Influenza has given us 2 opportunities within 5 years to critically evaluate our understanding of how infections caused by a relatively simple virus produce pathophysiologic mayhem and death. Concern about the potential devastating consequences of a species jump of H5N1 influenza virus from birds to humans stimulated extensive research on that virus, resulting in much new knowledge provided by investigators globally. In 2009, the world recognized another threatening viral event: the emergence and rapid spread of a reassortant H1N1 virus that has some genetic features of a swine flu virus and is the same serotype responsible for the 1918 flu pandemic blamed for the death of tens of millions. Because many who died in the 1918 pandemic had secondary lung infections caused by bacteria, as recently reaffirmed by painstaking analysis of autopsy records [1], it seemed prudent to gain enhanced understanding of the molecular mechanisms underlying this potentially devastating “one-two” microbial punch. It is against this backdrop that Lee et al [2] have produced the important work found in this issue of the *Journal*.

Influenza-associated mortality is among the leading causes of death in the United States [3]. However, influenza virus alone accounted for only 457 deaths in the United States in 2007, a death rate of merely 0.2 deaths per 100,000 persons [3]. By striking comparison, 52,847 deaths were attributed to combined influenza and pneumonia, which includes such comorbidity factors as bacterial pneumonia [3]. These data are consistent with a reemerging theme in our understanding of the pathogenesis of influenza. As noted above, the majority of deaths attributed to influenza during the 1918 pandemic were probably caused by secondary bacterial pneumonia [1], with streptococci, staphylococci, and *Haemophilus influenzae* being the most prominent pathogens recovered from patients who died [1]. Similarly, a study of 140 patients with influenza from Sheffield, England, who died in the 1957–1958 Asian influenza pandemic identified *Staphylococcus aureus* as being the most abundant pathogen isolated from sputum and necropsy material [4]. Louie et al [5] recently reported similar findings with the H1N1 influenza outbreak of 2009 and identified *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, and *H. influenzae* as key coinfecting pathogens present in postmortem lung specimens.

Previous studies in a mouse infection model demonstrated a lethal synergism between influenza virus and the pneumococcus [6]. Collectively, these data provide strong support for the idea that secondary bacterial infection is a significant cause of comorbidity and comortality among individuals with influenza. Although earlier work with animal infection models investigated comorbidity caused by influenza A virus and *S. pneumoniae* [6], there are few data that bear on similar comorbidity or comortality caused by *S. aureus*, the subject of the work reported by Lee and colleagues.

*S. aureus* is a leading cause worldwide of bacterial infections, manifested as a wide range of syndromes, including skin and soft-tissue infections and fatal necrotizing pneumonia [7]. The high prevalence of *S. aureus* disease is probably related in part to the tremendous ability of the organism to colonize humans. As many as ∼30% of healthy noninstitutionalized individuals are colonized with *S. aureus* in the anterior nares [8]. To make matters worse, *S. aureus* readily acquires resistance to antimicrobial agents [9]. Methicillin-resistant *S. aureus* (MRSA) is a significant problem in health care and/or community settings in virtually all industrialized countries. In the United States, community-associated MRSA (CA-MRSA) is the leading cause of bacterial infections outside of health care facilities [10]. In contrast to health care–associated
MRSA (HA-MRSA), which causes infections in individuals with predisposing risk factors, CA-MRSA strains typically cause disease in individuals with no such risk factors. Thus, the number of persons at risk for infection is very large. On the basis of these observations and data from animal infection models, the most prominent CA-MRSA strains in the United States appear to have enhanced virulence compared with typical HA-MRSA strains. These CA-MRSA strains are also known for their ability to cause severe disease, such as necrotizing pneumonia [11–14]. S. aureus necrotizing pneumonia is often associated with strains containing genes that encode Panton-Valentine leukocidin (PVL), as well as antecedent influenza A virus infection [11, 14–16]. The roles played by PVL and antecedent influenza virus infection in the pathogenesis of S. aureus pneumonia are unknown. Moreover, the high prevalence of CA-MRSA and influenza poses a serious health concern [17].

Lee and colleagues highlight the importance of antecedent influenza A virus infection in the pathogenesis of S. aureus pneumonia. Using a mouse pneumonia model, they discovered that antecedent influenza virus infection promotes rapid death in animals subsequently infected with CA-MRSA strains. Mice were infected with S. aureus, either alone or in combination with low or high doses of a mouse-adapted influenza virus (an H1N1 strain known as influenza virus A/Puerto Rico/8/34), followed 3 days later by low or high doses of CA-MRSA strains. Neither low-dose influenza virus alone nor low or high doses of bacteria alone caused death in mice. In contrast, all mice infected with high doses of both pathogens were dead within 2 days after coinfection, and animals infected with a low dose of influenza virus and a high dose of bacteria died 7 days after challenge with S. aureus. Thus, antecedent influenza virus infection significantly accelerated death after S. aureus coinfection. These mouse model findings are consistent with observations from human pandemic influenza [4] and the recent increase in mortality related to seasonal influenza virus–S. aureus coinfection among young children [17].

