance to benzylpenicillin in three representative populations of *Streptococcus pneumoniae* isolates from the coast of Kenya. Clin Infect Dis. 1998;27: 1442–50.

69. Levidiotou S, Vrioni G, Tzanakaki G, Pappa C, Gesouli H, Gartzonika C, et al. Serotype distribution of *Streptococcus pneumoniae* in north-western Greece and implications for a vaccination programme. FEMS Immunol Med Microbiol. 2006;48: 179–82. doi:10.1111/j.1574-695X.2006.00126.x

70. Azzari C, Cortimiglia M, Nieddu F, Moriondo M, Indolfi G, Mattei R, et al. Pneumococcal serotype distribution in adults with invasive disease and in carrier children in Italy: Should we expect herd protection of adults through

- 1212 infants' vaccination? Hum Vaccin Immunother. 2016;12: 344–350.
- 1213 doi:10.1080/21645515.2015.1102811
- 1214 71. Hanage WP, Kaijalainen TH, Syrjänen RK, Auranen K, Leinonen M, Mäkelä
- 1215 PH, et al. Invasiveness of serotypes and clones of *Streptococcus pneumoniae*
- 1216 among children in Finland. Infect Immun. 2005;73: 431–5.
- 1217 doi:10.1128/IAI.73.1.431-435.2005
- 1218 72. Cullotta AR, Kalter HD, Delgado J, Gilman RH, Facklam RR, Velapatino B, et
- 1219 al. Antimicrobial susceptibilities and serotype distribution of *Streptococcus*
- 1220 *pneumoniae* isolates from a Low socioeconomic area in Lima, Peru. Clin Diagn
- 1221 Lab Immunol. 2002;9: 1328–1331.
- 1222 73. Takala AK, Vuopio-Varkila J, Tarkka E, Leinonen M, Musser JM. Subtyping of
- 1223 common pediatric pneumococcal serotypes from invasive disease and
- 1224 pharyngeal carriage in Finland. J Infect Dis. 1996;173: 128–135.
- 1225 74. In HP, Pritchard DG, Cartee R, Brandao A, Brandileone MCC, Nahm MH.
- 1226 Discovery of a new capsular serotype (6C) within serogroup 6 of
- 1227 *Streptococcus pneumoniae*. J Clin Microbiol. 2007;45: 1225–1233.
- 1228 doi:10.1128/JCM.02199-06
- 1229 75. Browall S, Backhaus E, Naucler P, Galanis I, Sjöström K, Karlsson D, et al.
- 1230 Clinical manifestations of invasive pneumococcal disease by vaccine and non-
- 1231 vaccine types. Eur Respir J. 2014;44: 1646–57.
- 1232 doi:10.1183/09031936.00080814
- 1233 76. Vehtari A, Simpson D, Gelman A, Yao Y, Gabry J. Pareto Smoothed
- 1234 Importance Sampling. arXiv. 2021; 1507.02646v7.
- 1235 77. Stacey HL, Rosen J, Peterson JT, Williams-Diaz A, Gakhar V, Sterling TM, et
- 1236 al. Safety and immunogenicity of 15-valent pneumococcal conjugate vaccine

- 1237 (PCV-15) compared to PCV-13 in healthy older adults. Hum Vaccines
- 1238 Immunother. 2019;15: 530–539. doi:10.1080/21645515.2018.1532249
- 1239 78. Hurley D, Griffin C, Young M, Scott DA, Pride MW, Scully IL, et al. Safety,
- 1240 Tolerability, and Immunogenicity of a 20-Valent Pneumococcal Conjugate
- 1241 Vaccine (PCV20) in Adults 60 to 64 Years of Age. Clin Infect Dis. 2020;
- 1242 ciaa1045. doi:10.1093/cid/ciaa1045
- 1243 79. O'Brien KL, Shaw J, Weatherholtz R, Reid R, Watt J, Croll J, et al.
- 1244 Epidemiology of invasive *Streptococcus pneumoniae* among Navajo children
- 1245 in the era before use of conjugate pneumococcal vaccines, 1989-1996. Am J
- 1246 Epidemiol. 2004;160: 270–8. doi:10.1093/aje/kwh191
- 1247 80. Ladhani SN, Collins S, Djennad A, Sheppard CL, Borrow R, Fry NK, et al.
- 1248 Rapid increase in non-vaccine serotypes causing invasive pneumococcal
- 1249 disease in England and Wales, 2000–17: a prospective national observational
- 1250 cohort study. Lancet Infect Dis. 2018;18: 441–451. doi:10.1016/S1473-
- 1251 3099(18)30052-5
- 1252 81. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, et
- 1253 al. Generalized linear mixed models: a practical guide for ecology and
- 1254 evolution. Trends Ecol Evol. 2009;24: 127–135. doi:10.1016/j.tree.2008.10.008
- 1255 82. Chewapreecha C, Harris SR, Croucher NJ, Turner C, Marttinen P, Cheng L, et
- 1256 al. Dense genomic sampling identifies highways of pneumococcal
- 1257 recombination. Nat Genet. 2014;46: 305–309. doi:10.1038/ng.2895
- 1258 83. Feikin DR, Kagucia EW, Loo JD, Link-Gelles R, Puhon MA, Cherian T, et al.
- 1259 Serotype-Specific Changes in Invasive Pneumococcal Disease after
- 1260 Pneumococcal Conjugate Vaccine Introduction: A Pooled Analysis of Multiple
- 1261 Surveillance Sites. PLoS Med. 2013;10: e1001517.

- doi:10.1371/journal.pmed.1001517
84. Shiri T, Datta S, Madan J, Tsertsvadze A, Royle P, Keeling MJ, et al. Indirect effects of childhood pneumococcal conjugate vaccination on invasive pneumococcal disease: a systematic review and meta-analysis. *Lancet Glob Heal*. 2017;5: e51–e59. doi:10.1016/S2214-109X(16)30306-0
85. José RJ, Brown JS. Adult pneumococcal vaccination: advances, impact, and unmet needs. *Curr Opin Pulm Med*. 2017;23.
86. Harrow GL, Lees JA, Hanage WP, Lipsitch M, Corander J, Colijn C, et al. Negative frequency-dependent selection and asymmetrical transformation stabilise multi-strain bacterial population structures. *ISME J*. 2021;15: 1523–1538. doi:10.1038/s41396-020-00867-w
87. Fedson DS, Anthony J, Scott G. The burden of pneumococcal disease among adults in developed and developing countries: what is and is not known. *Vaccine*. 1999;17: S11–S18. doi:https://doi.org/10.1016/S0264-410X(99)00122-X
88. Torres A, Blasi F, Dartois N, Akova M. Which individuals are at increased risk of pneumococcal disease and why? Impact of COPD, asthma, smoking, diabetes, and/or chronic heart disease on community-acquired pneumonia and invasive pneumococcal disease. *Thorax*. 2015;70: 984 LP – 989. doi:10.1136/thoraxjnl-2015-206780
89. Kyaw MH, Lynfield R, Schaffner W, Craig AS, Hadler J, Reingold A, et al. Effect of Introduction of the Pneumococcal Conjugate Vaccine on Drug-Resistant *Streptococcus pneumoniae*. *N Engl J Med*. 2006;354: 1455–1463. doi:10.1056/NEJMoa051642
90. Mavroidi A, Aanensen DM, Godoy D, Skovsted IC, Kalltoft MS, Reeves PR, et

