Time and dose-dependent risk of pneumococcal pneumonia following influenza: a model for within-host interaction between influenza and *Streptococcus pneumoniae*

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A significant fraction of seasonal and in particular pandemic influenza deaths are attributed to secondary bacterial infections. In animal models, influenza virus predisposes hosts to severe infection with both *Streptococcus pneumoniae* and *Staphylococcus aureus*. Despite its importance, the mechanistic nature of the interaction between influenza and pneumococci, its dependence on the timing and sequence of infections as well as the clinical and epidemiological consequences remain unclear. We explore an immune-mediated model of the viral–bacterial interaction that quantifies the timing and the intensity of the interaction. Taking advantage of the wealth of knowledge gained from animal models, and the quantitative understanding of the kinetics of pathogen-specific immunological dynamics, we formulate a mathematical model for immune-mediated interaction between influenza virus and *S. pneumoniae* in the lungs. We use the model to examine the pathogenic effect of inoculum size and timing of pneumococcal invasion relative to influenza infection, as well as the efficacy of antivirals in preventing severe pneumococcal disease. We find that our model is able to capture the key features of the interaction observed in animal experiments. The model predicts that introduction of pneumococcal bacteria during a 4–6 day window following influenza infection results in invasive pneumonia at significantly lower inoculum size than in hosts not infected with influenza. Furthermore, we find that antiviral treatment administered later than 4 days after influenza infection was not able to prevent invasive pneumococcal disease. This work provides a quantitative framework to study interactions between influenza and pneumococci and has the potential to accurately quantify the interactions. Such quantitative understanding can form a basis for effective clinical care, public health policies and pandemic preparedness.

1. Introduction

In a typical year approximately 250 000–500 000 people die of influenza worldwide [1], with significantly more deaths occurring during pandemic years [2]. However, a large fraction of influenza deaths is attributable to bacterial infections, either simultaneously with or closely following influenza [3,4]. A common bacterial infection secondary to influenza is pneumonia, caused primarily by

Streptococcus pneumoniae and *Staphylococcus aureus*. Similar to influenza, these bacterial infections are seasonal and peak during the winter months in temperate countries [5,6]. Bacterial pneumonia causes significant morbidity and mortality, especially among very young children and the elderly [7]. Identifying the mechanisms underlying the association between influenza and the severity of pneumococcal infections, and determining their clinical and epidemiological consequences are a research priority because of their potentially important implications for prevention and control, and pandemic influenza preparedness.

Although an association between influenza and bacterial pneumonia has been observed—especially during pandemics—definitive evidence for an interaction has come from animal models. A relationship between influenza infection and subsequent bacterial colonization was noted in animal experiments as early as the 1940s [8]. Subsequent studies found that influenza infection predisposes an animal to a more severe pneumococcal infection [9]. The viral–bacterial interaction is not limited to influenza and *S. pneumoniae*: similar interactions have been noted between human metapneumovirus and *S. pneumoniae* [10] and between influenza and *S. aureus* [11–13].

Several mechanisms have been proposed to explain this phenomenon [9,14]. These include a range of viral mechanisms that facilitate bacterial adherence or proliferation, such as epithelial damage [15,16], the expression of viral neuraminidase [17,18], exposure of platelet-activating factor receptor [19–21] or a reduction in the efficiency of innate immunity, particularly alveolar macrophages, through the invoked expression of different cytokines (e.g. interferon γ (IFN-γ) [11,22], interleukin 10 (IL-10) [23], type I IFN [24,25]). Although not as numerous, mechanisms have been proposed to elucidate how bacterial infection might facilitate subsequent viral infection, including viral usage of bacterially derived protease for infectivity [26,27].

Focusing on the kinetics of within-host infection dynamics, together with a putative immune-mediated interaction mechanism, we integrate previously proposed models for influenza and *S. pneumoniae* to create a virus–bacterium model. Both the influenza [28] and pneumococcal pneumonia [29] models were developed as stand-alone models, parametrized with data from experimental challenge with the focal pathogen. We then coupled these models on the basis of one of the proposed mechanisms of viral–bacterial interaction, whereby cytokines expressed as a response to viral infection interfere with the functioning of macrophage-based response to subsequent bacterial exposure [22,25]. The complete model provides quantitative predictions of dual infections, which enables us to study the consequences of important features of the interactions, such as timing of the infection, dose–response and the antiviral treatment.

We find that predictions born out of the model successfully capture several key features of sequential infections observed in animal experiments. In particular, the model predicts that exposure to pneumococcal bacteria approximately 4–6 days following influenza infection can significantly enhance the risk of invasive pneumonia. Additionally, we find that antiviral treatment is expected to be efficacious only when administered during the early phase of influenza infection. We discuss how these results compare with experimental data, the implications of these results for clinical care, and expected differences in the association between the two during pandemic and non-pandemic influenza outbreaks.
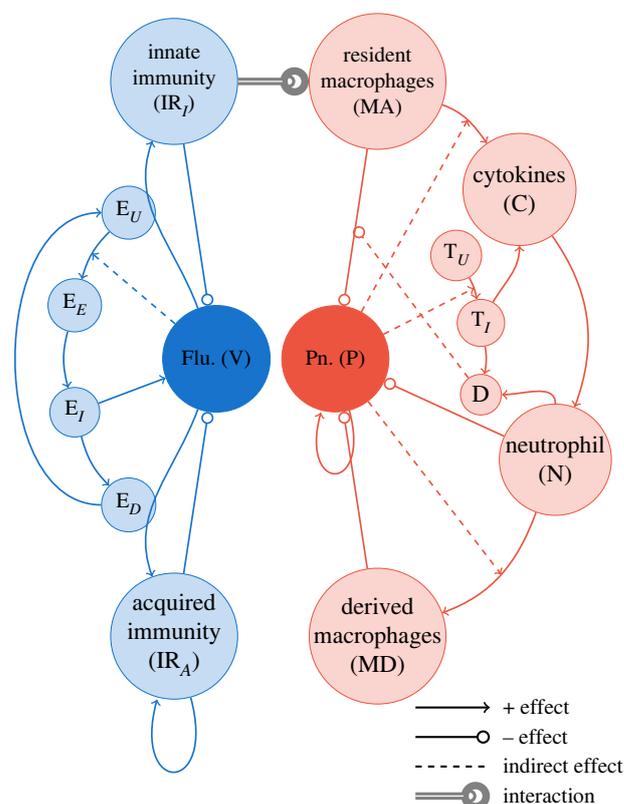
Figure 1. Schematic diagram of the within-host kinetics and the immune-mediated interaction. Schematic diagrams for within models for influenza infection (in blue) [28] and pneumococcal pneumonia infection (in red) [29]. The virus (V) targets epithelial cells (E_U), which become infected (E_I) after some latency (E_E). The infected cells produce more virus, before they die (E_D), and are replaced by new uninfected cells. Both the innate and the acquired immune responses are stimulated by the virus, which in turn inhibits the viral growth. The pneumococcal bateria (P) attach to cells (converting them from unattached T_U to attached T_I) which eventually die and form debris (D). The three parts of the innate immune response, resident alveolar macrophages (MA), neutrophils (N) and monocyte-derived macrophages (MD), all work to contain bacterial growth. When the host is co-infected with both pathogens (first with influenza and then with pneumococci), innate immune response illicited as a result of the viral infection can interfere with functionings of the resident alveolar macrophages [22,25]. These macrophages are the first line of defence and play an important role in limiting the bacterial growth in the initial stages of the bacterial infection. The resulting interaction is indicated by the thick grey arrow. A complete mathematical description of the model can be found in the electronic supplementary material.

