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Assessing pneumococcal meningitis association with viral respiratory infections and antibiotics: insights from statistical and mathematical models

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Pneumococcus is an important human pathogen, highly antibiotic resistant and a major cause of bacterial meningitis worldwide. Better prevention requires understanding the drivers of pneumococcal infection incidence and antibiotic susceptibility. Although respiratory viruses (including influenza) have been suggested to influence pneumococcal infections, the underlying mechanisms are still unknown, and viruses are rarely considered when studying pneumococcus epidemiology. Here, we propose a novel mathematical model to examine hypothetical relationships between *Streptococcus pneumoniae* meningitis incidence (SPMI), acute viral respiratory infections (AVRIs) and antibiotic exposure. French time series of SPMI, AVRI and penicillin consumption over 2001–2004 are analysed and used to assess four distinct virus–bacteria interaction submodels, ascribing the interaction on pneumococcus transmissibility and/or pathogenicity. The statistical analysis reveals strong associations between time series: SPMI increases shortly after AVRI incidence and decreases overall as the antibiotic-prescription rate rises. Model simulations require a combined impact of AVRI on both pneumococcal transmissibility (up to 1.3-fold increase at the population level) and pathogenicity (up to threefold increase) to reproduce the data accurately, along with diminished epidemic fitness of resistant pneumococcal strains causing meningitis (0.97 (0.96–0.97)). Overall, our findings suggest that AVRI and antibiotics strongly influence SPMI trends. Consequently, vaccination protecting against respiratory virus could have unexpected benefits to limit invasive pneumococcal infections.

1. Introduction

Streptococcus pneumoniae is a Gram-positive coccal bacterium that commonly colonizes the human nasopharynx, and is a leading cause of infectious disease and deaths worldwide, responsible for 1.6 million deaths per year

[1,2]. In developed countries, *S. pneumoniae* is the main cause of bacterial meningitis at any age [3]. In the USA, it is responsible for 50 per cent of meningitides in infants three months to 2 years old and more than 70 per cent of adults more than 40 years old [4]. Lethality fluctuates between 10 and 33 per cent depending on age, with neurological relapses for 30 per cent of cases all ages combined [4,5].

Over the past decades, the spread of antibiotic-resistant pneumococcal strains has made meningitis treatment more difficult, and generally increased the morbidity and mortality associated with severe pneumococcal infections [6]. The high antibiotic-resistance rates in these bacteria have led to policies of limiting antibiotic use and encouraging anti-bacterial vaccination, resulting in important changes of pneumococcal infection ecology [7–9].

Although much attention has been focused on pneumococcal meningitis, its epidemiological dynamics are not fully understood. For example, the seasonal fluctuations of *S. pneumoniae* meningitis incidence (SPMI) that peak during the winter remain poorly understood [10]. Understanding factors driving these dynamics is crucial to optimize public health programmes devoted to their prevention, for example, anti-pneumococcus vaccine programmes or limiting antibiotic prescriptions.

From an ecological point of view, the dynamics of transmission and evolution of *S. pneumoniae* in host populations are likely to be influenced by the bacteria's environment. The latter includes population drug exposure [11–13] and co-circulating species [14,15]. Potential interaction mechanisms between community respiratory viruses and *S. pneumoniae* have been proposed, based on incidence time-series analysis and animal *in vivo* studies [16,17]. The underlying hypotheses rely on within-host pathophysiological respiratory virus–bacterium interactions that could influence *S. pneumoniae* colonization, transmission and disease [10,16,18–21]. However, the underlying *in vivo* biological mechanisms are still unclear and cannot be directly inferred [10].

In this study, we propose a new mathematical model of pneumococcal colonization and meningitis infection to explore the hypotheses that community respiratory virus infections influence the epidemiology of pneumococcal meningitis by increasing strain transmissibility or pathogenicity. To assess these hypotheses, we first analysed chronological association in French epidemiological data in the pre-vaccine era, and we then fitted distinct mechanistic submodels to observational time series.

2. Methods

(a) Data
We used three distinct datasets over the 2001–2004 period in France: the SPMI, the incidence of acute viral respiratory infection (AVRI) and antibiotic-exposure rates (ATB; figure 1). Those data were provided by three different surveillance systems.

(i) Pneumococcal meningitis
Weekly SPMI came from surveillance within the French National Centre of Reference for Pneumococci (CNRP) [22], which systematically collects data on pneumococcal meningitis in collaboration with 22 regional observatories for pneumococci, covering around 60 per cent of French cases [24]. *Streptococcus pneumoniae* isolates with minimal inhibitory concentrations (MICs) more than $0.06 \, \mu\text{g ml}^{-1}$ were considered to be penicillin-non-susceptible

strains. In this study, both terms 'non-susceptible' or 'resistant' were used to design equally these strains. In France, during the 2001–2004 period, 85–95 per cent of the penicillin-non-susceptible strains responsible for diseases were also macrolide-resistant [25].

(ii) Winter viruses
Weekly AVRI incidence rate was acquired from the Euroflu Network (http://www.euroflu.org/cgi-files/graphs_public.cgi). French data were collected through the Groupe Regional d'Observation de la Grippe (GROG), a network of more than 500 general practitioners reporting for AVRI all over the country (see http://grog.org/presentation.html for more information on the GROG network). Data were extracted from the web page using GRAPHCLICK® (http://www.arizona-software.ch/graph-click). The Euroflu AVRI data are available only during winter season for epidemiological weeks 1–15 and 40–52. Additionally, no data were available at the country level for the 2000–2001 winter season. In total, 97 weeks of observation were available over the 2001–2004 period.