- 1287 al. Genetic relatedness of the *Streptococcus pneumoniae* capsular
- 1288 biosynthetic loci. J Bacteriol. 2007;189: 7841–7855. doi:10.1128/JB.00836-07
- 1289 91. Croucher NJ, Finkelstein JA, Pelton SI, Mitchell PK, Lee GM, Parkhill J, et al.
- 1290 Population genomics of post-vaccine changes in pneumococcal epidemiology.
- 1291 Nat Genet. 2013;45: 656–663. doi:10.1038/ng.2625
- 1292 92. Manso AS, Chai MH, Attack JM, Furi L, De Ste Croix M, Haigh R, et al. A
- 1293 random six-phase switch regulates pneumococcal virulence via global
- 1294 epigenetic changes. Nat Commun. 2014;5: 5055. doi:10.1038/ncomms6055
- 1295 93. Kwun MJ, Oggioni MR, De Ste Croix M, Bentley SD, Croucher NJ. Excision-
- 1296 reintegration at a pneumococcal phase-variable restriction-modification locus
- 1297 drives within- and between-strain epigenetic differentiation and inhibits gene
- 1298 acquisition. Nucleic Acids Res. 2018;46: 11438–11453.
- 1299 doi:10.1093/nar/gky906
- 1300 94. D'Aeth JC, van der Linden MPG, McGee L, de Lencastre H, Turner P, Song J-
- 1301 H, et al. The role of interspecies recombination in the evolution of antibiotic-
- 1302 resistant pneumococci. Elife. 2021;10: e67113. Available:
- 1303 <https://doi.org/10.7554/eLife.67113>
- 1304 95. Skwark MJ, Croucher NJ, Puranen S, Chewapreecha C, Pesonen M, Xu YY,
- 1305 et al. Interacting networks of resistance, virulence and core machinery genes
- 1306 identified by genome-wide epistasis analysis. PLoS Genet. 2017;13:
- 1307 e1006508. doi:10.1371/journal.pgen.1006508
- 1308 96. Lewnard JA, Givon-Lavi N, Tähtinen PA, Dagan R. Pneumococcal Phenotype
- 1309 and Interaction with Nontypeable *Haemophilus influenzae* as Determinants of
- 1310 Otitis Media Progression. Infect Immun. 2018;86: e00727-17.
- 1311 doi:10.1128/IAI.00727-17

- 1312 97. Epping L, van Tonder AJ, Gladstone RA, Bentley SD, Page AJ, Keane JA.
1313 SeroBA: rapid high-throughput serotyping of *Streptococcus pneumoniae* from
1314 whole genome sequence data. Microb Genomics. 2018;4:
1315 10.1099/mgen.0.000186. doi:10.1099/mgen.0.000186
- 1316 98. Kapatai G, Sheppard CL, Al-Shahib A, Litt DJ, Underwood AP, Harrison TG, et
1317 al. Whole genome sequencing of *Streptococcus pneumoniae*: development,
1318 evaluation and verification of targets for serogroup and serotype prediction
1319 using an automated pipeline. PeerJ. 2016;4: e2477. doi:10.7717/peerj.2477
- 1320 99. Li Y, Metcalf BJ, Chochua S, Li Z, Gertz RE, Walker H, et al. Penicillin-Binding
1321 Protein Transpeptidase Signatures for Tracking and Predicting β -Lactam
1322 Resistance Levels in *Streptococcus pneumoniae*. MBio. 2016;7: e00756-16.
1323 doi:10.1128/mbio.00756-16
- 1324 100. Huebner RE, Dagan R, Porath N, Wasas AD, T M, Klugman KP. Lack of utility
1325 of serotyping multiple colonies for detection of simultaneous nasopharyngeal
1326 carriage of different pneumococcal serotypes. Pediatr Infect Dis J. 2000;19:
1327 1017–1020. doi:10.1097/00006454-200010000-00019
- 1328 101. Turner P, Hinds J, Turner C, Jankhot A, Gould K, Bentley SD, et al. Improved
1329 detection of nasopharyngeal cocolonization by multiple pneumococcal
1330 serotypes by use of latex agglutination or molecular serotyping by microarray.
1331 J Clin Microbiol. 2011;49: 1784–1789. doi:10.1128/JCM.00157-11
- 1332 102. Nzenze SA, Von Gottberg A, Shiri T, Van Niekerk N, De Gouveia L, Violari A,
1333 et al. Temporal Changes in Pneumococcal Colonization in HIV-infected and
1334 HIV-uninfected Mother-Child Pairs Following Transitioning from 7-valent to 13-
1335 valent Pneumococcal Conjugate Vaccine, Soweto, South Africa. J Infect Dis.
1336 2015. doi:10.1093/infdis/jiv167

- 1337 103. Madhi SA, Nzenze SA, Nunes MC, Chinyanganya L, Van Niekerk N, Kahn K,
1338 et al. Residual colonization by vaccine serotypes in rural South Africa four
1339 years following initiation of pneumococcal conjugate vaccine immunization.
1340 Expert Rev Vaccines. 2020;19: 383–393.
1341 doi:10.1080/14760584.2020.1750377
- 1342 104. Nzenze SA, Shiri T, Nunes MC, Klugman KP, Kahn K, Twine R, et al.
1343 Temporal changes in pneumococcal colonization in a rural African community
1344 with high HIV prevalence following routine infant pneumococcal immunization.
1345 Pediatr Infect Dis J. 2013;32: 1270–1278.
1346 doi:10.1097/01.inf.0000435805.25366.64
- 1347 105. Statistics South Africa. Mid-year population estimates: 2009-2013. 2013.
- 1348 106. Chang Q, Stevenson AE, Croucher NJ, Lee GM, Pelton SI, Lipsitch M, et al.
1349 Stability of the pneumococcal population structure in Massachusetts as PCV13
1350 was introduced. BMC Infect Dis. 2015;15: 68. doi:10.1186/s12879-015-0797-z
- 1351 107. Centres for Disease Prevention and Control. Active Bacterial Core surveillance
1352 matrix. 2018. Available: [https://www.cdc.gov/abcs/downloads/abcs-](https://www.cdc.gov/abcs/downloads/abcs-surveillance-matrix.pdf)
1353 [surveillance-matrix.pdf](https://www.cdc.gov/abcs/downloads/abcs-surveillance-matrix.pdf)
- 1354 108. Enright MC, Spratt BG. A multilocus sequence typing scheme for
1355 *Streptococcus pneumoniae*: Identification of clones associated with serious
1356 invasive disease. Microbiology. 1998;144: 3049–3060. doi:10.1099/00221287-
1357 144-11-3049
- 1358 109. Syrjänen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal
1359 carriage of *Streptococcus pneumoniae* in Finnish children younger than 2
1360 years old. J Infect Dis. 2001;184: 451–459. doi:10.1086/322048
- 1361 110. Sleeman KL, Daniels L, Gardiner M, Griffiths D, Deeks JJ, Dagan R, et al.

- 1362 Acquisition of *Streptococcus pneumoniae* and nonspecific morbidity in infants
1363 and their families: A cohort study. *Pediatr Infect Dis J.* 2005;24: 121–127.
1364 doi:10.1097/01.inf.0000151030.10159.b1
- 1365 111. Normark BH, Christensson B, Sandgren A, Noreen B, Sylvan S, Burman LG,
1366 et al. Clonal Analysis of *Streptococcus pneumoniae* Nonsusceptible to
1367 Penicillin at Day-Care Centers with Index Cases, in a Region with Low
1368 Incidence of Resistance: Emergence of an Invasive Type 35B Clone among
1369 Carriers. *Microb Drug Resist.* 2003;9: 337–344.
1370 doi:10.1089/107662903322762761
- 1371 112. Galanis I, Lindstrand A, Darenberg J, Browall S, Nannapaneni P, Sjöström K,
1372 et al. Effects of PCV7 and PCV13 on invasive pneumococcal disease and
1373 carriage in Stockholm, Sweden. *Eur Respir J.* 2016;47: 1208–1218.
1374 doi:10.1183/13993003.01451-2015
- 1375 113. Mato R, Santos Sanches I, Simas C, Nunes S, Carriço JA, Sousa NG, et al.
1376 Natural history of drug-resistant clones of *Streptococcus pneumoniae*
1377 colonizing healthy children in Portugal. *Microb Drug Resist.* 2005;11: 309–322.
1378 doi:10.1089/mdr.2005.11.309
- 1379 114. Gibson C. Population of the 100 Largest Cities and Other Urban Places In The
1380 United States: 1790 to 1990. 1998. Available:
1381 [https://www.census.gov/library/working-papers/1998/demo/POP-](https://www.census.gov/library/working-papers/1998/demo/POP-twps0027.html)
1382 [twps0027.html](https://www.census.gov/library/working-papers/1998/demo/POP-twps0027.html)
- 1383 115. Gray BM, Converse GM, Dillon HC. Serotypes of *Streptococcus pneumoniae*
1384 causing disease. *J Infect Dis.* 1979;140: 979–83. doi:10.1093/infdis/140.6.979
- 1385 116. Sharma D, Baughman W, Holst A, Thomas S, Jackson D, Da Gloria Carvalho
1386 M, et al. Pneumococcal carriage and invasive disease in children before

