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The impact of antibiotic use on influenza vaccine effectiveness in children aged 6-59 months

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An abstract of
A thesis submitted to the Faculty of the
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Abstract

The impact of antibiotic use on influenza vaccine effectiveness

in children aged 6-59 months

By Jennifer L. Brosseau

Background: Recent studies in animals have suggested that immune response to the influenza virus may be impacted by the likely change in intestinal commensals associated with antibiotics. This study examined the relationship between antibiotic use and influenza vaccine effectiveness to determine whether the same altered immune response occurs in children.

Methods: Data were obtained from the Kaiser Permanente Georgia (KPGA) managed care organization (MCO) research databases, and included 6 influenza seasons from 2005-2011. Poisson regression analysis was conducted to determine the effect of antibiotic use and influenza vaccination on rates of MAARI among children aged 6-59 months. Logistic regression was used to evaluate confounding.

Results: We found no significant difference in the incidence rate ratios (IRRs) of MAARI among the vaccinated versus the unvaccinated, stratified by antibiotic use. The ratio of IRRs for non-antibiotic users comparing the period of widespread activity with the pre-influenza period was 0.98 (95% CI, 0.79-1.23). The ratio of IRRs for antibiotic users for the same comparison period was 0.90 (95% CI, 0.63-1.30). No significant differences existed in vaccine effectiveness (VE). During the pre-influenza period, we found VE to be 10% (95% CI, -9%-26%) among non-antibiotic users and 17% (95% CI, -12%-39%) among non-antibiotic users, even after adjustment for known confounders. During the period of widespread influenza activity, VE was 2% (95% CI, -23%-21%) among non-antibiotic users and 10% (95% CI, -30%-37%) among antibiotic users.

Conclusions: The presence of vaccine effectiveness in the pre-influenza period demonstrates that we were unable to eliminate unmeasured confounding, limiting our ability to draw conclusions. It does appear the likely change in intestinal commensals associated with antibiotic use does not reduce TIV effectiveness.. However, given evidence exists for the biological possibility of interaction between antibiotic use and immune response to influenza vaccination, further studies should be undertaken to address this topic.

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Table of Contents

Background/Literature Review

Influenza is transmitted through the airborne/droplet route, and viral shedding in respiratory secretions normally occurs for 5-10 days. Following transmission, the influenza virus replicates in the respiratory epithelial cells in the trachea and bronchi, causing coughing. The incubation period for influenza is typically 2 days, but may vary from 1 to 4 days (1). Major symptoms last an average of 7 days (2).

Symptoms of "classic" influenza disease include fever, myalgia, sore throat, nonproductive cough, and headache, but presentations vary widely and complications beyond viral illness may occur. These "classic" symptoms are developed by 50% of people (1). Other symptoms include anorexia, chills, extreme fatigue, photophobia, abdominal pain, diarrhea and pharyngitis. Approximately 30-50% of people who are infected with the influenza virus are asymptomatic, but still may transmit the virus to other individuals (2). While pneumonia due to the influenza virus is rare, it does have a high fatality rate when present (2, 3). The most frequent complication of influenza infection is secondary bacterial pneumonia, usually caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, or *Haemophilus influenzae* (1, 2). Treatment for pneumonia may include a hospital stay, intensive care unit admission, and/or mechanical ventilation, all of which expose the patient other health risks (4). Other complications include myocarditis, worsening of chronic respiratory disorders, congestive heart failure, bronchitis, and bacterial sinusitis (1, 2).

The presentation of influenza disease in children is often different from that of adult patients. Fevers are often higher in children, and may lead to febrile seizures (2, 3). A study of Danish children found that febrile seizures are a substantial burden of influenza-like illness, with hospital admissions for febrile illness increasing with the incidence of influenza-like illness (5). Abdominal pain, vomiting, diarrhea, croup, myositis, otitis media and conjunctivitis also occur more frequently in children than in adults (2, 3). A study of children presenting to the emergency department with febrile respiratory illness found that the triad of cough, headache, and pharyngitis was an accurate predictor of influenza infection (6). However, infants and young children may present with generalized febrile illness with no specific respiratory symptoms, or with respiratory symptoms that are indistinguishable from other childhood respiratory illnesses such as respiratory syncytial virus (3, 6).

Children also experience different complications of influenza infection than adults. The most common complication of influenza in children is otitis media (7). Influenza may also be associated with Reye's syndrome in children, though incidence has declined since warnings were issued regarding giving children aspirin, which is the major risk factor for the condition. Reye's syndrome is reported primarily in children infected with influenza B virus, but may also be present with influenza A virus (2). Encephalopathy may also develop in children, increasing risk of influenza mortality (2, 8).

Treatment options are mostly limited to attenuation of symptoms, and influenza is generally a self-limiting illness. There are several prescription medications available to treat and prevent influenza infection. These include the adamantanes (amantadine and rimantadine) and NA inhibitors (zanamivir and oseltamivir) (2). When used within 48 hours of symptom onset, the adamantanes decrease the severity and duration of illness (3). However, antimicrobial resistance has developed to amantadine, decreasing its usefulness as a treatment option (2).

There are three basic strains of influenza, which cause varying degrees of illness among human and animal hosts. Influenza viruses are part of the *Orthomyxoviridiae* family, with 3 separate genera. Influenza virus strains A, B, and C are differentiated by antigenic differences in two structural proteins, the nucleoprotein and the matrix protein (2). Influenza A is further subdivided into types based on serological and genetic differences in two major membrane glycoproteins, HA and NA (2, 3). Influenza A viruses are referred to by the type of HA and NA glycoproteins they have on their surface. Influenza A viruses cause the majority of disease in human populations, inducing moderate to severe respiratory illness in all age groups (1). Influenza A is also naturally found in animals, primarily wild aquatic birds, though subtypes have been isolated from pigs, poultry, dogs, horses, seals, and whales (1-3). Influenza A has been known to cause disease in animals, though the virus may also be asymptomatic. Interspecies transmission has been well documented and may cause severe

illness in humans, as does the highly pathogenic avian influenza H5N1, first documented in 1997 (2). Influenza B, found naturally only in humans, does not generally cause severe disease, but may cause mild disease, primarily in children (1, 2). Influenza C infection occurs only in humans and pigs and normally cause only localized or sporadic outbreaks of infection (1).

