Implications of Non-Pharmaceutical Interventions for Mitigating Influenza in Schools and the Role of Co-Infection in the University Setting

by

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Dedication

For my family and friends.

Acknowledgements

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Abstract

The 2009 influenza A (H1N1) pandemic resulted in a renewed focus on non-pharmaceutical interventions (NPI) due to a delay in the development of an appropriate vaccine. Having determined that co-occurrence with either bacterial or viral pathogens may influence susceptibility to acute respiratory illness (ARI), we conducted one methodological review of co-occurrence as well as three novel studies, using available data from NPI studies in the community setting. We sought to examine risk factors for co-occurrence and to assess associations between school closures and influenza-like illness (ILI), a type of ARI. This research furthers knowledge regarding common respiratory agents, providing estimates of the prevalence of common viruses and bacteria as well as co-occurrence in a college-aged population. Additionally, we utilized surveillance data from the state of Michigan to provide a quantitative analysis of the effects of school closure. Our findings indicated a high prevalence of human coronaviruses during the season of interest among otherwise healthy adults with ARI, with coronavirus infected students having a decrease in symptomatic cough and chills over the 6-day illness episode measured, and runny nose increasing. A second finding of an association between viral infection and *Streptococcus pneumoniae* colonization suggests that ARI symptoms in young adults can be indicative of a viral and bacterial co-occurrence, and the screening for both can provide a better understanding of the causes of ARI. The finding suggested that a variety of underlying infectious agents cause ARI among young adults living in residence halls, suggesting this environment is conducive to

ARI transmission. Finally, this dissertation found a minimal effect of reactive school closures on community levels of ILI during the 2009 H1N1 influenza A pandemic. While this finding does not disprove the effectiveness of school closure as an NPI, it does suggest that reactive school closures may not be effective once illness is already in the community. We discuss implications from these findings in order to better understand and address issues related to ARI in the real-world setting.

Chapter 1: Introduction

1.1 Introduction

This dissertation focuses on two areas relevant to the study of infectious disease within the field of public health: acute respiratory infections (ARIs) and non-pharmaceutical interventions. ARIs are common causes of illness during the winter months in the northern hemisphere [1]. Advances in molecular techniques, and the subsequent affordability of using these techniques, have allowed for an expansion beyond a single-agent, single-illness approach to ARIs. Using these techniques, this dissertation studies co-occurrence: the interaction between multiple agents detected from a single individual at a single time point. The second focus of this dissertation is analysis of the use non-pharmaceutical interventions, specifically the use of school closure, in order to prevent the spread of infectious agents. I specifically examined the effectiveness of steps taken by individual school districts during the 2009 H1N1 influenza A pandemic.

Overview of Co-Occurrence

Co-occurrence, also known as co-infection, is defined as an infection caused by more than one microorganism that interacts within a host [2-4]. In some cases, co-occurrence can either cause or prevent transmission of agents and subsequent infection. In other cases, it has no effect. As more viruses are being tested for, basic epidemiological research can be useful for identifying common co-circulating agents. Future research will be necessary to determine the effects of these interactions at the molecular or clinical level.

The concept of co-occurrence is not new. Deaths resulting from the 1918 influenza A pandemic have long been attributed to secondary bacterial pneumonia [5, 6]. However, studies of interactions at the molecular level, without evident clinical or population level outcomes, have become more common[2, 7-12]. The ability to describe underlying infections or carriage of agents can help to explain patterns of disease. We hope that disease pattern information can be used to prevent transmission in at-risk populations where evidence of clinical severity is apparent.

Overview of Non-Pharmaceutical Interventions

Non-pharmaceutical interventions (NPIs) are commonly thought of as actions implemented to prevent the spread of illnesses without the use of drugs [13]. For influenza, they have been practiced since at least the 1918 influenza pandemic [14, 15], and take a myriad of different approaches. The Centers for Disease Control and Prevention (CDC) distributed a list of recommended NPIs during the 2009 H1N1 influenza A pandemic, which included both individual and community prevention recommendations. Individual interventions included covering the mouth and nose during coughs and sneezes, increasing hand hygiene through frequent washing or use of alcohol-based hand sanitizer, or staying home when sick. Community-level interventions suggested included temporary school closures, flexible sick-leave policies for companies, and postponing mass gatherings [13]. While these interventions are suggested during any outbreak to reduce the risk of transmission, they are especially relevant during a novel pandemic, where the ability to vaccinate or treat with medication is limited [16-18].

School closure remains a popular option, primarily due to high rates of infection in children, who can then spread infection to caretakers in the household [17]. Several studies have modeled the effectiveness of school closure as an NPI to prevent the spread of ARIs, though few studies have taken a comprehensive look at the effects of school closure on community transmission [19]. Sequestration, or the voluntary isolation of an individual, has been utilized since 1918. The idea behind the intervention is to prevent sick individuals from being able to infect people with whom they would come in contact during the normal course of their day.

Taken together, the study of co-occurrence and NPIs are areas of growing concern for public health researchers. Using two different datasets, the purpose of this dissertation is to 1) provide a framework for thinking about co-occurrence; 2) explain the role of viruses and virus co-occurrence among young adults living in a university setting; 3) explain the role of viral and bacteria co-occurrence among young adults living in a university setting; and 4) quantify the effects of reactive school closure on circulating levels of influenza-like illness (ILI) in the community setting.

1.2 Specific Aims and Hypothesis

Aim 1: Provide a descriptive framework for conceptualizing co-infection between more than one agent at a molecular, clinical, and epidemiological level.

Aim 2: Examine epidemiological characteristics of human coronaviruses among young adults with acute respiratory tract illness and their acute respiratory tract negative social contacts living in residence halls.

Hypothesis 2a: We will observe a high prevalence of human coronaviruses and other respiratory in our young adult population.

Hypothesis 2b: The prevalence of the four human coronaviruses tested will be higher among subjects with acute respiratory illness compared to those without acute respiratory illness.

Hypothesis 2c: Symptoms associated with acute respiratory tract illness symptoms will be higher among our population with a human coronavirus infection compared to human coronavirus negative participants.

Aim 3: Examine the associates of bacteria and viral co-occurrence among young adults with acute respiratory tract illness and their healthy contacts living in residence halls.

Hypothesis 3a: The presence of a viral infection will be associated with an increase in the likelihood of bacterial colonization.

Hypothesis 3b: We will observe a positive association between symptomatic upper respiratory tract infection and the likelihood of bacterial colonization.

Aim 4: Estimate the effects of school closure on community levels of influenza-like illness in Michigan during the 2009 H1N1 influenza A pandemic.

Hypothesis 3a: For the period of three weeks leading to peak infection and four weeks following peak infection, school districts with a higher proportion of closure will result in a lower rate of influenza-like illness.

Hypothesis 3b: School closure during the 2009 H1N1 pandemic were reactive in nature based on peak rate of influenza-like illness within each school district.

1.3 Overview of Dissertation Studies

This dissertation uses two different data sources to address the research questions related to acute respiratory illness. Aims 2 and 3, covered in chapters 3 and 4, examine the detection of several ARI viruses, and ARI and bacterial co-occurrence. These chapters use data from the eX-FLU study, a prospective and novel study designed to assess the effect of sequestration on the transmission of infectious agents among social contacts living in university residence halls. The eX-FLU study was conducted within six residence halls on the University of Michigan campus.

Participants were recruited beginning in October of 2012 and continuing through the start of the study on January 17, 2013. Participants were eligible if they lived in an approved residence hall, were at least 18 years of age, and were willing to participate in the study. The students were assigned to one of 117 clusters, with each cluster having an equal probability of being assigned to either of the intervention groups. This study was funded by the Centers for Disease Control and Prevention (CDC) and approved by the University of Michigan IRB.

To answer the research questions for Chapters 3 and 4 on the burden of disease in college students, samples collected from symptomatic and asymptomatic eX-FLU participants were tested for common respiratory viruses and bacteria. If students experienced influenza-like illness, defined as cough plus one other constitutional symptom (body aches, feverishness, chills, or a temperature over 100.4°F), they were asked to provide three throat swabs over the course of their illness: the first within 24 hours of illness onset, the second within 72 hours of illness onset, and the final within 144 hours of illness onset. Using the most recent reported list of social contacts, an email invitation was sent automatically to a sick individual's healthy contacts (i.e., a participant who had not been ill in the past two weeks). These healthy contacts were invited to

provide three samples over six days to assess bacteria and viruses among asymptomatic participants.

Aim 4 of the dissertation, covered in Chapter 5, uses data collected during the 2009 influenza A (H1N1) pandemic in the state of Michigan. This dataset looks at the effects of reactive short-term school closure on community levels of influenza-like illness (ILI), a type of ARI, measured through surveillance. The Michigan Department of Community Health (MDCH), with the assistance of the CDC, collected real-time data on schools that closed during the Fall 2009 school semester. The location of the closed school, timing of closure and re-opening of the school was recorded for all known school closures.

These data were then combined with a publically available databases of schools and school districts provided by the Michigan Department of Education and the Center for Educational Performance and Information, containing school-level variables related to the status of each school (public, private, or public charter), grade levels taught, and school district information for each school in the state. Private schools and public charter schools were given a geographical public school district, which, combined with the public school districts, amounted to 551 school districts and over 4,300 active schools in the 2009-10 school year.

Finally, the outcome of interest in Chapter 5 came from surveillance data provided by the MDCH. Influenza-like illness reports were used from September 1 to December 31, 2009. The date of collection and date of onset were recorded, as was the zip code reporting the over 7,000 suspected influenza cases. These data were aggregated at the zip code level and week of illness reporting, and a rate was assigned to each school district.

School closure is considered an especially important NPI due to the ability for infection to spread within a school and then brought back into the household, and therefore the community

[13, 20]. The eX-FLU study was conceived to examine the effect of a NPI on reducing transmission without a pharmaceutical intervention, though was mined for this dissertation to provide an overview of the burden of viral and bacterial infection independent of the NPI. Taken together, both studies evaluate the role of NPIs with suggestions for preparing for future pandemic outbreaks where traditional vaccination and medication may be unavailable.

1.4 Infectious Agents

Chapters 3, 4, and 5 of this dissertation examine the role of infectious agents causing ARI. A general discussion of the main agents examined, as well as their clinical and epidemiological presentations, follows.

Influenza A

All aims of this dissertation report on influenza or influenza-like illness. Influenza-like illness is a constellation of respiratory symptoms, and a more specific type of ARI. Influenza-like illness is frequently used as a proximate measure for influenza used for surveillance in the community, or for defining a flu-like illness without laboratory confirmation [21-23]. Here, we describe the epidemiology of influenza virus and its public health importance. Influenza is transmitted by respiratory droplets through either direct or indirect contact. Each year, seasonal influenza infects hundreds of thousands of people worldwide and accounts for an estimated 36,000 deaths in the United States [24], with pandemic strains often causing greater mortality [25, 26]. Complications from influenza are most severe among the young, the elderly, and those with a compromised immune system [27]. In order to reduce morbidity and mortality associated with influenza, a seasonal vaccination is developed annually. Influenza vaccination aims to

protect individuals from the most common circulating strains of virus. However, the effectiveness of influenza vaccination has not approached levels that public health professionals would hope, and recent estimates suggest that age is a factor in effectiveness. Studies in young children show influenza vaccination to be efficacious in up to 90% [28, 29], though efficacy in young adults has ranged from 30 to 77%, depending on how the sample was identified and the type of vaccine (live attenuated compared to inactivated) [30]. The wide range in efficacy appears to depend on subtype and strain of influenza, method of identifying titers and positive samples, type of vaccine provided, and underlying health status of individuals in the study [31, 32].

At the molecular level, influenza is a ribonucleic acid (RNA) virus with eight core genomes: PB2, PB1, PA, HA, NP, NA, M, NS [33]. There are two regions of the influenza A virus that determine subtype: the hemagglutinin (HA) and neuraminidase (NA) regions. Variations in these regions as a result of viral shift, (a significant change in either the HA or NA region), or viral drift, (a seasonal change within the HA or NA region), can result in a reduced innate immune response and an increase in the risk of infection. Specific HA and NA sites have also been linked to severity of disease in human populations.

Influenza is an important global health problem, and one reason for this is the role of secondary infections. Severe complications from influenza include viral pneumonia or bacterial infections such as bacterial pneumonia, bronchitis, sinus infections, ear infections, or death [34, 35]. These risks are especially pronounced among the elderly population, young children, and individuals with chronic diseases.

Human Coronaviruses

Chapter 3 of this dissertation focuses on four predominantly respiratory human coronaviruses (HCoVs): 229E, HKU1, NL63, and OC43. Two strains, HCoV-229E and HCoV-OC43 were studied in the 1960's as part of human challenge studies [36, 37]. These viruses were linked to the common cold and were not thought of as particularly harmful [36]. In 2003, a novel HCoV, severe acute respiratory syndrome coronavirus (SARS-CoV) emerged, with a 10-50% mortality rate [37]. This lead to a renewed search for HCoVs, resulting in two more globally circulating viruses being identified: HCoV-NL63 found in the Netherlands in 2004 [38] and HCoV-HKU1 identified in China in 2005 [39]. Both viruses, subsequently discovered to circulate globally, had been present for years before identification [38-45].

Coronaviruses are enveloped RNA viruses from the family *Coronaviridae* ranging from 70 to 120nm and encircled by spiked glycoproteins [37]. The family can be found in a wide range of animal species, and the lethal SARS-CoV and Middle East respiratory syndrome (MERS-CoV) were both believed to have evolved to cause human illness from animal reservoirs [37].

The four HCoVs studied in this dissertation are often believed to cause the common cold in healthy individuals [36]. Recently, these viruses have been shown to cause pneumonia in immunocompromised patients [46]. They have also been associated with upper and lower respiratory tract infections including bronchitis, bronchiolitis, pneumonia and croup in hospitalized children and elderly patients [43, 47-49]. Concerns have been raised about the lack of vaccinations for the HCoVs, but without quality prevalence estimates it is difficult for policy makers to assess community level needs [37].

Upper Respiratory Tract Bacteria

Chapter 4 of this dissertation reports on the presence and co-occurrence with viruses of three different upper respiratory tract bacteria: *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. Below is a brief overview of the three bacterial species.

Haemophilus influenzae is a gram-negative coccobacillary bacteria frequently colonizing in nasopharaynx region of children. There are six currently identified subtypes of *H. influenzae*, with serotype b (HIB), for which there is a vaccination. However, all strains of HIB are linked to bacteremia and pneumonia, specifically among children [50]. Non-typable *H. influenzae* infections colonize the upper respiratory tract and can cause illness in children and adults. These nontypeable serotypes are often causes of disease post-HIB vaccine [50].

The pathogenesis of infection among nontypeable strains of *H. influenzae* is through contagious spread, often from subjects colonized in the upper respiratory tract [50]. More than one half of children are colonized by the age of five, and transmission often occurs in day care centers [50]. Once colonized in the upper respiratory tract, *H. influenzae* infection has been associated with acute otitis media [51], and sinusitis [52] among children. The advancement of the pneumococcal vaccine has resulted in an increase in sinusitis as a result of *H. influenzae* [53].

Staphylococcus aureus is a gram-positive coccal bacteria that can colonize both healthy and at-risk individuals. Longitudinal studies have suggested individual differences in colonization of the nares– 20 to 30% of individuals are persistently infected, 30% are occasionally colonized, and up to 50% are rarely colonized [54]. Colonization of the nares is common and of the greatest interest for our research purposes. Invasive illness, such as bacteremia, pneumonia, and meningitis can occur; however, illness risk is highest among young children, elderly adults, and immunocompromised individuals [55].

Streptococcus pneumoniae is one of the most common colonizers of the upper respiratory tract among children and adults, and the most frequent cause of bacterial pneumonia [56]. A gram-positive bacteria, it is a leading cause of meningitis, otitis media, and bacteremia in the United States. Severe illness often targets young children, the elderly, or those with a suppressed immune system. *S. pneumoniae* is carried by an estimated 11% of the U.S. population [57], with a higher percentage of children being carriers. While there are over 90 strains of *S. pneumoniae*, few have been linked to invasive disease [58]. The most common strains have been targeted through vaccinations, such as the 7-valent and 23-valent vaccinations.

1.5 Bacterial Co-Occurrence

Chapters 2 and 3 of this dissertation deal with viral and bacterial interactions. Chapter 2 lays out a method of conceptualizing bacterial and viral interactions using influenza and pneumonia as a model. In reviewing historical studies of 1918 pandemic, researchers have hypothesized that secondary bacterial infections played a large role in the high number of deaths recorded [5, 6]. However, the frequency and timing between infection has not been well established, which has implications for prevention and treatment, as well as policy recommendations for groups most at risk for co-occurrence [59]. The most common results of secondary bacterial infection following influenza include otitis media (OM) in children [2] and bacterial pneumonia in the elderly [60]. With over 90 serotypes identified, and multiple licensed vaccines*, S. pneumoniae* remains one of the best researched of the secondary infection bacteria and a good model organism to explain secondary bacterial infection following initial influenza infection (13).

