

Do specific virus–bacteria pairings drive clinical outcomes of pneumonia?

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Abstract

Bacterial pneumonia is a common contributor to severe outcomes of influenza. Epidemiological data suggest that the incidence, severity and associated bacterial pathogens differ between epidemics and by geographical location within epidemics. Data from animal models demonstrate that differences in both viral and bacterial strains alter the incidence and outcomes of pneumonia. For influenza viruses, evolutionary changes to specific virulence factors appear to alter the ability of viruses within particular lineages to prime the host for secondary bacterial infection. Although bacterial strains differ considerably in disease potential in the setting of viral co-infection, the bacterial virulence factors underlying this finding are currently unknown. The hypothesis that geographical variation exists in the prevalence of bacterial strains expressing factors that enable efficient disease potentiation during viral epidemics should be considered as one explanation for regional differences in severity. This would have implications for surveillance, vaccine development, and the conduct of clinical trials for the prevention or treatment of pneumonia.

Keywords: Influenza, mouse model, pneumonia, *Staphylococcus aureus*, *Streptococcus pneumoniae*

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History, Background, and Epidemiology

The first documentation of viral–bacterial superinfections has been attributed to the French physician R. T. H. Laennec, who practised in Paris at the dawn of the 19th century. He noted an increased incidence of typical lobar pneumonia occurring in patients suffering from influenza [1]. Although it is now well recognized that secondary bacterial pneumonia is a frequent cause of excess mortality during influenza epidemics, the epidemiology remains frustratingly unclear. This stems from two basic problems: mortality resulting from influenza and pneumonia cannot be disentangled within standard reporting structures; and it remains exceedingly difficult to accurately establish the aetiology of pneumonia [2,3]. Excess mortality, i.e. deaths occurring during an influenza epidemic in excess of the baseline deaths expected for the time of year, is used as an estimate of attributable mortality for the virus, because a definitive diagnosis of influenza is not available from death

certificates. Determining the proportion associated with pneumonia, or with another cause of death such as a cardiac complication, is difficult or impossible. In addition, excess mortality statistics only encompass the most severe cases and outcomes, and the greater burden of disease in outpatient and hospital settings goes largely undefined. The advent of nucleic acid-based diagnostics for viral pathogens is greatly expanding our knowledge of viral contributors to these interactions, and leading to an increased appreciation that many, if not most, pneumonias result from co-infections [3]. Diagnostics for bacterial causes of lower respiratory infection, however, remain severely limited, because of both sensitivity issues (an inability to easily access the lower respiratory tract and determine which pathogens are present) and specificity problems (some of the most common pneumonic bacterial pathogens also colonize the nasopharynx, limiting the utility of sampling of the upper respiratory tract to establish causation in the lower respiratory tract). In addition, the use of antibiotics prior to sampling may obscure a potential bacte-

rial aetiology when culture techniques are used for diagnosis [4].

The WHO has estimated that influenza epidemics cause 3–5 million severe illnesses and 250 000–500 000 deaths each year in industrialized countries [5]. The impact in the developing world is unknown. In typical epidemic years, approximately one-quarter of these deaths are thought to be caused by secondary bacterial pneumonia [6] (reviewed in [7]). However, these estimates are derived from large-scale population data that were not intended to separate out pneumonia deaths and that vary greatly by season and circulating strain. No definitive studies have been performed. Better data are available from the three influenza pandemics of the 20th century, in which bacterial aetiologies were identified in 50–95% of patients with fatal or life-threatening pneumonia [7–10]. In the modern era, the influenza A virus H1N1 pandemic of 2009 provided an opportunity to study the epidemiology of serious secondary infections in greater detail than had previously been possible. Bacterial co-infections were found as a complication of influenza in 25–56% of well-characterized severe or fatal cases in selected studies [4, 11–15]. Detection of bacteria such as *Streptococcus pneumoniae* was associated with a worse outcome [16]. Overall, these data suggest that secondary bacterial pneumonia is more common during the circulation of pandemic strains than during inter-pandemic influenza epidemics, although the dearth of data on complications of seasonal influenza makes exact comparisons difficult.