Surface antigens on influenza virus play a large role in immune response to the virus. The severity of illness depends on previous immunologic experience with the variant of the virus with which the person has been infected (1). For example, a person previously exposed to a variant of the H1N1 influenza A virus may have some immunity to a different H1N1 variant, providing some protection against illness. Some strains may provoke a greater immune response than others, leading to more severe symptoms and even death. During seasons when influenza A H3N2 virus was prominent, the mortality rate was 2.7 times higher than in seasons when H3N2 was not prominent (1).

Minor and major changes in genetic structure contribute to the infectivity and pathogenicity of the virus. Minor changes in genetic structure, known as antigenic drift, result from mutations in the RNA genes that encode the HA and NA antigens, creating new variants of the disease. As people develop immunity to older variants of influenza, new strains begin to infect susceptible individuals, allowing these variants to become the dominant circulating strains (2, 3). This cycle continues, allowing individuals to be infected with many different influenza variants in their lifetimes (1, 2). A major change in the genetic

structure of circulating influenza viruses is called antigenic shift, and occurs when an influenza virus with an HA and NA combination that has not recently infected humans is transmitted (2, 3). These strains may be reassortments of animal and human influenza viruses, or may be animal influenza viruses transmitted directly to humans (3). Because the majority of the population has no immunity to these new combinations of antigens, a pandemic is likely to develop (2, 3). These new pandemic strains may cause increased rates of illness and death compared with seasonal influenza (2). High attack rates and rates of hospitalization are seen, especially in adults with respiratory disease (1).

A definitive diagnosis of influenza requires laboratory testing, but often influenza is diagnosed based on clinical presentation. A diagnosis based on clinical findings alone is more likely when influenza is known to be circulating in the community (1). The influenza virus can be isolated from a swab of the throat or nasopharynx within 3 days of symptom onset, however, virus isolation and identification takes 18 hours to 4 days (1, 2). The relatively long processing time required limits the clinical usefulness of the results of these tests. Serologic confirmation of influenza infection is demonstrated by a rise in influenza IgG, with diagnosis requiring at least a fourfold increase in antibody titer (1). Rapid tests are available to diagnose influenza A and B viruses, but are generally have a sensitivity of 40-100% and a specificity of 52-100%, limiting their reliability in the clinical setting (2).

Epidemics of influenza are seen seasonally during the winter months in the Northern hemisphere, while pandemics may occur at any time and have a global impact. In the Northern hemisphere, influenza infection rates normally peak in January and February, but may peak as early as December or as late as May. In tropical climates, influenza occurs throughout the year (1, 2). The number of cases and severity of infection are related to the level of immunity existing in the population. If the predominant circulating strains are identical or similar to those circulating in previous years, more people will have some immune protection against the virus (1). However, if the virus is genetically rather different from that circulating in previous years, there will be more people susceptible to the virus and thus more infection. Worldwide pandemics occur when the influenza A virus undergoes antigenic shift and infects a large population of susceptible people. These pandemics occur infrequently, but often result in the circulation of a new type of influenza A virus. People generally have had no exposure to these viruses, and so immune response is low, increasing the rate of severe disease (2).

Influenza disease is most severe in the very young and the very old. The annual attack rate of influenza infection in children 0-4 years of age is estimated to be greater than 20%, where the annual attack rate for all other age groups ranges from 6% to just over 10% (7, 9). The attack rate for adults aged 65 and older is approximately 9% (9). Hospitalization rates are similar among these age groups. Estimated overall rates of hospitalization due to influenza among

children aged less than 5 years range from 17 to 253 per 100,000 population, with rates of 300 to 400 per 100,000 population in children under 2 (4, 10-12). During periods in which influenza virus was circulating, excess rates of hospitalization in children aged 6-59 months due to cardiopulmonary causes were 90 to 500 per 100,000 population (13). Among the elderly, annual hospitalization rates range from 71.1 per 100,000 person-years among 65-69 year olds to 628.6 per 100,000 person-years for those aged 85 and older. Comparatively, the influenza hospitalization rate among 5-49 year olds is only 6.8 per 100,000 person-years. Median length of stay also increases as age increases (14).

Influenza mortality occurs among young children, but people older than age 65 account for the majority of influenza-related deaths. A study of 2003-04 influenza season found a mortality rate of 0.21 per 100,000 among all children, with the highest mortality rate among children younger than 6 months and decreasing as age increased (8). National surveillance data shows an influenza and associated pneumonia mortality rate of 0.2-0.3 deaths per 100,000 person-years for children, while the rate for those aged 65 and older is 22.1 deaths per 100,000 person-years (15).

A substantial number of outpatient and emergency department visits are attributable to childhood infection with influenza virus. A study of children 6 months to 13 years of age found that 13.5% of total pediatric outpatient visits during influenza season were caused by influenza infection (16). Another study found influenza accounted for 23-35% of excess outpatient visits in children 0 to

35 months (13). A third study estimated the rate of outpatient visits for influenza in children 0-59 months at 50-95 influenza visits per 1,000 visits, whereas emergency department visits for influenza comprise 6-27 per 1,000 total visits (11).

Those with high-risk comorbid conditions are also at increased risk of influenza infection and complications. Several studies found that children with high-risk conditions made up one-third of children hospitalized for influenza infection, with the majority of those having a comorbid pulmonary condition such as asthma (4, 17). When modeling the impact of influenza infection, it is estimated high-risk individuals in all age groups would account for 85% of influenza deaths, 38% of all hospitalizations, and 20% of all outpatient visits (18).

Children typically have the highest attack rates during influenza epidemics and serve as major sources of transmission of influenza within the community. The annual attack rate in children is greater than 20%, by far the highest among all age groups (7, 9). Attack rates as high as 50% have been noted in some high-risk populations, such as day care attendees (7). Studies have shown that seroprevalence of antibodies against influenza A virus increases with increasing age, and that by the age of 6 years, most children have been infected with at least one influenza A virus (19, 20). Children are thought to be frequent spreaders of influenza infection among all age groups (21). Vaccinating children against influenza has benefits their contacts. A study of 10 day care facilities in San Diego found unvaccinated contacts of vaccinated children had 42% less

febrile respiratory illnesses than unvaccinated contacts of unvaccinated children (22). Modeling the impact of vaccinating children in the United States estimated that if 20% of children were vaccinated, the number of influenza cases among those 18 years of age and younger would be halved, and the number of influenza cases among adults would be reduced by 43% (23). Moreover, 20% vaccination coverage in children would reduce hospitalizations in the elderly from 42,800 to 24,600 and would reduce mortality in the elderly from 34,400 to 19,800 (23).