Within an individual, response to influenza is believed to play a role in susceptibility to bacterial infections that cause pneumonia [61]. This idea was originally observed by reports following the 1918 influenza pandemic. A viral infection that damaged the mucosal lining in the lungs, providing an opportunity for bacterial infection to take root, was cited as a potential reason for the widespread observation of bacterial pathogens found upon death. As laid out by McCullers (2006), these changes in the respiratory tract can occur through multiple pathways, such as epithelial damage, decrease in airway functioning, and changes in receptors within the tract [35].

Bacteria that cause community-acquired pneumonia (CAP) are transmitted through person-to-person direct contact, but the infectivity and progression to bacterial pneumonia are thought to be lower than influenza, and many of the CAP-causing bacteria are found in healthy individuals. The most common causes of CAP are *S. pneumoniae, S. aureus, H. influenzae, Mycoplasma pneumoniae,* and *Chlamydophila pneumoniae*, although there are regional variations [57].

A comprehensive understanding of co-occurrence requires examining the problem across different levels of measures: the molecular level, or what occurs between a virus and another virus or a virus and a bacteria within a system; the clinical level, or how two agents may interact to result in a more severe disease within an individual; and the population level, or how rates of disease in communities may be affected by the presence of multiple agents. Co-occurrence can also be thought of on axis of timing (whether one agent preceded another, of if the infection occurred simultaneously) and on an axis of effect (synergism or antagonism) [59].

1.6 Public Health Significance

Healthy young adults living in residence halls remain at risk for a variety of illness in the community setting. They are also transitioning from childhood, with high levels of bacterial and viral colonization and infections, to adulthood, where innate immunity reduces the risk of severe illness. Studying college-aged students will allow us to estimate prevalence of infectious agents in a previously understudied population. This dissertation will describe the rates of colonization and co-colonization observed in this population, with an impact in other university settings with large clusters of young adults, as well as other community situations in which young adults co-mingle.

Coronaviruses in particular are under study, in the community as well as among adults [37, 62]. As interest in severe strains of coronaviruses grow, basic epidemiology about carriage, as well as co-occurrence, can provide researchers with important information both about vaccination targets and usefulness [37], as well as enlighten any risks for a viral shift that may occur through co-occurrence [62].

Identifying the prevalence of common respiratory tract bacteria in the university setting is a goal relevant to public health. Indeed, colds and upper respiratory infections have been shown to cause significant morbidity in the university setting [63]. The close contact that occurs between students, both in residence halls, and in classrooms, provides both an interesting study design for the transmission of illness [64], as well as an effective laboratory for testing non-pharmaceutical interventions [65].

Studies on the link between school closure and community levels of influenza-like illness flourished following the 2009 influenza A (H1N1) pandemic, but no research has prospectively examined the extent of district level closure across a state. By identifying a novel approach to

measuring rates of ILI and school closures, we can use the natural experiment of school closures during the 2009 pandemic to assess the effectiveness of this NPI. Given the growing interest in school closure as a response to pandemic levels of influenza, this dissertation will provide invaluable information on reactive school closures that have major implications for containment of illness in a community environment.

To address these issues, Chapter 2, entitled *Influenza and Community-acquired Pneumonia Interactions: The Impact of Order and Time of Infection on Population Patterns*, describes a theoretical framework for thinking through bacterial and viral co-occurrence. The chapter discusses the importance of timing of infection, and at what levels of public health research information is needed to have a better understanding of co-occurrence research.

Chapter 3*, High Prevalence of Human Coronaviruses in a University Setting*, describes the prevalence of viral ARI infectious agents in a college setting. Using data collected over 6-days from the start of illness onset, this chapter also examined the symptoms associated with frequently identified viruses, and how those symptoms changed over the course of the illness.

Chapter 4, *Viral infection is associated with increased Streptococcus pneumoniae carriage among otherwise healthy college students*, used the premise discussed in Chapter 2 to look at the interaction with bacteria and viruses in college students, using measures of bacterial load and the presence or absence of viruses in the same college population.

Chapter 5, The Effect of Reactive School Closure on Community Influenza-Like Illness Counts in the State of Michigan during the 2009 H1N1 Pandemic, stepped back from the previous studies of individuals with infection to look at community level differences in ARI. Using data collected during the 2009 influenza pandemic, school closures were linked to surveillance data of ILI in the state of Michigan. This study expressively looked at how effective

a NPI, part of the national dialogue on dealing with pandemic influenza, was as it implemented in real-time.

Finally, Chapter 6 brings together the research and discusses future areas for research in ARI and NPIs.

Chapter 2: Influenza and Community-acquired Pneumonia Interactions: The Impact of Order and Time of Infection on Population Patterns

2.1 Introduction

Community-acquired pneumonia (CAP) often follows influenza infection. The hypothesized synergistic interaction resulting from co-infection with influenza and agents of CAP is thought to be a major factor in the severity of the 1918 influenza A pandemic [66, 67]. Today, up to 20% of persons who have CAP show evidence of recent exposure to the influenza virus [68], and pneumonia is a leading indicator of influenza severity [35]. Although *Staphylococcus aureus* is a relatively uncommon cause of CAP after influenza infection, methicillin-resistant *S. aureus* has been considered an important pathogen in deaths of co-infected pediatric patients. Data from the Centers for Disease Control and Prevention on the 2004–2007 influenza seasons showed that methicillin-resistant *S. aureus* was present in 60% of the 20 pediatric patients who died from *S. aureus* co-infection, with the highest rate during the 2006–2007 season [69], which suggests that the problem of antibiotic resistance among children with CAP in this age group is growing.

Influenza is transmitted via respiratory droplets, through either direct or indirect contact, and is highly infectious. Each year, seasonal influenza infects hundreds of thousands of people worldwide and accounts for an estimated 36,000 deaths in the United States [24], with pandemic strains often resulting in higher mortality rates [25, 26]. Complications from influenza are most severe in the young, the elderly, and persons with compromised immune systems [27]. Bacteria that cause CAP are transmitted through person-to-person direct contact; however, the rates of infectivity and progression to pneumonia are thought to be lower than those of influenza, and

many of the CAP-causing bacteria are found in healthy individuals. The most common causes of CAP are *Staphylococcus pneumoniae, S. aureus, Haemophilus influenzae, Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*, although there are regional variations [57]. Although investigators performing studies in which they used animals and experimental systems have begun to address how co-infection may enhance pathogenesis, there is little in the literature on the impact of co-infection on transmission of or susceptibility to bacteria. Perhaps most importantly, although population-level synergy has been observed, the timing of transmission related to the order of infection from each agent has not been definitively established [70, 71]. Understanding the order and timing of this synergistic relation and the resulting population-level effects is especially relevant to epidemiologists who are engaged in planning for pandemics.

In the present commentary, we use *S. pneumoniae*, the most common cause of CAP, as a model organism to explore the relation between influenza A and CAP. With over 90 identified serotypes and multiple licensed vaccines, *S. pneumoniae* remains one of the best researched of the pneumonia-causing bacteria [58]. We present 3 pathways to co-infection and discuss the impact of order and timing of co-infection on what epidemiologic patterns might be observed. We close with a discussion of outstanding research questions and their implications for CAP prevention.

2.2 Pathways to Co-Infection

There are 3 possible pathways to co-infection: 1) infections with both etiologic agents occur essentially simultaneously; 2) S. pneumoniae colonization precedes influenza infection; and 3) influenza infection precedes *S. pneumoniae* colonization (Figure 2.1). Each of these pathways is possible (although they likely occur at different frequencies); however, we expect

the resulting population patterns to differ depending on the relative frequency of each pathway, as will the implications for control measures (discussed below).

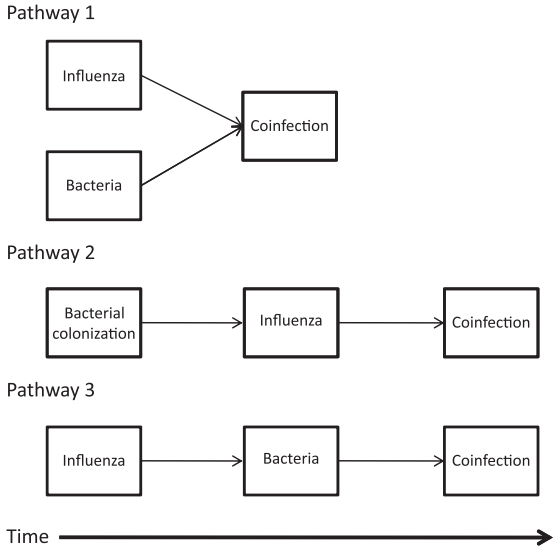


Figure 2-1: Schematic of 3 pathways of influenza and bacterial pneumonia exposure that lead to co-infection

Simultaneous acquisition of influenza and S. pneumoniae

Viruses and bacteria can be transmitted together through coughing or sneezing [71-74]. However, the extent to which simultaneous infections with influenza and *S. pneumoniae* lead to CAP is uncertain. In one of the few animal studies in which simultaneous infections were studied, investigators found no evidence of lethal synergy in mice infected with sub lethal titers of both influenza and *S. pneumoniae* [75]. In humans, influenza infection alone increases cough, potentially facilitating transmission of both viruses and bacteria [35]. Additionally, influenza replicates more rapidly than does bacteria, so even if the infections are acquired simultaneously, the clinical manifestations in an individual would appear sequentially. At the population level, we might expect little or no lag between peaks in influenza and pneumonia occurrence after simultaneous infection. Potential course-of-infection and research questions related to concurrent infection are presented in Table 2.1.

S. pneumoniae colonization preceding influenza

If *S. pneumoniae* colonization precedes influenza infection, changes in the upper and lower respiratory tracts resulting from the influenza infection would enable the colonizing strain of *S. pneumoniae* to successfully avoid a host immune response, invade the lung, and cause pneumonia. Although it is assumed that bacteria that colonize the nasopharynx frequently enter the lungs, the host immune response is usually sufficient to prevent infection. The introduction of influenza primes the lungs for bacterial colonization and adversely affects the host response, leading to secondary pneumonia [35, 61, 71]. In this case, the endogenous bacteria exploit an opportunity that may not have presented itself in the absence of an influenza infection. The prevalence of *S. pneumoniae* colonization ranges from 19% in children [7] to approximately 11% in adults in the United States [57]. Thus, for pneumonia caused by *S. pneumoniae*, the relative contribution of co-infection would be age-dependent and rely upon rates of colonization among the elderly or adult interaction with children who had an *S. pneumoniae* infection.

There have been few animal models in which this pathway was studied directly. In one mouse model, the introduction of influenza A 3 days after inoculation with *S. pneumoniae* led to an increase in the presence of pneumonia and facilitated influenza transmission to littermates [70]. However, in a second mouse model, investigators found that *S. pneumoniae* colonization before influenza infection was protective compared with influenza infection before *S. pneumoniae* colonization [75]. These studies seem to suggest that *S. pneumoniae* can facilitate transmission of influenza, but they provide insufficient evidence for any synergy in terms of co-infection.

At the population level, we would expect a limited time lag before the onset of bacterial

pneumonia (Table 2.1). Because the bacterial agent has had a chance to proliferate in the host, we would expect more rapid proliferation once the lungs were no longer able to clear the bacteria. However, because acquisition of a new bacterial isolate is required, we could anticipate that the onset of co-infection would be much more variable and would be associated with individuals with high risk of exposure, such as those who had contact with young children.

Influenza infection preceding S. pneumoniae colonization

Clinically, it is assumed that bacterial pneumonia associated with influenza results from a bacterial infection that follows the influenza infection. Within an individual, the typical course of secondary bacterial pneumonia involves initial recovery from influenza followed by secondary symptoms, such as cough or fever, 4–14 days later [76]. Results from animal models support this clinical observation. When mice are exposed to both wild-type and laboratory influenza strains and various strains of *S. pneumoniae* and *S. aureus*, there is an increased number of pneumonia infections, as well as an increased likelihood of death, compared with when any agent is given alone [71, 75, 77-79]. In the aggregate, these experiments strongly support the idea of a synergistic interaction between influenza and *S. pneumoniae* (and *S. aureus*) when infection occurs sequentially.

At the population level, we would expect to see a relatively long lag time between influenza infection and the presence of CAP because of the time needed for the influenza to reduce the ability of the lungs to clear a bacterial infection. This lag time would enable partial recovery before the bacteria could infect the individual. The bacterial agent would then have to proliferate within the host before the host began to show symptoms of CAP. This lag was observed when data from the 1918 pandemic were reanalyzed. A graph of the timing from

influenza infection to death found a lag period of 7–21 days [6], which suggests that affected individuals recovered before developing a more severe bacterial infection. Other research on data from the 1918 influenza A pandemic that suggests the role that secondary bacteria may have played has been supported by similar death curves created from data from infection with bacterial pneumonia alone through the 1920s and 1930s [5].

Pathway	Relevant Research Questions	Population Implications
Cotransmission	Is a cough or sneeze sufficient for simultaneous infection, or must fomites play a role?	Short or no lag observed between peak of influenza infection and peak of bacterial pneumonia infection
	Does bacterial survival depend upon droplet size?	
Bacterial colonization followed by influenza infection	Does the body exert more effort to keep certain bacteria in check? If so, is an individual with those bacteria more likely to become infected after influenza infection?	Short lag between peak of influenza infection and peak of bacterial pneumonia infection
	Can probiotics or prebiotics be used to prevent colonization with pneumonia-causing bacteria?	
Influenza infection followed by bacterial colonization	What role do children play in transmitting bacteria to influenza-infected adults?	Long lag between peak of influenza infection and peak of bacterial pneumonia infection
	How can we prime the immune system to prevent secondary infection from bacterial pathogens?	

Table 2-1: Examples of Relevant Research Questions for Each Pathway of Influenza and Bacterial Pneumonia Co-infection

2.3 Research Questions and Implications

Specifying the pathways to co-infection may help to identify new research questions about the interaction between influenza and CAP (Table 2.1) that can only be answered through basic science, clinical, and population studies. Basic laboratory science studies of the interactions within animal models have provided the most information to date for understanding the interactions between influenza and *S. pneumoniae* because unlike in human populations, experiments in laboratories can control the timing of exposure to influenza and secondary bacterial infection. Animal research has also shed light on specific cytokine pathways, such as mediation of infection with influenza by interleukin-10 [80] and inhibition of the pulmonary system's ability to fight infection by interferon-gamma [81, 82], that provide testable hypotheses for an inflammatory response that might occur in humans. Further, in animal studies, investigators can explore what combinations of influenza strains and *S. pneumoniae* serotypes

may result in greater risks to human populations [58, 71, 83, 84].

Seasonal influenza and pneumococcal vaccinations can protect elderly populations against hospitalization for either influenza or pneumonia, and the effects are additive. We see the fewest hospitalizations among individuals who have had both vaccinations, but persons with either the influenza vaccine or the Streptococcus vaccine have lower rates of hospitalization than do unvaccinated individuals [85]. The novel 2009 hemagglutinin type 1 and neuraminidase type 1 influenza (commonly known as H1N1 or swine flu) pandemic provided a natural experiment in a naive population not previously exposed to the influenza strain. Specifically, the lethality of the strain in an unexposed population and potential immunity among the elderly who had been previously exposed to an H1N1 strain provided information about risk of co-infection [26, 86, 87]. The pandemic also started a robust debate about when it is appropriate to provide antibiotics to prevent secondary infection and how to ration them [88-90]. Because etiology is determined in only 30%–50% of patients with CAP who are tested [72] and antibiotics are often given without identifying the bacterial agent, clinical research studies will need to include bacterial surveillance. Additionally, determining the effectiveness of neuraminidase inhibitors and specific age groups to target to prevent a secondary pneumonia infection is needed to provide evidence of which populations will benefit the most from limited resources.

Historical research into the role that bacterial infections played in the deaths from the 1918 influenza A pandemic provides a baseline for our understanding of co-infection at the population level [5, 6, 91] but little insight into the role of order and timing of the infections. Although current recommendations to stay home after influenza infection have been suggested to prevent secondary influenza infections, following the same advice may also prevent subsequent exposure to novel bacteria (pathway 3 in Figure 2.1) that can cause secondary pneumonia—a

major cause of death in 1918. More recent data on the timing between influenza infection and bacterial pneumonia are needed to determine whether antibiotic and antiviral treatments have changed the basic timing and relation at the population level.

We described 3 pathways to CAP associated with influenza and the biologic evidence that supports the potential for each of these pathways to lead to co-infection. By exploring the implications of each pathway and examining co-infection at the biologic, clinical, and population levels, we should be able to identify key signals for predicting and preventing CAP. Although most animal models are currently used to test and support a sequential infection with influenza followed by exposure to bacteria, translating that research to a human model is not straightforward. The interaction between colonization with bacteria and infection, as well as real-life variability in the timing to transmission, means that much further research needs to be conducted for a true understanding of how this co-infection occurs in humans. However, by thinking about the interaction between influenza and pneumonia-causing bacteria in terms of timing of transmission, we are able to raise questions about where to target future human research. Creation of public health recommendations to reduce transmission among at-risk populations and determination of the effectiveness of vaccinations and treatments can be guided by using results from current animal models with a goal of shaping population-level studies. With the majority of historical deaths from influenza pandemics attributable to bacterial infection [67], sorting out the relative contribution of each pathway to disease is a project too important to ignore.