If data are scant on the most studied pairing, influenza and *S. pneumoniae*, they are even more scarce on the association between other respiratory viruses and pneumococcal disease (reviewed in [8]). Large studies that have attempted to

comprehensively detect both viruses and bacteria have demonstrated that such viral–bacterial co-infections do occur, but detailed information on specific pairings has typically not been reported [17–20]. A recent study of community-acquired pneumonia in hospitalized children in Taiwan showed frequent *S. pneumoniae* co-infection in children with respiratory viruses, including respiratory syncytial virus (RSV), influenza A virus, parainfluenza viruses, and adenoviruses, although proven cases of pneumococcal infection were not reported separately from probable cases identified through examination of oropharyngeal specimens [21]. A study of RSV in hospitalized children in Brazil demonstrated a statistically significant association between infection with the virus and isolation of bacteria from sterile sites as compared with RSV-negative children, but many of the recovered bacteria would not be considered typical pneumonic pathogens [22]. Very few data on the frequency of respiratory virus co-infection with other bacterial causes of pneumonia are available. Overall, although such infections are known to occur, there is a paucity of data on the frequency of secondary bacterial infections in persons infected with viruses other than influenza virus. Likewise, the clinical significance of these co-infections is not known.

Staphylococcus aureus, *S. pneumoniae* and *Streptococcus pyogenes* (group A streptococcus) are the most common bacterial pathogens that complicate influenza (reviewed in [7, 23]). *Streptococcus pneumoniae* is the most frequently identified secondary pathogen in most case series, including those from the 1918 pandemic (Fig. 1) [9]. *Staphylococcus aureus* was the most prominent aetiological agent in the 1957 pandemic [24–26], and has been a more common cause of pneumonia since the emergence of the USA300 clonotypes in regions

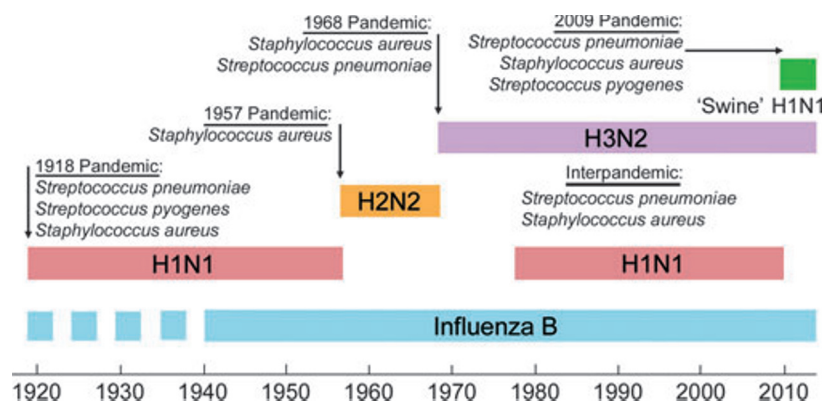


FIG. 1. Timeline of circulating influenza strains and related complications since 1918. The bacterial species most commonly isolated from serious or fatal cases of disease are listed for each pandemic. Notes: (i) significant geographical variation in the relative frequency of bacterial pathogens existed within pandemics, as discussed in the text; (ii) the broken blue line indicates that, although influenza B virus was not isolated until 1940, it is inferred to have circulated prior to this time; (iii) although the first isolation of an influenza A virus was in 1933, the 1918 pandemic strain has been resurrected from frozen material; (iv) the seasonal H1N1 strain that re-emerged in 1977 is most similar to viruses that circulated around 1950; the 2009 'swine' H1N1 has an HI haemagglutinin that differs phylogenetically from that in the previously circulating human H1N1 strains.

where these strains predominate [27]. Widespread use of pneumococcal vaccines in developed countries may be altering the relative frequency of these two bacteria, both by decreasing the absolute incidence of pneumococcal infections, and by changing the dynamics of staphylococcal colonization and invasion through alterations to the nasopharyngeal flora. Group A streptococcus is entirely absent from some case series, and is typically third in incidence when it appears [28]. *Haemophilus influenzae*, a frequent cause of secondary bacterial pneumonia in the early part of the 20th century, is less common, particularly in children, with the advent of the type B conjugate vaccine. Non-typeable strains are still significant causes of acute otitis media associated with infections by influenza virus and other respiratory viruses, and can be minor causes of virus-associated community-acquired pneumonia in adults [29] or in children where vaccination is not widespread [30]. The relative rank order of the top three secondary pathogens differs by influenza epidemic and between studies within an epidemic. Considerable regional variation was seen during the 1918 pandemic [31]. Although the precise reasons are presently unclear, this geographical variation in disease aetiology suggests that both strain-related differences in the ability to prime the host for bacterial infections and regional differences in the distribution and make-up of the participating pathogens contribute to the incidence and aetiology of secondary bacterial pneumonia.