(iii) Antibiotics
In France, the National Health Insurance refunds medical care provided by physicians in private practice, community clinics and hospitals to everyone. The antibiotic data used consisted of all ambulatory β-lactam prescribed, dispensed by outpatient pharmacies and reimbursed by two main French National Health Insurance agencies (CNAM-TS and RSI, covering 85% of the French population) from 2001 to 2004 [23]. Because antibiotics are prescription drugs in France, these data are nearly exhaustive. In the following, β-lactam, penicillin and antibiotics were used equally to design this dataset.

(iv) Population
Population demographic data for the different years came from the French National Institute of Statistics and Economical Studies (http://www.insee.fr/en/default.asp).

(b) Statistical analysis of the data
Weekly SPMIs were modelled using a generalized estimating equations (GEE) approach, with a Poisson distribution [26]. This model enables specification of overdispersion, and a first-order autoregressive structure that accounts for the autocorrelation of the weekly SPMI within each year and assumes the independence of the years. Data were subjected to univariate and multivariate analyses. For all weekly time lags k in [−10,10], regressions were defined by:

$$\ln(\text{E}[M(w)]) = \log(\text{pop}) + \text{season}(w) + r \times RX(w - k),$$

where $M(w)$ represents the SPMI at week w; 'pop' is the population estimate for the considered year; 'season' is a periodic trigonometric function and $RX(w - k)$ represents antibiotic use or AVRI, at week $(w - k)$, after removing seasonality using a trigonometric function of period 52 weeks:

$$X(w) = a + b \times \sin\left(2\pi\frac{w}{52}\right) + c \times \cos\left(2\pi\frac{w}{52}\right) + RX(w)$$

for week w, where $X(w)$ represents the antibiotic use (ATB) or AVRI time series, $RX(w)$ their weekly respective residuals and a, b, c the regression coefficients.

To determine which time-delay range has the strongest association, separate lagged Poisson regressions between $E[M(w)]$ and $RATB(w - k)$ or $RAVRI(w - k)$ were estimated for different k-values, $-10 \leq k \leq 10$, and t-test for the regression coefficients was performed [27].

Multivariate Poisson regressions were also performed and compared using the quasi-likelihood information criterion (QIC) [28].
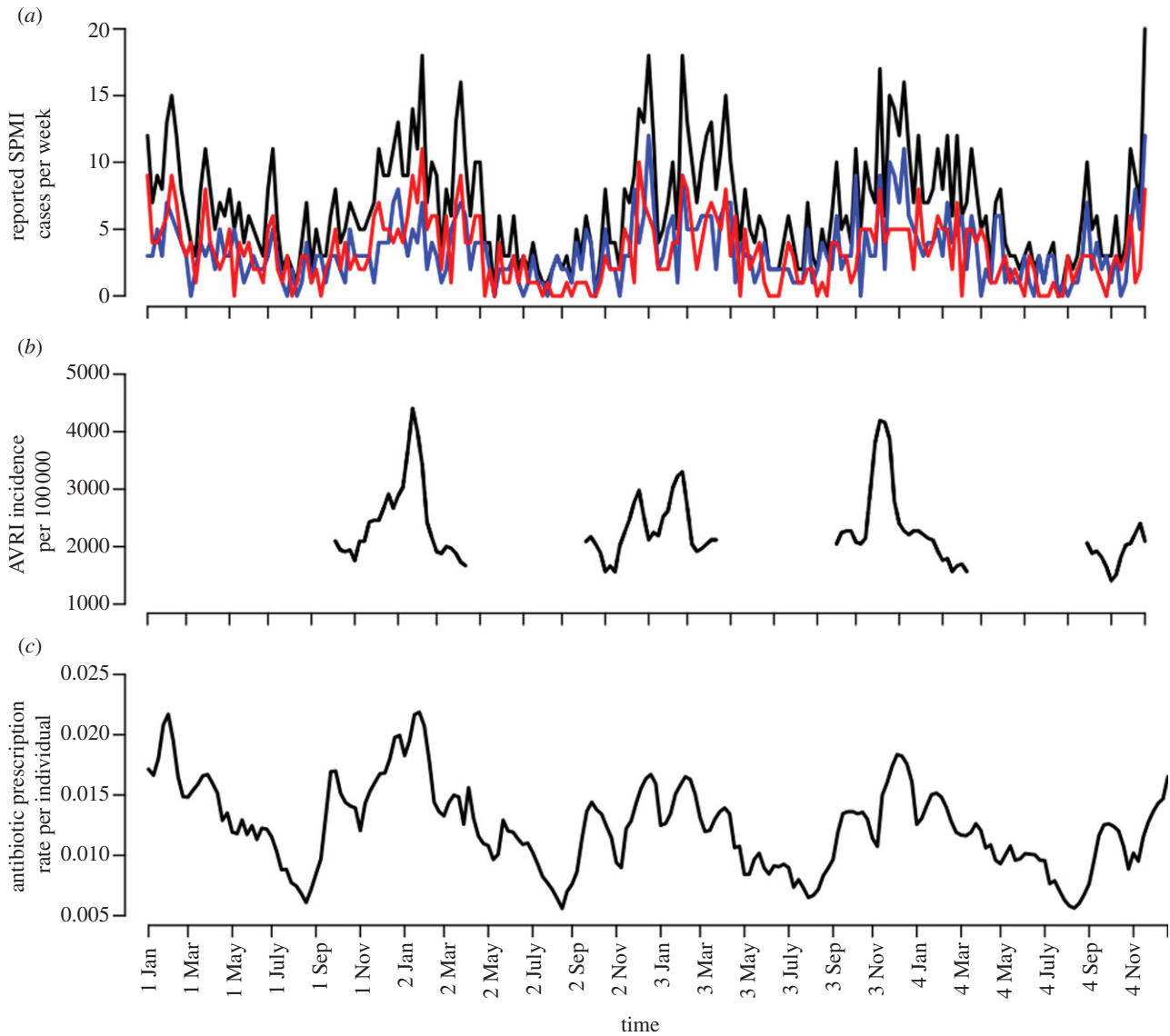
Figure 1. Observed *S. pneumoniae* meningitis reported cases (SPMI), acute viral respiratory infections (AVRI) and antibiotic use over seasons 2001–2004. (*a*) Weekly total (black curve), antibiotic-susceptible (blue) and -resistant SPMI (red), from surveillance within the CNRP [22]. Isolates with MICs more than 0.06 μg ml^{-1} were considered to be penicillin-non-susceptible, designated herein by 'non-susceptible' or 'resistant'. (*b*) Weekly AVRI incidence rate during winter seasons, acquired from Euroflu Network (collected only from October to March, epidemiological weeks 1–15 and 40–52 of each year). (*c*) Weekly antibiotic prescription rate per individual. Antibiotic use was based on β-lactam reimbursement data from the French National Health Insurance [23]. The datasets covered 208 weeks for SPMI and antibiotic use, and 97 weeks of AVRI observation. Weeks ending in a new month are indicated on the *x*-axis.