2. Material and methods

We proceed by first presenting mathematical models previously proposed to describe the within-host dynamics of influenza [28] and pneumococcal [29] infections in isolation. Each model attempts to capture the kinetics of pathogen proliferation, the resultant immune response and its impact on pathogen load during the course of an infection. Subsequently, we combine these pathogen-specific models via an interaction model, which enables an examination of the effect of interaction between these viral and bacterial pathogens. In figure 1, we present a schematic of our model.

2.1. The influenza model
Several mathematical models have been proposed to describe the kinetics of influenza virus and the impact of a subsequent

immune response [30–32]. The model we examine here is adapted from that proposed by Handel et al. [28]. Following infection, the virus proliferates via infection of 'target cells', which then generate more viral particles. The immune response is separated into two broad categories: (i) an innate immune response that is activated early in the course of infection and mostly works to contain the viral population in the host and (ii) an acquired immune response that acts later and is crucial in eventual viral clearance. In figure 1, we present a schematic of the model. The mathematical equations describing the system and the parameter estimates are presented in the electronic supplementary material, equations S1 and table S1, respectively.

Handel et al. [28] parametrized the within-host influenza model by statistical fitting to data from two separate experimental studies: (i) the study by Kris et al. [33], which observed viral loads in both wild-type (normal) and nude, athymic (no antibody response) mice during an H3N2 infection, and (ii) the study by Iwasaki & Nozima [34], which documented viral loads, IFN and antibody levels, as well as lung lesions in mice with H1N1 infection under various permutations of treatments. We illustrate representative viral titres and different measures of immune response over the course of the infection, as predicted by the model, in the electronic supplementary material, figures S1 and S2. Note that, in the electronic supplementary material, we examine the sensitivity of our findings to this choice of influenza model.

2.2. The *Streptococcus pneumoniae* model

The pathogenesis of a pneumococcal infection depends on the expression of several virulence factors of the bacterium, together with a series of host immune responses [35]. Furthermore, the dynamics of the interaction between the pathogen and the immune response depend on (i) *location*: for instance, the host relies on serum and mucosal antibodies for clearance when the bacteria reside in the nasopharynx [36–38], whereas macrophages and cytokines play a crucial role in containing bacterial growth in the lungs [39–41], and (ii) *timing*: for instance, resident macrophages are available at the beginning of the infection, whereas neutrophils recruited via cytokines arrive a few hours after infection [42]. The approach we adopt is to focus on pathogenesis once the bacterium has progressed to the lower respiratory tract and the lungs, where macrophages and cytokines of the innate immune response are the main protagonists [40].

The best available description of the progression of pneumococcal infection in the lungs is afforded by the model developed by Smith et al. [29]. This model assumes a three-pronged innate immune response to infection with *S. pneumoniae* (figure 1). Resident alveolar macrophages act as the first line of defence and are critical in controlling bacterial growth during the initial phase of the infection [39]. These macrophages trigger a neutrophil response via cytokines, which are active within a few hours of the infection [41]. The neutrophil response may not be sufficient to clear the bacteria and in turn triggers monocyte-derived macrophages that appear 24–48 h following infection. Depending on the initial bacterial dose, the three-pronged innate immune response can either (i) clear the pathogen without establishment, (ii) lead to an acute infection, with eventual clearance, or (iii) fail to control the bacterium.

Smith et al. [29] confirmed their model's veracity by carrying out extensive experimental studies, where normal mice are challenged with different inocula of *S. pneumoniae*, and the bacterial load in the lung tracked through time. Dynamics vary with inoculum sizes: a small inoculum of 10^3 colony-forming units (CFUs) is rapidly cleared from the lung; an intermediate inoculum of 10^4 CFUs is cleared but only after a peak; and finally a large inoculum of 10^5 CFUs is not cleared. The full description of the mathematical model, its parametrization and examples of different infection outcomes can be found in the original paper [29] and are reproduced in the electronic supplementary material, figure S3, table S2 and equations S2, respectively.

Table 1. Parameter values for the two-pathogen model.

parameter	description	value
K	maximal interference	0
I_{max}	maximum level of virus-specific innate immune response	$10^{2.4}$
σ	shape of the saturation	1

2.3. A model for influenza–pneumococcus interaction

Our integrated model focuses on the effects of influenza infection on subsequent invasion by *S. pneumoniae*. Alveolar macrophages are a critical part of host immunity against bacterial infections, as they carry out phagocytosis and produce cytokines to recruit neutrophils [22,39–41]. Experimental studies have shown that viral infection interferes with the functioning of alveolar macrophages in subsequent bacterial clearance, either in the intracellular phagocytic process (phagosome–lysosome fusion) [43,44] or in both the ingestion and killing of bacteria [45]. We assume that the operating efficiency of the resident alveolar macrophages is reduced by the expression of interferons resulting from viral infection. This is in line with observations made by Sun & Metzger [22] in a mouse model that IFN-γ produced after viral infection inhibits the functioning of resident alveolar macrophages, which are crucial for containing the growth of bacteria in the initial phase of infection.

We model the efficiency of the resident alveolar macrophages, Φ, by the following equation:

$$\Phi(t) = 1 - \left[\frac{\mathrm{IR}_I(t - \tau_V)}{I_{max} + K}\right]^{\sigma}.$$

Here, the efficiency of the resident macrophage at time t, $\Phi(t)$, is assumed to be inversely related to the innate immunity mounted in response to the preceding influenza infection, IR_I, with a time delay of τ_V. This delay is taken to be 1.3 days, the same as that associated with innate immunity's action on the reduction of viral reproduction [28]. The parameter K determines the strength of interference: when $K = 0$, the inhibitory effect is maximal, while larger K results in lower interference effects. The exponent σ determines the shape of the interference: $\sigma = 1$ implies a linear relationship between the cytokine levels and inhibition, $\sigma < 1$ implies a saturating relationship. I_{max} is the maximum level of virus-specific innate immune response. The values of the parameters used for the main results are given in table 1. Sensitivity to variation in the parameters is explored in the electronic supplementary material, figures S6, S7 and S11.