- 1387 introduction of the 13-valent conjugate vaccine: Comparison with the era
- 1388 before 7-valent conjugate vaccine. *Pediatr Infect Dis J.* 2013;32: e45-53.
- 1389 doi:10.1097/INF.0b013e3182788fdd
- 1390 117. Parra EL, De La Hoz F, Díaz PL, Sanabria O, Realpe ME, Moreno J. Changes
- 1391 in *Streptococcus pneumoniae* serotype distribution in invasive disease and
- 1392 nasopharyngeal carriage after the heptavalent pneumococcal conjugate
- 1393 vaccine introduction in Bogotá, Colombia. *Vaccine.* 2013;31: 4033–8.
- 1394 doi:10.1016/j.vaccine.2013.04.074
- 1395 118. Instituto Nacional de Estadística Venezuela. Censo Nacional de Población y
- 1396 Vivienda. 2011. Available:
- 1397 <http://www.redatam.ine.gob.ve/Censo2011/index.html>
- 1398 119. Rivera-Olivero IA, Del Nogal B, Sisco MC, Bogaert D, Hermans PWM, De
- 1399 Waard JH. Carriage and invasive isolates of *Streptococcus pneumoniae* in
- 1400 Caracas, Venezuela: The relative invasiveness of serotypes and vaccine
- 1401 coverage. *Eur J Clin Microbiol Infect Dis.* 2011;30: 1489–95.
- 1402 doi:10.1007/s10096-011-1247-5
- 1403 120. Czech Statistical Office. Age Distribution of the Population of the Czech
- 1404 Republic - 2005. 2005. Available: [https://www.czso.cz/csu/czso/4003-06-2005-](https://www.czso.cz/csu/czso/4003-06-2005-01n)
- 1405 [01n](https://www.czso.cz/csu/czso/4003-06-2005-01n)
- 1406 121. Zemlickova H, Jakubu V, Urbaskova P, Motlova J, Musilek M, Adamkova V.
- 1407 Serotype-specific invasive disease potential of *Streptococcus pneumoniae* in
- 1408 Czech children. *J Med Microbiol.* 2010;59: 1079–83.
- 1409 doi:10.1099/jmm.0.018390-0
- 1410 122. Trotter CL, Waight P, Andrews NJ, Slack M, Efstratiou A, George R, et al.
- 1411 Epidemiology of invasive pneumococcal disease in the pre-conjugate vaccine

- era: England and Wales, 1996-2006. J Infect. 2010;60: 200–8.
- doi:10.1016/j.jinf.2009.12.008
123. Papua New Guinea National Statistical Office. National Population and Housing Census Ward Population Profile: Highlands Region. 2011. Available: <https://www.nso.gov.pg/index.php/population-and-social/other-indicators#highlands-region>
124. Plan H-C au. Annuaire statistique de la région Rabat-Salé-Zemmour-Zaër, 2010. 2012. Available: https://www.hcp.ma/downloads/Annuaire-statistiques-regionaux_t11956.html
125. Jroundi I, Mahraoui C, Benmessaoud R, Moraleda C, Munoz Almagro C, Seffar M, et al. *Streptococcus pneumoniae* carriage among healthy and sick pediatric patients before the generalized implementation of the 13-valent pneumococcal vaccine in Morocco from 2010 to 2011. J Infect Public Health. 2017;10: 165–170. doi:10.1016/j.jiph.2016.02.012
126. United Nations Department of Economic and Social Affairs. Population by age groups - both sexes. 2020. Available: <https://population.un.org/wpp/Download/Standard/Population/>
127. Vissers M, Wijmenga-Monsuur AJ, Knol MJ, Badoux P, van Houten MA, van der Ende A, et al. Increased carriage of non-vaccine serotypes with low invasive disease potential four years after switching to the 10-valent pneumococcal conjugate vaccine in The Netherlands. PLoS One. 2018;13: 1–15. doi:10.1371/journal.pone.0194823
128. Statistics Canada. Data tables, 1996. 1996. Available: <https://www.statcan.gc.ca/>
129. Instituto Nacionalde Estadistica Portugal. Censos 2001. 2002.

- 1437 130. Statistics Sweden. Population by region, marital status, age and sex. Year
1438 1968 - 2018. Available:
1439 [http://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__BE__BE0101__](http://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__BE__BE0101__BE0101A/BefolkningNy/?rxid=e550e75b-4cef-4e77-b5e5-83adcc09c97e)
1440 [BE0101A/BefolkningNy/?rxid=e550e75b-4cef-4e77-b5e5-83adcc09c97e](http://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__BE__BE0101__BE0101A/BefolkningNy/?rxid=e550e75b-4cef-4e77-b5e5-83adcc09c97e)
- 1441 131. Sandgren A, Christensson B, Samuelsson A, Olsson-Liljequist B, Sjöström K,
1442 Kronvall G, et al. Effect of Clonal and Serotype-Specific Properties on the
1443 Invasive Capacity of *Streptococcus pneumoniae*. J Infect Dis. 2004;189: 785–
1444 96. doi:10.1086/381686
- 1445 132. del Amo E, Brotons P, Monsonis M, Triviño M, Iñigo M, Selva L, et al. High
1446 invasiveness of pneumococcal serotypes included in the new generation of
1447 conjugate vaccines. Clin Microbiol Infect. 2014;20: 684–9. doi:10.1111/1469-
1448 0691.12422
- 1449 133. Institut National de la Statistique et des Etudes Economiques. Age pyramid
1450 2020 – France and metropolitan France. 2008.
- 1451 134. Varon E, Cohen R, Béchet S, Doit C, Levy C. Invasive disease potential of
1452 pneumococci before and after the 13-valent pneumococcal conjugate vaccine
1453 implementation in children. Vaccine. 2015;33: 6178–85.
1454 doi:10.1016/j.vaccine.2015.10.015
- 1455 135. Yildirim I, Hanage WP, Lipsitch M, Shea KM, Stevenson A, Finkelstein J, et al.
1456 Serotype specific invasive capacity and persistent reduction in invasive
1457 pneumococcal disease. Vaccine. 2010/10/30. 2010;29: 283–288. doi:S0264-
1458 410X(10)01502-1 [pii]10.1016/j.vaccine.2010.10.032 [doi]
- 1459 136. Scott JR, Millar E V., Lipsitch M, Moulton LH, Weatherholtz R, Perilla MJ, et al.
1460 Impact of more than a decade of pneumococcal conjugate vaccine use on
1461 carriage and invasive potential in native American communities. J Infect Dis.

1462 2012. doi:10.1093/infdis/jir730

1463 137. Statistics Finland. Tilastokeskus. 2021.

1464 138. Office for National Statistics. Mid-1998 Population Estimates: Single year of

1465 age and sex for local authorities in England and Wales; estimated resident

1466 population. 2004.

1467 139. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K,

1468 Pelton SI, et al. Continued Impact of Pneumococcal Conjugate Vaccine on

1469 Carriage in Young Children. Pediatrics. 2009;124: e1-11.

1470 doi:10.1542/peds.2008-3099

1471 140. Wroe PC, Lee GM, Finkelstein JA, Pelton SI, Hanage WP, Lipsitch M, et al.