Modelling Co-infections in Animals

The difficulties in precisely establishing the aetiology of pneumonia in humans and associated problems in understanding the epidemiology of secondary bacterial infections have led to the use of animals to investigate virus–bacteria synergism. A variety of small-animal models have been used since the 1918 pandemic for this purpose (reviewed in [7]). Most recent research on secondary bacterial pneumonia has been performed in mice, with variations on a model of pneumococcal superinfection following influenza published in 2002 [32]. A variety of viral and bacterial pathogens have been utilized in these sequential infection models, including influenza viruses, paramyxoviruses [33–35], *Staphylococcus aureus* [36,37], *S. pyogenes* [28], and *H. influenzae* [38]. These models have been useful platforms for the study of prevention of disease by vaccines [23], as well as treatment with antiviral [8], antibacterial [39] and anti-inflammatory drugs [40]. Although mice have been very useful for exploring the basic mechanisms underlying the pathogenesis of co-infections, most studies have been performed with a very limited set of mouse-adapted laboratory strains, leading to questions about how well the

results can be generalized to more clinically relevant strains. The use of genetic approaches to isolate specific virulence factors and study variations in sequence and related function has begun to answer some of these questions [41].

In the case of influenza viruses, evolutionary changes that occur during adaptation to mammalian hosts appear to greatly alter the propensity of viruses to support secondary bacterial infections. All influenza A viruses are zoonotic pathogens, sporadically entering humans from wild-bird reservoirs, either directly or through intermediate hosts such as pigs [42]. Several virological features common in the avian form of these strains act to promote bacterial superinfections. These include low levels of glycosylation on the main surface glycoprotein haemagglutinin (HA), high neuraminidase activity to complement the decrement in receptor binding affinity associated with increased HA glycosylation, and expression of an inflammatory PB1-F2 protein [43–46]. As these viruses adapt to the mammalian lung, either in humans or in pigs, they tend to lose their disease-associated phenotypes, with increased glycosylation of the surface proteins, lower neuraminidase activity, and either mutation to a non-functional form of PB1-F2 or loss of the active site through introduction of a stop codon and truncation of the resulting protein (Fig. 2)

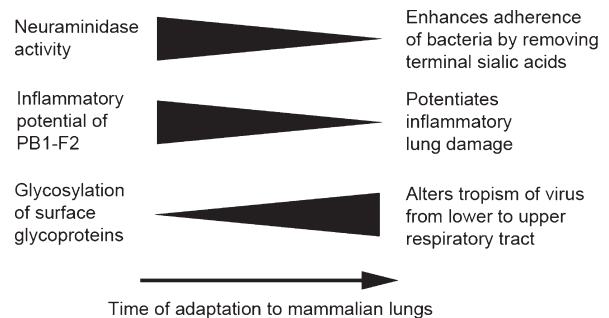


FIG. 2. Evolution of viral virulence factors that support secondary bacterial pneumonia. The schematic shows the typical changes over time of three virulence factors that support bacterial infections in animal models. During adaptation to mammalian hosts, influenza viruses tend to lose neuraminidase activity, which normally supports bacterial adherence through removal of sialic acids, which can block access to bacterial receptors. Similarly, the inflammatory activity of PB1-F2, which potentiates pneumonia, decreases through either mutation of key C-terminal amino acids or through truncation of the protein. The presence of key glycans on the surface proteins allows clearance of viruses from the lower respiratory tract through binding of collagenous lectins; these accumulate over time, altering the tropism of viruses from the lower to the upper respiratory tract, and diminishing viral-mediated effects on bacteria in the deep lung. Overall, the net effect of these changes is to decrease support for secondary bacterial infections.