The GEE model was successively tested for total, susceptible and resistant SPMI, using ATB and AVRI as covariates.

The regressions were computed using the GENMOD procedure in SAS.

(c) Mathematical modelling

We developed a new mathematical model of pneumococcal colonization and meningitis infection, and used it to identify the mechanisms responsible for the chronological associations among AVRI, antibiotic use and SPMI. A series of dynamic submodels in which pneumococcal transmissibility and pathogenicity could change as a function of earlier AVRI were formulated. The models were then fitted to SPMI data to assess their explanatory power.

We built a deterministic susceptible–colonized–susceptible model of *S. pneumoniae* transmission in the French community under β-lactam exposure (outlined in figure 2), using French-context parameters (table 1). We used demographic data from the French National Institute of Statistics and Economical Studies (http://www.insee.fr/en/default.asp) for the birth and mortality rates.

Model compartments were structured with respect to colonization status and antibiotic exposure (figure 2*a*). Uncolonized individuals (NC) could become colonized at rate β through contacts with colonized individuals. Two stages of *S. pneumoniae* penicillin susceptibility, corresponding to different levels of MIC, were modelled: susceptible (S) for strains with MIC < 0.06 μg ml^{-1}; and intermediate or resistant (R) for strains with MIC ≥ 0.06 μg ml^{-1}. Emergence of resistance or increase of its level was not considered in the model. Independently of their carriage status, individuals could be unexposed (U_{NC}, U_S, U_R) or exposed (P_{NC}, P_S, P_R) to antibiotics.