Vaccination with trivalent inactivated influenza virus is generally an effective measure for preventing influenza infection in children and adults, though some studies have failed to show statistically significant effectiveness. In studies of vaccine effectiveness in fully vaccinated children, TIV has been shown to be from 52-86% effective against medically attended, laboratory confirmed influenza illness (24-28). Vaccine effectiveness against severe influenza-like illness in children has been estimated at 25% (29). However this effectiveness varies by season and, by association, antigenic match of the vaccine strain to the circulating strain. A study of children aged 6 to 59 months found no significant vaccine effectiveness against laboratory confirmed influenza for the 2003-04 influenza season (25). A systematic review of studies of healthy adults aged 18 to 49 found TIV effectiveness ranged from 50-68% (30). Few studies have been conducted on the effectiveness of influenza vaccination in the elderly (31). One study in the Netherlands found that vaccination with TIV halved risk of influenza infection among those aged 60-69 years, but did not have enough

power to detect an effect in those 70 years and older (32). TIV is not highly effective in the elderly; however, it does provide protection against complications and death. In a study of a nursing home population during the 1982-83 influenza outbreak, unvaccinated residents were four times more likely to die than were vaccinated residents (1).

Strains contained within the influenza vaccine change annually, based on predictions of which strains are likely to circulate during the coming influenza season. Yearly global surveillance of circulating influenza strains inform the decision on which three strains to include in the vaccine, generally two influenza A virus strains and one influenza B virus strain. The need for accurate and timely surveillance must be balanced against the need for adequate manufacturing time for the vaccine. It generally takes 4-6 months from the time strains are identified to produce a vaccine (2).

The two most common types of influenza vaccine in the United States are trivalent influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV). TIV is an inactivated vaccine made from subvirion preparations, which retain the antigens required for immune response but are not as reactogenic as whole virus vaccines. It is administered intramuscularly (2). TIV is approved for people aged 6 months and older, who have not had a severe adverse reaction to a previous dose of TIV. TIV may be administered to pregnant and breastfeeding women, and the immunocompromised (1). LAIV is a live virus vaccine, which is sprayed into the nose using a syringe-like device (33). Because it is a live virus

vaccine, LAIV only is approved for people from 2-49 years of age, without chronic medical conditions, including pregnancy and immunosuppression (1).

The influenza vaccine is now almost universally recommended. In 2010, the Advisory Committee on Immunization Practices recommended influenza vaccination for all people over the age of 6 months. In order to permit an adequate immune response to occur before circulation of the virus, practitioners are recommended to administer the vaccine as soon as it becomes available each year. Because TIV is manufactured using chicken eggs, those who have had mild allergic reactions to eggs in the past should be vaccinated only with extra safety precautions, and those with severe allergic reactions to eggs should be vaccinated only after consultation with an appropriate specialist (34).

Influenza vaccine recommendations for children aged 6-59 months have changed over the last decade. Until the 2003-04 influenza season, there was no direct recommendation regarding vaccination for healthy children, however children vaccinated for the first time were recommended to receive an additional dose one month after receiving the first dose (35). Beginning in the 2004, vaccination was recommended for all healthy children aged 6 to 23 months, with children vaccinated for the first time receiving two doses one month apart. Children having received only one dose of influenza vaccine in the previous season were not required to receive a second dose (36). In 2006, this recommendation was expanded to include all healthy children from 6 to 59 months of age, with the recommendations on number of doses remaining the

same (37). In 2007, the recommendations regarding dosing were amended to recommend children who received only one dose of vaccine in a prior season receive two doses of vaccine during the current season (38). In 2008, the recommendation for children was expanded to include those up to 18 years of age (39). During the 2009 H1N1 pandemic, two doses of monovalent vaccine were recommended for all people aged 6 months to 24 years (40). Recommendations for seasonal trivalent vaccine remained the same (41). In 2010, the current recommendation for universal vaccination of those over 6 months of age was made and continues to the present (42).

Trivalent influenza vaccine (TIV) includes two influenza A strains and one influenza B strain, thought to be most likely to circulate in the approaching influenza season. It has been available since the 1940s. The current vaccine is composed of a strain of influenza A H1N1, a strain of influenza A H3N2, and an influenza B virus strain (1). Global surveillance of circulating influenza viruses helps vaccine developers choose which strains to include in the vaccine by March of each year. Vaccine viruses are obtained from World Health Organization (WHO) isolates. TIV also generally contains an adjuvant which increases immune response to the antigens in the vaccine. In addition to the aluminum-based adjuvants used in many commercially available vaccines, two additional adjuvants have been licensed in Europe, though these may also increase reactogenicity to the vaccine (2).

Trivalent influenza is an inactivated, subvirion preparation manufactured through the use of egg or mammalian cells. While each manufacturer uses a slightly different process, all of these result in a standardized amount of HA antigen per dose. From WHO isolates, reference strains are developed and distributed manufacturers for use in creating seed viruses. Because most wild-type strains are unable to flourish in chicken eggs, antigenically similar strains are examined to find qualities necessary for large scale production. These vaccine strains are isolated in either eggs, primary chick kidney cultures, or mammalian cells. After an adequate amount of virus has been grown, it is purified and the HA level is adjusted to target levels. Viral proteins are further purified through disruption of the lipid envelope, decreasing the reactogenicity of the final product (2).

Adverse reactions to TIV are generally not severe, though some significant side effects have been noted rarely. The most common side effects are local reactions, including soreness, erythema and induration at the injection site. These are reported in 15-20% of those vaccinated (1). The most common systemic reactions include fever, myalgia, arthralgia, and headache (1, 2). Sometimes a severe neurological reaction may occur after influenza vaccination. This condition, called Guilliane-Barre Syndrome (GBS) is an acute, immune-mediated disorder of the peripheral nervous system that causes progressive paralysis (2, 43). GBS occurs in only 4 out of every 10,000,000 influenza vaccinees

(43). In children, an increased rate of febrile convulsions has been associated with the influenza vaccine (5).