Chapter 3: High Prevalence of Human Coronaviruses in a University Setting

3.1 Introduction

As demonstrated by the 2012 discovery of the Middle East Respiratory Syndrome coronavirus (MERS-CoV) in Saudi Arabia, [92] human coronaviruses continue to emerge and may become significant public health problems. MERS-CoV followed closely on the 2003 identification of severe acute respiratory syndrome coronavirus (SARS-CoV) [93]. Both viruses originated from animal reservoirs and cause significant mortality [37, 93, 94]. By contrast, four other human coronaviruses (HCoVs) 229E, HKU1, NL63 and OC43 - already circulate globally, but generally have low fatality rates [43, 47-49, 95, 96]. These four HCoVs also are believed to have originated from zoonotic sources, including bats (NL63, 229E) or cattle (OC43), although the origins of HKU1 remain uncertain [97-99].

Human coronavirus 229E, HCoV-NL63, and HCoV-OC43 [38, 39, 95, 96] are linked to common cold symptoms, while HCoV-HKU1 is associated with respiratory and, less definitively, gastrointestinal symptoms [45, 100]. HCoV-HKU1 and HCoV-NL63 can cause severe diseases, including bronchitis, bronchiolitis, and/or croup [43, 47-49, 101, 102], among pediatric and adult hospitalized patients. However, due to the relatively mild course of illness in the majority of otherwise healthy individuals, HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43 are thought to be underreported [103].

Our current understanding of the epidemiology of HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43 outside of clinics is extremely limited. The prevalence, severity, and co-occurrence of HCoVs with other respiratory viruses are not yet established [37]. Data are

primarily from outbreak reports, case studies, and clinical studies focusing predominantly on children [38, 42, 45, 47, 49]. Here, we begin to address this gap by estimating the prevalence, shedding duration, symptom progression, and codetection with other respiratory viruses - HCOV-229E, HKU1, NL63 and OC43 - among a cohort of college-aged students.

3.2 Methods

We collected demographic, clinical data, and throat and anterior nasal specimens from students as part of a previously described large social network study of acute respiratory infection (ARI) among university students [64]. In brief, a total of 590 students living in one of six on-campus residence halls were recruited through a chain referral method between October 2012 and January 17, 2013. For a 10-week period from January 17 until April 9, 2013, enrolled participants experiencing respiratory symptoms were asked to complete an online screening survey to self-report illness symptoms. All enrolled participants were asked to identify other enrolled social contacts through searching a list of enrolled contacts or through suggestions based on the underlying social network on a weekly online survey.

Participants reporting symptoms meeting the ARI case definition (cough plus at least one of: body aches, chills, or fever/feverishness) were asked to provide up to three specimens over a 6-day period following ARI onset. In order to reduce the likelihood that any two-illness episodes were linked to the same etiology, symptom-onset dates were required to be *at least* two weeks apart for an ARI participant to provide more than one set during the study period. This allowed us to consider each illness episode as an independent event.

Social Contacts

Once an ARI case was identified through our online screening survey, an email was automatically sent out to the individual's network contacts, inviting presumed "healthy" social contacts to provide a specimen. The social network was identified through a list of contacts that each enrollee generated over the course of the study. Social contacts were eligible if: 1) they had recent face-to-face contact within the previous calendar week with an ARI participant, and 2) they had not been an ARI participant during the previous two weeks. Social contacts that elected to provide specimens were scheduled for up to three specimen collections: 1) day 0 specimen captured as close as possible to the illness onset of their linked cased; 2) specimen captured 3 days after the first; and 3) final specimen captured 6 days after the first specimen.

Although healthy social contacts were not experiencing ARI when they were asked to provide a specimen, some of the social contacts reported symptoms of illness, such as cough or sneezing, at the time of specimen collection. Changes in symptoms among social contacts were calculated as the time from the first specimen collection to illness onset. Any social contact symptomatic on any one or more of the specimen collection days was defined as a "social contact with symptoms." Any social contact remaining healthy on specimen collection days 0, 3, and 6 was defined as an "asymptomatic social contact."

The University of Michigan Institutional Review Board (IRB) (HUM00054432) approved the study protocol and the Centers for Disease Control and Prevention's Human Subjects Research Office reviewed and approved deferral to the University of Michigan's IRB.

Symptom Assessment

All participants providing specimens reported information on 13 acute symptoms: abdominal pain, body aches, chills, cough, diarrhea, ear ache, feverishness, headache, nasal

congestion, runny nose, sneezing, sore throat, and vomiting. Symptoms were collected using a standardized questionnaire administered by trained staff during the sample collection visit, and severity was reported as: not present, mild, moderate, or severe.

Specimen Collection and Testing

For each ARI illness participant and invited social contact, we aimed to collect up to three samples from each study participant as follows:

ARI Participants

Day 0 specimen – Within 24 hours of illness onset

Day 3 specimen – Between 25 and 96 hours after illness onset

Day 6 specimen – Between 97 and 144 hours after illness onset

Social Contacts

Day 0 specimen – Time of first specimen collected, as close to illness onset for ARI contact as possible

Day 3 specimen – Approximately 72 hours after initial specimen collection

Day 6 specimen – Approximately 144 hours after initial specimen collection

If a social contact reported symptoms consistent with our ARI definition, either through the online screening survey or during specimen collection, they were considered an ARI participant and their next scheduled specimen was considered a day 0 ARI specimen. The collection of any combination of day 0, day 3, and day 6 specimens for any participant was defined as a "set" of specimens.

Trained staff collected specimens at each participant's residence. Swabs were taken from two locations: the anterior nares and along the uvula. Both specimens were placed in Copan

Universal Transport Media (Copan, Murrieta, California) and then stored at -70° C prior to testing.

All specimens were tested for 13 respiratory viruses: coronaviruses 229E, HKU1, NL63, and OC43; adenovirus; human metapneumovirus (hMPV); influenza A and B; parainfluenza 1, 2, and 3; rhinovirus; and respiratory syncytial virus (RSV). For all viruses except influenza A/B, aliquots from the throat and nasal swab were combined prior to testing. Influenza A/B testing was performed separately on throat and nasal swabs, and participants were considered positive for influenza if either swab tested positive.

The number of specimens collected per episode ranged from 1-3 per set. For each illness episode, participants and each of their social contacts received an incentive of $15 for their first specimen, $20 for their second, and $25 for their third specimen within a collection period.

Tests for all respiratory viruses were performed in the laboratory using real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Primers and probes were developed by the Centers for Disease Control and Prevention (CDC) and obtained from the Division of Viral Disease, Gastroenteritis, and Respiratory Viruses and the Influenza Division. Additional information about the RT-PCR process and RNA/DNA extraction can be found elsewhere [104]. We assessed the type and number of viral pathogens in each of the day 0, 3, and 6 specimens. A participant was considered positive for a particular virus (or viruses) if at least one of the three specimens within an illness episode had a positive RT-PCR result.

Statistical Analysis

We used Fisher's χ^2 tests and t-tests to compare demographic differences between study participants providing and not providing specimens, as well as the virus prevalence between

three groups: 1) ARI participants, 2) social contacts with symptoms and 3) healthy social contacts. Symptoms were analyzed as present or absent, except for cough, which, as a required symptom for the ARI case definition, was defined as absent/mild compared to moderate/severe. To assess changes in symptoms over time, we compared the proportion of participants who reported each symptom on day 0, 3, and 6 for each illness episode, testing for trends by virus with the Cochran-Armitage test. We assessed the change in illness symptoms over the 6-day period separately for ARI participants (with a defined symptom-onset date) and social contacts with symptoms (with no defined symptom-onset date). Due to sample size constraints, the four human coronaviruses were combined for symptom analysis. All statistical analyses were calculated using SAS 10.1 (Cary, NC).

3.3 Results

Of the 590 enrolled participants, 176 (29.8%) provided specimens as an ARI participant, a social contact, or as both an ARI participant and social contact. A total of 250 sets, the collection of 1 to 3 specimens over an illness episode, were collected: 81/176 (46.0%) participants provided 96 sets of specimens after meeting the ARI case definition; 70/176 (39.8%) participants provided 88 sets of specimens as social contacts; and 25/176 participants (14.2%) provided 66 sets of specimens (31 sets as an ARI case and 35 sets as social contacts); 115 ARI reports were eligible for specimen collection, of those 96/115 (83.5%) provided a specimen. A mean of 1.6 specimens were collected per set. Compared to enrolled students who did not report ARI or did not provide specimens as a social contact, those providing specimens were slightly older (19.5 years vs. 19.1 years; p=0.0006), had parents who were less well-educated (p=0.04), and were less likely to have received a 2011/12 seasonal influenza vaccine (37.7% vs. 51.2%; p=0.01) (Table 3-1).

Table 3-1: Demographic Information for the 590 Participants Enrolled in the eX-FLU Study.

	Participants Providing Specimens (N=176)	Participants Not Providing Specimens (N=414)	p-value
Male	75 (42.6)	160 (41.9)	0.87
Age; Mean, SD	19.5 (1.2)	19.1 (0.9)	0.0009
Race			0.36
White	110 (64.7)	254 (68.7)	
Black	13 (7.7)	34 (9.2)	
Other	47 (27.7)	82 (22.2)	
Parental Education			0.04
<College	43 (25.0)	62 (16.7)	
College	49 (28.5)	99 (26.6)	
>College	80 (46.5)	211 (56.7)	
Seasonal Influenza Vaccination 2012-13	58 (37.7)	104 (51.2)	0.01

Virus Prevalence

Half (127/250; 50.8%) of the specimen sets were from ARI participants, 78 (31.2%) from social contact with symptoms, and 45 (18.0%) from asymptomatic social contacts. Overall, 76 (30.4%) of the 250 sets were positive for at least one of the 13 viruses included in our assay; a total of 101 viruses were identified (11 dual infections, one triple infection). The overall prevalence of virus from ARI participants was 46.5%, compared to 28.3% for social contacts with symptoms, and 13.3% for asymptomatic social contacts (p=0.001). The most common virus identified was HCoV-NL63 (10.0%; 25/250), followed by rhinovirus (7.6%; 19/250), influenza A (6.4%; 16/250), and RSV (3.2%; 8/250). Influenza A was the only virus that appeared statistically significantly by a test of heterogeneity among the three classes more frequently in ARI cases than social contacts with symptoms or asymptomatic social contacts (ARI participants 10.2%; social contact with symptoms 2.6%; and asymptomatic social contacts 2.2%; p=0.05). No specimens tested positive for parainfluenza 2 (Table 3-2).

Table 3-2: Prevalence of RT-PCR Viral Detection Among 176 Participants with 250 Specimen Sets Using Symptom Status from the eX-FLU Study in the University Setting.

Identified Virus	ARI Participant[a] n=127		Social Contacts: With Symptoms n=78		Social Contacts: Asymptomatic n=45		p-value
HCoV-229E	5	3.9%	2	2.6%	1	2.2%	0.90
HCoV-HKU1	1	0.8%	2	2.6%	0	0.0%	0.58
HCoV-NL63	17	13.4%	6	7.7%	2	4.4%	0.20
HCoV-OC43	4	3.1%	1	1.3%	0	0.0%	0.58
Influenza A	13	10.2%	2	2.6%	1	2.2%	0.05
Influenza B	2	1.6%	0	0.0%	0	0.0%	0.68
Adenovirus	2	1.6%	1	1.3%	0	0.0%	1.00
Human Metapneumovirus	4	3.1%	1	1.3%	1	2.2%	0.86
Parainfluenza 1	0	0.0%	1	1.3%	0	0.0%	0.49
Parainfluenza 2	0	0.0%	0	0.0%	0	0.0%	--
Parainfluenza 3	1	0.8%	4	5.1%	0	0.0%	0.09
Respiratory Syncytial Virus	6	4.7%	2	2.6%	0	0.0%	0.40
Rhinovirus	13	10.2%	4	5.1%	2	4.4%	0.38
Any detected virus	59	46.5%	22	28.2%	6	13.3%	0.0001

[a]ARI: Acute respiratory illness consists of a cough plus at least one of: body aches, chills, and fever/feverishness

Viral Co-Detection

The overall prevalence of co-detection (i.e., detection of > 1 virus per illness episode) in our population was 4.8% (12/250) (Table 3-3). There were 11 two-virus codetections and one triple codetection in our population (positive for HCoV-HKU1, influenza A, and rhinovirus). Rhinovirus occurred most frequently as a codetected agent (8/12 specimens; 66.7%), while HCoV-NL63 was present in 50% of the codetected specimens (6/12). The viral positive counts in any one group were too small to draw conclusions about the statistical associations between codetection and clinical symptoms.

Table 3-3: Frequency of 12 Laboratory-identified Codetected Viruses within a Single Specimen among 250 Specimen Sets Collected from the eX-FLU Study in the University Setting.

	Human Coronaviruses					
Identified Virus	229E	NL63	OC43	Influenza A	RSV	Rhinovirus
HCoV-229E	--	2	0	0	0	1
HCoV-NL63		--	0	1	1	2
HCoV-OC43			--	0	0	1
Influenza A				--	0	1
Respiratory Syncytial Virus					--	2
Rhinovirus						--

[a]One specimen tested positive for HCoV-HKU1, influenza A, and rhinovirus

Persistence of Virus Shedding Over Time

Among ARI participants, the prevalence of all viruses detected decreased from time of symptom onset to follow-up. Influenza A (16.9%) was the most frequently detected virus on the day of illness onset, followed by HCoV-NL63 (15.3%). Human coronavirus NL63 was the most frequent virus detected 6 days following illness onset (8.9%), followed by rhinovirus (6.7%). Parainfluenza viruses 1 and 2 were not detected in any specimens collected from ARI participants (Table 3-4).

Table 3-4: Persistence of Virus Detection by RT-PCR among 127 Specimen Sets from Participants with ARI[a] from the ex-FLU Study in the University Setting.

	Day 0 (n=59)		Day 3 (n=98)		Day 6 (n=90)	
Identified Virus[b]	Viral Positive	% Positive	Viral Positive	% Positive	Viral Positive	% Positive
HCoV-229E	2	3.4%	4.1%	7.3%	1	1.1%
HCoV-HKU1	1	1.7%	0.0%	0.0%	0	0.0%
HCoV-NL63	9	15.3%	15.3%	23.8%	8	8.9%
HCoV-OC43	2	3.4%	2.0%	3.6%	3	3.3%
Influenza A	10	16.9%	10.2%	10.2%	3	3.3%
Influenza B	2	3.4%	1.0%	1.0%	1	1.1%
Adenovirus	2	3.4%	1.0%	1.8%	1	1.1%

Human Metapneumovirus	0	0.0%	2.0%	3.7%	2	2.2%
Parainfluenza 3	0	0.0%	1.0%	1.9%	1	1.1%
Respiratory Syncytial Virus	3	5.1%	4.1%	7.0%	3	3.3%
Rhinovirus	7	11.9%	10.2%	16.7%	6	6.7%

[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom: body aches, chills, and feverishness
[b]No ARI participants tested positive for parainfluenza 1 or parainfluenza 2

Symptoms Present During Specimen Collection

Of the 127 participants with ARI, 56 provided a specimen on day 0, 98 provided a specimen on day 3, and 90 provided a specimen on day 6. The most frequent symptoms on day 0 were moderate/severe cough (87.5%) and sore throat (83.9%). By day 3, the most frequent symptoms were moderate/severe cough (80.6%), nasal congestion (73.5%), and runny nose (72.4%). Finally, six days following illness onset, the most frequent symptoms were nasal congestion and runny nose (both 73.3%) (Figure 3-1).