Because antibiotic exposure varies over the year, we used the observed time series ATB(w) to parametrize the rates of exposure each week w of the period considered: $\alpha_P(w) =$ ATB(w). In the absence of antibiotic use, natural immunity was defined as inducing carriage clearance in colonized individuals with an average of three weeks in the population [29–32]. The impact of antibiotic exposure, characterized by σ, depended on the colonizing strain's levels of resistance: antibiotics cleared susceptible bacteria in one week on average ($\sigma_S = 0$), but had no effect on colonization with resistant bacteria ($\sigma_R = 1$).
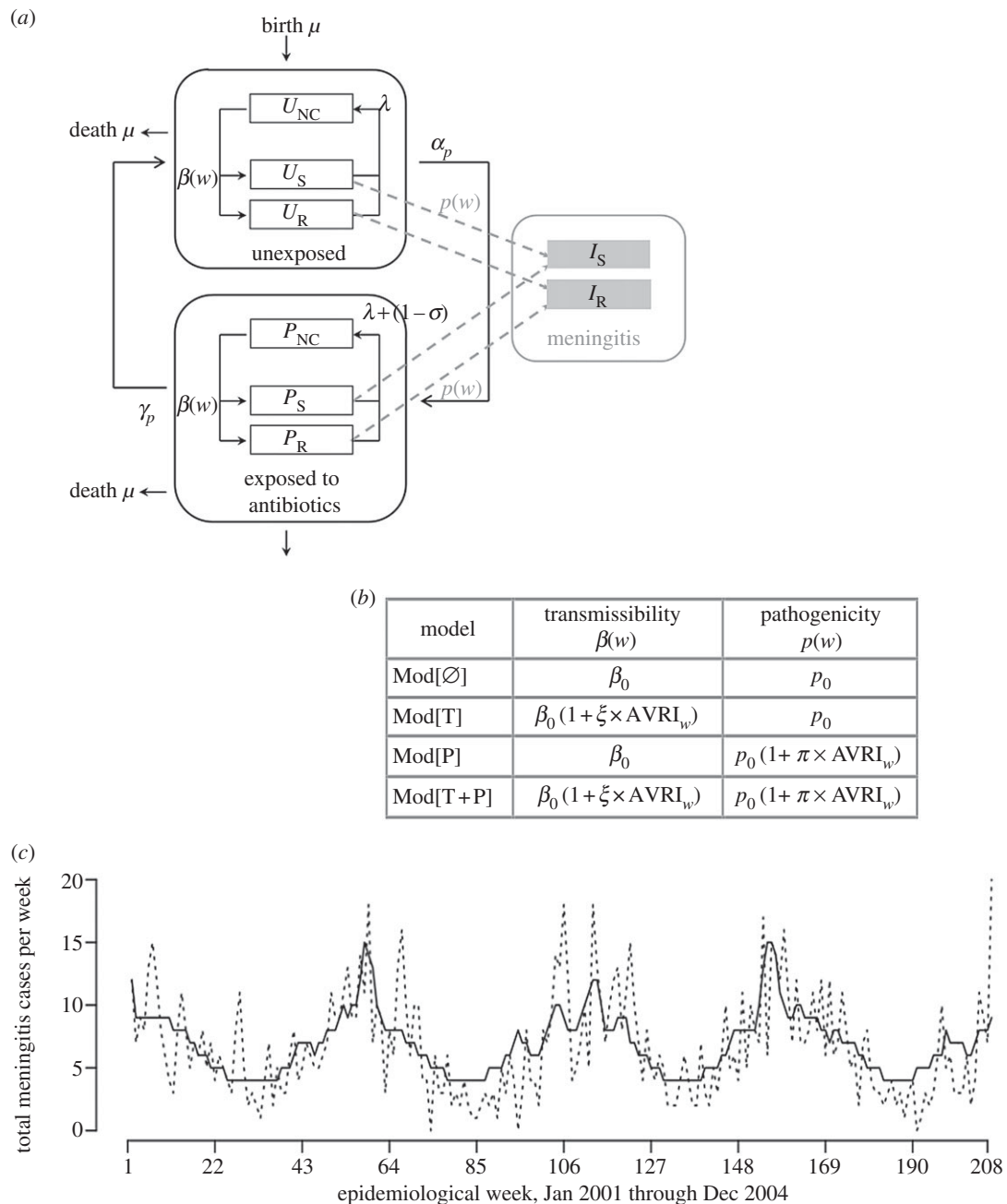
Figure 2. Outline and description of the four tested models. (*a*) Pneumococcal transmission model. The population was divided into several compartments, according to *S. pneumoniae* colonization status and antibiotic exposure, with *U*, *P* and *I*, respectively, representing unexposed, penicillin-exposed and meningitis-infected compartments; NC, S and R subscripts designating, respectively, non-colonized, colonized with susceptible and resistant strains. Uncolonized individuals (U_{NC}, P_{NC}) could become colonized at rate $\beta(w)$ per contact with colonized individuals (U_S, U_R, P_S, P_R) independently of antibiotic exposure or antibiotic resistance of colonizing strains. $\beta(w)$ is defined as a time-varying function depending on β_0, the natural pneumococcal transmission rate and AVRI incidence over time. Independently of their carriage status, individuals could be exposed during an average time $1/\gamma_P$ to antibiotics at frequency α_P, for which the population weekly antibiotic exposure rate was used (for all weeks w, $\alpha_P(w) = \text{ATB}(w)$). Decolonization could result either from natural immunity (after average time $1/\lambda$) or antibiotic consumption (with $\sigma = (\sigma_S, \sigma_R)$ being the probability of decolonization depending on colonizing strain susceptibility). Finally, meningitis infection (I_S, I_R) could occur in carriers at rate $p(w)$, regardless of the level of susceptibility to penicillin. (*b*) Description of the four distinct models of SPMI−AVRI interactions. Because the link between AVRI and SPMI fluctuations has been ascribed to different factors, AVRI effects were modelled within transmission (T, i.e. pneumococcal transmissibility or acquisition) and pathogenicity (P) parameters. (*c*) Observed (dashed line) global SPMI (January 2001−December 2004) versus the simulated one (solid line), using the median set of parameters obtained with the best-fitting model Mod[T+P]. Note that parameters were estimated using SPMI data only from weeks when AVRI were available. The simulation for the whole period is presented here, using an extrapolated AVRI incidence rate when not available.

Antibiotic exposure duration was assumed to have a mean of $1/\gamma_P = 8$ days.

The duration of colonization by susceptible strains was fixed ($1/\lambda_S = 3$ weeks on average). Possible epidemic fitness differences between penicillin-susceptible and -resistant pneumococcal strains were included in the model by introducing different clearance rates for each strain. Resistance-associated fitness cost was defined as the mean ratio of carriage durations between resistant and susceptible strains: $f_{R/S} = \lambda_S/\lambda_R$. Other parameters being equal, this quantity is equivalent to the ratio of resistant to susceptible pneumococci reproductive numbers. $f_{R/S}$ was numerically estimated to maximize the log-likelihood of the model to reproduce observed susceptible and resistant SPMI data.