Immune memory protective against influenza virus is generated through generation of antibodies. After administration of the influenza vaccine, B cells are activated in the lymph nodes by migrating dendritic cells (44). Antigens activate both B cells and T cells, resulting in a B cell differentiation pathway through germinal centers, in which antigen specific B cells reproduce rapidly and differentiate into antibody-secreting plasma cells or memory B cells (44). However, very few non-live virus vaccines induce lasting immune memory after a single dose (44). This, in addition to yearly antigenic drift, means that the influenza vaccine should be administered every year regardless of any previous doses received.

Influenza vaccination stimulates production of antibodies against the major surface antigens of the virus, with the robustness of the response dependent on age and pre-existing antibody levels. After vaccination, antibodies are produced mainly against HA and NA glycoproteins, though in some cases, production of antibodies against the NP and M1 proteins may also occur. In previously vaccinated individuals or those who have had influenza, the primary response is development of anti-HA IgG. In previously unvaccinated children who have not experienced illness with influenza, anti-HA IgM may be more prominent. Numbers of virus-specific antibody cells in peripheral blood peak after 1 week, whereas serum antibody levels normally peak between 2-4 weeks.

The development of these antibodies takes longer in children, the elderly, and others not previously exposed to influenza virus (2).

Antibodies against surface antigens are generally thought to be the most accurate correlate of protection against the virus, however, research shows that local neutralizing antibodies and secretory immunoglobulin A (IgA) are also associated with protection. As defined by Plotkin, a correlate of protection is "a specific immune response A specific immune response to a vaccine that is closely related to protection against infection, disease, or other defined end point" (45). For influenza, the correlate of protection is most generally thought to be a hemagglutination-inhibition antibody titer of 1/40 dilution (2, 3, 45, 46). Challenge studies using live virus, such as that found in LAIV, have also found that neutralizing antibodies and IgA found on mucosal surfaces are also correlated with protection from influenza infection, and that these responses last longer than those produced by TIV (2, 47).

Recently published work in animal models has shown that the presence of intestinal flora is related to the immunogenicity of the influenza virus. These commensals may serve to keep the mucosal immune system, including antigen-presenting cells, aware of non-self antigens (48). Activation of innate immune cells may require some form of commensal-derived signals, such as those activating toll-like receptors (TLRs), in order to induce robust adaptive immune responses against the influenza virus (49). The influenza vaccine may also

require these commensal-derived signals in order to create an adequate immune response.

Antibiotic use is known to alter the amount and diversity of intestinal commensals, and may impede immune response to pathogens, including the influenza virus. Frequently used antibiotics such as amoxicillin, ampicillin, azithromycin, clindamycin, ciprofloxacin, levofloxacin, metronidazole, neomycin and sulfamethoxazole/trimethoprim are all known to alter intestinal flora (48, 50-53). A study of mice by Ichinohe et al. found that oral antibiotic treatment resulted in a decrease in CD4 T-, CD8 T-, and B-cell immunity after inoculation with live attenuated influenza virus (48). In another study, young mice with altered gastrointestinal tract flora inoculated with ovalbumin demonstrated decreased immunogenicity at 7 days of age, but immunogenicity was not significantly different from control mice at 14 days of age. Germ free mice showed significantly decreased immunogenicity at all developmental ages (52). In addition, unpublished studies in mice show that the decrease in intestinal commensals associated with antibiotic use may adversely affect immunogenicity of the inactivated influenza vaccine.

Manuscript

The impact of antibiotic use on influenza vaccine effectiveness in children aged 6-59 months

Abstract

Background: Recent studies in animals have suggested that immune response to the influenza virus may be impacted by the likely change in intestinal commensals associated with antibiotics. This study examined the relationship between antibiotic use and influenza vaccine effectiveness to determine whether the same altered immune response occurs in children.

Methods: Data were obtained from the Kaiser Permanente Georgia (KPGA) managed care organization (MCO) research databases, and included 6 influenza seasons from 2005-2011. Poisson regression analysis was conducted to determine the effect of antibiotic use and influenza vaccination on rates of MAARI among children aged 6-59 months. Logistic regression was used to evaluate confounding.

Results: We found no significant difference in the incidence rate ratios (IRRs) of MAARI among the vaccinated versus the unvaccinated, stratified by antibiotic use. The ratio of IRRs for non-antibiotic users comparing the period of widespread activity with the pre-influenza period was 0.98 (95% CI, 0.79-1.23). The ratio of IRRs for antibiotic users for the same comparison period was 0.90 (95% CI, 0.63-1.30). No significant differences existed in vaccine effectiveness

(VE). During the pre-influenza period, we found VE to be 10% (95% CI, -9%-26%) among non-antibiotic users and 17% (95% CI, -12%-39%) among non-antibiotic users, even after adjustment for known confounders. During the period of widespread influenza activity, VE was 2% (95% CI, -23%-21%) among non-antibiotic users and 10% (95% CI, -30%-37%) among antibiotic users. **Conclusions:** The presence of vaccine effectiveness in the pre-influenza period demonstrates that we were unable to eliminate unmeasured confounding, limiting our ability to draw conclusions. It does appear the likely change in intestinal commensals associated with antibiotic use does not reduce TIV effectiveness.. However, given evidence exists for the biological possibility of interaction between antibiotic use and immune response to influenza vaccination, further studies should be undertaken to address this topic.

Introduction

The burden of influenza infection among children less than 5 years old is higher than that among the general population. The annual attack rate of influenza infection in children 0-4 years of age is estimated to be greater than 20%, where the annual attack rate for all other age groups ranges from 6% to just over 10% (7, 9). Influenza accounts for over 30% of medically-attended acute respiratory illness (MAARI) in children less than 14 (54). Influenza infection results in increased hospitalization (4, 10-12), outpatient visits (11, 13, 16), and emergency department visits (11) in this age category. Given this burden,

effectively preventing influenza in this population is a high public health priority.