In the absence of a preceding viral infection, the virus-specific innate immune response is absent, i.e. $\mathrm{IR}_I = 0$, and resident alveolar macrophages are fully functional, i.e. $\Phi = 1$. In this case, the pneumococcal infection proceeds independently, as described by the *S. pneumoniae* model, whereas, in the presence of an on-going viral infection, the efficiency of the resident alveolar macrophages depends inversely on the level of the virus-induced innate immune response, IR_I. The ensuing pneumococcal infection in the presence of preceding viral infection is fully detailed in the electronic supplementary material, equations S4. Note that, for these parameter values, the interaction Φ decreases linearly from 1 when $\mathrm{IR}_I = 0$ to 0 when $\mathrm{IR}_I = I_{max}$.

2.4. Antiviral treatment

We establish the patient care consequences of the influenza–pneumococcal interaction by examining the role of antiviral

treatment on the severity of bacterial infection. Antiviral treatment is modelled to block viral production that occurs via infected target cells, the effect being representative of oseltamivir treatment. For antiviral drugs administered at time τ_{AV}, the fractional reduction of the viral production via infected target cells, AV, is modelled as follows:

$$AV = \begin{cases} 0 & \text{if } t < \tau_{AV} \\ 0.9 & \text{if } t \geq \tau_{AV}. \end{cases}$$

In this formulation, the viral production is reduced by 90% after the introduction of antiviral drugs. The ensuing effect of antivirals on the viral kinetics is detailed in the electronic supplementary material, equation S5. By varying parameter τ_{AV}, we explore the effect of antivirals administered at different times following influenza infection.

2.5. Computation

The resulting model is a system of coupled delayed differential equations, which are presented in the electronic supplementary material in their entirety. We numerically solve this model while systematically varying inoculum sizes, and the timing of co-infection and antiviral treatment in order to understand the epidemiological impact of influenza–pneumococcal interaction. All of the computations were conducted using freely available software R [46]. The systems of delayed differential equations were solved numerically using R package PBSddesolve. The resulting pneumococcal infections are classified into three qualitatively distinct categories, as follows. (i) Severe invasive infections that are not cleared (coloured dark red in the figures). The criterion used for this classification is: bacterial load is not cleared up to 15 days after inoculation with pneumococcal pneumonia. (ii) Acute infections that are eventually cleared (coloured orange in the figures). The criterion used for this classification is: bacterial load is cleared, but only after it first increases by 10-fold. (iii) Transient infections that are cleared rapidly (coloured pink in the figures). The criterion used for this classification is: bacterial load is cleared before it can increase by 10-fold.

3. Results

3.1. Immune interaction leading to enhanced susceptibility

We find two key predictions resulting from immune-mediated pathogen interaction. First, the timing of bacterial infection determines its invasion success and clinical severity. In the absence of a recent history of influenza infection, the introduction of pneumococcus will lead to a severe outcome only when the inoculum size is sufficiently large (figure 2). Resident alveolar macrophages acting in concert with other parts of the immune response are able to clear infections at low dosage. Only when confronted with a large dosage do they fail to clear the bacteria. In contrast, when the bacterial inoculation coincides with the proliferation of IFNs, there is an inhibitory effect that impairs the functioning of macrophages. As a result, initial bacterial growth is unchecked, leading to severe consequences. The model predicts that enhanced susceptibility is observed during a brief window of time, approximately between 4 and 6 days following influenza infection. This is consistent with results of mouse experiments in which the severity of co-infections depends critically on the exact time schedule of the two infections [19] (also reproduced in the electronic supplementary material, figure S4). Co-infections in which the bacterial

infection lags viral infection by 5–7 days were more severe, resulting in greater bacterial growth [22,25] and contributing to increased mortality (decreased survival and mean survival time [19]) and morbidity (increased weight loss [10]). When the order of infections was reversed or when they were simultaneously inoculated, the infections were not as severe [19].

The second prediction of the model is a significant dose dependence. In the absence of influenza infection, a pneumococcal dose in excess of 10^5 CFUs is required for successful invasion. As shown in figure 2, if exposure occurs in the critical period between 4 and 6 days after viral infection, inoculum sizes significantly less than 10^5 are sufficient to generate substantial bacterial growth, consistent with empirical results [19]. Both predictions are robust to variations in the shape and the extent of the model for interaction. In the electronic supplementary material, we demonstrate the robustness of our findings by carrying out extensive sensitivity analyses.

3.2. Implications for antiviral treatment

In figure 3a,b, we present the model output when antivirals are administered 2 (figure 3a) and 4 (figure 3b) days after influenza infection. The window of enhanced susceptibility is eliminated if antiviral treatment commences only 2 days into a viral infection, whereas by day 4 antivirals will have a negligible impact. This point is further illustrated by figure 3c, where we systematically explore the effect of varying the timing of antiviral treatment on the severity of pneumococcal infection when the bacterium invasion occurs 5 days after viral infection. Treatments need to be administered within the first 4 days of influenza infection in order to prevent the increased susceptibility to secondary pneumococcal infection. A less optimistic assumption, comprising 50% antiviral efficacy, shows similar results (see the electronic supplementary material, figure S12), suggesting that our findings are more sensitive to the timing of the therapy rather than the efficacy of antivirals.