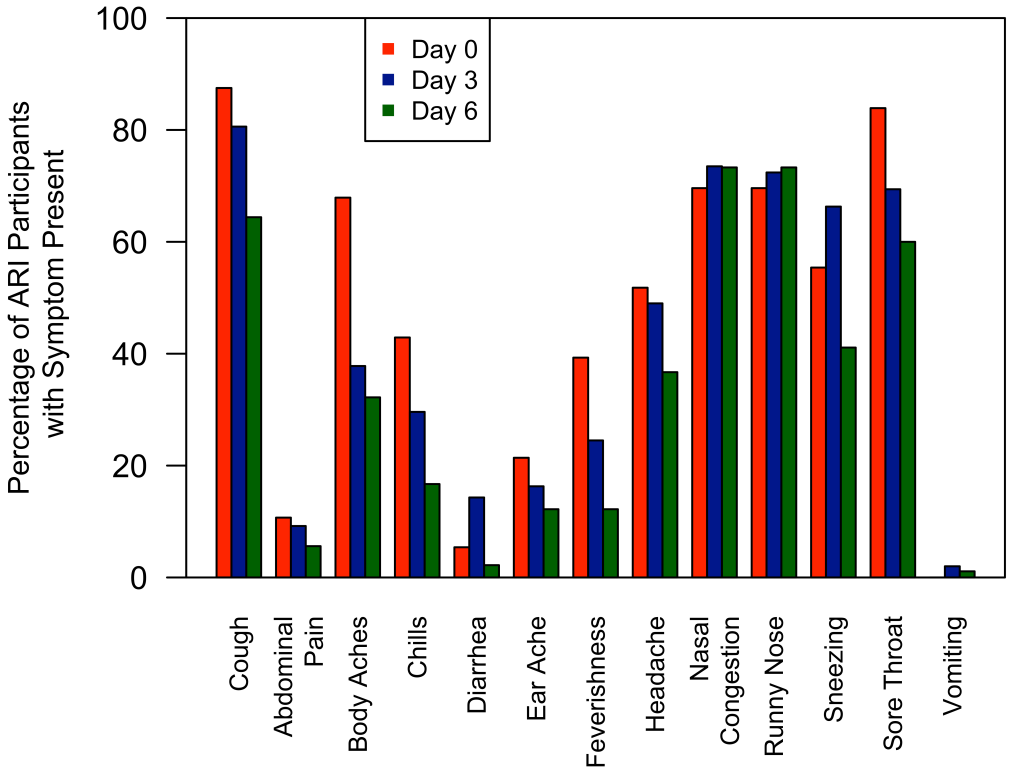


Figure 3-1: Frequency of Symptoms Present Among ARI[a] Participants (N=127) on Day 0 (n=56 specimens), Day 3 (n=98 specimens), and Day 6 (n=90 specimens).
[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom : body aches, chills, and feverishness.

Of the 78 social contacts with symptoms, 78 provided a specimen on day 0, 67 on day 3, and 60 on day 6. The most frequent symptoms across the 6-day specimen collection time frame were runny nose (43.4% on day 0, 43.3% on day 3, and 50.0% on day 6) and nasal congestion (39.5% on day 0, 41.8% on day 3, and 45.0% on day 6) (Figure 3-2).

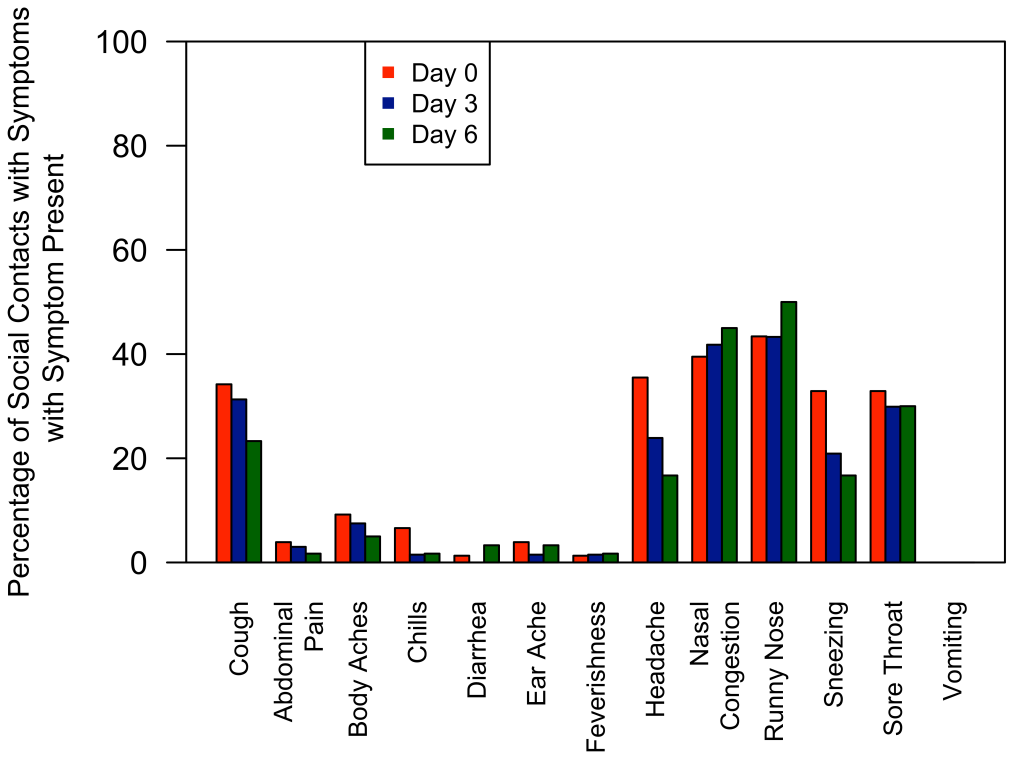


Figure 3-2: Frequency of Symptoms Present among Social Contacts with Symptoms (N=78) on Day 0 (n=78 specimens), Day 3 (n=67 specimens), and Day 6 (n=60 specimens) following the Initial Specimen Collection[a].
[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom : body aches, chills, and feverishness.

Looking over all the specimens collected in a set, 67.2% (203 out of 302 social contact specimens) of specimens collected from social contacts were associated with at least one symptom and 32.8% (98 out of 302 social contact specimens) were associated with no symptoms.

Change in symptoms over time

Among ARI participants with HCoV and multiple specimens (n=19), the most common symptom within 24 hours of symptom onset was moderate/severe cough (12/12; 100%), followed by sore throat (11/12; 91.7%) and nasal congestion (9/12; 75.0%). Three days

following symptom onset, moderate/severe cough (17/18; 94.4%) and sore throat (15/18; 83.3%) were the most common symptoms. Six days following symptom onset, the most common symptoms among ARI patients with HCoV were runny nose (16/17; 94.1%) and nasal congestion (14/17; 82.4%). Moderate/severe cough (p = 0.04), chills (p = 0.01), and headache (p = 0.03) decreased in prevalence from day 0 to day 6. Only the reports of rhinitis (p = 0.02) increased over the 6-day period (Figure 3-3).

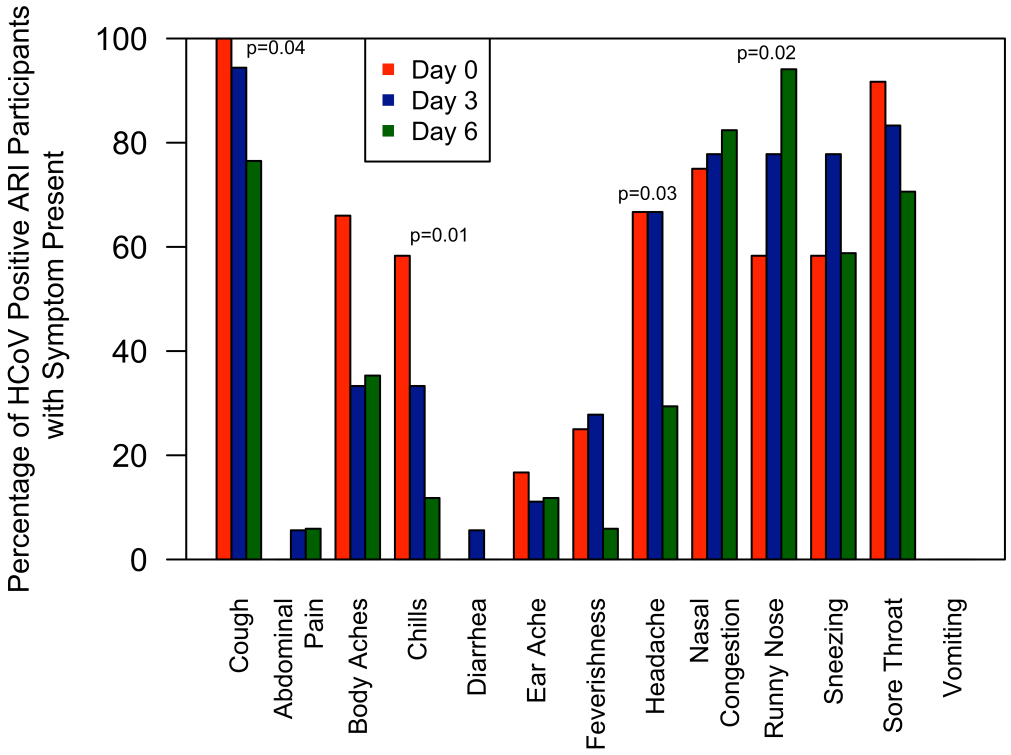


Figure 3-3: Frequency of Symptoms Present among 19 ARI[a] Participants Positive for at Least One of the Four HCoVs on Day 0 (n=12), Day 3 (n=18) and/or Day 6 (n=16) Following Illness Onset[b,c].

[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom : body aches, chills, and feverishness.

[b]Cough defined as moderate or severe vs. mild or absent; all other symptoms were either present or absent.

[c]P-values calculated by the Cochran–Armitage test for trend over the day 0, 3, and 6 specimens.

For ARI patients with influenza A and multiple specimens (n=12), moderate/severe cough at was the most prevalent symptom during the illness episode, followed by sore throat on day 0 and nasal congestion and runny nose on days 3 and 6 of the illness. Body aches (p=0.02) and feverishness (p=0.02) were the only symptoms with a significant difference in the prevalence of symptoms over time (Figure 3-4).

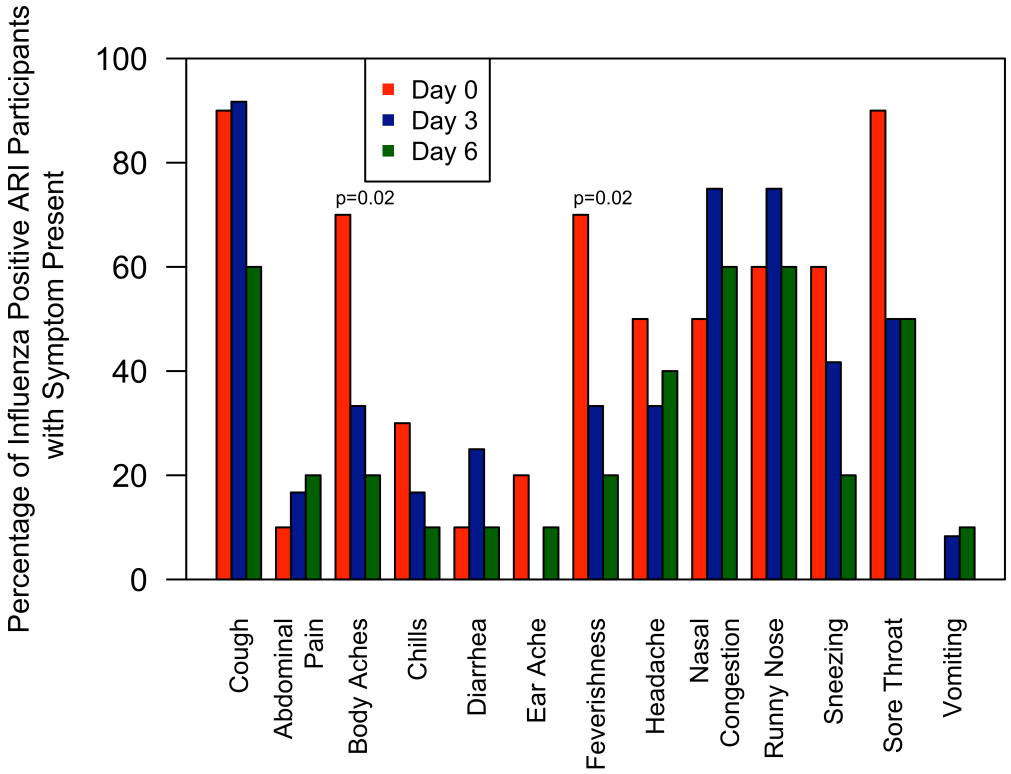


Figure 3-4: Frequency of Symptoms Present among 12 ARI[a] Participants Positive for Influenza A on Day 0 (n=10), Day 3 (n=12) and/or Day 6 (n=10) Following Illness Onset[b,c].
[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom : body aches, chills, and feverishness.
[b]Cough defined as moderate or severe vs. mild or absent; all other symptoms were either present or absent.
[c]P-values calculated by the Cochran–Armitage test for trend over the day 0, 3, and 6 specimens.

Among ARI participants with rhinovirus and multiple specimens (n=9), nasal congestion was present in all participants at all three collection times. Runny nose was the second most

common symptom, decreasing over the illness period from 100% on day 0 to 71.4% 6 days after symptom onset; there were no significant changes in the prevalence of symptoms over time among ARI participants with rhinovirus (Figure 3-5).

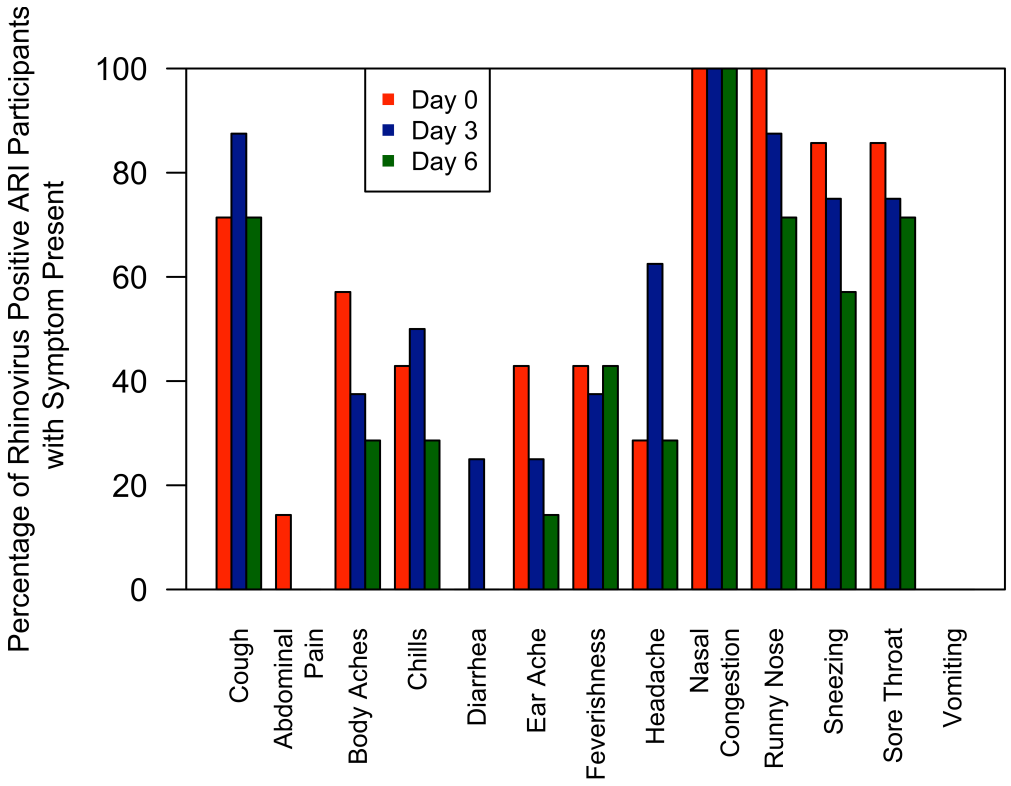


Figure 3-5: Frequency of Symptoms Present among Nine (ARI[a] Participants Positive for Rhinovirus on Day 0 (n=7), Day 3 (n=8) and/or Day 6 (n=7) Following Illness Onset[b].
[a]ARI: acute respiratory illness is defined as a cough plus at least one additional symptom : body aches, chills, and feverishness.
[b]Cough defined as moderate or severe vs. mild or absent; all other symptoms were either present or absent.

Symptoms among social contacts were compared at day 0, 3, and 6 for HCoV (n=9 participants), as this was the most prevalent type of virus identified in this group. Moderate/severe cough, nasal congestion, and sore throat were the most frequent symptoms on day 0 and day 3 of specimen collection. Six days after the initial specimen collection, nasal congestion (37.5%; 3/8) was the most common symptom, followed by sore throat (25%; 2/8)

among HCoV-positive social contacts with symptoms. There were no symptoms with significant changes in the prevalence over time among HCoV-positive social contacts with symptoms (Figure 3-6).