Trivalent influenza vaccination (TIV) is the most effective form of prevention of influenza infection. In studies of children 6 to 59 months of age, TIV has an effectiveness of 44-86% against laboratory-confirmed influenza (24, 25, 27). Routine, annual influenza immunization is recommended for all people ≥ 6 months of age (34). In addition, a recent study determined that influenza vaccination prevented 1.1 million to 5 million cases of influenza per season from 2005-2011 (55).

Children are frequent consumers of antibiotics and may be at risk for decreased TIV effectiveness. Antibiotics are prescribed at 21% of pediatric ambulatory care visits, and 50% of these are broad spectrum (56), and likely to have the most impact on intestinal commensals. Because recommendations allow for mildly ill children to receive the influenza vaccine (1), there are occasions when children may be both prescribed antibiotics and given influenza vaccine within a short time period. If, as the authors hypothesized, antibiotics affect the immune response to TIV, this combination could result in an altered risk of influenza in these children.

While studies have been undertaken to address concurrent antibiotic use and influenza vaccination in animals, no studies have been conducted in humans. Recent work in animals has shown that the presence of intestinal flora is related to the immunogenicity of the influenza virus (48). Such flora may serve

to keep the mucosal immune system, including antigen-presenting cells, active and aware of foreign antigens. Activation of innate immune cells may require some form of commensal-derived signals, such as those activating toll-like receptors (TLRs), in order to induce robust adaptive immune responses against the influenza virus (49). It is possible that influenza vaccination works in a similar way. In both published and unpublished data, mice exposed to antibiotics demonstrated initially reduced immunogenicity, but eventually mounted an immune response similar to unexposed mice (48, 52).

This study addresses whether antibiotic-exposed children follow the same pattern of delayed response to the influenza vaccine as antibiotic-exposed mice did. An improved understanding of how antibiotic use affects influenza vaccine immunogenicity will inform future recommendations on administration of TIV to children.

Methods

Data were obtained from the Kaiser Permanente Georgia (KPGA) managed care organization (MCO) databases, which include inpatient, outpatient, immunization, and pharmacy records for KPGA enrollees. The study population included children who were aged 6-59 months as of September 1st of each influenza season from 2005-2011 and were continuously enrolled (no gaps in coverage greater than 2 weeks) during the entire influenza season, from September 1st until May 31st of the following year. During the 2009-2010 pandemic, the influenza season was defined as June 1, 2009 – May 31, 2010, and

the same rule was applied. Children were eligible for inclusion if they received TIV during any season, received monovalent H1N1 vaccine during the 2009-2010 season, or did not receive an influenza vaccine. Children were eligible to be included for multiple seasons. Each observation represented not an individual child, but an individual influenza season for a child, hereafter referred to as child-seasons. The study population consisted of 45,325 child-seasons contributed by 24,866 unique subjects (Table 1).

Children were excluded from an individual season if they were not continuously enrolled during the entire influenza season as defined above or if they received live attenuated influenza vaccine (LAIV). These children were still eligible to be included for previous or future seasons provided they met the inclusion criteria. Children with a diagnosis of cancer, HIV/AIDS or other immunodeficiency, history of organ transplantation, use of immunoglobulin or immunosuppressant, were excluded for the season in which they were diagnosed and all future seasons. 450 child-seasons were excluded because subjects received LAIV, and 428 child-seasons were excluded due to diagnosis of cancer, HIV/AIDS or other immunodeficiency. 14 child-seasons were excluded due to organ transplantation, 629 child-seasons were excluded due to treatment with immunoglobulin or immunosuppression, and 35 child-seasons were excluded due to some combination of the above.

A child was considered exposed to influenza vaccination if he/she received a dose of TIV during the influenza season of the observation year.

Because immune response to TIV is not immediately protective, a child was not considered exposed until 7 days after vaccination. A child was considered exposed to antibiotics if pharmacy records indicated that he/she was prescribed any doses of the pre-specified antibiotics (Table 2) within 14 days prior to and 4 days after administration of TIV, or if he/she was prescribed any doses of the pre-specified antibiotics within 14 days prior to and 4 days after a well-child visit or an ill visit for anything other than respiratory illness.

Because laboratory information was not available to the authors, we were unable to calculate vaccine effectiveness against laboratory-confirmed influenza, and instead used cases of medically-attended acute respiratory illness (MAARI) as our outcome measure. Analysis identified cases of MAARI using relevant ICD-9 codes for acute respiratory infection, influenza, otitis media, and viral pneumonia. Cases of MAARI occurring ≤ 7 days after vaccination were excluded.

SAS 9.2 software and an alpha of 0.05 was used for all analysis. Analysis of cohort data was performed using Poisson regression to analyze the effect of antibiotic use and influenza vaccination on rates of MAARI among children aged 6-59 months. Vaccine effectiveness was calculated from rate ratios. For analysis, children were divided into four exposure groups based on all combinations of vaccine and antibiotic exposure. For vaccinated children, person-time was calculated based on the date of influenza vaccination (plus 7 days) until the end

of the influenza season. For unvaccinated children, person-time was calculated from the start of the influenza season until the end of the influenza season. In order to determine antibiotic exposure status in the unvaccinated, the first well-child or illness visit of the influenza season was used. If children had no available vaccination, well-child visit, or other healthcare visit on which to calculate exposure, they were excluded from the cohort for that season. The total number of child-seasons excluded for this reason was 5,463, for a final total number of child-seasons of 45,325. Children in the vaccinated groups contributed both vaccinated and unvaccinated person-time. Some children contributed person-time to multiple seasons, creating dependency among the observations. Generalized estimating equations were used to account for this dependency.

To account for confounding due to differences between vaccinated and unvaccinated groups, a ratio of ratios approach was used. It is well recognized that confounding due to differences between vaccinated and unvaccinated individuals exists in observational studies of influenza vaccine effectiveness. We accounted for this confounding by using the approach described by Jackson et al (57), Nelson et al (31), and Omer et al (58). This involved first choosing a period when the influenza vaccine was available, but influenza was not circulating locally, according to Council of State and Territorial Epidemiologists (CSTE) reports. This was designated the pre-influenza control period. During this period, there should be no vaccine effect, and any effects observed are assumed

to be due to confounding. We identified a group of covariates that moved the incidence rate ratios (IRRs) of association between vaccination and MAARI towards the null. These covariates were then used to control for the confounding caused by differences between the vaccinated and unvaccinated groups. Confidence intervals for the ratio of incidence rate ratios and associated vaccine effectiveness were calculated using methods described by Altman and Bland (59).