1472 Pneumococcal carriage and antibiotic resistance in young children before 13-

1473 valent conjugate vaccine. Pediatr Infect Dis J. 2012;31: 249–254.

1474 doi:10.1097/INF.0b013e31824214ac

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1476 **Figures**

Figure 1 Comparison of observed and predicted counts of each serotype within each study of *S. pneumoniae* invasiveness in children. The points are coloured by the study to which they correspond, and show the observed value on the horizontal axis, and the mean predicted value on the vertical axis. The error bars show the 95% credibility intervals. The red dashed line shows the line of identity, corresponding to a perfect match between prediction and observation. The left column shows the correspondence for carriage data (values of $c_{i,j}$), and the right column shows the correspondence for disease isolates (values of $d_{i,j}$). Each row corresponds to a different model: (A) null Poisson model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-specific negative binomial model; (E) study-adjusted Poisson model; (F) study-adjusted negative binomial model; (G) study-adjusted type-specific Poisson model; (H) study-adjusted type-specific negative binomial model.

Figure 2 Invasiveness estimates for *S. pneumoniae* serotypes from the study-adjusted type-specific Poisson model applied to the full dataset of serotypes from child carriage and disease. Points represent the mean estimate, and are coloured according to the vaccine formulation in which they are found; all higher valency formulations encompass the serotypes found in lower valency formulations, with the exception of 6A not being present in PPV23. The point shape represents the sample size, summed across disease and carriage isolates from all studies, on which the estimates are based. The error bars show the 95% credibility intervals. (A) Estimates for all 72 serotypes in the full dataset. (B) Estimates when distinguishing between vaccine-type serotypes in unvaccinated and vaccinated populations. Vaccine serotypes in vaccinated populations are denoted with the suffix “_pv”.

Figure 3 Comparison of the serotype invasiveness estimates from the study-adjusted type-specific Poisson model with those from meta-analyses of the same dataset calculated as logarithmic odds ratios combined using (A) fixed effects models and (B) random effects models. Points are coloured based on the vaccine formulations in which serotypes are found. The horizontal error bars are the 95% credibility intervals from the Bayesian model, and the vertical error bars show the 95% confidence intervals from the logarithmic odds ratio analyses. Each plot is divided based on the sample size associated with each serotype, calculated as the sum of isolates from carriage and disease across all studies. The correlation between the two estimates is summarised as Kendall's τ on each panel, with an associated p value.

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Figure 4 Invasiveness estimates for *S. pneumoniae* serotypes from the study-adjusted type-specific Poisson model applied to the full dataset of serotypes from child carriage and adult disease. Points represent the mean estimate, and are coloured according to the vaccine formulation in which they are found; all higher valency formulations encompass the serotypes found in lower valency formulations, with the exception of 6A not being present in PPV23. The error bars show the 95% credibility intervals. (A) Estimates for all 53 serotypes in the full dataset. The point shape represents the sample size, summed across disease and carriage isolates from all studies, on which the estimates are based. (B) Scatterplot comparing the invasiveness estimates for adults and children.

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Figure 5 Estimates of within- and between-strain variation in invasiveness for strain and serotype combinations represented by at least ten isolates across the studies with genotype information. Points represent the mean estimate, and are coloured by the vaccine formulations in which the corresponding serotype is present. The shape of the point represents the sample size on which the estimate is based. Estimates are grouped by strain. Only strains for which estimates of multiple strain and serotype combinations were possible are displayed.

Figure 6 Estimates of within- and between-serotype variation in invasiveness for strain and serotype combinations represented by at least ten isolates across the included studies. Points represent the mean estimate, and are coloured by the vaccine formulations in which the corresponding serotype is present. The shape of the point represents the sample size on which the estimate is based. Estimates are grouped by serotype. Only serotypes for which estimates of multiple strain and serotype combinations were possible are displayed.

1546 **Tables**

Parameter	Description	Value or prior distribution
i	Index denoting study	1..(number of studies)
j	Index denoting type	1..(number of types)
k	Index denoting strain	1..(number of strains)
x	Generic index for type or strain	-
y	Generic index of type or strain that modifies the invasiveness of a genotype	-
c	Number of isolates detected in carriage study	-
d	Number of isolates detected in disease surveillance	-
ρ	Carriage prevalence	$\rho \sim \text{Beta}(1,1)$
η	Total number of positive and negative samples in carriage study	From previous publications
N	Number of individuals of the specified demographic under disease surveillance	From previous publications
t	Duration of disease surveillance	From previous publications
v	Progression rate	$\log_{10}(v) \sim U(-6,1)$ or truncated Cauchy($\mu_{\text{mod}}, \tau_{\text{mod}}$); see below
γ	Study-specific scale parameters	1 or $\log_{10}(\gamma) \sim \text{Cauchy}(\mu_{\text{study}}, \tau_{\text{study}})$
ϕ	Precision parameter of negative binomial distribution	$\log_{10}(\phi) \sim U(-3,3)$
Γ_i	Random variable for sampling from the Cauchy distribution for the study-specific scale parameters	$\Gamma_i \sim U(-0.5\pi, 0.5\pi)$
μ_{study}	Location parameter of the Cauchy distribution for the study-specific scale parameters	0
τ_{study}	Scale parameter of the Cauchy distribution for the study-specific scale parameters	2
Y_y	Random variable for sampling from the truncated Cauchy distribution for the invasiveness modification terms	$Y_y \sim U(-1.25, 1.25)$
μ_{mod}	Location parameter of the truncated Cauchy distribution for the invasiveness modification terms	0
τ_{mod}	Scale parameter of the truncated Cauchy distribution for the invasiveness modification terms	0.5

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1548 **Table 1** Summary of parameters used in the models.

1549 **Supporting Information**

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1552 **Analysing pneumococcal invasiveness using Bayesian models of pathogen**

1553 **progression rates**

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1555 Alessandra Løchen, James E. Truscott and Nicholas J. Croucher

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1557 **Text S1**

1558 **Figures S1-S31**

1559 **Tables S1-11**

1560 **Datasets S1-S3 (legends only)**

Text S1: Sample descriptions for genomic and genotyped datasets

The *S. pneumoniae* population was previously divided into strains by the Global Pneumococcal Sequencing project [31] using PopPUNK [5]. Previous estimation of the effect of strain background on invasiveness by Gladstone *et al* [35] used two study populations. The first combined two carriage studies from Agincourt and Soweto, in South Africa, with nationwide surveillance of IPD by the National Institute for Communicable Diseases. After excluding carriage and disease isolates from individuals reported as being HIV positive, genomes were available for 1,089 carriage isolates and 855 IPD isolates from children under seven years old. Model fitting required calculating the number of swabs taken from HIV negative children under seven in the carriage studies. In Soweto, the reported age stratification meant this number could only be calculated for children under five [102], and therefore four carriage isolates from children aged five or six were excluded from the study. In Agincourt, the reported age stratification meant 33 isolates from children aged six were excluded [103]. As the prevalence of HIV was low in infants in the Agincourt study [104], and the exact number of swabs from HIV positive children was not reported, no adjustment was made to the sampling based on HIV status. The population of children under surveillance for IPD was estimated from nationwide census data between 2009-2013 [105]. All samples were stratified into the post-PCV7 (2009-2011) and post-PCV13 (2012-2013) periods [31].

The second study population combined a carriage survey from Massachusetts, USA and isolates from the Active Bacterial Core Surveillance (ABCS) of IPD across several states. Isolates were collected over the sampling periods of 2000-2001, 2003-2004, 2006-2007 and 2008-2009. As only 51 IPD samples were available for

the earliest two periods, and the population was changing rapidly over these years [91], only samples from the more stable post-PCV7 2006-2009 period were studied [106]. This included 405 IPD isolates, modelled as coming from a time interval of two years (as samples only came from 2007 and 2009) from a population size calculated from the ABCS population matrix [107]. Of the carriage isolates, 280 were assigned to strains from genomic data, while 291 were only associated with multi-locus sequence typing (MLST) genotypes [108]. Gladstone *et al* assigned these isolates to strains using the close relationship between MLST and PopPUNK population classifications [5,35].