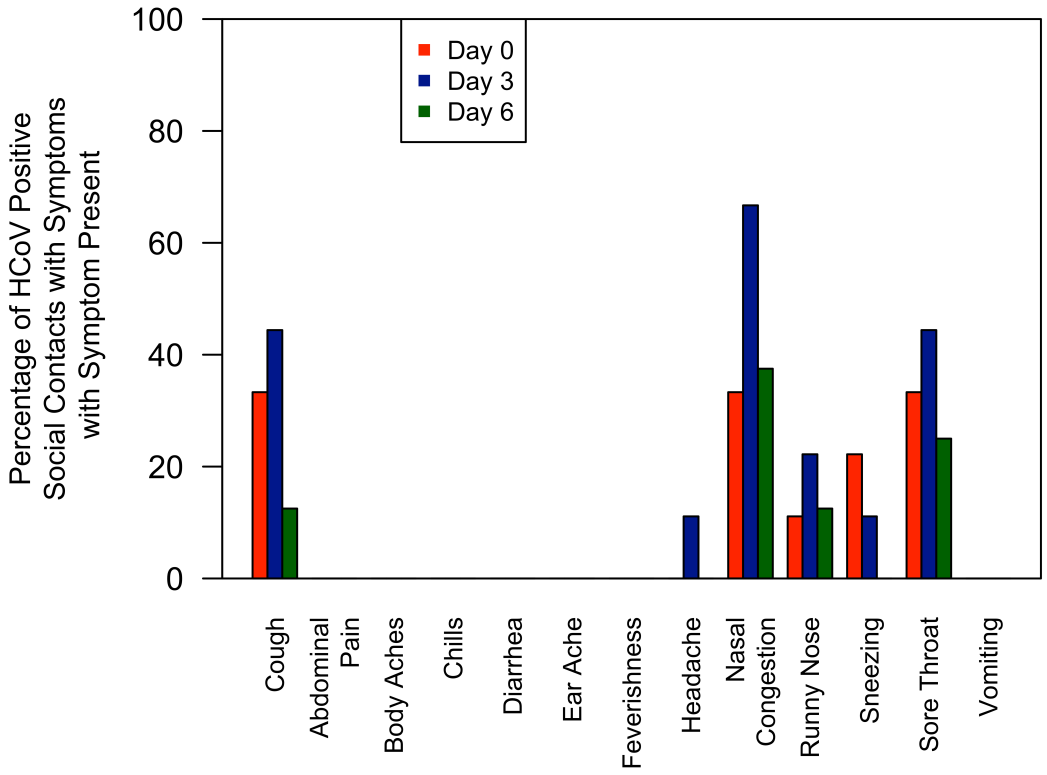


Figure 3-6: Frequency of Symptoms Present among Nine Social Contact Participants Positive for at Least One of the Four HCoVs on Day 0 (n=9), Day 3 (n=9), and/or Day 6 (n=8) Following Initial Specimen Collection[a].
[a]Cough defined as moderate or severe vs. mild or absent; all other symptoms were either present or absent.

3.4 Discussion

There are few prospective non-clinic-based studies describing the epidemiology of human coronaviruses 229E, HKU1, NL63 and OC43 and the changes in symptoms over time. Among the otherwise healthy young adults with ARI symptoms and a sample of their social contacts participating in this study, the prevalence of the four HCoVs combined was 19.7% among specimens from participants with ARI, 14.1% among social contacts with symptoms, and

6.7% among asymptomatic social contacts. Codetection of viruses was found in 12 specimens collected during the study period, including one triple codetection with HCoV-HKU1, influenza A, and rhinovirus. Influenza A was the most commonly detected virus among specimens collected from ARI participants, while HCoV-NL63 was the most frequent virus detected 6 days following illness onset. We found that moderate/severe cough, chills, and headache decreased in frequency over the 6-day period among students with HCoV infections, while runny nose increased in frequency over the 6-day period; no similar frequency trends were observed among symptomatic social contacts with HCoV.

Our prevalence estimates are higher than estimates for a previously conducted study examining these four HCoVs in adult and asymptomatic populations, potentially due to the close contact within the residence halls. In that retrospective study conducted over 9 years in São Paulo, Brazil, the prevalence of HCoVs tested by RT-PCR was 8% among 50 adults living in the community with influenza-like illness [10]. An additional 50 asymptomatic adults were tested, and no positive HCoV specimens were detected. By contrast, we found that 6.7% of our asymptomatic contacts were positive for HCoVs. A household study that used similar RT-PCR methods conducted over the same period as our study in southeast Michigan found a prevalence of 16% of HCoVs among individuals with ARI, but they did not examine the prevalence among non-ARI contacts [104].

The high prevalence of HCoV, compared to the 12 other viruses in our testing panel, could be attributed to the timing of our study. Human coronaviruses are most frequently found during December through May, and long-term cohort studies suggest a cyclical pattern in the presence of the four HCoVs over multiple years [105]. However, without multi-year data, we are unable to determine whether the high prevalence of the HCoVs found was due to the cyclical

nature of the virus or a result of testing ill individuals in close quarters. Unpublished data from a pilot study conducted among an independent sample of 574 students followed from February-April 2011, resulted in few patients with ARI providing specimens (25), but we found a similar prevalence for HCoVs (16%; 4/25) in a similar young adult population. Further long-term annual studies of HCoVs in this community are needed to determine whether there is a seasonal effect or whether there is consistently higher prevalence among young adults in the university setting.

A total of 4.8% (12/250) of specimens were positive for more than one virus, and coronaviruses were found in 44% of the detected codetection. Due to the small sample size, we were unable to assess which characteristics contributed to co-detection, including the one individual with three detected viruses. Other clinic-based studies, predominantly among children, have reported the occurrence of codetected viruses [42, 49, 106, 107]. However, studies outside of the clinical setting are rare. A study of healthy preschool-aged children in Australia reported twice the prevalence of codetection (56%), but their sample size was smaller (n=18) and young children tend to have higher rates of respiratory illness than young adults [62]. These studies suggest that viral codetection is frequent in children. More research is needed on adults to determine risk factors for co-infections among relatively healthy individuals with developed immune systems.

HCoV-NL63 and rhinovirus had the highest proportion of specimens positive after illness onset. A study examining the viral load of HCoV in children in a daycare setting found an average shedding duration of 6.4 days, with a range of 2.8-10.1 days [108], while a previous rhinovirus challenge study reported patients shedding for at least 4 days, suggesting our findings are not unusual [109]. These findings could influence infection control practices in schools, as well as elsewhere in the community.

Our findings of persistently high prevalence of runny nose over the 6-day period in ARI cases with HCoV corresponds with common symptoms found in historical challenge studies of these viruses [105]. However, we were unable to find any other studies presenting a change in symptoms observed over time for the four globally circulating HCoVs outside of human challenge trials. The statistically significant decrease in cough, chills, and headache and increase in runny nose over the 6-day period for the HCoV observed in our study suggest that symptoms change significantly over the course of natural infection, making it difficult to delineate between viral etiologies associated with common ARI. The similarity of our findings with those of another study conducted in the region during the same season [104] suggests that university students were under similar regional viral pressure. Due to the low level of severe illness, and the lack of treatment sought among the students, screening for these viruses in a university setting does not seem necessary. However, it does seem likely that increased testing in the university setting, even among those with mild symptoms, would result in a high number of viruses detected.

Because we used a chain-referral methodology for enrollment, our study population was not randomly recruited. It is unlikely that this would bias the estimates of viral prevalence among those with ARI; however, it is possible that the estimates for seropositivity from healthy contacts may be elevated compared to the prevalence found in the general population. Further, our testing for viruses was not exhaustive; the 13 viruses included were selected for their frequency of appearance as upper respiratory viruses in the population, as well as their clinical importance. However, additional respiratory viruses may have been present; as a result, the number of codetected viruses identified in this study is likely underestimated. Finally, seasonality may have

influenced our findings. By recruiting and testing patients January-April of 2012, we were more likely to see respiratory viruses compared to other circulating viruses [1].

HCoVs are common, even among those without respiratory symptoms, and specific symptoms may change over the course of an illness that can mirror symptoms ranging from influenza to rhinovirus. Further social contact studies are needed in community settings to better understand the epidemiology and clinical significance of codetection within large prospective studies, helping to uncover important transmission characteristics that could inform measures for addressing more deadly coronavirus outbreaks in the community setting, should they emerge.

Chapter 4: Viral Infection is Associated with Increased *Streptococcus pneumoniae* Carriage among Otherwise Healthy College Students

4.1 Background

Commensal bacteria colonize the upper respiratory tract, and occasionally cause invasive disease. One such occasion is otitis media or pneumonia due to commensals *Streptococcus pneumoniae, Haemophilus influenzae,* and *Staphylococcus aureus* secondary to an acute respiratory infection (ARI) caused by a virus [110]. While the mechanisms of this viral-bacterial interaction have been explored in animal models [71, 111], relatively few studies have examined how human nasopharyngeal commensal bacteria change in response to viral infection [12, 112].

We address this gap in the literature by estimating the colonization rates and relative abundance of three common upper respiratory bacteria in an otherwise healthy college-aged population with and without ARI due to a virus. We compared the prevalence of *H. influenzae* (HI)*, S. aureus* (SA)*, S. pneumoniae* (SP) colonization by presence of ARI symptoms, timing between symptom onset and timing of specimen collection, detection of selected viruses, and bacterial load by viral status.

4.2 Methods

Study population

We used samples collected as part of the eX-Flu Study, a randomized controlled intervention study designed to estimate the effects of sequestration on influenza transmission to social contacts [64]. Briefly, 578 college students aged 18 and older living in one of six

dormitories were enrolled using a social network referral model during the 2012-13 influenza season. Participants completed weekly surveys about their enrolled social contacts and reported respiratory symptoms using a separate survey, available 24 hours a day, at the time of symptom onset. We limited this analysis to the 39.4% of the enrolled population that provided specimens during the course of the study (a total of 176 participants; 99 participants who met the case definition for ARI and 77 non-ARI participants).

The study protocol was approved by the university's IRB (clinical trial number: HUM00054432). The Centers for Disease Control and Prevention's IRB approved deferral to the university's IRB for this study.

ARI case definition

Participants were considered ARI positive if they had cough, and one of the following: fever/feverishness, chills, or body aches.

Specimen collection

Trained study staff collected throat and nasal swabs from participants with ARI on date of onset or as close to symptom onset as possible, and on day 3, and day 6 following ARI onset. Using the same collection methods, throat and nasal swabs were collected from social contacts of ARI patients who had not been ill in the previous 14 days (i.e., non-ARI participants) as close to the date of onset of symptoms in their ill social contact as possible. Swabs were stored in universal transport media (Copan, Murrieta, California). The current analysis is limited to results from the first sample provided.

Bacterial testing

Total bacterial DNA from throat specimens were extracted using a commercially available protocol (Qiagen DNeasy) with modifications and stored at -20°C. We performed quantitative PCR (qPCR) using species-specific probes designed to target specific genes for each species of interest using the SYBR green fluorescent detection system. qPCR reactions were carried out using a commercially available SYBR green PCR master mix (Ssofast EvaGreen Supermix, Bio-Rad, CA), primers specific to each species, and genomic DNA as template. For each bacterial species, a gene fragment obtained from PCR was cloned into a commercial vector (TOPO TA cloning kit, Invitrogen, CA) and used to generate standard curves. A 10-fold dilution series of the cloned plasmid insert from the different bacteria was generated and qPCR was determined to be linear between 10^2 *rrn* copies/ul and 10^8 *rrn* copies/ul. Specific genes for each of the bacteria were targeted: the housekeeping gene fumarate reductase iron–sulfur gene B (*frdB*) was used for HI, the pneumolysin gene (*ply*), which is a species-specific protein toxin unique to SP produced by nearly all clinical isolates and which has been shown to be well conserved was targeted, and the well conserved and specific *nuc* gene for *S. aureus* detection [113]. The qPCR assays were performed as previously described [114, 115].

Viral testing

Aliquots from the nasal and throat swab were combined for viral testing except for influenza A/B. Influenza A/B were tested separately on throat and nasal swabs; a positive sample for either was used to define a participant as influenza positive. Samples were considered influenza positive based on the TaqMan System (Applied Biosystem, Foster City, CA) using primers and probes developed by the CDC influenza branch and approved for use in our lab

[104]. A total of eleven other viruses were tested in this study on a combined sample: adenovirus; coronaviruses 229E, HKU1, NL63, and OC43; human metapneumovirus (hMPV); parainfluenza 1, 2, and 3; rhinovirus; and respiratory syncytial virus (RSV). Additional information about the viral testing and primers were previously described [104].

Data analysis

To assess whether differences between groups were statistically significant we used chi-square for categorical variables and student's t test for continuous variables. We used time of symptom onset and self-reported severity of illness measured across 13 individual symptoms to evaluate the association between viral status and bacterial colonization. To assess whether there were differences in presence of a particular bacterial species by time of symptom onset we used the Cochran–Armitage test for trend. All analyses were conducted in SAS (version 10.1, Cary, North Carolina).

4.3 Results

Study participants were relatively young (mean of 19 years), predominately Caucasian, with highly educated parents (Table 4-1). Among the 176 participants, 60 (34.1%) tested positive for at least one of the 13 viruses in our screen, most commonly coronavirus NL63 (8.5%), influenza A/H3N2 (5.7%), and rhinovirus (4.5%)(Table 4-2). Overall, 51 (29.0%) tested positive for at least one of the three bacteria of interest, most commonly SP (15.9%) (HI: 7.4%; SA: 11.9%)(Table 4-1). More than half of the viral-positive and viral-negative participants were vaccinated against influenza for the 2012-13 season.

Table 4-1: Demographic and pathogen status of project participants from large university during the 2012-13 influenza season, comparing laboratory confirmed infection by a respiratory virus participants to laboratory confirmed negative participants.

	Laboratory Confirmed Infection by a Respiratory Virus* (n,%) n=60 (34.1)	Laboratory Confirmed Negative* (n,%) N=116 (65.9)	p-value
Age (mean, SD)	19.2	19.7	0.006
Male	28 (46.7)	14 (40.5)	0.43
Race			0.43
White	38 (66.7)	72 (63.7)	
African American	6 (10.5)	7 (6.2)	
Other	13 (22.8)	34 (30.1)	
Parental Education			0.76
<College	16 (26.7)	27 (24.1)	
College	15 (25.0)	34 (30.4)	
Post Graduate	29 (48.3)	51 (45.5)	
Flu Shot 2012-13	37 (71.2)	59 (57.8)	0.11
Contact Type			0.008
Participant meeting ARI** definition	42 (70.0)	57 (49.1)	
Participant not meeting ARI** definition	18 (30.0)	59 (50.9)	
H. influenzae	6 (10.0)	7 (6.0)	0.37
S. aureus	11 (18.3)	21 (18.1)	0.97
S. pneumoniae	14 (23.3)	14 (12.1)	0.05

*Tested for influenza A/B; adenovirus; coronaviruses 229E, HKU1, NL63, and OC43; human metapneumovirus; parainfluenza 1, 2, and 3; rhinovirus; and respiratory syncytial virus

**Acute respiratory infection (ARI) defined as cough plus at least one of the following: fever/feverishness, chills, or body aches

Those who met our case definition of ARI were significantly more likely than those without ARI to test positive for a respiratory virus (p=0.008), and laboratory confirmation of a respiratory virus was significantly associated with an increase in SP carriage (p=0.05) but not HI or SA carriage (Table 4-1). Among the 42 participants with ARI and a laboratory confirmation of a respiratory virus, 13 (31.0%) were SP positive, compared to only 7 (12.3%) among the 57 with ARI who tested negative for virus (p=0.04). Of the 18 in the non-ARI group with laboratory confirmation of a respiratory virus, 1 (5.6%) was SP positive compared to 7 (11.9%) of the 59 in

the non-ARI group who tested negative for virus (p=0.74). HI and SA prevalence were not associated with ARI. The numbers of individuals positive for any one virus were too small for meaningful analysis (Table 4-2).

Table 4-2: Frequency and percent of the 13 laboratory confirmed respiratory viruses participants tested positive for in the eX-FLU study based on their first samples provided. Total positive specimens = 60. Samples collected from 99 ARI participants and 77 non-ARI participants from a large university during the 2012-2013 influenza season.

Identified Virus	Positive Specimens (n=60)	Prevalence of Virus
Influenza A/H3N2	10	5.7
Influenza B/Yamagata	1	0.6
Adenovirus	2	1.1
Coronavirus 229E	3	1.7
Coronavirus HKU1	1	0.6
Coronavirus NL63	15	8.5
Coronavirus OC43	2	1.1
Human Metapneumovirus	4	2.3
Parainfluenza 1	1	0.6
Parainfluenza 2	0	--
Parainfluenza 3	3	1.7
Rhinovirus	8	4.5
Respiratory Syncytial Virus	2	1.1
Coronavirus 229E and Coronavirus NL63	2	1.1
Coronavirus NL63 and Influenza A/H3N2	1	0.6
Coronavirus NL63 and Rhinovirus	2	1.1
Coronavirus NL63 and Respiratory Syncytial Virus	1	0.6
Influenza A/H3N2 and Rhinovirus	1	0.6
Rhinovirus and Respiratory Syncytial Virus	1	0.6

To assess if there is an association between bacterial colonization and ARI symptoms, we examined the time between symptom onset and collection of a specimen positive for HI, SA or SP for participants that met our ARI case definition (n=60). There were no detectable trends in symptom onset date and collection of a specimen positive or HI or SA (Cochran-Armitage test, p=0.22; p=0.26). However, a higher proportion of day 0 samples (collected on the day that

symptoms were first reported) were positive for SP than samples collected 3 or 6 days after symptom onset (p=0.01) (Figure 4-1). The proportion of ARI participants positive for SP on day 0 (35.1%), was significantly higher than the proportion of ARI negative participants positive for SP (12.1%; p=0.02).