Because confounders in the pre-influenza period may be different from those in the influenza activity period, we also undertook an additional approach to identifying confounders. We developed secondary multivariate models using a more traditional approach to confounder adjustment. The covariate list was developed based on evidence in the literature as well as data available in the KPGA databases. The covariate list included the following: age, in months, at time of exposure classification, history of environmental allergies, history of asthma, previous pneumococcal vaccination, influenza vaccination in a previous season, influenza season, sex, number of well-child visits per season, and number of illness visits per season. Logistic regression was used calculate the odds ratios for association of the various covariates with vaccination and antibiotic exposure status.

Based on initial estimates of numbers of subjects in each exposure group in the KPGA databases, power was computed for an estimated sample size of 128,855 child-seasons using the SWOG Interaction Binomial online calculator

(60). Power calculations assumed a gross attack rate of approximately 20%, with antibiotics reducing influenza vaccine effectiveness without affecting influenza risk in the absence of vaccine. Given our estimated sample size, the power to detect a 10% or greater decrease in influenza vaccine effectiveness caused by antibiotic use was greater than 0.99.

Results

The total number of eligible child-seasons with uninterrupted enrollment in KPGA during the study period was 45,325. Among these child-seasons, influenza vaccine was received in 18,053 (39.8%). In 6,237 (13.8%) child-seasons, the subject received a course of antibiotics during the defined exposure window 14 days prior to and 4 days after vaccination, an illness visit, or a well child visit. In 2,087 (4.6%) child-seasons, the subject received both influenza vaccination and a course of antibiotics. Among the study cohort, vaccination coverage varied by season, from a low of 13.6% during the 2005-06 season to a high of 20.4% during the 2006-07 season. Of the 246 weeks of the study period, influenza was circulating at least locally during 133, with widespread influenza activity during 51 of those weeks. 48.0% of the study population experienced at least 1 episode of MAARI per season during the study period. Of these, 51.4% experienced 2 or more episodes per season.

The odds of having received influenza vaccination were higher among children 24-35 months of age (OR = 1.19; 95% CI, 1.14-1.24) when compared with children 6-11 months of age. The odds of influenza vaccination were also higher

among children with a history of environmental allergies (OR = 1.07; 95% CI, 1.03-1.10), children with a history of asthma (OR = 1.17; 95% CI, 1.13-1.20), those with previous pneumococcal vaccination (OR = 4.71; 95% CI, 4.30-5.15), those who received influenza vaccination in a previous season (OR = 1.96; 95% CI, 1.92-1.99), and those who received antibiotics prior to their exposure classification date (OR=1.45; 95% CI, 1.41-1.49). The odds of vaccination were significantly associated with influenza season, with the likelihood of vaccination increasing over time. Children who had more illness visits and more well child visits were also significantly more likely to receive an influenza vaccination. Sex was not associated with likelihood of vaccination (Table 3).

The odds of receiving a course of antibiotics during the specified exposure window were lowest among those 48-59 months of age (OR = 0.76; 95% CI, 0.67-0.87) when compared with children 6-11 months of age, though in all other age categories there was no statistically significant difference compared with the youngest children. History of environmental allergies (OR = 1.29; 95% CI, 1.19-1.39) and history of asthma (OR = 1.47; 95% CI, 1.37-1.58) made a child more likely to receive a course of antibiotics. Vaccination for influenza in a previous influenza season decreased a child's likelihood of receiving antibiotics (OR = 0.79; 95% CI 0.74-0.84). Children were more likely to have received antibiotics if they had received prior courses of antibiotics (OR = 1.71; 95% CI, 1.56-1.87). Children were less likely to receive antibiotics during the 2007-08 influenza season (OR = 0.88; 95% CI 0.81-0.97), 2008-09 influenza season (OR = 0.77; 95%

CI, 0.70-0.84), 2009-10 influenza season (OR = 0.58; 95% CI, 0.52-0.65), and 2010-11 influenza season (OR = 0.63; 95% CI, 0.57-0.70), indicating a decline in the use of antibiotics over time. Children with the greatest number of visits due to illness per season were also the most likely to receive antibiotics (OR = 2.71; 95% CI, 2.53-2.90). Those with 2 well child visits were less likely to receive antibiotics (OR = 0.90; 95% CI, 0.84-0.98) than children with ≤ 1 well child visit, however this benefit did not hold for children with > 2 well child visits per season (OR = 1.06; 95% CI, 0.98-1.15). Sex and previous history of pneumococcal vaccination were not associated with antibiotic use (Table 4).

In the pre-influenza period, vaccinated non-antibiotic users contracted MAARI at a 36% higher rate (IRR = 1.36; 95% CI, 1.30-1.43) than those who were not vaccinated using a model including vaccination status, antibiotic use, and an interaction term containing vaccination status and antibiotic use, indicating uncontrolled confounding. Among antibiotic users, the rate of MAARI was nearly 2.5 times (IRR = 2.47; 95% CI, 2.32-2.64) higher in the vaccinated versus the unvaccinated. After adjusting for all measured confounders, the model that moved the IRR among non-antibiotic users the closest to the null contained age at time of exposure classification, history of asthma, number of illness visits, in tertiles, and number of well child visits, in tertiles. After this adjustment, vaccinated non-antibiotic users were 23% more likely to experience MAARI than unvaccinated non-antibiotic users (IRR = 1.23; 95% CI, 1.18-1.29), indicating the presence of unmeasured confounding. This was also true among vaccinated

antibiotic users, who had a rate of MAARI 77% higher than unvaccinated antibiotic users (IRR = 1.77; 95% CI 1.66-1.87) (Table 5).