As these studies were estimating odds ratios across samples stratified by year, Gladstone *et al* randomly subsampled isolates to ensure an even ratio of disease and carriage at each sampling timepoint [35]. This correction is unnecessary with the models described in this study, as the γ_i scaling parameters can adjust for differing levels of surveillance between samples (incorporating variation in both the samples collected, and the subset sequenced). Furthermore, such subsampling would distort the inferred $\rho_{i,x}$.

MLST genotypes were also available for isolates from the studies of serotype invasiveness from Stockholm [75], Finland [71,109] and Oxford, UK [29]. Where possible, these were assigned to GPS strains using the isolate's MLST data, or the clonal complex to which they belonged at the time of publication [4]. If an isolate could not be assigned to a GPS strain, then its MLST sequence type was used to designate its genotype instead. Isolates that could not be unequivocally assigned a strain, serotype and source (carriage or disease) were omitted.

1611
 1612 For the Oxford study, these criteria meant 84 of the 150 disease isolates, and 113 of
 1613 the 351 carriage isolates, could be analysed. The carriage isolates were assembled
 1614 from three different surveys, only one of which could be linked to a more detailed
 1615 published description of the total carriage sample size [110]. As the isolates in this
 1616 study were recovered from 213 individuals, and the Oxford study attempted to
 1617 minimise multiple sampling from any one host [29], the overall carriage sample size
 1618 for the three studies was approximated as three times the number of individuals in
 1619 this published study (639).

1620
 1621 For the Finland study, the criteria described above meant 143 of the 224 disease
 1622 isolates, and 102 of the 217 carriage isolates, could be analysed. The carriage
 1623 isolates were recovered from ten longitudinal samples from each of 329 individuals
 1624 [33]. As the Finland study sought to avoid multiple sampling of the same carriage
 1625 episode [71], the host population size was used as the carriage sample size.

1626
 1627 For the Stockholm study, the criteria described above meant 65 of the 165 disease
 1628 isolates, and 178 of the 550 carriage isolates, could be analysed. The carriage
 1629 isolates were recovered from studies conducted between 1997 and 2004. Two
 1630 published descriptions of the original carriage studies reported the recovery of 506
 1631 isolates from 1,330 individuals [111,112]. As this accounted for over 90% of the
 1632 nasopharyngeal isolates, this number was used as the carriage sample size.

1633
 1634 An additional study from Portugal used a collection of disease isolates primarily
 1635 recovered from adults [52]. Applying the same criteria as to the child disease studies

1636 allowed unambiguous assignation of genotype and serotype to 463 of the 769
1637 carriage isolates, and 152 of the 475 of the disease isolates. The carriage isolates
1638 were a subset of a previous study [113], and originated from 1,170 nasopharyngeal
1639 swabs (R. Sá-Leão, personal communication).

Supplementary Figures

Figure S1 Properties of the child serotype dataset. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S2 Properties of the child serotype dataset after removing datapoints in which fewer than five isolates of a serotype were recorded from both carriage and disease in a given study. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S3 Line plots showing the post-warmup MCMC traces for the logarithmic posterior probabilities across two independent chains for models fitted to the filtered serotype data from child carriage and disease. The horizontal axis shows the generation of the MCMC, with values for the two chains shown by orange and purple lines. Each panel corresponds to a different model: (A) null Poisson model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-specific negative binomial model; (E) study-adjusted Poisson model; (F) study-adjusted negative binomial model; (G) study-adjusted type-specific Poisson model; (H) study-adjusted type-specific negative binomial model.

Figure S4 Histograms of Rhat values calculated from the paired MCMCs for models fitted to the filtered serotype data from child carriage and disease. Each panel corresponds to a different model: (A) null Poisson model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-specific negative binomial model; (E) study-adjusted Poisson model; (F) study-adjusted negative binomial model; (G) study-adjusted type-specific Poisson model; (H) study-adjusted type-specific negative binomial model.

Figure S5 Violin plots showing the root mean square error between observed and predicted values across model fits to the filtered serotype data from child carriage and disease. Blue crosses represent the individual observations.

Figure S6 Graph showing the values of the negative binomial distribution's precision parameter, ϕ , from model fits to the filtered serotype data from child carriage and disease. The points represent the mean estimate from the MCMCs, and the error bars show the 95% credibility interval.

Figure S7 Plots validating the fit of the study-adjusted type-specific Poisson model to the full serotype data from child carriage and disease. (A) Histogram showing the distribution of Rhat values. (B) Post-warmup MCMC traces of the log posterior probability.

Figure S8 Study-adjustment scale factors from the study-adjusted type-specific Poisson model fitted to the full serotype data from child carriage and disease. The reference study, for which the value was fixed at one, was the Navajo post-PCV7 dataset, which had the greatest sample size in this meta-analysis (Fig. S1).

Figure S9 Plots validating the fit of the study-adjusted type-specific Poisson model to the full serotype data from child carriage and disease, when distinguishing between vaccine-type serotypes pre- and post-PCV. (A) Histogram showing the distribution of Rhat values. (B) Post-warmup MCMC traces of the log posterior probability.

Figure S10 Study-adjustment scale factors from the study-adjusted type-specific Poisson model fitted to the full serotype data from child carriage and disease, when distinguishing between vaccine-type serotypes pre- and post-PCV. The reference study, for which the value was fixed at one, was the Navajo post-PCV7 dataset, which had the greatest sample size in this meta-analysis (Fig. S1).

Figure S11 Relationship between serotype invasiveness in children (as shown in Fig. 2) and carriage duration in a study of infant colonisation in Maela, Thailand. The carriage duration estimates were derived from a multi-variate lasso regression that included both serotype and antibiotic resistance phenotypes. Values were available for 14 serotypes, all relative to the carriage duration of serotype 6A/C, which was therefore assigned a value of zero days.

Figure S12 Properties of the adult serotype dataset. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S13 Properties of the adult serotype dataset after removing datapoints in which fewer than five isolates of a serotype were recorded from both carriage and disease in a given study. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S14 Line plots showing the post-warmup MCMC traces for the logarithmic posterior probabilities across two independent chains for models fitted to the filtered serotype data from child carriage and adult disease. The horizontal axis shows the generation of the MCMC, with values for the two chains shown by orange and purple lines. Each panel corresponds to a different model: (A) null Poisson model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-specific negative binomial model; (E) study-adjusted Poisson model; (F) study-adjusted negative binomial model; (G) study-adjusted type-specific Poisson model; (H) study-adjusted type-specific negative binomial model.

Figure S15 Histograms of Rhat values calculated from paired MCMCs for models fitted to the filtered serotype data from child carriage and adult disease. Each panel corresponds to a different model: (A) null Poisson model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-specific negative binomial model; (E) study-adjusted Poisson model; (F) study-adjusted negative binomial model; (G) study-adjusted type-specific Poisson model; (H) study-adjusted type-specific negative binomial model.

1 **Figure S16** Comparison of observed and predicted counts of each serotype within
2 each adult study. The points are coloured by the study to which they correspond,
3 and represent the observed value on the horizontal axis, and the mean predicted
4 value on the vertical axis. The error bars show the 95% credibility intervals. The red
5 dashed line shows the line of identity, corresponding to a perfect match between
6 prediction and observation. The left column shows the correspondence for carriage
7 data (values of $c_{i,j}$), and the right column shows the correspondence for disease
8 isolates (values of $d_{i,j}$). Each row corresponds to a different model: (A) null Poisson
9 model; (B) null negative binomial model; (C) type-specific Poisson model; (D) type-
10 specific negative binomial model; (E) study-adjusted Poisson model; (F) study-
11 adjusted negative binomial model; (G) study-adjusted type-specific Poisson model;
12 (H) study-adjusted type-specific negative binomial model.