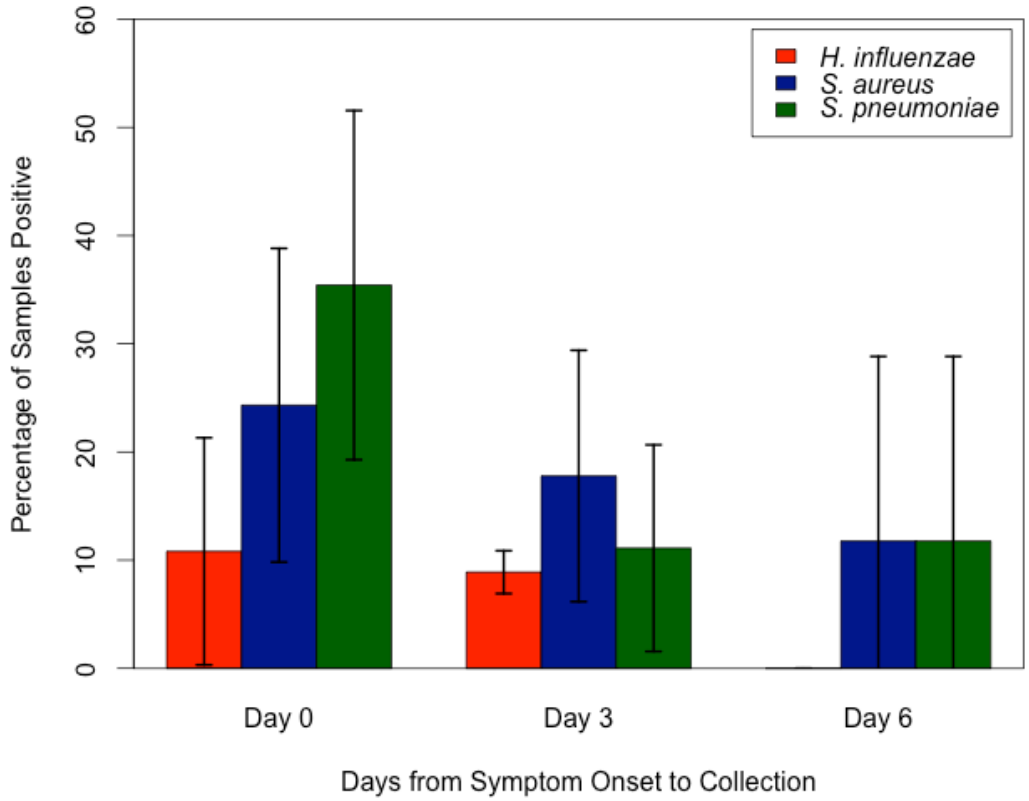


Figure 4-1: Proportion of each throat specimen positive for bacteria based on time from onset for ARI participants, and p-value comparing each bacteria over the three time points (Day 0: n=27; Day 3: n=44; Day 6: n=17) based on Cochran-Armitage test for trend, among 176 participants from large university during 2012-2013 influenza season. There were no statistically significant differences based on time from onset and positive samples for *H. influenzae* (p=0.22) or *S. aureus* (p=0.26), but a higher likelihood of positive *S. pneumoniae* closer to illness onset (p=0.01).

SP and HI bacterial loads were similar between participants with and without laboratory confirmation of a respiratory virus. By contrast, SA bacterial load was significantly lower among those with a laboratory confirmation of a respiratory virus (4.1 log genomic copies/mL vs. 5.1/ log genomic copies/mL p=0.03) (Figure 4-2). We observed some evidence of competition between SA and SP: the mean SA bacterial load was significantly higher in the absence of SP (n=27; mean = 4.83 log genomic copies/mL) than in the presence of SP (n=5; mean = 4.12 log genomic copies/mL; p=0.05). But the mean SP load did not differ by presence of SA: (SA

positive, n=5; mean = 5.15 log genomic copies/mL; SA negative, n=23; mean=4.86 log genomic copies/mL; p=0.39).

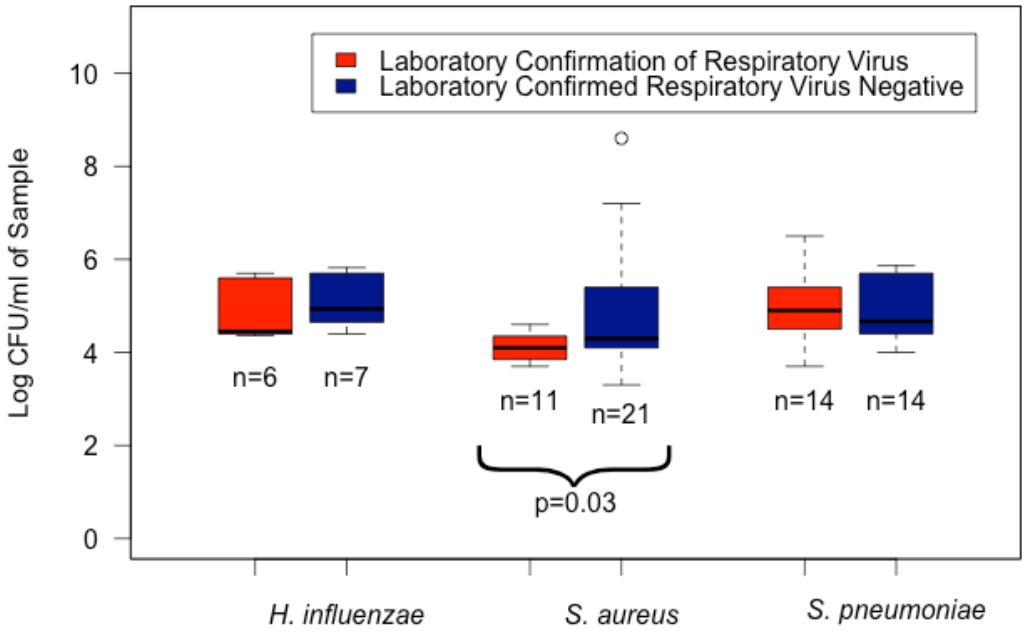


Figure 4-2: Relative abundance of *Haemophilus influenzae, Staphylococcus aureus*, and *Streptococcus pneumoniae,* in Log CFU/ml of sample by laboratory confirmation status of a respiratory viral infection.* Difference between viral load for *S. aureus* was significant (p=0.03).

4.4 Discussion

SP colonization, but not HI or SA colonization, was more common in the presence of ARI (cough, and one of the following: fever/feverishness, chills, or body aches) and in the presence of a respiratory virus. This association was strongest for specimens collected on the date of symptom onset. By contrast, SA bacterial load was decreased in the presence of virus, and in the presence of SP, but there was no association of SP bacterial load with viral or SA colonization.

In this cohort of college students, we found that the prevalence of three common bacteria was similar to those found among young adults using RT-PCR methods [116]. A study

conducted among Portuguese children 4 months to 6 years old in the community setting looked at the association between symptoms, bacterial carriage, and viral status. The authors showed an increase in SP with increasing symptom severity, and with viral detection [12]. However, the study did not measure or compare bacterial load.

Many models of co-infection assume that viral infection primes the immune system, in part by changes in the lung, increased inflammation, or immune system modulation [117]. Using influenza as a model, bacteria are hypothesized to proliferate over the first three days of infection, as the immune system responds the viral threat. Due the cross sectional nature of our study, we were unable to directly address this model. However, the three day period for bacterial proliferation falls within the two to five day incubation period for influenza, which could explain the why the highest proportion of SP colonization found from participants came from those who provided specimens on the day of symptom onset [117]. A second study directly examined the effect of rhinovirus inoculation on nasopharynx microbiota, and found that rhinovirus did not consistently change the prevalence or bacterial loads during infection compared to the microbiota during the wellness period [112]. Building on these findings, additional studies with serial samples are needed to firmly establish the timing of infection, while also further understanding how different viruses interact with colonizing bacteria. Further research into the effect that HI, SA, or SP can have on the immune response when colonizing an individual is also needed [117].

We relied on a chain-referral sample method for recruitment of students into our study, limiting our ability to generalize our results to the broader young-adult community. The social network was used to identify non-ARI participants, meaning not all non-ARI participants were given the opportunity to provide specimens. However, the high level of colonization observed in

the non-ARI population suggests, at least in regard to bacterial colonization, that the targeted sampling method was not dependent upon ARI symptoms.

In conclusion, we identified an increased SP presence by laboratory confirmed infection with a respiratory virus and ARI presence among young adults. These findings add to a growing literature on the role of co-infection between viruses and bacteria in human disease. Further research should establish the temporal course of viral and bacterial co-infection, how specific bacteria are able to flourish with viral infection, and the role of the microbiome in the acquisition of co-infections [117]. If confirmed in larger studies, these findings have both clinical and public health implications, including changes in therapy and vaccination strategies to target co-occurring agents.

Chapter 5: The Effect of Reactive School Closure on Community Influenza-Like Illness Counts in the State of Michigan during the 2009 H1N1 Pandemic

5.1 Introduction

At the start of the 2009 influenza A (H1N1) pandemic, the Centers for Disease Control and Prevention (CDC) recommended proactive school closures as a non-pharmaceutical intervention (NPI) whenever a confirmed or probable case of 2009 influenza A (H1N1) was identified in a school [118]. On May 5, 2009, the CDC modified its guidelines, emphasizing local decision-making and recommending school closures only when high absenteeism interfered with a school's educational mission [119]. Over 3,000 schools in the United States closed during the spring and fall waves of the 2009 influenza A (H1N1) pandemic.

We studied retrospective data on 559 school closures in the state of Michigan during the fall wave of the 2009 influenza A (H1N1) pandemic. Most were reactive and occurred late in these school districts' pandemic experience [120]. We hypothesized that late school closures would not result in a significant difference in influenza-like illness (ILI) rates in these communities.

5.2 Methods

We used data from the Michigan Department of Community Health (MDCH) collected during the fall of 2009. The MDCH proactively recorded information on school closures from 559 public traditional, public charter and private K-12 schools during the fall term in response to 2009 influenza A (H1N1). Several schools issued multiple closures during the period, for a total

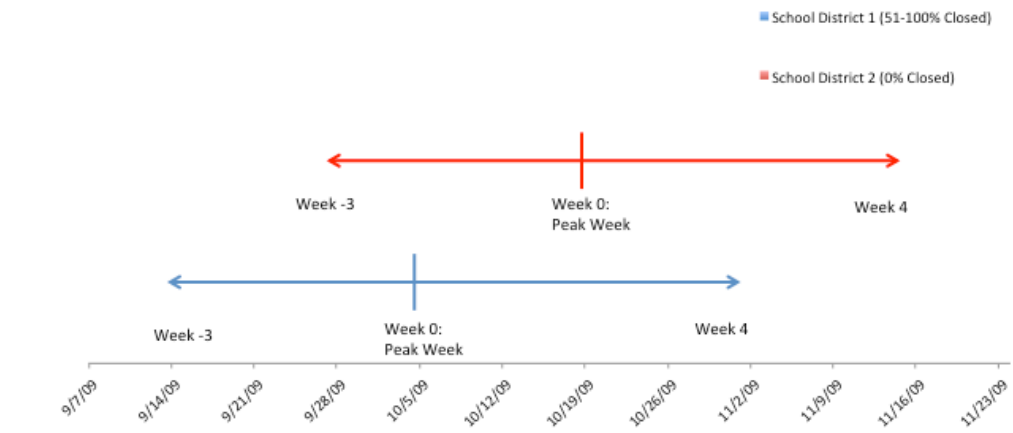
of 567 separate school closure incidents. This study was considered an activity not regulated by the University of Michigan Health Sciences and Behavioral Sciences Institutional Review Board (HUM #00091632).

Influenza-like Illness Outcomes

The primary outcome of interest was the weekly ILI count for each school district. In addition to schools closed, MDCH provided a list of all ILI cases reported from September 1 through December 31, 2009. Of 7,000 reports to the Michigan Disease Surveillance System (MDSS) containing zip codes, 1,248 had dates for both referral and symptom onset. To more accurately reflect what the unreported onset may have been for the 5,752 individuals without onset date, we calculated the median days between referral and onset among the 1,248 with complete information. We then subtracted that value from those with only a referral date, by week, to estimate the date of onset. We adjusted our reported onset time to allow for an assessment of how additional epidemiological information over that time period may have changed reporting practices.

To determine the time period for analysis, we calculated a weekly ILI rate for each district by summing the ILI cases per week and dividing them by the total population in each school district. The peak ILI rate was based on two different methods: districts that had three or more weeks of ILI data were assigned a peak week based on the maximum rate of ILI per 100,000 persons. Districts with less than three weeks of ILI data, or a peak week occurring prior to October 10th, were assigned a peak week value based on their public health region. After accounting for a one-week lag to assess closure impact on ILI, we set the peak week for each

school district as our 0 week, and looked at ILI counts three weeks preceding and four weeks following peak ILI rate in our models (See Figure 5-1).



Example Model Estimate for Week -2 comparing
Category 3: 51-100% Closed to Category 1: 0% Closed

$$RR_{\substack{51-100\% \\ 0\%},W-2} = \frac{R_{51-100\%,9/21/09}}{R_{0\%,10/5/09}} = \frac{R_{W-2}}{R_{W-2}}$$

Figure 5-1: Illustration of Centering of Our Peak Week of Influenza-Like Illness for Comparisons, as well as Example of our Relevant Outcome.

School Districts

Information about individual schools and districts were accessed electronically from the Michigan Department of Education's Center for Educational Performance and Information (CEPI). During the 2009 – 2010 academic year, all schools in the CEPI database could be geographically assigned to 551 public school districts. One district contained no open and eligible schools during our study period, and two school districts contained population sizes that skewed ILI rates; these were dropped from analysis. The final dataset analyzed contained 548 districts. We considered a school district "closed" if at least one school closed and "open" if no schools in the district closed at any point during the study period.

Closed Schools

The CEPI database contains information on 10,145 school districts, non-classroom facilities, non-school recipients, unique education providers, and public, public charter, and private schools [121]. We limited our analysis to schools only, leaving us with 6,469 unique state institutions. We removed from our dataset 2,038 schools that were not in session during the fall 2009 semester; 100 schools that encompassed special education, adult education, juvenile detention and other facilities not under normal closure pressure; 28 schools with no grade information; and 8 online/virtual schools. Of these 4294 remaining schools, 559 reported at least one closure during the fall wave of 2009 influenza A (H1N1).

Statewide Geographical Data

We used two statewide geographical shape files to assign population size and outcome of ILI counts, and to assess peak ILI rates in the greater community. We used the US 2010 Census, which contained school district information with associated housing density. Next, a shape file for each of the 988 Zip Code Tabulation Areas (ZCTAs) in Michigan was accessed to delineate population by age; ZCTAs are used to combine census blocks with US Postal Service data and are often coterminous with zip code-defined areas, although boundary differences sometimes occur in rural areas [122]. This allowed us to calculate the proportion of the school-aged population (5-17 years of age) and total population within each ZCTA, and provide a geographic area for our ILI cases.

We then overlaid a statewide shape file (provided by the Geodata Services section of the Michigan Department of Technology, Management, and Budget) containing polygons

representing each of the public school districts in Michigan. The shape file represented school districts as of 2011, when 551 school districts covered the state.

These two shape files were read into ArcGIS 10.1 (Redlands, CA) in order to determine which ZCTAs were contained within each school district. An intersect merge of the two layers enabled an identification of ZCTAs for each school district that provided the school-aged and total populations. These data were then exported to the statistical software package for additional analysis.

Statistical Methods

School district level data from the CEPI dataset was summarized using SAS 10.1 (Cary, NC). Select data was aggregated across individual school levels into district-wide variables: proportion of students eligible for the National School Lunch Program (NSLP), which we used as a proxy measurement for district socio-economic level; type of school (public, private, public charter); and eligible grade levels. We derived the main predictor – the proportion of schools closed in a district varying over time – using the total number of schools closed by week over the total number of schools in the district. Due to the asymmetric distribution of our raw data describing the proportion of schools closed in each district, we consolidated this value into three levels of closure:

Level 1: 0% of all schools in a district close for a given week;

Level 2: 1 to 50% of all schools in a district closed for a given week;

Level 3: 51 to 100% of schools in a district closed for a given week.

To assess the effect of closure timing, we examined characteristics of individual schools and districts. Grade level and number of days closed were assessed among districts that closed prior to the week of peak infection and districts that closed at or following peak infection, determined by measuring the weeks of closure for each school district and subtracting that week value from the peak week of infection. Differences between categorical and quantitative variables were calculated using chi-squared and t-tests.