There was no statistically significant difference in the incidence rate ratios of MAARI among the vaccinated versus the unvaccinated, stratified by antibiotic use, when comparing the various analysis periods to the pre-influenza period. No significant differences existed between the pre-influenza period and the period of at least local circulation, the pre-influenza period and the period of at least regional circulation, and the pre-influenza period and the period of widespread influenza activity. Using the ratio of ratios approach, during the period of widespread influenza activity, vaccine effectiveness was 2% (95% CI, -23%-21%) among non-antibiotic users and 10% (95% CI, -30%-37%) among antibiotic users (Table 6).

Discussion

Our study was limited by our ability to control for confounding. It is well-recognized that, in observational studies of influenza, there is a strong possibility of confounding due to differences in the vaccinated and unvaccinated groups (31). Our results show those who were vaccinated were more likely to experience MAARI. However, this may be because those who are more likely to be vaccinated are also more likely to seek medical treatment when they become ill, whereas those who are unvaccinated may be less likely to utilize the healthcare system overall. It is also likely that antibiotic users are different from non-antibiotic users. Our results showed that there were a variety of

confounding variables significantly associated with both vaccination and antibiotics. These differences are found even in the pre-influenza period, where the adjusted rate of MAARI among the vaccinated was 24% higher than among the unvaccinated for non-antibiotic users and 77% higher among the vaccinated than among the unvaccinated for antibiotic users. This indicates that there is unmeasured confounding that we were unable to account for. By using the ratio of ratios approach we were able to adjust for some of this unmeasured confounding, but a substantial amount of bias remained.

Though we were unable to make a strong conclusion, this study demonstrates that antibiotic use concurrent with influenza vaccine administration likely does not have an effect on vaccine effectiveness against medically-attended acute respiratory illness. There was no significant difference in the rate of MAARI or vaccine effectiveness among vaccinated antibiotic users and vaccinated non-antibiotic users during the pre-influenza period, the period of at least local activity, the period of at least regional activity, or the period of widespread activity. Thus, the decrease in intestinal commensals related to antibiotic use does not likely have a meaningful impact on immune response to influenza virus antigens in children. These findings support current Advisory Committee on Immunization Practices (ACIP) recommendations that state that influenza vaccination may be administered to children suffering from mild illness (1).

Moreover, this study emphasizes the importance of separating bias from true effects in studies of influenza vaccine effectiveness. Other retrospective cohort studies of influenza vaccine effectiveness have successfully used the ratio of ratios approach to eliminate confounding (31, 58). However, given the likely presence of a number of unmeasured confounding covariates in databases used for large, retrospective studies of vaccine effectiveness, researchers should use caution when interpreting results.

Given that evidence exists for the biological possibility of interaction between antibiotic use and the immune response to influenza vaccination, further studies should be undertaken to address this topic. A prospective, observational cohort study that uses laboratory-confirmed influenza as the outcome may better control for confounding than did our retrospective study using MAARI as the outcome. While we were limited by the data available in the KPGA databases, a prospective study could collect information on factors thought to create differences in the vaccinated versus unvaccinated groups.

Tables

Table 1. Description of study population, by exposure group status, 2005-2011.

	Antibiotic - Vaccine -		Antibiotic - Vaccine +		Antibiotic + Vaccine -		Antibiotic + Vaccine +	
	n	%	n	%	n	%	n	%
Total	**23,122**	**51.0**	**15,966**	**35.2**	**4,150**	**9.2**	**2,087**	**4.6**
Sex								
Male	11,795	51.0	8,137	51.0	2,142	51.6	1,069	51.22
Female	11,327	49.0	7,829	49.0	2,008	48.4	1,018	48.78
Age[a]								
6-11 months	1,423	6.2	1,273	8.0	202	4.9	228	10.9
12-23 months	5,124	22.2	5,542	34.7	780	18.8	831	39.8
24-35 months	5,764	24.9	3,874	24.3	1,203	29.0	475	22.8
36-47 months	6,196	26.8	3,491	21.9	1,270	30.6	365	17.5
48-59 months	4,615	20.0	1,786	11.2	695	16.8	188	9.0

[a]Age in months at time of exposure classification

Table 2. List of pre-specified antibiotics included in exposure definition, by number of prescriptions, 2005-2011 influenza seasons

Generic Name	Prescriptions	
	n	%
Amoxicillin	3,482	55.8
Amoxicillin/Potassium Clavulanate	1,546	22.8
Azithromycin	669	10.7
Sulfamethoxazole/Trimethoprim	452	7.3
Clindamycin	39	0.6
Penicillin	38	0.6
Ciprofloxacin	8	0.1
Metronidazole	2	<0.1
Levofloxacin	1	<0.1
Total	**6,237**	

Table 3. Influenza vaccination by child characteristics

Characteristic	OR[a]	95% CI		p-value
Age				
6-11 months of age	referent			
12-23 months of age	1.18	1.13	1.23	<.0001
24-35 months of age	1.19	1.14	1.24	<.0001
36-47 months of age	1.16	1.11	1.21	<.0001
48-59 months of age	0.99	0.94	1.04	0.55
History of environmental allergies	1.07	1.03	1.10	0.0004
History of asthma	1.17	1.13	1.20	<.0001
Previous pneumococcal vaccination	4.71	4.30	5.15	<.0001
Previous influenza vaccination	1.96	1.92	1.99	<.0001
Antibiotics prior to exposure classification date	1.45	1.41	1.49	<.0001
Season				
2005-2006	referent			
2006-2007	1.88	1.82	1.95	<.0001
2007-2008	1.76	1.69	1.84	<.0001
2008-2009	1.90	1.83	1.98	<.0001
2009-2010	2.35	2.25	2.45	<.0001
2010-2011	2.27	2.17	2.38	<.0001
Male	1.00	0.98	1.03	0.83
Illness Visits				
≤ 2 illness visits per season	referent			
3-4 illness visits per season	1.06	1.03	1.09	<.0001
> 4 illness visits per season	1.19	1.16	1.22	<.0001
Well-Child Visits				
≤ 1 well child visit per season	referent			
2 well child visits per season	1.25	1.22	1.29	<.0001
> 2 well child visits per season	1.25	1.22	1.29	<.0001

[a]Ratio of odds of having received influenza vaccination by category, for example, the odds of being vaccinated in those with history of asthma is 17% greater than the odds of being vaccinated in those without asthma