Figure S17 Plots validating the fit of the study-adjusted type-specific Poisson model to the full serotype data from child carriage and adult disease. (A) Histogram showing the distribution of Rhat values. (B) Post-warmup MCMC traces of the log posterior probability.

Figure S18 Study-adjustment scale factors from the study-adjusted type-specific Poisson model fitted to the full serotype data from child carriage and adult disease. The reference study, for which the value was fixed at one, was the Navajo post-PCV7 dataset, which had the greatest sample size in this meta-analysis (Fig. S11).

Figure S19 Properties of the child serotype and strain dataset. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S20 Properties of the child serotype and strain dataset after removing datapoints in which fewer than five isolates of a serotype were recorded from both carriage and disease in a given study. (A) Stacked bar plot showing the distribution of carriage and disease isolates between studies. (B) Stacked bar plot showing the distribution of carriage and disease isolates between serotypes.

Figure S21 Line plots showing the post-warmup MCMC traces for the logarithmic posterior probabilities across two independent chains for models fitted to the filtered strain and serotype data from child carriage and disease. Each panel corresponds to a model with a different method of associating isolates with an invasiveness estimate: (A) serotype-determined, Poisson-distributed invasiveness; (B) serotype-determined, negative binomially-distributed invasiveness; (C) strain-determined, Poisson-distributed invasiveness; (D) strain-determined, negative binomially-distributed invasiveness; (E) serotype-determined, strain-modified Poisson-distributed invasiveness; (F) serotype-determined, strain-modified negative binomial model; (G) strain-determined, serotype-modified Poisson-distributed invasiveness; (H) strain-determined, serotype-modified negative binomially-distributed invasiveness; (I) strain- and serotype-determined Poisson-distributed invasiveness; (J) strain- and serotype-determined negative binomially-distributed invasiveness.

Figure S22 Histograms of Rhat values calculated from paired MCMCs for models fitted to the filtered strain and serotype data from child carriage and disease. Each panel corresponds to a different model: (A) serotype-determined, Poisson-distributed invasiveness; (B) serotype-determined, negative binomially-distributed invasiveness; (C) strain-determined, Poisson-distributed invasiveness; (D) strain-determined, negative binomially-distributed invasiveness; (E) serotype-determined, strain-modified Poisson-distributed invasiveness; (F) serotype-determined, strain-modified negative binomial model; (G) strain-determined, serotype-modified Poisson-distributed invasiveness; (H) strain-determined, serotype-modified negative binomially-distributed invasiveness; (I) strain- and serotype-determined Poisson-distributed invasiveness; (J) strain- and serotype-determined negative binomially-distributed invasiveness.

Figure S23 Comparison of observed and predicted counts of isolates, categorised by both their serotype and strain background, within each study. The points are coloured by the study to which they correspond, and represent the observed value on the horizontal axis, and the mean predicted value on the vertical axis. The error bars show the 95% credibility intervals. The red dashed line shows the line of identity, corresponding to a perfect match between prediction and observation. The left column shows the correspondence for carriage data (values of $c_{i,j,k}$), and the right column shows the correspondence for disease isolates (values of $d_{i,j,k}$). Each row corresponds to a different model: (A) serotype-determined, Poisson-distributed invasiveness; (B) serotype-determined, negative binomially-distributed invasiveness; (C) strain-determined, Poisson-distributed invasiveness; (D) strain-determined, negative binomially-distributed invasiveness; (E) serotype-determined, strain-modified Poisson-distributed invasiveness; (F) serotype-determined, strain-modified negative binomial model; (G) strain-determined, serotype-modified Poisson-distributed invasiveness; (H) strain-determined, serotype-modified negative binomially-distributed invasiveness; (I) strain- and serotype-determined Poisson-distributed invasiveness; (J) strain- and serotype-determined negative binomially-distributed invasiveness.

Figure S24 Graph showing the values of the negative binomial distribution's precision parameter, ϕ , from model fits to the filtered strain and serotype data from child carriage and disease. The points represent the mean estimate from the MCMCs, and the error bars show the 95% credibility interval.

Figure S25 Violin plots showing the root mean square error between observed and predicted values across model fits to the filtered strain and serotype data from child carriage and disease. Blue crosses represent the individual observations.

Figure S26 Study-adjustment scale factors from the study-adjusted type-specific Poisson model fitted to the full serotype and strain data from child carriage and disease, with the combination of both serotype and strain determining an isolate's invasiveness. The reference study, for which the value was fixed at one, was the South Africa post-PCV7 dataset, which had the greatest sample size in this meta-analysis, and was associated with a known carriage sample size (Text S1).

Figure S27 Plots validating the fit of the study-adjusted type-specific Poisson model to the full strain and serotype data from child carriage and disease, with the combination of both serotype and strain determining an isolate's invasiveness. (A) Histogram showing the distribution of Rhat values. (B) Post-warmup MCMC traces of the log posterior probability.

Figure S28 Invasiveness estimates for all strain and serotype combinations in the full strain and serotype dataset from child carriage and disease.

Figure S29 Bar plot showing the distribution of carriage and disease isolates between serotypes for the dataset from Portugal comparing child carriage with primarily adult disease.

Figure S30 Plots validating the fit of the type-specific Poisson model to the strain and serotype data from child carriage and adult disease, with the combination of both serotype and strain determining an isolate's invasiveness. (A) Histogram showing the distribution of Rhat values. (B) Post-warmup MCMC traces of the log posterior probability.

Figure S31 Invasiveness estimates for strain and serotype combinations represented by at least ten isolates in the study of child carriage and adult disease. Points represent the mean estimate, and are coloured by the vaccine formulations in which the corresponding serotype is present. The shape of the point represents the sample size on which the estimate is based. (A) Estimates are shown grouped by strain. Only strains for which estimates of multiple strain and serotype combinations were possible are displayed. (B) Estimates are shown grouped by serotype. Only serotypes for which estimates of multiple strain and serotype combinations were possible are displayed.

1 **Supplementary Tables**

2

Population	Vaccine period	Carriage study time interval	Carriage isolate source	Disease isolate source	No. of swabs	Population of children	No. disease isolates from children	Population of adults	No. disease isolates from adults	References
Alabama	Pre-PCV	July 1975 – December 1978	Unvaccinated sick and healthy children <18 years old from Alabama	Unvaccinated children with IPD <18 years old from Alabama and unvaccinated adults hospitalised with pneumonia or IPD in Alabama	827	19,316	114	232,373	86	[114,115]
Atlanta	Pre-PCV	January – December 1995	Unvaccinated children with recent URI in Atlanta <5 years old	Unvaccinated children <5 years old with IPD in Atlanta	231	204,680	202	-	-	[116]
Bogota	Pre-PCV	May 2005 – November 2006	Healthy unvaccinated children <18 months old in Bogota	IPD in children <2 years old in Bogota	197	357,200	353	-	-	[117]
Caracas	Pre-PCV	December 2006 – January 2008	Unvaccinated healthy children <6 years old in Caracas	Unvaccinated children with IPD <6 years old in Caracas	1,004	146,125	36	-	-	[118,119]
Czech	Pre-PCV	1996 – 2005	Unvaccinated healthy children 3 - 5 years old across the Czech Republic	Unvaccinated children <6 years old with IPD across the Czech Republic	425	478,177	138	-	-	[120,121]
England & Wales	Pre-PCV	July 1996 – June 2006	Unvaccinated healthy children	Unvaccinated children <5	3,752	3,091,000	461	48,702,414	1,876	[122]