An important question remains: What is the molecular basis of this increased mortality after influenza and S. aureus coinfection? Is death due to enhanced bacterial colonization of the lungs or, alternatively, an overwhelming inflammatory response? There was a trend toward increased numbers of bacteria in lungs after antecedent influenza compared with bacterial infection alone, although the difference was significant at only 1 of the 4 time points tested. This trend is consistent with the presence of bacteria in autopsy material from patients who died of influenza virus–bacteria coinfection [1, 4]. By comparison, there was a dramatic increase in the ability of S. aureus to disseminate from the lungs to spleen, kidney, and liver in animals with antecedent influenza, compared with those who had bacterial infection alone. These findings are in accordance with the capacity of S. aureus to disseminate to sterile sites in humans during influenza coinfection [17] and suggest that the lung histologic architecture was significantly disrupted by coinfection. Indeed, infection with influenza virus followed by S. aureus caused increased acute inflammation and lung tissue injury compared with bacterial infection alone, and pathology was localized to the trachea in animals infected with influenza virus alone.

More neutrophils were present in infected lungs than in those infected with bacteria alone. Neutrophils are the primary cellular defense against bacterial infections, and patients with neutrophil defects have enhanced susceptibility to S. aureus infections. On the other hand, neutrophils generate or release microbial components that are not specific for invading microorganisms. As a result, these cells can cause significant host tissue damage and further inflammation—a type of “friendly fire” pathology. Influenza virus has multiple effects on neutrophils, including up-regulation of adhesion molecules [18], activation via Toll-like receptors [19], altered apoptosis and turnover [20], and enhanced recruitment to the lungs [21]. In addition, death caused by S. aureus pneumonia in animal models involves recruitment of neutrophils by a mechanism involving chemokines elicited by S. aureus α-hemolysin (Hla) [22]. Accordingly, transcripts encoding Hla and protein A, molecules known to be involved in the pathogenesis of S. aureus pneumonia [23, 24], were significantly increased in mice coinfected with influenza virus and S. aureus, compared with those infected with bacteria alone [2]. Thus, a constellation of factors probably contribute to the unchecked inflammatory response, which ultimately leads to death in animals coinfected with influenza virus and S. aureus.

Another noteworthy finding by Lee and colleagues was the lack of a significant difference in the number of wild-type and isogenic PVL-negative CA-MRSA strains recovered from lungs of animals with antecedent influenza. These data indicate that PVL fails to promote bacterial survival in this animal model. Furthermore, expression of lukS-PV, a gene encoding 1 of the 2 PVL subunits, was very low during coinfection. These data are surprising, given the epidemiologic association of PVL with S. aureus necrotizing pneumonia and antecedent influenza [16]. One possible explanation is that mouse neutrophils are less susceptible than human cells to PVL-mediated cytolysis, a notion supported by in vitro data [25]. Alternatively, PVL may have a limited role in most infections, or host susceptibility factors may play a prominent role in determining the relative contribution of PVL to pathogenesis in human infections.

Coinfection studies are technically challenging, and we applaud the investigators for providing new information about how antecedent influenza promotes S. aureus pathogenesis in mice. Nonetheless, a few caveats are in order. First, although mouse infection models are widely used to study
bacterial pathogenesis, they do not fully reflect events occurring in humans, in part owing to differences between these species in neutrophil distribution and function. Mice are also relatively resistant to infection with *S. aureus*, and mouse infection models use relatively high doses to cause disease. Another factor to consider is the timing used by Lee and colleagues for coinfection with *S. aureus* (72 h). Although 72 h worked well to show cooperation between the 2 organisms, one wonders how important timing is for human coinfection and related mortality. It will be important in future studies to resolve the timing issue and quantify virus at the various points of the infection cycle, because such knowledge may dramatically affect effective treatment.

Combined influenza and pneumonia, which largely involves influenza with bacterial coinfection or secondary infection, remains a leading cause of mortality in the United States (ranked eighth in 2007) [3]. Future research in this area is critical if we are to develop new approaches for the prevention and treatment of influenza and pneumonia. Such efforts should include a refinement of current coinfection models, development of new animal models that better approximate the human condition, and the application of cutting-edge technologies, including high-throughput whole-genome sequencing and systems biology approaches.

To summarize, the findings of Lee and colleagues are important for several reasons. First, they strongly support the idea that although influenza virus infection alone produces significant morbidity, coinfection or sequential infection with influenza virus and a common pathogenic bacterium may produce devastating disease and death. Second, the data suggest that early diagnosis and treatment of bacterial pneumonia are critical to decreasing the number of influenza-related deaths. Third, given that many studies have shown that a significant proportion of influenza-related deaths are linked to pneumonia caused by *S. pneumoniae*, one must conclude that enhanced emphasis on deployment of the available vaccines against this bacterium is warranted. Accordingly, renewed emphasis on the formulation of novel therapeutics or vaccines against *S. aureus* and *S. pyogenes* (group A *Streptococcus*) is critical, because these organisms also contribute significantly to influenza-related deaths [1, 4, 5].

References