4. Discussion

Understanding how viral infections affect immunity and susceptibility to subsequent invasion by other pathogens remains an important challenge. This is particularly true for influenza virus, since it is known to be responsible for a significant number of deaths annually, many of which are due to secondary bacterial infections. To examine this phenomenon, we formulated a model of viral and bacterial infection kinetics in the lower respiratory tract and the lungs. In these organs, alveolar macrophages are a key component of the innate immune system in orchestrating bacterial clearance [39–41]. Viral infection interferes with the routine functioning of these macrophages, reducing early bacterial clearance and increasing the risk of more severe infection [22,43–45]. Since viral interference is modulated via cytokines, the timing and duration of cytokine production is paramount in defining susceptibility to bacterial invasion. Our model prediction concerning the window during which susceptibility to bacterial infection is amplified is consistent with studies of co-infection in animal models [19]—the interaction is asymmetric (virus affects bacterium) and only operational during the 4–6 day period following influenza infection.
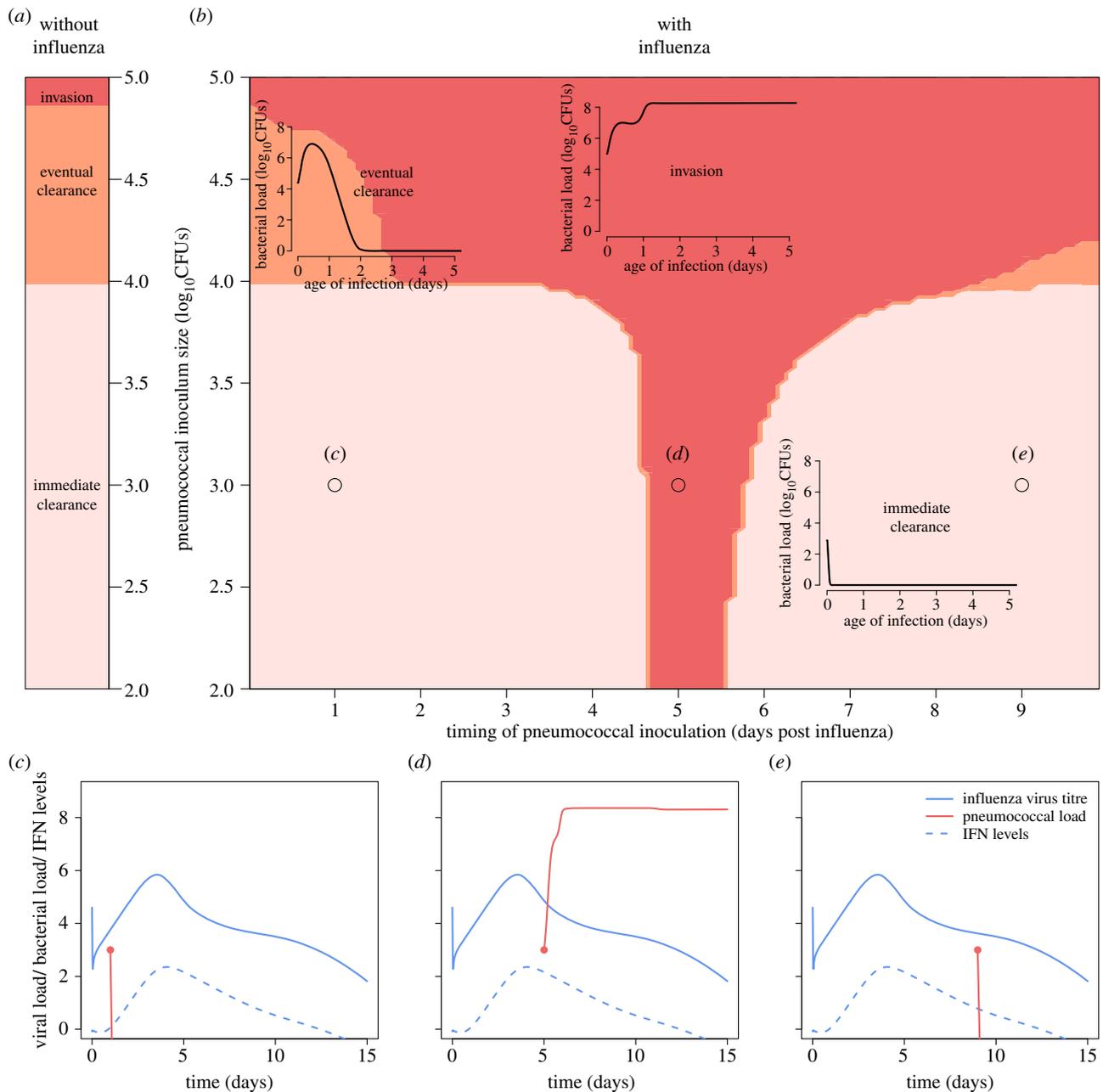
Figure 2. Impact of interaction between influenza and pneumococcus. (*a*) The strip labelled 'without influenza' shows the dose–response of a pneumococcal infection without prior infection with influenza [29]. The three colours represent qualitatively distinct infection outcomes: (i) severe invasive infections (dark red), (ii) acute infections that are eventually cleared (orange) and (iii) transient infections that are cleared rapidly (pink). (*b*) The model-predicted clinical outcome of pneumococcal infection as a function of the timing of introduction (horizontal axis) and inoculum size (vertical axis). The three inset graphs, placed in the three colour-coded regions, show the approximate time profiles of bacterial load for three qualitatively different categories. (*c*–*e*) Time profiles of influenza viral titre, pneumococcal load and IFN levels in the three scenarios, when pneumococcus is introduced, respectively, 1, 5 and 9 days post influenza.

Our treatment of secondary bacterial pneumonia has considered the timing of exposure to pneumococci relative to influenza infection, but it is also applicable to instances of prior colonization with the bacterial pathogen. Asymptomatic carriage of both pneumococcal and staphylococcal bacteria in the nasal cavity and upper respiratory tract is common, and thought to be the source of subsequent invasive pneumonia [47]. Influenza infection may also enhance bacterial colonization of the upper respiratory tract [48], and thereby increase the risk of secondary bacterial pneumonia. Coughing induced by viral infection may aid in aspiration of bacteria from the nasal cavity into the lungs. However, not all bacteria are equally likely to cause

pneumonia. The risk of pneumococcal pneumonia is serotype dependent [49].