[43,44,47]. Thus, most pandemic strains that enter humans directly from birds or after only a short adaptation period in pigs would be predicted to efficiently prime for bacterial superinfections, whereas well-adapted seasonal strains, including influenza B viruses, would be expected to engender less secondary disease. It is of note that the 2009 H1N1 pandemic strain coded for a truncated, non-functional PBI-F2 protein, which may have contributed to the lower death toll in the recent pandemic than that associated with bacterial infections complicating a strain such as the 1918 virus [45,48]. As more molecular signatures of virulence in the context of the ability to support secondary bacterial pneumonia are discovered, surveillance efforts should focus on identifying strains carrying these signatures as targets for public health concern [49]. Neuraminidase activity has been identified as a contributor to secondary bacterial pneumonia following paramyxovirus infections [33], but specific predictors of the ability to support bacterial superinfections for other viruses are currently unknown.

Variations in the relative contribution to disease during co-infections involving other viruses or bacteria are less well established. Animal data suggest that there is strain specificity in the ability of *S. pneumoniae* to cause pneumonia during influenza; isolated strains selected from different serotypes and genotypes had different growth kinetics in the lungs following influenza virus infection, resulting in differences in mortality [50]. Interestingly, these differences were not apparent in single-agent infections, suggesting a specificity for the interaction with the virus rather than simple amplification of inherent virulence. This implies that specific bacterial virulence factors, present in some strains but not in others, are responsible for genotype-related differences in the ability to interact with influenza viruses in the lung. These factors may also contribute to disease in the absence of a viral co-infection, or they may impact on pathogenesis only in the specific situation of superinfection. In the latter case, these virulence factors are likely to have been missed by traditional virulence screens in animals, as such screens are performed in 'clean' systems, with efforts being made to ensure that no adventitious agents are present. Understanding what these factors are and how they contribute to virus–bacteria synergism could form the basis for rational vaccine or drug-related approaches to the prevention or treatment of pneumonia. Similar data in mice suggest strain-related differences in the ability of *Staphylococcus aureus* to work with influenza [36]. It is of note that the USA300 strains that are now commonly causing fulminant pneumonia in association with influenza [27] were highly lethal in association with influenza in the mouse model as compared with other strains [36]. Strain-related differences in other pathogens associated with co-infections have not yet

been studied in animal models or in broader human epidemiological studies.

Implications for Human Disease

On the basis of animal model data, we can form a set of testable hypotheses about the role of co-infections in human disease. Expression of specific virulence factors appears to drive differences in co-infection models in mice. Although we know that different bacterial strains differentially code for and differentially express many virulence factors, little is known about the spatio-temporal distribution of bacteria by genotype and the frequency of expression of particular proteins. If there is significant geographical variation in expression of the bacterial virulence factors associated with secondary pneumonia during viral infections, then one might expect to see different rates of pneumonia in different locales during influenza or other viral epidemics. As a single example, although there was a high incidence of secondary bacterial pneumonia in Memphis during the first wave of the 2009 pandemic, including five deaths in children (unpublished data), a careful study in Milwaukee, a US city of similar size only 620 miles away, demonstrated that bacterial superinfections were very infrequent and typically mild in the same time frame [51]. Could differences in the strain distributions of *S. pneumoniae* and *Staphylococcus aureus* account for these disparate experiences during the same pandemic?

Addressing these and associated questions will require significant reorganization of bacterial surveillance efforts to account for both spatial and temporal patterns of bacterial prevalence. Longitudinal, multi-city and multi-country studies are necessary, and characterization of isolates must include advanced sequencing methods to establish genotypes beyond the simplistic categorizations currently utilized. An improved understanding of the specific virulence factors that underlie the synergism with viruses will also be necessary to guide this effort, as will the impact of specific single-nucleotide polymorphisms within virulence genes on pathogenesis. Databases that curate these data must be capable of capturing the relevant sequence, demographic and geographical information in a form that is usable for research. Once available, however, these data will be able to direct vaccine, antiviral and antibacterial development and application efforts by allowing multiple-pathogen interactions to be considered, to avoid some of the pitfalls associated with the single-pathogen paradigm. These factors should also be taken into account during clinical trials or preclinical efficacy studies. Secondary bacterial infections, particularly otitis media and pneumonia, should be considered as relevant endpoints for the study of both bacterial and viral

vaccines, and trials of antivirals and antibiotics should consider the role of viruses and the potential for different outcomes in single-bacterium infections than in co-infections [39]. Finally, strain diversity may be a key variable confounding many of these analyses, and may account for geographical differences in outcomes and reduce the ability to generalize from one study site to another.

Transparency Declaration

The authors declares no conflicts of interest.

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