Table 4. Antibiotic use by child characteristics

Characteristic	OR[a]	95% CI		p-value
Age				
6-11 months of age	referent			
12-23 months of age	0.90	0.80	1.02	0.09
24-35 months of age	0.94	0.84	1.06	0.34
36-47 months of age	0.89	0.79	1.01	0.06
48-59 months of age	0.76	0.67	0.87	<.0001
History of environmental allergies	1.29	1.19	1.39	<.0001
History of asthma	1.47	1.37	1.58	<.0001
Previous pneumococcal vaccination	0.99	0.91	1.09	0.89
Previous influenza vaccination	0.79	0.74	0.84	<.0001
Antibiotics prior to exposure classification date	1.71	1.56	1.87	<.0001
Season				
2005-2006	referent			
2006-2007	0.94	0.86	1.02	0.14
2007-2008	0.88	0.81	0.97	0.01
2008-2009	0.77	0.70	0.84	<.0001
2009-2010	0.58	0.52	0.65	<.0001
2010-2011	0.63	0.57	0.70	<.0001
Male	0.98	0.93	1.04	0.55
Illness Visits				
≤ 2 illness visits per season	referent			
3-4 illness visits per season	1.88	1.75	2.03	<.0001
> 4 illness visits per season	2.71	2.53	2.90	<.0001
Well Child Visits				
≤ 1 well child visit per season	referent			
2 well child visits per season	0.90	0.84	0.98	0.01
> 2 well child visits per season	1.06	0.98	1.15	0.14

[a]Ratio of odds of receiving antibiotics by category, for example, the odds of receiving antibiotics in those with history of asthma is 47% greater than the odds of receiving antibiotics in those without asthma

Table 5. Adjusted incidence rate ratios comparing the vaccinated versus the unvaccinated, stratified by antibiotic use

	Non-Antibiotic Users				Antibiotic Users			
Analysis Period	IRR	95% CI		p-value	IRR	95% CI		p-value
Entire influenza season	1.06	1.04	1.09	<.0001	1.43	1.38	1.48	<.0001
Pre-influenza activity period	1.23	1.18	1.29	<.0001	1.77	1.66	1.87	<.0001
Period of at least local influenza activity	1.11	1.08	1.14	<.0001	1.46	1.39	1.53	<.0001
Period of at least regional influenza activity	1.12	1.09	1.15	<.0001	1.45	1.38	1.53	<.0001
Period of widespread influenza activity	1.21	1.17	1.26	<.0001	1.59	1.48	1.71	<.0001

[a]Adjustment variables included age at time of exposure classification, history of asthma, number of illness visits per season, and number of well child visits per season, and were selected because dropping them from a model including vaccination status, antibiotic use, and an interaction term containing vaccination status and antibiotic use moved the IRR in the preinfluenza period towards 1

Table 6. Ratio of incidence rate ratios and vaccine effectiveness, comparing the preinfluenza period to the period of at least local spread, the period of at least regional spread, and the period of widespread influenza activity

	Ratio of IRRs	95% CI		Vaccine Effective-ness	95% CI		p-value
Local/Pre-influenza							
Antibiotics	0.83	0.61	1.12	17%	-12%	39%	0.22
No Antibiotics	0.90	0.74	1.09	10%	-9%	26%	0.28
Regional/Pre-influenza							
Antibiotics	0.82	0.60	1.13	18%	-13%	40%	0.22
No Antibiotics	0.91	0.51	1.61	9%	-61%	49%	0.74
Widespread/Pre-influenza							
Antibiotics	0.90	0.63	1.30	10%	-30%	37%	0.58
No Antibiotics	0.98	0.79	1.23	2%	-23%	21%	0.88

Public Health Implications and Future Directions

Despite limitations imposed by unmeasured confounding in our analysis, this study still has implications for public health practice. These results reinforce the recommendation that mild or moderately ill children may receive influenza vaccination. Children who are acutely ill or recovering from a mild illness that required outpatient treatment with a course of antibiotics will have no significant difference in influenza vaccine effectiveness than children who were not concurrently on antibiotics. This is especially true for children who have completed their primary series of vaccinations. These findings provide reassurance to providers who may be concerned about vaccinating and treating mild illness with antibiotics at the same time. Our results support using not only well child but also illness visits to provide influenza prevention through vaccination to children. This has broad reaching public health implications as it increases the opportunities available to vaccinate, and these increased opportunities will ultimately boost the influenza vaccination rate among children aged 6 to 59 months.

This study supports previous research that describes limitations in accurately estimating influenza vaccine effectiveness. While these previous studies have effectively eliminated confounding using the ratio of ratios approach, we were unable to do so. Because of this, future researchers should be aware that unmeasured confounders in retrospective studies using large cohort databases might limit the usefulness of this technique. Our study had several

key differences from previous work that may have made eliminating confounding more difficult. Previous studies undertaken in seniors used community-acquired pneumonia, which is easily diagnosable, as a proxy for influenza infection. Using MAARI, which is a more vague diagnosis, as opposed to pneumonia or laboratory-confirmed influenza, probably increased confounding in our study. This is especially likely given our study population was made up of children, among whom the respiratory synticial virus season closely matches the influenza virus season. After conducting this study we can conclude that while a ratio of ratios approach to reducing bias may be appropriate for studies in the elderly, it may not be as effective in studies of children.

Two avenues of future research are suggested by this study. First, new techniques for eliminating confounding in studies of influenza vaccine effectiveness in children should be developed in order to yield more accurate results. Second, a prospective study of the impact of antibiotic use on TIV effectiveness in children should be undertaken. This study could address some of the limitations we faced when performing a retrospective analysis. While we were limited by the unmeasured covariates in the Kaiser Permanente HMO database, a prospective study could collect information on these potential confounders, which might include more detailed measures of health status, socioeconomic information, birth outcomes, or type of illness for which antibiotics were prescribed. In addition, a prospective study could use

laboratory-confirmed influenza as an outcome measure, decreasing the likelihood that the outcome would be incorrectly misclassified. Given that the change in intestinal flora caused by antibiotic use has been demonstrated to decrease immune response to the influenza virus in mice, it remains biologically plausible that antibiotic use may decrease influenza vaccine effectiveness. Further studies are needed to more fully examine this association.

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