It is worth emphasizing that, although our model is based on influenza A virus and *S. pneumoniae*, the cytokine response is a general mechanism that is likely to be broadly triggered. The timing and the intensity of the generated cytokine response are key in understanding the outcome of other viral–bacterial interactions. However, these interactions probably vary with viral subtypes and bacterial strains. In general, the mechanisms underpinning enhanced susceptibility and subsequent pathogenesis should hold for a range of other viral–bacterial infections. The fact that murine experiments based on different viral and bacterial systems find
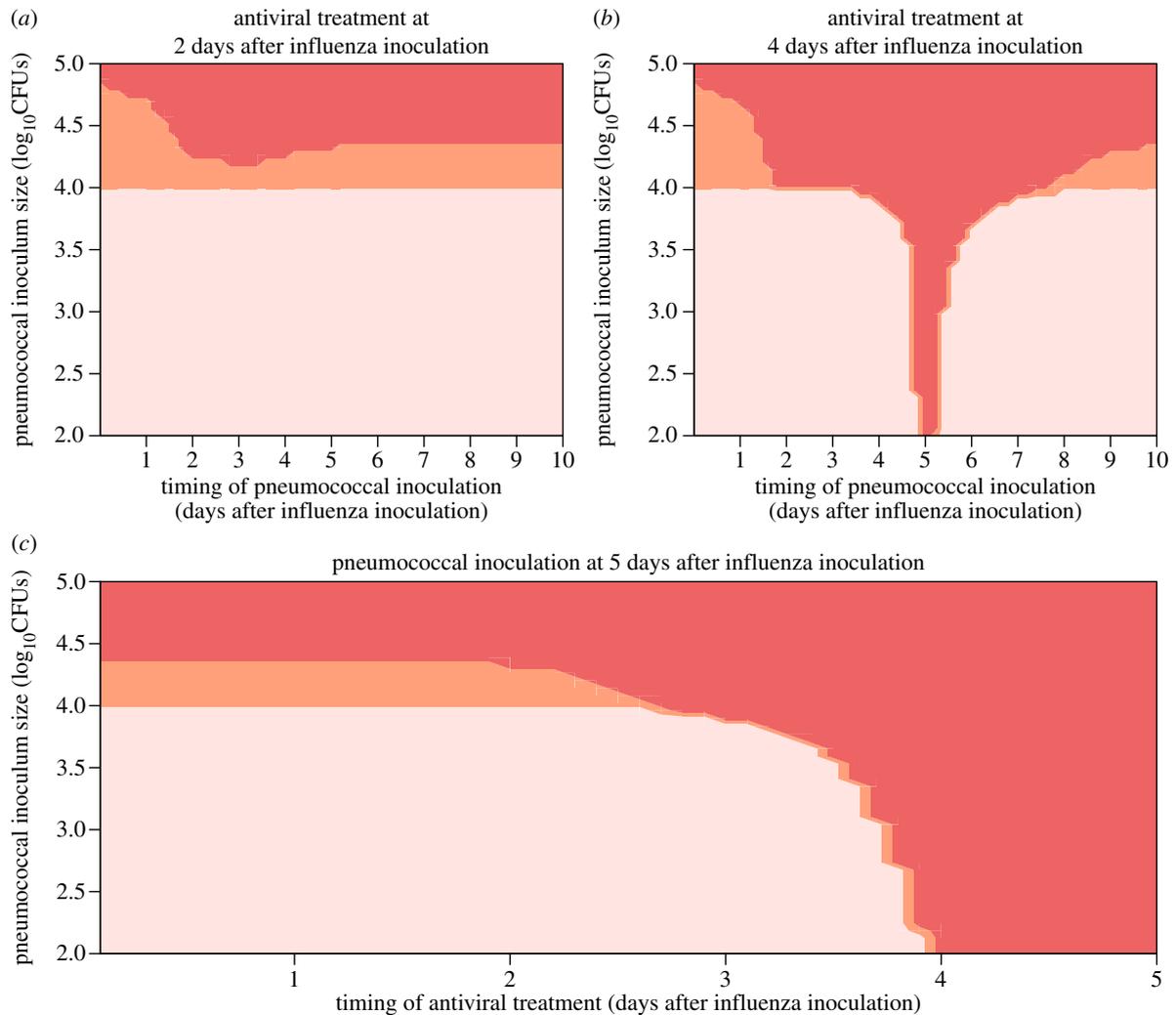
Figure 3. Limitation of antiviral treatment in preventing severe secondary pneumococcal infection. (*a,b*) Pneumococcal infections (as shown in figure 2) with antiviral treatments administered at days 2 and 4 post influenza, respectively. While the treatment administered at day 2 is able to prevent severe manifestation of the pneumococcal infection, its effect is significantly reduced when administered at day 4. (*c*) The consequences of administering treatments at various times (plotted on the horizontal axis) on the worst manifestation of the interaction (as it would at day 5 without treatment) at different intervals (plotted on the horizontal axis). The three colours represent qualitatively distinct infection outcomes: (i) severe invasive infections (dark red), (ii) acute infections that are eventually cleared (orange) and (iii) transient infections that are cleared rapidly (pink). Treatment administered on and after day 4 is unable to prevent secondary pneumococcal infection.

similar viral–bacterial interactions [10,43,50] (especially, influenza A with *S. aureus* [11–13]) testify to the potential generality of this interaction. Model formulation using the kinetics of different pathogens will help to generalize the interactions across different systems as well as elucidate key differences between them.

The sensitivity of our conclusions to our choice of within-host models is important to address. Other than the model by Smith *et al.* [29], we are not aware of any other experimentally confirmed model of the progression of *S. pneumoniae*. There are, however, several proposed models for influenza [31,51,52]. Of these, the model developed by Handel *et al.* [28] is best suited for our purposes for three key reasons: (i) in contrast to models based on human [30] or equine [31] influenza, the model by Handel *et al.* is based on a murine challenge system, with which all experiments on viral–bacterial interactions are studied; (ii) it explicitly considers innate immunity; and (iii) it is statistically fitted to experimental data. That said, we have explored the robustness of our findings to alternative formulations of the Handel *et al.* model, concerning the shape and strength of interference

(electronic supplementary material, figure S6) and the potential dependence of immune response on viral load (electronic supplementary material, figure S7). It is important to emphasize that we have additionally examined the sensitivity of results presented in figures 2 and 3 by implementing the alternative influenza model proposed by Baccam *et al.* [30]. As shown in the electronic supplementary material, figures S8–S10, our conclusions are qualitatively unaffected by the precise choice of influenza infection model.

Our work only focused on one of the several proposed pathways of influenza–pneumococcal interactions—that cytokines expressed as a part of the innate immune response to preceding viral infection interfere with the macrophage-based clearance of subsequent pneumococcal infection [22,25]. Our results show that this hypothesis can generate model outcomes that are consistent with experimental data on sequential infections. Bearing in mind that the pathogen-specific models of influenza and pneumococcal pneumonia were formulated to explain the course of infection in the absence of any interaction with other pathogens, our results underline the surprising consistency of the proposed

immune-modulated interaction. However, it is important to note that this model does not consider other hypothesized pathways of interaction, such as neuraminidase-based mechanisms [17,18] or the effects of epithelial damage [15,16]. The model also ignores potential competition between virus and bacterium for epithelial cells, or the role of target cell limitations on pneumococcal infections, which may have important consequences for the dynamics of the interaction. Meaningful evaluation of these critical questions was not possible in this study owing to the absence of appropriately parametrized mechanistic models at the time of this study. As such, an important area for future research remains the development of models that allow for the incorporation of alternative hypothesized pathways of interaction, as well as distinguishing between type-I- [25] and type-II-based [22] immune modulation. The predictions and the understanding of the viral–bacterial interaction could be further enhanced by tailoring the pathogen-specific models to specifically study interaction. The ability of this model to predict the consequences of the interaction between them, when the pathogen-specific parts were originally proposed without this intention, suggests that this framework bears promise to serve as a starting template.

By necessity, our mathematical framework is based on murine models. The differences in the immune components as well as the kinetics of viral and bacterial growth between humans and mice may be important. Although there seems to be very strong agreement between the two for influenza [30,53], we are not aware of any experiments or observational studies that have explored the relevant kinetics of pneumococcal infections in humans. Furthermore, the mice in the experiments were devoid of any immune history. Therefore, understanding how immune history may potentially affect our results in a natural population represents an important research question.

Limitations aside, the proposed mathematical model provides several insights. Susceptibility to secondary pneumococcal infection is dramatically increased during a critical window between 4 and 6 days after influenza infection. The pathway of pneumococcal infection could be either asymptomatic carriage prior to influenza infection and subsequent aspiration into the lungs or acquisition following exposure. During this critical period, a low dose of pneumococci that would otherwise be cleared from the lung can result in severe infection. This finding is supported by animal experiments demonstrating that pre-exposure to influenza increased the probability of pneumococci invasion [8,54,55]; relevant experimental data are reproduced in the electronic supplementary material, figures S4 and S5.