			<5 years old in Hertfordshire	years old across and predominantly unvaccinated individuals >4 years old across England & Wales						
Goroka	Pre-PCV	1981 – 1987	Unvaccinated children attending clinics near Goroka Town	Unvaccinated children with IPD in Goroka hospital	2,844	96,207	56	-	-	[28,123]
Morocco	Pre-PCV	November 2010 – December 2011	Unvaccinated healthy children in Rabat	Children hospitalised with severe pneumonia in Rabat <5 years old	200	212,566	118	-	-	[124,125]
Netherlands	Pre-PCV	June 2004 – May 2006	Unvaccinated healthy children <2 years old in Noord-Holland, Zuid-Holland and Utrecht	Children <5 years with IPD across Netherlands	321	250,924	100	-	-	[126,127]
Ontario	Pre-PCV	1995	Unvaccinated healthy children primarily <4 years old in Toronto	Unvaccinated children <18 years old with IPD in Toronto and surrounding area	1,139	580,507	89	-	-	[128]
Portugal	Pre-PCV	January 2001 – December 2003	Unvaccinated healthy children <7 years old in Lisbon and Oeiras	Unvaccinated children (<18 years) and adults (>18 years) across Portugal	1,170	2,071,223	90	8,284,894	378	[52,129]

Stockholm	Pre-PCV	1997	Unvaccinated healthy children <7 years old in Stockholm	Unvaccinated adults (and a small number of children) hospitalised with IPD in Stockholm	611	-	-	2,004,152	273	[130,131]
Atlanta	Post-PCV7	June 2008 – May 2009	PCV7-vaccinated sick Georgia-resident children <5 years old	PCV7-vaccinated children <5 years old with IPD in Atlanta	451	298,831	47	-	-	[116]
Barcelona	Post-PCV7	2007 – 2011	PCV7-vaccinated healthy children <7 years old in Barcelona	PCV7-vaccinated children <7 years old with IPD in Barcelona	209	228,000	159	-	-	[132]
Bogota	Post-PCV7	June – November 2011	PCV7-vaccinated healthy children <18 months old in Bogota	IPD in children <2 years old in Bogota	246	357,200	91	-	-	[117]
France	Post-PCV7	January 2008 – December 2009	PCV7-vaccinated healthy children <2 years old across France	PCV7-vaccinated children with IPD <2 years old across France	1,212	838,866	388	-	-	[133,134]
Massachusetts	Post-PCV7	2001 – April 2009	PCV7-vaccinated children visiting physicians <7 years old across Massachusetts	PCV7-vaccinated children <7 years with IPD old across Massachusetts	2,969	820,000	206	-		[135]
Navajo	Post-PCV7	March 2006 - March 2008	PCV7-vaccinated Navajo or White Mountain	Active IPD surveillance of PCV7-vaccinated	6,541	65,048	132	201,553	514	[37,39,136]

			Apache native American children <9 years old	Navajo or White Mountain Apache native American children <7 years old and people at least 18 years old						
Netherlands	Post-PCV7	June 2008 – May 2012	Vaccinated healthy children <2 years old in Noord-Holland, Zuid-Holland and Utrecht	IPD in children (<5 years) across Netherlands	660	232,251	73	-	-	[126,127]
France	Post-PCV13	January 2012 – December 2013	PCV13-vaccinated healthy children <2 years old across France	PCV13-vaccinated children with IPD <2 years old across France	1,212	842,076	181	-	-	[133,134]
Netherlands	Post-PCV10	June 2012 – May 2016	Vaccinated healthy children <2 years old in Noord-Holland, Zuid-Holland and Utrecht	IPD in children (<5 years) across Netherlands	659	222,671	47	-	-	[126,127]

Table S1: Study populations used for the joint analysis of strain and serotype invasiveness.

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Model	ELPD difference	ELPD difference standard error
study-adjusted type-specific Poisson	0	0
study-adjusted type-specific negative binomial	-11.61731	13.49535
type-specific negative binomial	-179.09155	23.2183
study-adjusted negative binomial	-239.06206	25.67848
null negative binomial	-283.58518	25.5161
type-specific Poisson	-825.94846	138.34479
study-adjusted null Poisson	-920.16588	112.08258
null Poisson	-1774.3198	213.90718

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Table S2 Model fit comparison with leave-one-out (LOO) cross-validation using the child serotype data on carriage and disease. These values were generated using the logarithm of the likelihoods calculated within the models. Models are ranked by their expected log pointwise predictive density (ELPD), calculated from the individual pointwise log predictive densities across all observed data points. The ELPD difference column shows the difference between the ELPD of a model and that of the most likely model (this value is zero for the first row, corresponding to the most likely model given the data). The ELPD difference standard error is calculated from the distribution of individual pointwise log predictive densities from the same comparison.

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Model	ELPD difference	ELPD difference standard error
study-adjusted type-specific negative binomial	0	0
study-adjusted type-specific Poisson	-16.68564	10.50462
type-specific negative binomial	-148.19801	13.72573
study-adjusted negative binomial	-203.23604	16.36764
null negative binomial	-249.59283	17.21276
type-specific Poisson	-729.33726	112.9739
study-adjusted null Poisson	-821.7134	96.65528
null Poisson	-1638.1251	190.51644

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Table S3 Model fit comparison with LOO cross-validation using the child serotype data on carriage and disease. These values were generated using the logarithm of the likelihoods calculated for the observations of isolates from disease only, as these were more constrained than the modelling of isolate counts from carriage. The table is displayed as described for Table S2.

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Model	Log(Bayes factor) relative to most likely model
study-adjusted type-specific Poisson	0.0000
type-specific Poisson	-406.5216
type-specific negative binomial	-459.7201
null negative binomial	-600.7347
study-adjusted type-specific negative binomial	-666.8982
study-adjusted null Poisson	-848.4240
study-adjusted negative binomial	-876.0923
null Poisson	-1445.7665

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Table S4 Comparison of model fits to child carriage and disease serotype data using Bayes factors calculated with bridge sampling. Models are ranked by their logarithmic marginal likelihoods. The logarithmic Bayes factors are calculated for each model relative to the most likely given the data; hence the value is zero for the first row.

Model	Log(Bayes factor) relative to most likely model
study-adjusted type-specific Poisson	0.0000
type-specific negative binomial	-131.7431
study-adjusted type-specific negative binomial	-185.9657
null negative binomial	-205.1582
study-adjusted negative binomial	-286.0869
type-specific Poisson	-362.1535
study-adjusted null Poisson	-1010.5423
null Poisson	-1787.1696

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2 **Table S5** Comparison of model fits to child carriage and adult disease serotype data using Bayes factors calculated with bridge
3 sampling. The table is displayed as described for Table S4.

Population	Vaccine period	Carriage study time interval	Carriage isolate source	Disease isolate source	No. of swabs	Population of children	No. disease isolates from children	Population of adults	No. disease isolates from adults	References
Finland	Pre-PCV	1994 - 1996	Unvaccinated children <2 years old in Tampere	Unvaccinated children <2 years across Finland	329	120,238	143	-	-	[71,137]
Oxford	Pre-PCV	1994 - 2001	Unvaccinated healthy children <5 years old in Oxford	Unvaccinated children <5 years old with IPD in Oxford	639	37,467	84	-	-	[29,138]
Portugal	Pre-PCV	January 2001 - December 2003	Unvaccinated healthy children <7 years old in Lisbon and Oeiras	Unvaccinated children (<18 years) and adults (>18 years) across Portugal	1,170	2,071,223	-	8,284,894	152	[52,129]
Stockholm	Pre-PCV	1997 - 2004	Unvaccinated children <7 years old attending day care centres in Stockholm County	Unvaccinated children <18 years old from the Stockholm area	1,330	397,289	65	-	-	[75,111,112,130]
South Africa	Post-PCV7	2009 - 2010	HIV negative children <13 years old in Soweto and Agincourt	HIV negative children <7 years old in South Africa	2,674	7,187,314	515	-	-	[35,102,104,105]
USA	Post-PCV7	2006 - 2009	Children <7 years of age in Massachusetts	Children <7 years old in the USA ABCS regions	1,983	1,931,331	405	-	-	[35,91,107,139,140]

South Africa	Post-PCV13	2011 - 2013	HIV negative children <13 years old in Soweto and Agincourt	HIV negative children <7 years old in South Africa	2,023	7,329,006	340	-	-	[35,102,104,105]
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Table S6: Study populations used for the joint analysis of strain and serotype invasiveness. The disease isolates from Portugal came from a mixture of infants and adults, but are tabulated based on them primarily arising from the latter age category.