Meningitis infection (I) could occur in all pneumococcal carriers. We assumed that the natural probability p_0 of developing meningitis was constant throughout carriage duration and was independent of

Table 1. Model parameters.

parameter	notation	value
birth rate, death rate	μ	0.00025 individual^{-1} week^{-1}
infectious contact rate:		
intrinsic pneumococcal transmission	β_0	estimated
impact of AVRI on transmission	ξ	estimated
time-varying transmission	$\beta(w) = f(\beta_0, w)$	
colonization duration:		
for susceptible strains	$1/\lambda_S$	three weeks
for resistant strains	$1/\lambda_R$	estimated
antibiotic exposure duration	$1/\gamma_P$	8 days
decolonization after one week of antibiotic use:		
probability for susceptible strains	$1 - \sigma_S$	1
probability for resistant strains	$1 - \sigma_R$	0
antibiotic exposure rate	$\alpha_P(w)$	ATB(w)
pathogenicity in colonized individuals:		
natural probability of meningitis	p_0	estimated
impact of AVRI on pathogenicity	π	estimated
time-varying pathogenicity	$p(w) = g(p_0, w)$	
fitness cost for resistant strains	$f_{R/S}$	estimated

antibiotic susceptibility. In France, vaccination with a pneumococcal conjugate vaccine (PCV) was introduced for children in 2003. Because vaccination recommendations were restrictive before 2005 [33], immunization was considered to be marginal during the period of study, and PCV was not considered in the model.

Four interaction submodels were derived, assuming different mechanisms of virus-induced modified transmissibility and pathogenicity (figure 2):

— MOD[Ø], in which AVRI has no impact on SPMI
— MOD[T], for which we assumed that, during AVRI epidemic periods, transmission of pneumococcal strains to non-carriers could be promoted when either these non-carriers or the pneumococcal carriers were infected with the virus. The pneumococcal transmission rate at week w was modelled as

$$\beta(w) = \beta_0 \times (1 + \xi \times \text{AVRI}(w)),$$

where β_0 is the intrinsic pneumococcal transmission rate, AVRI(w) is the virus-infection incidence in the population at week w and ξ is a factor representing the impact of a respiratory virus infection on pneumococcal strains transmission, reflecting either a modification of the chance of transmission, or of the rate of acquisition.

— MOD[P], for which we assumed that the probability of colonized individuals developing meningitis at week w, $p(w)$ (=P[meningitis|colonized]) could be increased in people with AVRI:

$$p(w) = p_0 \times (1 + \pi \times \text{AVRI}(w)),$$

where π is a factor representing the impact of a respiratory virus infection on the pathogenicity of pneumococcal strains and p_0 the natural pneumococcal pathogenicity.
— MOD[T + P], which combined the assumed impacts on transmissibility and pathogenicity of models MOD[T] and MOD[P].

The reported time series of AVRI from the GROG were directly used in the model to parameterize the rate of AVRI per individual each week. Reporting fidelity to AVRI was assumed to be equal to 100 per cent.

(d) Statistical inference and model fitting

Model compartments were initialized to describe a population in which penicillin-resistant pneumococcal rates were equivalent to the French 2001 data (CNRP) with more than 50 per cent of penicillin-non-susceptible strains [25]. Global pneumococcal carriage was set to 10 per cent on average, with a rate of non-susceptible strains initialized to 55 per cent. The meningitis infection compartments (I) were initialized by directly using meningitis observed incidence at the beginning of the study period.

The model was numerically simulated over the 2001–2004 period. To study the role of AVRI, the parameters of the four interaction models (MOD[Ø], MOD[T], MOD[P] and MOD[T + P]) were estimated to obtain the best-fitting model with the observed susceptible and resistant SPMI data, using a Markov chain Monte Carlo (MCMC) algorithm. Only the 97 weeks during which AVRI incidence was available were used for the calculation of the likelihood. The Metropolis–Hasting algorithm was used to sample over the parameter space. For each model, parameters were selected to maximize the Poisson log-likelihood for the susceptible and resistant meningitis time series (with expression detailed in electronic supplementary material, §S3). Posterior estimates were obtained using 3×10^6 MCMC iterations with sampling every 1000 and a burn-in of 100 000 iterations to allow for the chain convergence. Uninformative priors were used, and several starting points were tested. The best-fitting model was determined using the deviance information criterion (DIC; table 2). This criterion has the advantage of taking into account the model complexity associated with the number of parameters. The selected model is the one that presents the lowest DIC.

The model and estimation framework were programmed in C. The R statistical software (www.r-project.org) was used for the statistical analysis of the model outputs and graphics.

Table 2. Posterior median estimates (95% credibility interval) for the four fitted models. Four distinct models of SPMI−AVRI interactions were tested: Mod[∅], AVRI had no influence whatsoever on SPMI; Mod[T], AVRI affected only pneumococcal transmission, whereas pathogenicity remained constant; Mod[P], AVRI influenced only pneumococcal pathogenicity; and Mod[T+P], AVRI could affect both pneumococcal pathogenicity and transmission (transmissibility or acquisition). Here, β_0 defines natural pneumococcal transmission rate, ξ defines virus impact on pneumococcal transmission, p_0 defines the natural pathogenicity rate, π defines the natural pathogenicity rate, π defines virus impact on pathogenicity and $f_{R/S}$ defines resistance-associated fitness cost, which was defined as the mean ratio of carriage duration between susceptible and resistant strains $f_{R/S} = \lambda_S/\lambda_R$. In the best-fitting model (highlighted in grey), resulting *S. pneumoniae* transmission $\beta(w)$ and pathogenicity $p(w)$ ranged, respectively, from 0.36 to 0.47 per week per person and from 6×10^{-7} to 1.8×10^{-6}.

model	β_0	ξ	p_0	π	$f_{R/S}$	LL	DIC[a]
Mod[∅]	0.38 (0.37–0.39)	—	1.37×10^{-6} (1.06–1.72×10^{-6})	—	0.97 (0.969–0.975)	455 (452–456)	−906
Mod[T]	0.05 (0.02–0.20)	654 (109–1831)	2.67×10^{-7} (2.27–3.37×10^{-7})	—	0.97 (0.968–0.974)	475 (472–477)	−947
Mod[P]	0.38 (0.37–0.40)	—	2.2×10^{-7} (1.06–5.53×10^{-7})	208 (65–495)	0.97 (0.969–0.975)	476 (472–477)	−948
Mod[T+P]	0.34 (0.31–0.36)	8.7 (4.6–14.4)	3.6×10^{-7} (1.3–7.0×10^{-7})	92 (28–361)	0.97 (0.969–0.975)	485 (480–487)	−964

[a]We used the following expression of the deviance information criterion: DIC $= -4 \times$ LL $+ 2 \times$ max(LL), where LL is the mean of the log-likelihood and max(LL) the mode value for the MCMC sample. Model minimizing the DIC was preferred.