Our findings have implications for understanding the epidemiology of pneumococcal infections and targeting interventions. First, the identification of risk groups for targeted prevention efforts may need to take into account differential pneumococcal colonization rates. Specifically, cohorts that are associated with higher colonization rates may be at greater risk of developing severe secondary bacterial pneumonia when exposed to influenza. While the link between pneumococcal carriage and invasive pneumonia is not well understood, our conclusion is supported by the recent finding that detection of pneumococcal bacteria in nasopharyngeal swabs was a predictor of severe pneumonia during the 2009 H1N1 pandemic [56].

Second, the detectability of an association between influenza and invasive pneumonia is predicted to depend on the size of the influenza epidemic. Because the immune-mediated interaction is only operational over a brief window of time, the contribution of seasonal influenza to invasive pneumococcal disease risk may be difficult to discern from incidence data. However, during unusually large influenza outbreaks, such as a pandemic, a larger and a more observable frequency of invasive pneumococcal disease is predicted. Although the documentation of a high incidence of pneumococcal infection following lung autopsies of influenza victims during past influenza pandemics [3] is consistent with this prediction, a conclusive test requires access to longitudinal incidence data, which have proved elusive to date. Accounts of bacterial pneumonia cases in army camps across the USA during the 1918 pandemic [57] and hospital admissions during the Asian [58,59] and the Hong Kong [60,61] pandemics indicate high rates of bacterial co-infections in influenza patients. A significant impact on pneumococcal pneumonia was also observed during the 2009 influenza pandemic. Crucially, the variance in the magnitude of this effect between age groups correlated with the variations in influenza activity [62]. These, taken together with recent studies that find a low detectable association between seasonal influenza and invasive pneumococcal disease [63,64], suggest that there is a stronger association during pandemic years. The inability to detect a pronounced epidemiological correlation between the two during non-pandemic years is, we suggest, more likely to be a reflection of the brief duration of this interaction, rather than definitive evidence against the possibility that influenza affects pneumococcal transmission. The fact that a sensitive probe such as a vaccine trial using conjugate pneumococcal vaccine detects a larger impact of the pneumococcus on influenza-associated pneumonia even during seasonal influenza [65] provides more support to this hypothesis.

An important clinical implication from the model concerns when antiviral administration is effective in preventing severe invasive pneumonia in influenza patients. By day 4 of influenza infection, the cytokine response elicited by the virus has begun interfering with the macrophage response against bacteria. Consequently, after day 4, antivirals, even when they are highly efficacious, are ineffective in preventing severe invasive pneumonia. Further studies of antiviral treatments are urgently needed to verify this prediction, but it is in line with at least one study in mice that explored the time sensitivity of antiviral treatment [66] (also reproduced in the electronic supplementary material, figure S5). Another barrier to invasive pneumococcal disease prevention using antivirals is the timing of influenza symptomatology, typically observed 3–4 days into the infection [67,68]. Furthermore, experimental work on a primate model indicates that the interaction may proceed even earlier [69]. Hence, in the absence of concerted contact-tracing efforts (unfeasible during influenza epidemics or pandemics), attempts to reduce severe secondary pneumococcal infections via clinical diagnosis and therapeutic treatment of influenza patients appear doomed to failure. This conclusion underlines the importance of alternative control measures, such as influenza and pneumococcal immunization, antibiotic treatment and social distancing, aimed at reducing transmission in the community [70].

References

1. World Health Organization (WHO). 2009 Influenza (seasonal). Fact sheet no. 211. See http://www.who.int/mediacentre/factsheets/fs211/en.

2. Taubenberger J, Morens D. 2006 1918 influenza: the mother of all pandemics. *Emerg. Infect. Dis.* **12**, 15–22. (doi:10.3201/eid1201.051442)

3. Morens D, Taubenberger J, Fauci A. 2008 Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J. Infect. Dis.* **198**, 962–970. (doi:10.1086/591708)

4. Klugman KP, Chien Y, Madhi SA. 2009 Pneumococcal pneumonia and influenza: a deadly combination. *Vaccine* **27S**, C9–C14. (doi:10.1016/j.vaccine.2009.06.007)

5. Dowell S, Whitney C, Wright C, Rose Jr C, Schuchat A. 2003 Seasonal patterns of invasive pneumococcal disease. *Emerg. Infect. Dis.* **9**, 574–579. (doi:10.3201/eid0905.020556)

6. Walter N, Taylor Jr T, Dowell S, Mathis S, Moore M. 2009 Holiday spikes in pneumococcal disease among older adults. *N. Engl. J. Med.* **361**, 2584–2585. (doi:10.1056/NEJMc0904844)

7. Obaro S, Adegbola R. 2002 The pneumococcus: carriage, disease and conjugate vaccines. *J. Med. Microbiol.* **51**, 98–104.

8. Glover R. 1941 Spread of infection from the respiratory tract of the ferret. II. Association of influenza A virus and streptococcus group C. *Br. J. Exp. Pathol.* **22**, 98–107.

9. McCullers JA. 2006 Insights into the interaction between influenza virus and pneumococcus. *Clin. Microbiol. Rev.* **19**, 571–582. (doi:10.1128/CMR.00058-05)

10. Kukavica-Ibrulj I, Hamelin M, Prince GA, Gagnon C, Bergeron Y, Bergeron MG, Boivin G. 2009 Infection with human metapneumovirus predisposes mice to severe pneumococcal pneumonia. *J. Virol.* **83**, 1341–1349. (doi:10.1128/JVI.01123-08)

11. Lee M, Arrecubieta C, Martin F, Prince A, Borczuk A, Lowy F. 2010 A postinfluenza model of *Staphylococcus aureus* pneumonia. *J. Infect. Dis.* **201**, 508–515. (doi:10.1086/650204).