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Model	ELPD difference	ELPD difference standard error
strain- and serotype-specific Poisson	0	0
strain-specific serotype-modified Poisson	-3.7248	5.477322
type-specific strain-modified Poisson	-6.287393	5.468617
serotype-specific Poisson	-37.42351	18.306771
serotype-specific negative binomial	-46.514338	12.630727
strain-specific serotype-modified negative binomial	-56.069404	7.98463
type-specific strain-modified negative binomial	-57.304676	7.837755
strain- and serotype-specific negative binomial	-67.860004	7.923821
strain-specific Poisson	-90.523468	29.006827
strain-specific negative binomial	-98.704763	13.503281

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3 **Table S7** Model fit comparison with LOO cross-validation using the child strain and serotype data on carriage and disease. These
4 values were generated using the logarithm of the likelihoods calculated for the observations of isolates from carriage and disease.
5 The table is displayed as described for Table S2.

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Model	ELPD difference	ELPD difference standard error
strain- and serotype-specific Poisson	0	0
strain-specific serotype-modified Poisson	-6.069704	4.036103
type-specific strain-modified Poisson	-6.667989	4.181882
serotype-specific negative binomial	-33.721585	10.603748
serotype-specific Poisson	-43.800528	15.81467
type-specific strain-modified negative binomial	-48.519279	6.091823
strain-specific serotype-modified negative binomial	-50.379496	6.308592
strain- and serotype-specific negative binomial	-58.830455	6.314365
strain-specific Poisson	-78.341963	24.643501
strain-specific negative binomial	-83.515938	11.810553

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3 **Table S8** Model fit comparison with LOO cross-validation using the child strain and serotype data on carriage and disease. These
4 values were generated using the logarithm of the likelihoods calculated for the observations of isolates from disease only. The table
5 is displayed as described for Table S2.

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Model	Log(Bayes factor) relative to best-fitting model
strain- and serotype-specific Poisson	0
serotype-specific Poisson	-77.5832
strain-specific Poisson	-86.35369
strain-specific serotype-modified Poisson	-258.18324
type-specific strain-modified Poisson	-260.87589
strain- and serotype-specific negative binomial	-334.62845
strain-specific negative binomial	-371.46834
serotype-specific negative binomial	-388.66057
type-specific strain-modified negative binomial	-575.30246
strain-specific serotype-modified negative binomial	-575.70213

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Table S9 Comparison of model fits to child carriage and disease strain and serotype data using Bayes factors calculated with bridge sampling. The table is displayed as described for Table S4.

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Model	Log(Bayes factor) relative to best-fitting model
strain- and serotype-specific Poisson	0.00000
serotype-specific Poisson	-77.67605
strain-specific Poisson	-86.35044
type-specific strain-modified Poisson	-257.60470
strain-specific serotype-modified Poisson	-267.16176
strain- and serotype-specific negative binomial	-334.11318
strain-specific negative binomial	-371.16642
serotype-specific negative binomial	-388.88025
type-specific strain-modified negative binomial	-574.35959
strain-specific serotype-modified negative binomial	-577.58130

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Table S10 Comparison of model fits to child carriage and disease strain and serotype data using Bayes factors calculated with bridge sampling. In this analysis, the carriage sample size for three studies (Finland pre-PCV, Oxford pre-PCV and Stockholm pre-PCV) was increased 100-fold, to test for the sensitivity of the model comparisons to the uncertainty in this parameter (Text S1). The table is displayed as described for Table S4.

Previous publication	Serotype	Low invasiveness genotype	Low invasiveness strain	High invasiveness genotype	High invasiveness strain	Results from this study
Gladstone <i>et al</i> 2019	6A	CC172	GPSC5	CC1094	GPSC41	GPSC41-6A invasiveness significantly higher than GPSC5-6A
Gladstone <i>et al</i> 2019	14	CC63	GPSC9	CC15	GPSC18	GPSC18-14 invasiveness significantly higher than GPSC9-14
Gladstone <i>et al</i> 2019	16F	CC4088	GPSC33	CC30	GPSC46	Not tested
Gladstone <i>et al</i> 2019	19F	CC347	GPSC21	CC320	GPSC1	GPSC1-19F invasiveness significantly higher than GPSC21-19F
Gladstone <i>et al</i> 2019	23B	CC439	GPSC7	CC172	GPSC5	Not tested
Sá-Leão <i>et al</i> 2011	3	ST180	GPSC12	ST156	GPSC6	GPSC6 found to have significantly higher invasiveness
Sá-Leão <i>et al</i> 2011	6A	ST460, ST315, ST1879	GPSC47, GPSC64, ST1879	-	-	GPSC64 does not exhibit significantly divergent invasiveness; others not tested
Sá-Leão <i>et al</i> 2011	6B	ST176, ST315	GPSC24, GPSC47	-	-	Other GPSCs of this serotype not tested
Sá-Leão <i>et al</i> 2011	11A	ST62	GPSC3	-	-	Not tested
Sá-Leão <i>et al</i> 2011	14	-	-	ST156	GPSC6	More invasive than GPSC9-14
Sá-Leão <i>et al</i> 2011	19A	ST81	GPSC16	ST193, ST230	GPSC11, GPSC10	Not tested
Sá-Leão <i>et al</i> 2011	19F	ST177	GPSC44	-	-	Not tested
Sá-Leão <i>et al</i> 2011	22F	-	-	ST443	ST443	Not tested
Sá-Leão <i>et al</i> 2011	23F	ST176, ST439	GPSC24, GPSC7	-	-	GPSC7-23F has lower invasiveness than GPSC5-23F
Sá-Leão <i>et al</i> 2011	34	ST1439	GPSC45	-	-	Not tested
Brueggemann <i>et al</i> 2003	14	-	-	ST9	GPSC18	More invasive than GPSC9-14

Brueggemann <i>et al</i> 2003	14	-	-	ST124	GPSC39	More invasive than GPSC9
Brueggemann <i>et al</i> 2003	18C	-	-	ST113	GPSC50	Higher invasiveness than GPSC68-18C, but with overlapping credibility intervals
Hanage <i>et al</i> 2005	14	-	-	ST156	GPSC6	More invasive than GPSC9-14
Hanage <i>et al</i> 2005	19A	-	-	ST482	ST482	Higher invasiveness than GPSC17-19A
Hanage <i>et al</i> 2005	7F	-	-	ST191	GPSC15	Not tested
Hanage <i>et al</i> 2005	14	-	-	ST124	GPSC39	More invasive than GPSC9-14
Hanage <i>et al</i> 2005	6B	-	-	ST138	GPSC24	More invasive than other 6B GPSCs
Hanage <i>et al</i> 2005	19F	ST485	GPSC175	-	-	Low invasiveness, with overlapping credibility intervals with other GPSCs
Hanage <i>et al</i> 2005	11A	ST62	GPSC3	-	-	Similar to GPSC22

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2 **Table S11** Comparison of previously-identified within-serotype differences in strain invasiveness with those supported by this
3 analysis. The exclusion of strain and serotype combinations represented by fewer than ten isolates, summed across carriage and
4 disease, meant that some within-serotype tests between strains were not undertaken in this analysis.

Supplementary Datasets

Dataset S1 Invasiveness estimates for serotypes in children. The mean estimates, and lower and upper bounds of the 95% credibility interval, are listed for each serotype.

Dataset S2 Invasiveness estimates for serotypes in adults. The mean estimates, and lower and upper bounds of the 95% credibility interval, are listed for each serotype.

Dataset S3 Invasiveness estimates for serotype-strain combinations in children. The mean estimates, and lower and upper bounds of the 95% credibility interval, are listed for each serotype-strain combination.