The equations of the model are provided in the electronic supplementary material, §S2.

3. Results

(a) French data and statistical analysis

The data from January 2001 to December 2004, corresponding to a total of 208 weeks for SPMI and β-lactam use, and 97 weeks of AVRI observation, are given in figure 1. A total of 1383 SPMI episodes were reported. All three time series (SPMI, AVRI and ATB) present a 52-week seasonality with epidemics peaking during the winter.

Figure 3 shows plotted *t*-ratios between SPMI and residual ATB or residual AVRI, computed for all time lags *k* between −10 and +10 weeks. Detailed estimates are provided in the electronic supplementary material, §S1.

As shown in figure 3, the *t*-ratios plotted versus the time lag between AVRI or antibiotic and SPMI were highly asymmetric (none of the *t*-ratios for the forward lags was significant), suggesting that the SPMI during a given week is linked to AVRI incidence or antibiotic consumption over the previous weeks, but not to those of subsequent weeks. This asymmetry reinforces the plausibility of direct links among AVRI and antibiotic consumption and SPMI. By contrast, a symmetrical plot would have indicated that common confounders acted simultaneously on the different factors.

(i) Acute viral respiratory infection versus *Streptococcus pneumoniae* meningitis incidence

We found that, regardless of pneumococcal strain susceptibility, SPMI followed AVRI incidence by up to two weeks, indicative of positive short-term dependency ($p < 0.01$). Negative long-term dependency ($w-7$) was observed for total and susceptible meningitides, but associations were only marginally significant.

(ii) Antibiotic versus *Streptococcus pneumoniae* meningitis incidence

Total and antibiotic-resistant SPMIs were also positively linked with antibiotic consumption over the two preceding weeks. By contrast, total and antibiotic-susceptible SPMI were negatively significantly linked to penicillin consumption over the preceding five to nine weeks ($p < 0.02$), indicating long-term dependency.

(iii) Multivariate analysis

In countries with high antibiotic exposure, such as France, antibiotic use is strongly associated with AVRI dynamics, meaning that the short-term SPMI−antibiotic association could be an artefact of the data, a hypothesis consistent with our multivariate analysis results. Including residual RAVRI($w-2$) or RATB($w-2$) in the multivariate analyses of total, susceptible and resistant meningitides gave similar QIC, indicating that AVRI could act as a confounder in the short-term SPMI−antibiotic association (see the electronic supplementary material, §S1).