12. Small C et al. 2010 Influenza infection leads to increased susceptibility to subsequent bacterial superinfection by impairing NK cell responses in the lung. *J. Immunol.* **184**, 2048–2056. (doi:10.4049/jimmunol.0902772)

13. Iverson A, Boyd K, McAuley J, Plano L, Hart M, McCullers J. 2011 Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *J. Infect. Dis.* **203**, 880–888. (doi:10.1093/infdis/jiq113)

14. van der Sluijs K, van der Poll T, Lutter R, Juffermans N, Schultz M. 2010 Bench-to-bedside review: bacterial pneumonia with influenza-pathogenesis and clinical implications. *Crit. Care* **14**, 219. (doi:10.1186/cc8893)

15. Harford C, Leidler V, Hara M. 1949 Effect of the lesion due to influenza virus on the resistance of mice to inhaled pneumococci. *J. Exp. Med.* **89**, 53–69. (doi:10.1084/jem.89.1.53)

16. Sanford B, Shelokov A, Ramsay M. 1978 Bacterial adherence to virus-infected cells: a cell culture model of bacterial superinfection. *J. Infect. Dis.* **137**, 176–181. (doi:10.1093/infdis/137.2.176)

17. McCullers JA, Bartmess KC. 2003 Role of neuraminidase in lethal synergism between influenza virus and *Streptococcus pneumoniae*. *J. Infect. Dis.* **187**, 1000–1009. (doi:10.1086/368163)

18. Peltola V, McCullers J. 2004 Respiratory viruses predisposing to bacterial infections: role of neuraminidase. *Pediatr. Infect. Dis. J.* **23**, S87. (doi:10.1097/01.inf.0000108197.81270.35)

19. McCullers JA, Rehg JE. 2002 Lethal synergism between influenza virus and *Streptococcus pneumoniae*: characterization of a mouse model and the role of platelet-activating factor receptor. *J. Infect. Dis.* **186**, 341–350. (doi:10.1016/j.tree.2008.05.009).

20. van der Sluijs K et al. 2006 Involvement of the platelet-activating factor receptor in host defense against *Streptococcus pneumoniae* during postinfluenza pneumonia. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **290**, L194–L199. (doi:10.1152/ajplung.00050.2005)

21. McCullers J, Iverson A, Mckeon R, Murray P. 2008 The platelet activating factor receptor is not required for exacerbation of bacterial pneumonia following influenza. *Scand. J. Infect. Dis.* **40**, 11–17. (doi:10.1080/00365540701477568).

22. Sun K, Metzger DM. 2008 Inhibition of pulmonary antibacterial defense by interferon-γ during recovery from influenza infection. *Nat. Med.* **14**, 558–564. (doi:10.1038/nm1765)

23. van der Sluijs K et al. 2004 IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. *J. Immunol.* **172**, 7603–7609.

24. Navarini AA et al. 2006 Increased susceptibility to bacterial superinfection as a consequence of innate antiviral responses. *Proc. Natl Acad. Sci. USA* **103**, 15 535–15 539. (doi:10.1073/pnas.0607325103)

25. Shahangian A, Chow E, Tian X, Kang J, Ghaffari A, Liu S, Belperio J, Cheng G, Deng J. 2009 Type I IFNs mediate development of postinfluenza bacterial pneumonia in mice. *J. Clin. Invest.* **119**, 1910–1920. (doi:10.1172/JCI35412).

26. Tashiro M, Ciborowski P, Klenk H, Pulverer G, Rott R. 1987 Role of *Staphylococcus* protease in the development of influenza pneumonia. *Nature* **325**, 536–537. (doi:10.1038/325536a0)

27. Tashiro M, Ciborowski P, Reinacher M, Pulverer G, Klenk H, Rott R. 1987 Synergistic role of staphylococcal proteases in the induction of influenza virus pathogenicity. *Virology* **157**, 421–430. (doi:10.1016/0042-6822(87)90284-4)

28. Handel A, Longini I, Antia R. 2010 Towards a quantitative understanding of the within-host dynamics of influenza A infections. *J. R. Soc. Interface* **7**, 35–47. (doi:10.1098/rsif.2009.0067)

29. Smith AM, McCullers JA, Adler FR. 2011 Mathematical model of a three-stage innate immune response to a pneumococcal lung infection. *J. Theor. Biol.* **276**, 106–116. (doi:10.1016/j.jtbi.2011.01.052)

30. Baccam P, Beauchemin C, Macken CA, Hayden FG, Perelson AS. 2006 Kinetics of influenza A virus infection in humans. *J. Virol.* **80**, 7590–7599. (doi:10.1128/JVI.01623-05)

31. Saenz R et al. 2010 Dynamics of influenza virus infection and pathology. *J. Virol.* **84**, 3974. (doi:10.1128/JVI.02078-09).

32. Smith AM, Adler FR, McAuley JL, Gutenkunst RN, Ribeiro RM, McCullers JA, Perelson AS. 2011 Effect of 1918 PB1-F2 expression on influenza A virus infection kinetics. *PLoS Comput. Biol.* **7**, e1001081. (doi:10.1371/journal.pcbi.1001081)

33. Kris RM, Yetter RA, Cogliano R, Ramphal R, Small PA. 1988 Passive serum antibody causes temporary recovery from influenza virus infection of the nose, trachea and lung of nude mice. *Immunology* **63**, 349–353.

34. Iwasaki T, Nozima T. 1977 Defense mechanisms against primary influenza virus infection in mice. I. The roles of interferon and neutralizing antibodies and thymus dependence of interferon and antibody production. *J. Immunol.* **118**, 256–263.

35. Kadioglu A, Weiser J, Paton J, Andrew P. 2008 The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat. Rev. Microbiol.* **6**, 288–301. (doi:10.1038/nrmicro1871)

36. Musher D. 1992 Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. *Clin. Infect. Dis.* **14**, 801–807. (doi:10.1093/clinids/14.4.801)

37. Watson D, Musher D, Verhoef J. 1995 Pneumococcal virulence factors and host immune responses to them. *Eur. J. Clin. Microbiol. Infect. Dis.* **14**, 479–490. (doi:10.1007/BF02113425)

38. McCool T, Cate T, Moy G, Weiser J. 2002 The immune response to pneumococcal proteins during experimental human carriage. *J. Exp. Med.* **195**, 359–365. (doi:10.1084/jem.20011576)

39. Broug-Holub E, Toews G, Van Iwaarden J, Strieter R, Kunkel S, Paine 3rd R, Standiford T. 1997 Alveolar macrophages are required for protective pulmonary

defenses in murine *Klebsiella* pneumonia: elimination of alveolar macrophages increases neutrophil recruitment but decreases bacterial clearance and survival. *Infect. Immun.* **65**, 1139–1146.

40. Dockrell D, Marriott H, Prince L, Ridger V, Ince P, Hellewell P, Whyte M. 2003 Alveolar macrophage apoptosis contributes to pneumococcal clearance in a resolving model of pulmonary infection. *J. Immunol.* **171**, 5380–5388.

41. Knapp S, Leemans J, Florquin S, Branger J, Maris N, Pater J, van Rooijen N, van der Poll T. 2003 Alveolar macrophages have a protective antiinflammatory role during murine pneumococcal pneumonia. *Am. J. Respir. Crit. Care Med.* **167**, 171–179. (doi:10.1164/rccm.200207-698OC)

42. Bergeron Y, Ouellet N, Deslauriers A, Simard M, Olivier M, Bergeron M. 1998 Cytokine kinetics and other host factors in response to pneumococcal pulmonary infection in mice. *Infect. Immun.* **66**, 912–922.