The outcome of interest for our analyses was weekly ILI counts per district. We used a log population per district offset to account for differences in the size and population density of the school districts, with a one-week lag to allow assessment of closure impact. To accommodate the ILI count data and offset while accounting for clustering within school districts over time, we fit a longitudinal model examining changes in the counts of ILI using PROC GENMOD with a negative binomial link statement in SAS 10.1. Negative binomial models are often appropriately used for count data when the variance exceeds the mean, as was the case in our study [123]. The full model was adjusted for additional covariates, including whether the school was public or private, the percentage of NSLP-eligible students, the number of enrolled students, and the density of houses per square mile in the district. We calculated estimates for differences between school districts, comparing level 1 (0% closed), level 2 (1-50% closed) and level 3 (51-100% closed). Figure 5.1 illustrates the time frame and comparison groups used to estimate ILI rate ratios (RR).

5.3 Results

We analyzed 559 schools that closed in 548 school districts active during the 2009 academic year; 170 school districts had at least one school that closed, compared to 378 districts

in which all schools remained open. These districts accounted for 1434 and 2882 closed and open schools, respectively. Closed districts had a high percentage of Junior/Senior high schools and K-12 schools, more private schools, but fewer houses per square mile (Table 5-1). We found no statistically significant difference between average total students per district or our proxy socioeconomic measure of NSLP eligibility ratio.

Table 5-1: Descriptive Information About School Districts with at Least One School Closure Compared to No School Closure During the Fall 2009 Term in the State of Michigan.

	Closed Districts N=170	Open Districts N=378	P-value
Total Number of Schools	1434	2882	
School Level			0.003
Elementary	841 (58.7)	1695 (58.8)	
Jr. High School	151 (10.5)	329 (11.4)	
Jr. Sr. High School	97 (6.8)	156 (5.41)	
Sr. High School	221 (15.4)	536 (18.6)	
K-12	124 (8.7)	166 (5.8)	
School Type			0.002
Public School	1057 (73.7)	2186 (75.9)	
Public Charter School	79 (5.5)	208 (7.2)	
Private School	298 (20.8)	488 (16.9)	
Average Number of ILI Cases Over Study Period	5.42	6.33	0.06
Average Houses per Sq. Mile per District	182.9	397.3	<0.0001
Average Proportion of Population 5-17 per District	0.167	0.174	0.0002
Average Total Students per District	3056.3	3179.2	0.80
Average Free and Reduced Lunch Ratio per District	0.43	0.42	0.45
Average Schools per District	8.44	7.62	0.48
Average Closed Schools per District	3.28	--	--
Overall Proportion of Closed Schools per District	0.64	--	--

Open districts had a slightly, though not statistically significant, higher average number of cases than closed districts (6.33 versus 5.42, p=0.06), and a statistically significant higher proportion of the total population that were school-aged (17.4 versus 16.7, p = 0.0002). On

average, there were 8.44 schools in closed districts (districts with at least one school that closed), compared to 7.62 schools per open district (p=0.48). Among closed school districts, there was an average of 3.28 closed schools, or 64% of the schools per district. This value was skewed due the large number of school districts (n=67) where all schools closed (Table 5-1). A map of open and closed districts, peak week of infection, and public health regions can be found in Figure 5-2.

In the district-specific analysis, the rate of ILI peaked for public health regions 1, 2S, 3, 5 and 6 during the week of October 17, 2009. Regions 7 and 8 peaked the week of October 24, while region 2N peaked the week of October 31. Regions 1, 6 and 8 had the highest attack rate (approximately 4 cases per 100,000 persons), with the smallest peak for 2S, with approximately 2 cases per 100,000. A high of 250 Michigan schools closed the week of October 24. An animated graphic of changes in the rate of ILI per school district can be accessed online (http://cid.oxfordjournals.org/content/suppl/2015/04/17/civ182.DC1/civ182supp_video1.mp4).

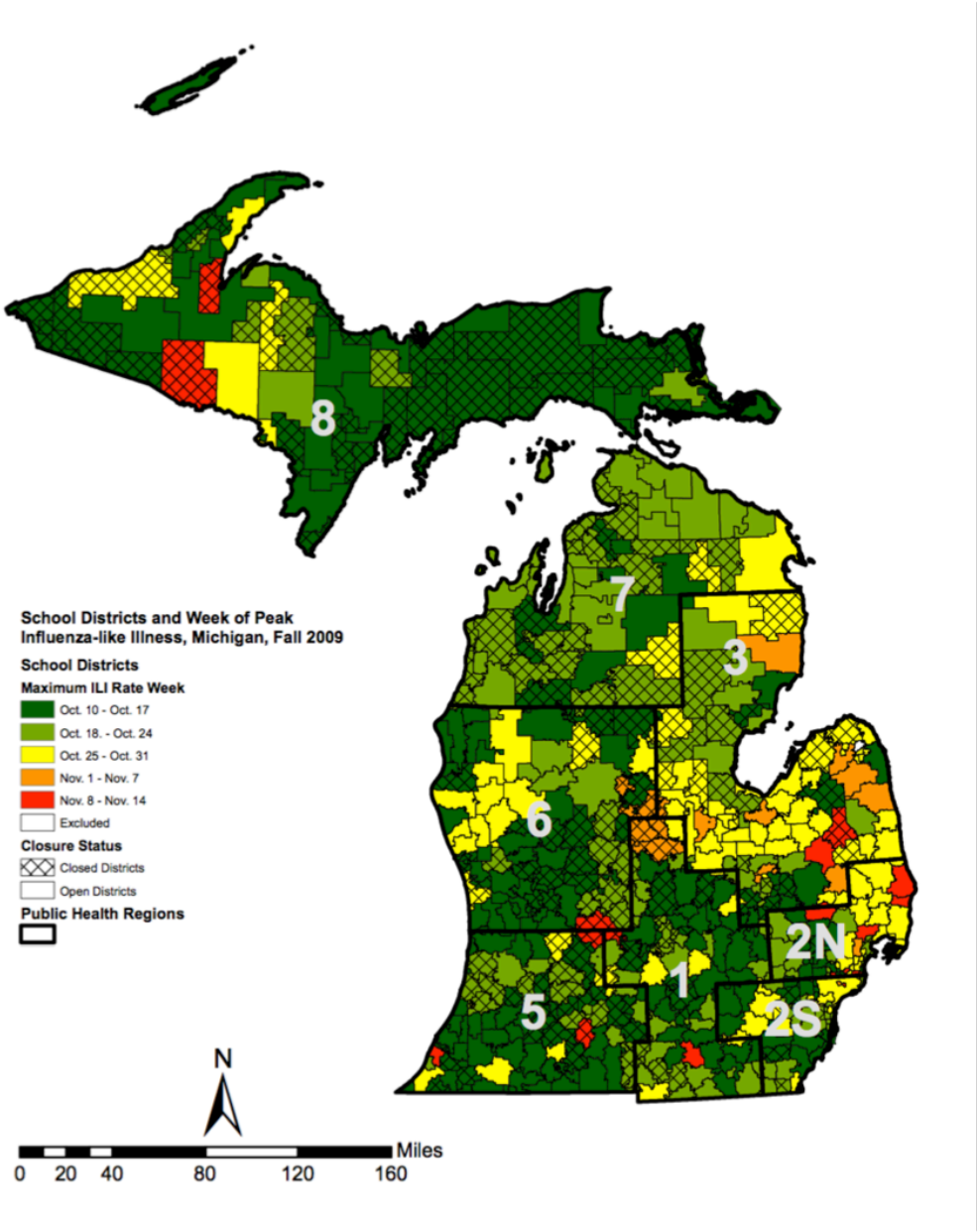


Figure 5-2: Map of 8 Public Health Regions and 551 School Districts in the State of Michigan with District Level Peak Week of Infection

The results from our demographic analysis of factors related to school closure are outlined in Table 5-2. We found no statistically significant differences between closed and open schools with respect to school grade level or school type, though public schools had a higher ILI rate per 100,000 individuals than e private schools or charter schools. Housing density was found

to be statistically significant, with a reduction in the number of houses per square mile associated with a higher rate of ILI (Rate per 100,000 = 0.88; p<0.0001).

Table 5-2: Demographic Factors at the District Level Related to School District Closures and Rates of Influenza-like Illness.

	Estimated Rate per 100,000 persons	95% Confidence Interval		p-value
Percent of Schools Closed by Week				
School Level				
Elementary	1.14	0.69	1.87	0.61
Jr. High School	0.98	0.34	2.83	0.97
Jr. Sr. High School	1.06	0.37	3.06	0.91
Sr. High School	1.12	0.43	2.91	0.81
K-12	1.00	0.36	2.82	1.00
School Type				
Private School	0.96	0.77	1.20	0.73
Public Charter School	0.96	0.91	1.01	0.15
Public School	1.30	0.32	5.24	0.71
Average Houses per Sq. Mile per District	0.88	0.86	0.91	<.0001
Average Proportion of Population 5-17 per District	1.15	0.62	2.13	0.66
Average FR Ratio* per District	1.08	0.95	1.22	0.31

*FR ratio is the ratio of students receiving free or reduced lunch out of all students in a school

We observed similar patterns in the time-varying unadjusted model and the model adjusted for grade levels, school type, and housing density, although the fully adjusted model was attenuated towards the null RR or 1.0 (Table 5-3). No significant differences were observed across any of the weeks in relation to the peak week of infection, comparing level 1 (0% closed), level 2 (1-50% closed) and level 3 (51-100% closed). However, level 2 had a lower RR when compared to level 1 or level 3 over the study period. In contrast, level 3 showed higher RR over the study period compared to both level 1 and level 2.

Table 5-3: Adjusted Model Predicting Rates of Influenza-Like Illness based on Proportion of Schools Closed per Week.

	Unadjusted	Fully Adjusted

	Rate Ratio	95% CI		P-value	Rate Ratio	95% CI		P-value
Peak Week -3								
1-50% Closed v. 0%	0.60	0.07	5.31	0.64	0.75	0.08	6.83	0.80
51-100% Closed v. 0%	2.19	0.13	35.74	0.58	1.73	0.13	23.82	0.68
51-100% Closed v. 1-50%	3.13	0.09	110.61	0.53	1.78	0.06	57.44	0.75
Peak Week -2								
1-50% Closed v. 0%	0.53	0.14	2.02	0.35	0.60	0.16	2.31	0.46
51-100% Closed v. 0%	2.15	0.33	14.20	0.43	1.75	0.30	10.31	0.54
51-100% Closed v. 1-50%	4.06	0.40	41.17	0.24	2.90	0.31	26.88	0.35
Peak Week -1								
1-50% Closed v. 0%	0.53	0.22	1.25	0.15	0.59	0.24	1.42	0.24
51-100% Closed v. 0%	1.85	0.54	6.36	0.33	1.55	0.49	4.95	0.46
51-100% Closed v. 1-50%	3.52	0.78	15.86	0.10	2.64	0.61	11.31	0.19
Peak Week 0								
1-50% Closed v. 0%	0.59	0.31	1.09	0.09	0.69	0.38	1.28	0.24
51-100% Closed v. 0%	1.61	0.63	4.09	0.32	1.36	0.57	3.29	0.49
51-100% Closed v. 1-50%	2.75	0.89	8.43	0.08	1.97	0.67	5.73	0.22
Peak Week 1								
1-50% Closed v. 0%	0.65	0.37	1.14	0.13	0.82	0.47	1.45	0.49
51-100% Closed v. 0%	1.40	0.71	2.76	0.33	1.20	0.62	2.31	0.58
51-100% Closed v. 1-50%	2.14	0.89	5.15	0.09	1.47	0.62	3.46	0.38
Peak Week 2								
1-50% Closed v. 0%	0.65	0.36	1.17	0.15	0.80	0.43	1.47	0.47
51-100% Closed v. 0%	1.21	0.74	1.97	0.46	1.06	0.65	1.74	0.81
51-100% Closed v. 1-50%	1.86	0.88	3.95	0.11	1.33	0.62	2.87	0.47
Peak Week 3								
1-50% Closed v.	0.58	0.15	2.18	0.42	0.64	0.16	2.60	0.54

0%								
51-100% Closed v. 0%	1.03	0.28	3.83	0.96	0.94	0.27	3.29	0.93
51-100% Closed v. 1-50%	1.79	0.28	11.38	0.54	1.47	0.23	9.43	0.69
Peak Week 4								
1-50% Closed v. 0%	0.46	0.03	6.73	0.57	0.43	0.02	7.64	0.56
51-100% Closed v. 0%	0.88	0.06	13.21	0.92	0.84	0.07	10.92	0.90
51-100% Closed v. 1-50%	1.91	0.04	85.41	0.74	1.98	0.04	92.24	0.73

*Adjusted for grade level, school type, free and reduced lunch ration, and housing density

The timing of district and school closures in relation to peak ILI is displayed in Table 5.5. Of 171 school districts with at least 1 closure, only 19 (11.2%) closed prior to the peak week of infection. This represented a total of 60 (10.8%) of all closed schools, with an average closed duration of 3.74 days (Table 5-4). Most school closures occurred during either the ILI peak in the corresponding state public health region, or within the two weeks following peak infection; 363 individual schools (65.1%) closed one or two weeks following the highest ILI rate.

Table 5-4: Average Duration, in Weeks, Between Peak ILI for a Public Health Region Where for Each School District and When School Districts and Schools Were Closed.

Weeks Between Max ILI Rate for region and School Closure	Total Districts Closed	Total Closed Schools	Average Days Closed	Proportion of Total Schools Closed
-3	1	3	4.67	1.00
-2	4	14	6.57	0.77
-1	14	43	4.79	0.63
0	32	96	4.75	0.62
1	73	249	4.45	0.62
2	38	114	4.52	0.53
3	18	31	5.35	0.52
4	7	8	4.75	0.34

An assessment of the schools that closed prior to the peak week of ILI compared to those that closed the week of peak infection or after is shown in Table 5-5. We found no differences

between school districts with closure before peak infection and closure during or after peak infection among school level, school type, total number of students enrolled, or NSLP eligibility ratio. We did observe a statistically significant greater number of days closed among schools that closed early compared to schools that closed at or following peak infection (pre-peak closure mean days closed 3.74, peak or post peak closure mean days closed 2.95, $p=0.04$).

Table 5-5: Characteristics of closed schools based on early closure (anytime before peak week of infection) and reactive closure (from peak week of infection to four weeks following peak week of infection).

	Pre-Peak Closure	Peak or Post-Peak Closure	P-value
Total Number of Schools	57	501	
School Level			0.36
Elementary	28	277	
Jr. High School	8	54	
Jr. Sr. High School	8	42	
Sr. High School	11	87	
K-12	2	41	
School Type			0.60
Public School	51	50	
Public School Academy	3	29	
Private School	3	422	
Average Total Students	334.40	346.70	0.71
Average Free and Reduce Lunch Ratio	0.48	0.48	0.93
Average Days Closed	3.74	2.95	0.04

In the district-specific analysis, the rate of ILI peaked for public health regions 1, 2S, 3, 5 and 6 during the week of October 17, 2009. Regions 7 and 8 peaked the week of October 24, while region 2N peaked the week of October 31. Regions 1, 6 and 8 had the highest attack rate (approximately 4 cases per 100,000 persons), with the smallest peak for 2S, with approximately 2 cases per 100,000. Around 250 schools across the state closed the week of October 24, the highest in our study.

5.4 Discussion

We retrospectively analyzed 559 Michigan schools that closed at least once during the 2009 influenza A (H1N1) fall wave. An analysis of the timing of school closure compared to peak ILI suggests that closure was a reactive decision in 83% of the schools. Based on previous studies, it is likely that the remaining school districts had ILI cases that were not reported

through MDSS and that all closures were reactive in nature [120, 124]. We found that schools that closed earlier did so, on average, for a slightly higher number of days than those that closed during the peak week of infection or thereafter (Table 5-4). This may reflect either the severity of illness within the schools that closed early (resulting in a longer duration of school closure), or uncertainty related to district closure guidelines during the fall term.

Our findings suggest that districts with 1 to 50% of schools closed had a lower ILI RR than school districts with 0% of schools closed or 51 to 100% of schools closed. This could be explained by differing demographics of 1-50% closed versus open districts (Table 5-1). School districts with 1-50% closures may have experienced lower ILI rates than open schools due to 1) differences in underlying population size or density, at-risk populations; or 2), timing of the ILI peak wave.

In contrast, districts with schools closed at the highest level (51-100%) had the highest ILI rate ratio. While more ILI cases occurred in open school districts than closed, these also had a higher proportion of school-aged children. ILI rates better address the underlying influence of population; a significant association of lower housing density with higher ILI correlates with findings that less-populated areas of the state were heavily impacted and had high rates of reactive school closures. These ILI rates also suggest that the school closures may have been implemented too late to be an effective NPI. Indeed, a recent survey of Michigan school closures during the fall wave of 2009 influenza A (H1N1) found that the most likely reason given for school closure was high absenteeism, suggesting a reactive rather than proactive intervention [120].

Brief reports, modeling, historical epidemiological analyses, and observational studies of seasonal and pandemic influenza events support our findings. A growing number influenza

outbreak studies suggest that, although proactive school closures may help slow the course of a pandemic [125-127], reactive or short-term closure show little to no effect [128, 129] on ILI, or reduce illness only in school-aged populations [130, 131].