(b) Mathematical modelling and mechanisms testing

For each of the four tested models, posterior estimates of the parameters are given in table 2. Among the four possibilities,
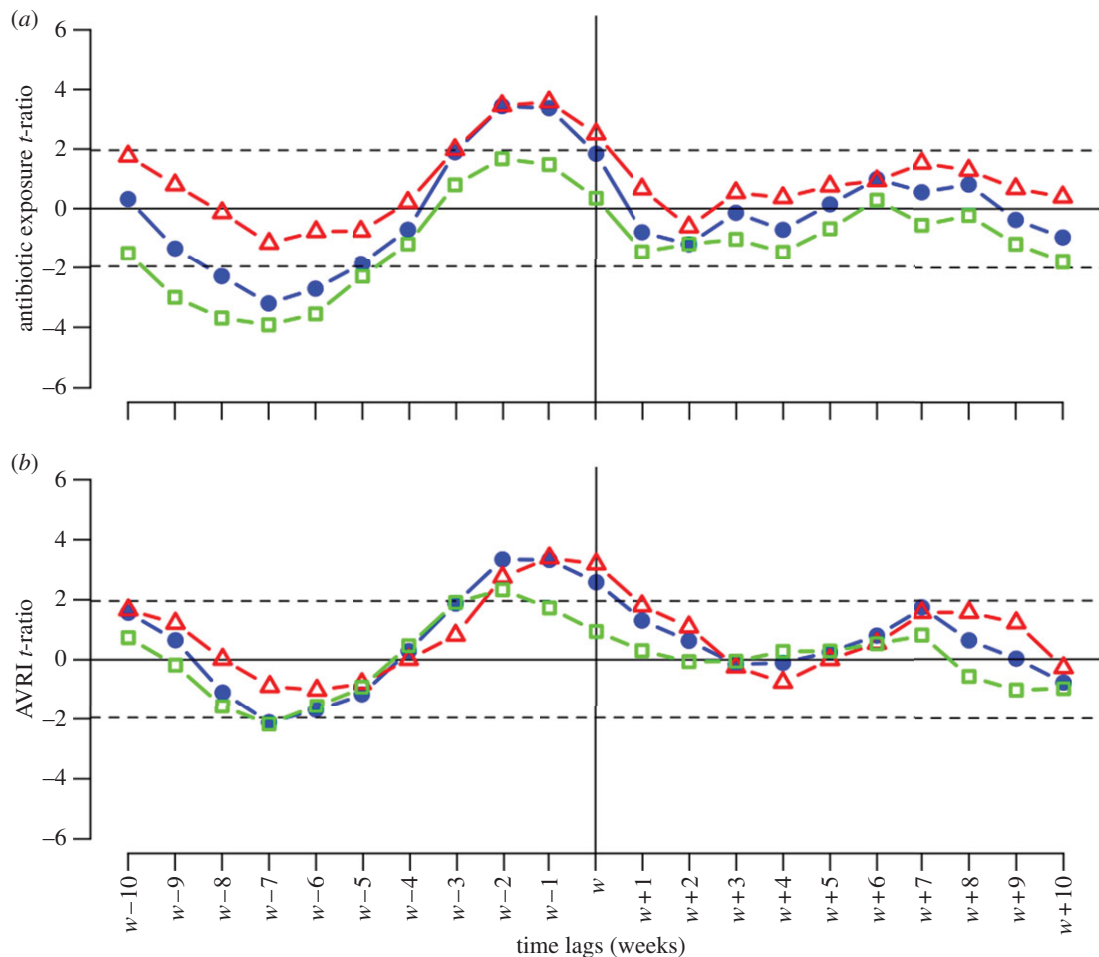
Figure 3. Statistical association between SPMI and AVRI or antibiotic exposure. Antibiotic and AVRI time series were detrended, and seasonality removed to create 'residual time series'. Lagged regression coefficient *t*-ratios between numbers of meningitis cases and residual antibiotic or residual AVRI are plotted for all lags *k* between weeks -10 and $+10$. (*a*) SPMI association with antibiotics. Total (blue circles) and resistant meningitides (red triangles) are positively significantly linked to antibiotic use at weeks -1 and -2. Long-term relationships were observed between total and susceptible meningitides (green squares) and antibiotic use, with a negative significant link for weeks -5 to -8, with a peak at -7. (*b*) SPMI association with AVRI. Regardless of strain susceptibility, increased SPMI was significantly associated with high AVRI incidence during the same and the preceding 2 weeks, indicating positive short-term dependency. Negative long-term dependency (week -7) was observed for total and susceptible meningitides, but associations were only marginally significant. Like the antibiotics analysis, resulting *t*-ratios were markedly asymmetric.

we found that the best fit was obtained when both transmissibility and risk of infection were allowed to depend on observed AVRI (table 2).

For the selected model, the posterior median estimates were $\xi = 8.7$ (95% credibility interval (4.6–14.4)) for virus impact on pneumococcal transmission/acquisition; $\pi = 92$ (28–361) for virus impact on pathogenicity; and $f_{R/S} = 0.97$ (0.969–0.975) for the antibiotic-resistance-associated fitness cost estimate. In a typical simulation using the median posterior estimates, pneumococcal transmissibility ranged from 0.36 to 0.47 per week per person (reaching a 1.3-fold increase) and pneumococcal pathogenicity ranged from 6×10^{-7} to 1.8×10^{-6} (a threefold increase). Although low, the resistance-associated fitness cost was significantly lower than 1, suggesting that resistant strains are slightly less competitive than susceptible ones.

Lastly, one can directly interpret the posterior estimates of ξ and π at the individual level (see the electronic supplementary material, §S2 for details on the mathematical development and interpretation). From that point of view, the model predicts that AVRI substantially increases a colonized patient's absolute risk of meningitis (by a factor of 90) and also increases the individual's risk of acquisition/transmissibility of pneumococci (by a factor of 9).

The 2001–2004 SPMI simulated using the selected model and the median estimated parameters are depicted in figure 2*c* (see also the electronic supplementary material, figure S2), along with the observed SPMI over the same period.

4. Discussion

Pneumococcal meningitis incidence (SPMI) was found to be highly associated with the incidence of AVRIs and with antibiotic use: SPMI increased shortly after AVRI incidence (the two following weeks) and decreased overall as the antibiotic-prescription rate rose (six to seven weeks after). In addition, our modelling study discarded the hypothesis of a unique interaction between AVRI and pneumococci. Model simulations required a combined AVRI impact on both pneumococcal transmissibility and pathogenicity to reproduce the data accurately, along with diminished epidemic fitness of resistant pneumococcal strains causing meningitis.

Vaccination with PCV has been shown to widely modify pneumococcal epidemiology [7,9]. In France, PCV vaccination was initiated in 2003, targeting a specific population (at-risk children less than 2 years old), with recommendations extended

in 2006 to every child less than 2 years old. In the same period, a campaign to decrease antibiotic consumption was launched [23]. In this study, we focused on years 2001–2004, in order to obtain stable meningitis ecology, with a limited effect, if any, of vaccination.

For clarity purposes, the model developed here used several simplifications regarding *S. pneumoniae* epidemiology. First, the model was not age-stratified. Pneumococcal colonization is known to decrease with age [34], and children have generally higher rates of pneumococcal meningitis and antibiotic consumption. Although this simplification should not affect our findings on the association between AVRI and SPMI, extending the model and examining the effect of age on such an association will be of interest in the future. Second, the model included only penicillin resistance, neglecting other drug resistances (and multiresistances). In France, most penicillin-resistant pneumococcal strains also carry a macrolide resistance gene. The inclusion in the model of the 2001–2004 macrolide exposure rates, in addition to the β-lactams ones, would increase the co-selection of resistant strains under antibiotic exposure [35]. Therefore, it is predictable that the estimated epidemic fitness difference between susceptible and resistant *S. pneumoniae* would be increased to compensate for this selective advantage.