43. Jakab G, Warr G, Sannes P. 1980 Alveolar macrophage ingestion and phagosome–lysosome fusion defect associated with virus pneumonia. *Infect. Immun.* **27**, 960–968.

44. Abramson J, Lewis J, Lyles D, Heller K, Mills E, Bass D. 1982 Inhibition of neutrophil lysosome–phagosome fusion associated with influenza virus infection *in vitro*. Role in depressed bactericidal activity. *J. Clin. Invest.* **69**, 1393–1397. (doi:10.1172/JCI110580)

45. Warshauer D, Goldstein E, Akers T, Lippert W, Kim M. 1977 Effect of influenza viral infection on the ingestion and killing of bacteria by alveolar macrophages. *Am. Rev. Respir. Dis* **115**, 269–277.

46. R Development Core Team. 2009 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

47. Bogaert D, De Groot R, Hermans P. 2004 *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. *Lancet Infect. Dis.* **4**, 144–154. (doi:10.1016/S1473-3099(04)00938-7)

48. Nakamura S, Davis K, Weiser J. 2011 Synergistic stimulation of type I interferons during influenza virus coinfection promotes *Streptococcus pneumoniae* colonization in mice. *J. Clin. Invest.* **121**, 3657–3665. (doi:10.1172/JCI57762)

49. Weinberger DM *et al*. 2010 Association of serotype with risk of death due to pneumococcal pneumonia: a meta-analysis. *Clin. Infect. Dis.* **51**, 692–699. (doi:10.1086/655828)

50. Jamieson A, Yu S, Annicelli C, Medzhitov R. 2010 Influenza virus-induced glucocorticoids compromise innate host defense against a secondary bacterial infection. *Cell Host Microbe* **7**, 103–114. (doi:10.1016/j.chom.2010.01.010)

51. Smith AM, Perelson AS. 2011 Influenza A virus infection kinetics: quantitative data and models. *WIREs Syst. Biol. Med.* **3**, 429–445. (doi:10.1002/wsbm.129)

52. Beauchemin C, Handel A. 2011 A review of mathematical models of influenza A infections within a host or cell culture: lessons learned and challenges ahead. *BMC Public Health* **11**, S7. (doi:10.1186/1471-2458-11-S1-S7)

53. Hayden FG, Fritz RS, Lobo MC, Alvord WG, Strober W, Straus SE. 1998 Local and systemic cytokine responses during experimental human influenza A virus infection. Relation to symptom formation and host defense. *J. Clin. Invest.* **101**, 643–649. (doi:10.1172/JCI1355)

54. McCullers JA, McAuley JL, Browall S, Iverson AR, Boyd KL, Normark BH. 2010 Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. *J. Infect. Dis.* **202**, 1287–1295. (doi:10.1086/656333).

55. Diavatopoulos D, Short K, Price J, Wilksch J, Brown L, Briles D, Strugnell R, Wijburg O. 2010 Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *FASEB J.* **24**, 1789–1798. (doi:10.1096/fj.09-146779).

56. Palacios G *et al*. 2009 *Streptococcus pneumoniae* coinfection is correlated with the severity of H1N1 pandemic influenza. *PLoS ONE* **4**, e8540. (doi:10.1371/journal.pone.0008540)

57. Brundage J. 2006 Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect. Dis.* **6**, 303–312. (doi:10.1016/S1473-3099(06)70466-2)

58. Louria D, Blumenfeld H, Ellis J, Kilbourne E, Rogers D. 1959 Studies on influenza in the pandemic of 1957–1958. II. Pulmonary complications of influenza. *J. Clin. Invest.* **38**, 213–265. (doi:10.1172/JCI103791)

59. Robertson L, Caley J, Moore J. 1958 Importance of *Staphylococcus aureus* in pneumonia in the 1957 epidemic of influenza A. *Lancet* **272**, 233–236. (doi:10.1016/S0140-6736(58)90060-6)

60. Lindsay Jr M, Herrmann Jr E, Morrow Jr G, Brown Jr A. 1970 Hong Kong influenza. Clinical, microbiologic, and pathologic features in 127 cases. *JAMA* **214**, 1825–1832. (doi:10.1001/jama.1970.03180100019004)

61. Schwarzmann S, Adler J, Sullivan Jr R, Marine W. 1971 Bacterial pneumonia during the Hong Kong influenza epidemic of 1968–1969. *Arch. Intern. Med.* **127**, 1037–1041. (doi:10.1001/archinte.1971.00310180053006)

62. Weinberger D, Simonsen L, Jordan R, Steiner C, Miller M, Viboud C. 2012 Impact of the 2009 influenza pandemic on pneumococcal pneumonia hospitalizations in the United States. *J. Infect. Dis.* **205**, 458–465. (doi:10.1093/infdis/jir749)

63. Walter N, Taylor T, Shay D, Thompson W, Brammer L, Dowell S, Moore M. 2010 Influenza circulation and the burden of invasive pneumococcal pneumonia during a non-pandemic period in the United States. *Clin. Infect. Dis.* **50**, 175–183. (doi:10.1086/649208).

64. Kuster SP, Tuite AR, Kwong JC, McGeer A, the Toronto Invasive Bacterial Diseases Network, Fishman DN. 2011 Evaluation of coseasonality of influenza and invasive pneumococcal disease: results from prospective surveillance. *PLoS Med.* **8**, e1001042. (doi:10.1371/journal.pmed.1001042)

65. Madhi S, Klugman K, the Vaccine Trialist Group. 2004 A role for *Streptococcus pneumoniae* in virus-associated pneumonia. *Nat. Med.* **10**, 811–813. (doi:10.1038/nm1077)

66. McCullers JA. 2004 Effect of antiviral treatment on the outcome of secondary bacterial pneumonia after influenza. *J. Infect. Dis.* **190**, 519–526. (doi:10.1086/421525).

67. Carrat F, Vergu E, Ferguson N, Lemaitre M, Cauchemez S, Leach S, Valleron A. 2008 Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am. J. Epidemiol.* **167**, 775–785. (doi:10.1093/aje/kwm375).

68. Tuite A *et al*. 2010 Estimated epidemiologic parameters and morbidity associated with pandemic H1N1 influenza. *Can. Med. Assoc. J.* **182**, 131. (doi:10.1503/cmaj.091807)

69. Berendt RF, Long GG, Walker JS. 1975 Influenza alone and in sequence with pneumonia due to *Streptococcus pneumoniae* in the squirrel monkey. *J. Infect. Dis.* **132**, 689–693. (doi:10.1093/infdis/132.6.689)

70. Chien Y-W, Levin BR, Klugman KP. 2012 The anticipated severity of a '1918-like' influenza pandemic in contemporary populations: the contribution of antibacterial interventions. *PLoS ONE* **7**, e29219. (doi:10.1371/journal.pone.0029219)