Due to the nature of the data, there were some limitations to our research. First, there is the probability of underreporting of ILI cases and incomplete MDSS surveillance data. The high sensitivity of ILI definition increases the number of cases identified. However, ILI is an imprecise measure of influenza, and select individuals with symptoms matching the ILI case definition may not be infected with influenza. Further, a majority of the cases were identified through school reporting, and were not medically-attended. Second, we were unable to assess the effect of absenteeism in this study. We had limited access to the number of absent students among schools that closed, and no information on schools that remained open, resulting in our inability to control for the number of number of students out of school. Previous studies have shown that absenteeism was an important factor in school closure [120]. Third, our data is limited by a lack of complete information for available zip codes. Fourth, the small number of districts with school closures prior to peak week of infection made it difficult to assess whether any of these closures were truly preemptive, or whether early school closures can translate to a reduction in rates of illness in the population. Fifth, the categorization of school districts as "open" versus "closed" is likely too coarse to address actual differences between the schools; however, the designation was chosen to simplify complications related to the time-varying nature of the school closures, and to attempt to determine if there were any non-varying demographic differences between open and closed districts. Finally, the 2009 pandemic may not be an appropriate context to test the effectiveness of school closures as an NPI; the CDC deemphasized

proactive school closures when it became clear that the 2009 influenza A (H1N1) pandemic was less severe than initially feared.

Still, our study is the first to link ILI surveillance data with reports of school closures captured in real time across an entire state. Our findings suggest that school closures employed in Michigan during the fall wave of 2009 influenza A (H1N1) pandemic had little effect on circulating levels of ILI. Our findings also demonstrate that reactive school closures did not significantly reduce the rate of illness compared to schools that remained open. We observed a small number of districts that implemented closure prior to peak infection, as measured by surveillance data, also fit this trend. Whether this was a result of undetected circulating disease or late reporting is unknown; more intensive disease surveillance in the community setting is required. Nevertheless, this study combined information at the school district level with ILI surveillance data to provide a quantitative analysis of the effect of reactive school closure during fall of 2009. Further studies that explore the impact of school closure as an NPI on ILI are recommended.

Chapter 6: Conclusion

This dissertation identified novel finding regarding the co-occurrence of human coronaviruses and other viruses as well as co-occurrence between bacteria and viruses. We also addressed the impact of school closure by analyzing a real-time response to pandemic influenza.

In order to provide a common language for discussing co-occurrence in this population, this dissertation established a framework for the study of co-occurrence. Specifically, we described the various ways in which pathogens may interact across three different axes: timing of the interaction between pathogens, the level at which the interaction is observed (i.e., molecular, clinical, or epidemiological), and whether the observed inaction resulted in a synergistic or antagonistic outcome. The commentary also included a discussion of future steps to be taken in research, from the laboratory to the epidemiological level, to provide a better understanding of co-occurrence that occur.

6.1 Acute Respiratory Illness in College Students

Key Findings

Chapters 3 and 4 described the burden of ARI among otherwise healthy young adults living in residence halls. The first of which examined the prevalence and co-occurrence of four human coronaviruses (HCoV-229E, HKU1, NL63, and OC43) and 9 other viruses (influenza A/B; adenovirus; human metapneumovirus; parainfluenza 1,2, and 3; rhinovirus; and respiratory syncytial virus). The prevalence of the four HCoVs combined among participants that provided specimens ranged from 6.7% of completely asymptomatic participants to 19.7% among

participants with meeting our case definition of ARI, and HCoV-NL63 was the most frequent virus detected among all participants. This finding was likely a result of the seasonality of viruses in our population. Co-occurrence of viruses was found in 12 specimens collected during the study period, including one participant with three viruses co-occurring at the same time. We found that moderate/severe cough, chills, and headache decreased in frequency over the 6-day period among students with HCoV infections, while runny nose increased in frequency over the 6-day period.

The second paper using the eX-FLU dataset examined co-occurrence among the eX-FLU study between viruses and three bacteria that commonly cause disease (*Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*). We found that viral presence was significantly associated with an increase in *S. pneumoniae* carriage, but not with the other bacteria in our study. We also found that these results persisted among participants who were experiencing symptomatic illness, but not among those without symptomatic illness. Finally, we found that a viral positive sample statistically significantly reduced the viral load of *S. aureus*, but not for any of the other viruses.

Implications and Future Directions

These novel findings offer a timely contribution to the current epidemiological literature because they establish estimates of common diseases in previously understudied populations and age groups. Research from this dissertation highlights the limitations in our knowledge of the human coronaviruses. Previous epidemiological studies examining HCoV outside of the clinical or outpatient setting have not found high levels of coronaviruses among completely asymptomatic individuals, making this finding especially noteworthy. Symptoms associated with

the viruses followed along with earlier studies: feverishness has previously been associated with influenza [132-134], and upper respiratory symptoms have been associated with rhinovirus [135, 136]. We observed too few positive specimens to make any conclusions about co-occurrence of viruses within this population.

The bacterial co-occurrence study was the first to report on bacteria and viruses among young adults living in close quarters. A recent review of co-occurrence has found a cooperative relationship between *S. aureus* and influenza [137]. This relationship, in which influenza enhances the adherence and growth of *S. aureus*, may account for the higher prevalence of *S. aureus* that we observed among viral positive specimens. However, additional research is needed to confirm our findings. In terms of the association between virus and *S. pneumoniae*, while there is strong evidence of an increased risk of severe clinical disease, there is increasing evidence that colonization with *S. pneumoniae* can protect against influenza infection [138]. Due to the temporal relationship that we were able to assess, we were unable to test any specific mechanisms that occur between the viruses we tested for and colonization. We were, however, able to report on an association between viral infection and colonization. The finding suggests that bacteria take advantage of a viral attack to proliferate within a host. While we did not observe severe illness as a result, this finding could provide a biological pathway for the observation of severe bacterial illness, such as pneumonia, following viral infections. From a public health perspective, this could provide additional evidence of the importance of viral vaccinations to prevent infection, therefore assisting with reducing severe bacterial illness, and to alert clinicians working with at-risk populations of the need to monitor patients diagnosed with viral disease for an opportunistic secondary bacterial infection.

Both of these studies suffer from temporal ambiguity. Since we had insufficient sample size to examine individuals who provided specimens while not experiencing ARI symptoms, we were unable to make any conclusions about what likely caused transmission in this population. Additionally, among the few co-occurrences that we identified, we were unable to determine which of the agents infected an individual first, or whether both were transmitted at the same time. This limitation was especially true when looking at associations between bacteria and viruses. As we only assessed bacterial colonization at a single time point, we were unable to determine whether an individual was infected with a virus prior to colonization, or vice versa. As such, we can provide information on associations observed, but we are unable to causally test the relationship with the available data.

Methodological Considerations for Co-Occurrence

Additional methodological research could advance the field of co-occurrence research. While were we able to test a large number of the most common known causes of ARI, the list of 13 viruses and 3 bacteria were specifically targeted based on *a priori* assumptions of what causes symptomatic illness in young adults. However, certain agents, such as human bocavirus, have been identified in large populations of symptomatic individuals, and may have driven some of the illnesses observed in our population. Additionally, the availability for in-depth sequencing would allow for the identification of agents that are not commonly tested in our population.

This study design did not allow for us to determine whether an infection could have occurred prior to the testing nor how long shedding of HCoVs apply. As a descriptive study, we are unable to ascertain any causal relationship in our data. As such, questions remain about why the prevalence of coronaviruses was so high among this group. Previous studies have pointed to

the HCoVs as being underrepresented outside of the clinical setting [104]. Since these viruses often cause illness no more severe than the common cold, infected individuals tend not to seek treatment in traditional health care systems; as a result, they are rarely tested for an identifiable agent. Given that we found such high levels of coronavirus in our population, future studies should consider testing for HCoVs as a likely cause of acute respiratory illness.

Future Research on Co-Occurrence

The question of co-occurrence and the interaction between bacteria and viruses is continually being studied. In aim 1 of this dissertation, we examined the different levels at which co-occurrence may happen. This study was able to show a synergistic effect in college-aged students at the epidemiological level. The presence of a viral infection was associated with a higher prevalence of *S. pneumoniae*. We were unable to address the two other axes of co-occurrence in this study. Due to the cross-sectional nature of the study, we were unable to determine the timing of co-occurrence. As such, we cannot tell whether symptomatic disease increased the likelihood of either viral infection or bacterial colonization, or whether the co-occurrence led to symptomatic illness at a higher level. We were also unable to determine whether colonization occurred prior to viral infection, whether the virus and bacteria were transmitted together, or whether *S. pneumoniae* took hold after viral infection. We were also unable to assess whether the interaction occurred at the molecular level. Molecular based studies are needed to determine whether, for example, *S. pneumoniae* and influenza directly interact with each other independent of the host response. Finally, since our population consisted of otherwise healthy young adults and our sample size was too small, we did not observe any clinical

outcomes that could elucidate whether disease was more severe among those with co-occurrence compared to those without co-occurrence.

The university setting has been tied to multiple disease outbreaks in the past [139, 140], but these findings may not be applicable to all young adults. As such, future epidemiological studies among young adults are needed in order to see whether virus and *S. pneumoniae* colonization remains linked. Future studies would also benefit from the ability to ascertain the timing of the co-occurrence. A study design that would allow for the sampling of asymptomatic adults at the beginning of the study, and following them until they get sick may shed some light on the timing of infection.

Interesting corollaries remain as areas of research, including the questions of what pathogens are, as raised by Pirofski and Casadevall [141]. In their commentary on the nature of a pathogen, they touched on questions of opportunistic infections, commensals, and the importance of host response in defining a pathogen, including pathogenicity caused by immune response rather than pathogens. In doing so, they highlighted the importance of understanding pathogens across different levels of interactions, as well as how host-microbe interactions are key to understanding pathogenicity. Taken together, these commentaries have implications in the fields of both bacteriology and virology.

Future virology studies are needed to examine the relationship among viruses within asymptomatic individuals, the effects of secondary viral assault, and timing of infection. However, recently published articles have raised the question of human bocavirus, which may occur in humans with no symptoms, or only cause more severe symptomatic illness when present with a second pathogen [142]. We did not have the sample size necessary to examine this relationship; future community based studies which examine coronaviruses in the community

over multiple years, such as those published when identifying the spread of HCoV globally, will be necessary to determine whether HCoV may fall under similar aspects.

6.2 School Closures during the 2009 H1N1 Pandemic

Key Findings

The final paper of this dissertation examined school closure responses to the 2009 H1N1 influenza A pandemic. We found that most of the school closures were reactive compared to the peak week of infection determined by surveillance data. Within the reactive closures, we identified three categories for each school district – 0 percent of schools closed during a given week, 1-50% of schools closed for a given week, or 51-100% of schools closed for a given week. Across these three categories, we observed no reduction in the rate of ILI as observed through surveillance data. This lack of significant results persisted with both our unadjusted and fully adjusted models.

Implications and Future Directions

This study was the first study that we found to link ILI surveillance outcomes with school closures across such a large geographical region. Our findings supported previous research, including hypothetical models, that reactive school closures would not be effective in reducing the spread of ILI. Brief reports, modeling and observational studies of season and pandemic influenza outbreaks in the United States, Japan and Hong Kong suggested that while proactive school closures may result in effective slowing of a pandemic [125-127], reactive and short-term closure show little to no effect [128, 129], or school-aged specific effects [130, 131]. Other natural experiments have supported these findings. For example, school closures due to seasonal

influenza Hong Kong in 2008 had no effect on community transmission [143]. However, studies in Israel [144] and France [130] related to school closures for holidays and teacher strikes found a reduction in transmission, specifically among school-aged children. Historical analyses of the 1918 influenza pandemic suggested early, sustained, and layered NPI had a significant impact on pneumonia and influenza mortality [14].

Results from modeling studies examining school closures have been mixed. At least one study has found that longer school closures (up to 16 weeks) and coordinated area-wide closures of multiple schools have been found to be effective in preventing intensive care units from reaching capacity [127]. Another model found that closures of entire school systems were not more effective than closures of individual schools, but added that longer closures (8 weeks) would be most effective [128]. Estimates using a reproduction number of 1.5 (consistent with early findings from Mexico of an R_0 of 1.4 to 1.6) found that a one-week school closure had a minimal effect on the attack rate of influenza [129]. The authors concluded that school closures should be combined with other NPIs such as reducing close contacts and household prophylaxis to provide the best prevention to reduce the impact of a pandemic. Finally, one study based on school holiday data suggests that closures during a pandemic could reduce the transmission of illness among children, but would have little effect on adults [130]. Additionally, the sustained social distancing of children required to make the NPI effective would be difficult to maintain. Taken as a whole, modeling studies seem to support our findings that both reactive and short-term school closures are likely to have little impact on community levels of influenza.

Methodological Considerations for School Closure

There are two main methodological areas in which the findings from the school closure study could be strengthened. The first problem relates to reporting of the illness and the second to the timeliness of decisions to close schools. In terms of illness reporting, there were two limitations to the Michigan Department of Community Health's Michigan Disease Surveillance System (MDSS) surveillance data that were used. The first of these was the use of ILI as an outcome instead of confirmed influenza cases. Among the cases that were reported to the surveillance system, it seems likely that other agents could be causes at least some of the cases. As such, not all of the reported cases of ILI were likely attributable to pandemic influenza. However, the second problem is likely a larger factor that results in under-counting of influenza in the community: most of the cases that are reported to MDSS were medically attenuated. As a result, a number of probably influenza cases were not captured in our data, resulting in a limited sample size, especially when stratifying over time.

The second methodological consideration to consider for school closures is the timeliness of available data for making decisions to preemptively close schools. Multiple modeling studies have suggested that early and prolonged school closures are the most effective method of slowing down transmission. However, without timely surveillance data to indicate the extent of the spread of infection, school administrators will be unable to make timely decisions regarding school closure. A potential solution is discussed below.

Future Research on School Closure

School closure as a NPI remains controversial. School closures may burden caregivers who work outside the home [19, 127, 145]. Moreover, approximately 21.5 million children receive free or reduced-price meals at school, an important source of nutrition for this

population. School closures, particular lengthy ones, interfere with students' educations, and closures that extend past the allotted emergency days represent a significant cost to school districts (e.g., salaries, building maintenance, supplies, and energy). Furthermore, the information necessary to make a decision about when the timing of a closure could be most effective is difficult to predict in real time as an influenza pandemic is unfolding [19, 127, 145-147].

A recent study from sentinel data suggested a potential way forward. The Chinese region of Hubei set up a school absenteeism surveillance system in 17 primary schools. Over two and a half years, the system generated 52 signals from school absenteeism and successfully three different outbreaks, resulting in excess protection rates for two of them [148]. Paired with timely surveillance data collected by the state, school based absenteeism surveillance may be an effective method for preventing the transmission of infectious diseases within the community setting.

6.3 Final Thoughts

Using prospective data from the eX-FLU study, this dissertation reported on the prevalence of respiratory viruses and bacteria among University of Michigan students during the 2012-2013 influenza season. This is the first study to report on HCoVs among young adults in the community setting, and also addressed the carriage of common bacteria in the same population. Further studies can build on this research using the social network design to examine the mechanisms that influence transmission in close quarters.

The design of the eX-FLU provided the opportunity to answer some of the questions raised in the commentary about how co-occurrence occurs. Through the use of social network

information, we were able to sample study participants who did not meet our case definition for an acute illness, a difficult population to sample from. The prospective study design, and multiple days of sample collection for each individual, also allowed for us to assess changes in laboratory status of specimens over time. However, even with a study design that would allow for answering questions about transmission and timing, the limited number of participants and specimens impeded our ability to make strong conclusions about shedding or co-occurrence. We see a great deal of promise in the social network model used in this study. However, future studies of this type might be improved by a larger population size and the ability to sample all participants prior to the illness season.

Using retrospective surveillance data from the 2009 H1N1 influenza A pandemic in Michigan, we were able to look at the effect of school closure on community ILI. This is the first study to look at community level effects at the state level. This wider scope allowed us to assess more clearly the larger-scale geographical effects of school closure. Future research can build on this approach to study the effectiveness of school closures as a NPI, though the recommended approach from modeling studies of early and extended closures are likely to remain politically untenable.

The school closure study also suffered from sample size issues. As described in the limitations above, the data did not capture all of the cases of ILI in Michigan during the timeframe of the study. Further, when breaking down the reported cases by week, and looking at so many counties, we ran into model fitting issues when looking at our data. In future studies, more comprehensive reporting from the state could address that issue. A second lesson from the study was the difficulty of matching data that comes from different sources. Since individual school districts do not make available either absenteeism information or the total number of

cases in a district, we had to combine many different sources of data at different levels, such as summing county level information within each district.

This dissertation has been a multi-disciplinary effort, incorporating a wide range of data including laboratory results and surveillance information. Throughout the process, I have had the opportunity to strengthen my analytical skills, ranging from basic descriptive epidemiology to more complex models. Future studies can build upon the basic descriptive information to incorporate the complex study design, and further the available research on NPIs and co-occurrence.

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