The model and data used here did not allow estimating any reporting fidelity in AVRI. The AVRI reporting rate was therefore assumed to be equal to 100 per cent in the model. In practice, reporting fidelity in AVRI is usually imperfect. This simplification should not impact the significance of our conclusions regarding the role of AVRI on pneumococcus epidemiology. It would, however, affect the amplitude of this effect: a lower reporting rate would reduce the quantitative estimation of ξ and π by the same rate. The presented estimates can therefore be interpreted as a maximization of AVRI's impact on pneumococcal pathogenicity and transmissibility.

Some data limitations should also be mentioned. Two independent surveillance systems report viral respiratory infections in France: the Sentinelle network, which reports influenza-like syndrome incidence; and the GROG surveillance programme, which combines virological testing and sentinel indicators to report AVRI (including influenza-like illness and respiratory syncytial virus, RSV) [36]. In order to include AVRI with the largest definition, data from the GROG network were used here. As mentioned earlier, during the study period, the GROG network reported only viral infections occurring during winter seasons. As a consequence, our statistical and inference analyses were restricted to these periods, excluding summertime incidences and limiting the power of our analysis. Furthermore, other respiratory viral pathogens, not reported by the GROG, together with other environmental factors, such as air pollution, daily light or temperature, were not included in this study. Despite these limitations, the short-term associations found between AVRI and SPMI are in accordance with previous findings [10,17,37].

High incidences of bacterial respiratory infections during influenza pandemics have been reported in the past [19,38], and an increased incidence of these infections (e.g. pneumonia) in potentially coming pandemics is of concern [39]. The temporal relationship among circulating winter virus infections (influenza virus, RSV) and invasive pneumococcal disease has also been reported in several studies [10,17–20,37]. Herein, we highlight two distinct mechanisms underlying such associations and suggest that the existence of a unique interaction mechanism is very unlikely.

AVRIs are known to increase the risk of invasive bacterial infection [16]. Several biological mechanisms have been suggested to explain such phenomena. First, the idea that daily light and temperature exposure modifications altered host immune responses was advanced to explain the seasonal variations of infection incidences [40]. This hypothesis does not agree with our statistical results. Such a mechanism would tend to influence AVRI and SPMI simultaneously. Herein, the time-series relationships were strongly asymmetric. Second, virus infection might facilitate pneumococcal infection by damaging the airway mucosa and epithelial lining [10,41,42], increasing pneumococcal adherence to epithelia [41,43] and/ or impairing innate immune responses to pneumococcal infections [42,44–46].

However, our results suggest that AVRI-induced increased pathogenicity of pneumococci is not enough to explain the observed trends of pneumococcal meningitis. The AVRI should also modify between-host interactions by emphasizing *S. pneumoniae*'s transmissibility. Such an increased epidemic potential could actually reflect two distinct mechanisms. First, AVRI infection could enhance the transmission probability of the bacteria in colonized individuals (and therefore increase pneumococcus's reproductive number) by increasing bacterial load in the host or creating new transmission routes. Second, virus infection could increase host susceptibility to pneumococcal colonization (e.g. enhance the acquisition risk) in AVRI-infected individuals. Our model did not allow discriminating between the two. A more complex model, in which the dynamics of virus infection in the population is explicitly modelled, will be necessary in the future to explore in details this enhancing effect on pneumococcal transmissibility. To date, this phenomenon of 'cloud humans', in which virus-infected individuals transmit more bacteria, has been little studied in human populations [47,48].

Based on the influenza-like illness incidence reported by the Sentinelles network, it is probable that influenza viruses are responsible for the second winter peak shape of AVRI. Here, the lack of virological information impeded the separate quantification of the role of the distinct viruses on pneumococcal epidemiology. However, assessing the role of the viruses individually will be of great interest in the future.

A distinct but related aspect is that of the significant resistance-associated fitness cost. In this study, antibiotic-driven selection on its own was not sufficient to reproduce the observed trends, and significantly lower fitness for resistant pneumococci was found (estimated resistance-associated fitness cost of 0.97 (0.969–0.975)). Reduced transmissibility of antibiotic-resistant pneumococcal strains has already been suggested [49,50], but biological evidence in humans is still lacking. Although resistance-associated fitness cost was a secondary consideration here, this parameter is of importance in measuring the selective power associated with antibiotic exposure pressure. Our finding provides some insights into the influence of antibiotic exposure on the selection of circulating pneumococcal strains: susceptible strains would be disadvantaged under antibiotic exposure but become more epidemic than resistant strains when antibiotic use declines. A possible consequence of fewer antibiotic prescriptions might be an increased incidence of severe pneumococcal infections caused by antibiotic-susceptible strains.

The results presented here bring to light, at the epidemiologic level, the potential means for respiratory viruses and *S. pneumoniae* interactions. They suggest that respiratory viruses

actually increase the incidence of invasive pneumococcal infections by both enhancing *S. pneumoniae* dissemination in human populations and increasing the risk of invasive infections in colonized individuals. This finding could be of importance when considering priorities for anti-bacterial and anti-viral vaccine policies, as it may lead to an indirect public health impact of anti-viral vaccines conferring immunity against influenza (and in the near future RSV) on the spread of *S. pneumoniae* strains and on meningitis risk [51]. This impact will probably have to be considered when elaborating vaccine combination strategies aimed at further decreasing SPMI.

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