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[Jonathan Kolsin] Date

Evaluating prior antibiotic use as a risk factor for acute gastroenteritis among children in Davidson County, Tennessee - 2014-2015

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Evaluating prior antibiotic use as a risk factor for acute gastroenteritis among children in Davidson County, Tennessee - 2014-2015

By

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Bachelor of Science, Molecular Biology
Certificate in Global Health
University of Wisconsin at Madison
2014

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An abstract of
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Abstract

Evaluating prior antibiotic use as a risk factor for acute gastroenteritis among children in Davidson County, Tennessee - 2014-2015

By Jonathan Kolsin

Background: Among children under 5 years of age in the U.S., acute gastroenteritis (AGE) accounts for >1.5 million outpatient visits, 200,000 hospitalizations and approximately 300 deaths each year. Most AGE in children is caused by viruses, with norovirus and rotavirus being the most prevalent pathogens. Despite growing biologic evidence that the intestinal microbiota is involved in norovirus and rotavirus infections, there is a lack of epidemiologic studies which evaluate the relationship between antibiotic use and AGE caused by these pathogens.

Methods: We analyzed AGE cases and healthy controls from the Vanderbilt University School of Medicine site of the New Vaccine Surveillance Network from December 1, 2014 to November 30, 2015. Four outcome groups were defined: overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE. Multiple logistic regression was performed to evaluate the association between prior antibiotic use and the four AGE outcomes as well as with AGE severity.

Results: The percentage of patients with reported antibiotic use in the 3 months prior to enrollment was similar across the four AGE outcomes (overall: 21.1%, norovirus-associated: 23.4%, rotavirus-associated: 26.0%, and non-norovirus/non-rotavirus: 22.2%), and was higher than reported among healthy controls (9.4%). Compared to healthy controls, overall AGE cases were 4.1 (95% confidence interval [CI]: 1.6, 10.3) times more likely to have antibiotic use in the 3 weeks prior to enrollment and 2.7 (95% CI: 1.7, 4.3) times more likely to have antibiotic use within 3 months prior to enrollment. Similar results were found for the other specific AGE outcomes. For the overall AGE group, the odds of antibiotic use in the 3 months prior to illness onset was 4.4 (95% CI: 1.7, 3.4) times higher for inpatient compared to outpatient cases and 2.2 (95% CI: 1.5, 3.2) times higher for emergency department compared to outpatient cases.

Conclusions: This study provides evidence that prior antibiotic use among children in Davidson County, Tennessee was associated with increased odds of AGE, irrespective of etiology, and this association was stronger for more recent antibiotic use. Prior antibiotic use was also associated with more severe AGE. These data may suggest that intestinal microbiota play a protective role in AGE among children, providing additional support for the judicious use of antibiotics.

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Chapter 1: Literature Review

Introduction

The three primary objectives of this literature review are to (1) provide an overview of the burden of acute gastroenteritis (AGE) among children in the United States, specifically that due to norovirus and rotavirus, (2) describe the role of the intestinal microbiota in human health, particularly research related to norovirus and rotavirus, and (3) describe the prevalence of antibiotic use and misuse in the United States.

1. Burden of Acute Gastroenteritis in Children

Acute gastroenteritis is defined as inflammation and/or irritation of the digestive tract that can cause nausea, vomiting, diarrhea, and/or abdominal pain that lasts less than 14 days (1). Worldwide, diarrheal diseases are a leading cause of pediatric morbidity and mortality, with 1.5 billion episodes and 1.5-2.5 million deaths estimated to occur annually among children <5 years (2). Among children under 5 years of age in the United States, gastroenteritis accounts for >1.5 million outpatient visits, 200,000 hospitalizations, approximately 300 deaths, and a cost of around 1 billion dollars each year (2). Most cases of acute gastroenteritis in children are caused by viruses (~70%), with norovirus and rotavirus being the most prevalent pathogens (3).

1.1- Norovirus

Globally, norovirus is estimated to be the most common cause of acute gastroenteritis across all age groups, being associated with approximately one-fifth of all cases of acute gastroenteritis (4). Worldwide, norovirus is estimated to be responsible for 685 million cases of acute gastroenteritis every year, 200 million of these cases are among children younger than 5 years old, leading to an estimated 50,000 child deaths each year (5). Each year in the United States, norovirus causes an estimated 19-21 million cases of acute gastroenteritis, leading to 1.7-

1.9 million outpatient visits and 400,000 emergency department visits, primarily among young children. Norovirus also contributes to an estimated 56,000-71,000 hospitalizations and 570-800 deaths each year in the United States, mostly among young children and the elderly (6).

1.2- Rotavirus

Globally, rotavirus is the leading cause of severe diarrhea in infants and young children (7). In 2008, rotavirus caused an estimated 453,000 deaths worldwide in children younger than 5 years of age. Rotavirus was the leading cause of severe diarrhea among infants and young children in the United States before the vaccine was introduced in 2006. Prior to the introduction of the vaccine, among children younger than 5 years of age in the US, rotavirus led to more than 400,000 doctor visits, more than 200,000 emergency room visits, 55,000-70,000 hospitalizations, and 20-60 deaths each year (7)

1.3- Transmission of Rotavirus and Norovirus

Norovirus and rotavirus are highly contagious pathogens that are spread by the fecal-oral route, meaning the virus is shed by an infected person and then enters a susceptible person's mouth to cause infection (46, 47). Most outbreaks of norovirus and rotavirus occur in closed places like daycare centers, nursing homes, schools, and cruise ships and happen in winter or spring months (46, 47). Young children (0-4 years old) and the elderly (65+ years old) are the most commonly affected age groups for norovirus, while rotavirus primarily affects young children (46, 47).

2. The Human Microbiota and its Role in Human Health

2.1- Definition of the Human Microbiota

We have only recently started to appreciate the vital role the microbiota plays in human health. On average, an individual human being is home to at least 100 trillion (10^{14}) microbial cells (8) and a quadrillion viruses (9). Collectively, the microbial associates that reside in and on the human body constitute our microbiota, and the genes they encode are known as our microbiome. The microbiota contains taxa from across the tree of life, including bacteria, eukaryotes, viruses, and at least one archaeon. Only a small minority of these microbes can be cultured, and recently, culture independent high-throughput sequencing has greatly expanded the repertoire of known microbes in our bodies (10).

2.2- Composition of the Human Intestinal Microbiota

Microbes colonize all the surfaces of the human body that are exposed to the environment, with the majority residing in the intestinal tract, which is now known to comprise of over 1,000 different species. There is a large variability in the composition of the intestinal microbiota in healthy individuals, with twins sharing less than 50% of their species-level bacterial taxa and even fewer viral sequences (11). Even though there is a huge range of variation in the taxa present in the gut and interindividual variability in microbial composition, the microbiota of most individuals can be categorized into one of three variants based on the dominant genera (*Bacteroides, Prevotella, or Ruminococcis*) (12).

2.3- Factors that Affect the Intestinal Microbiota

The establishment of the infant microbiota is mainly influenced by the type of delivery (vaginal vs. caesarian) and the subsequent feeding practices, including whether the infant was breastfed or given formula and the age at solid food introduction. The microbiota of infants (up to 2-3 years) is more unstable and has a lower diversity compared with that of adults. In healthy

conditions, microbial diversity and richness increase with age and reach their highest complexity during adulthood, at which time the gut microbiome is practically stable (13). The human gut microbiota is also strongly influenced by diet, since the food we consume also provides nutrients to the microbes (14).

2.4- Effect of Antibiotics on the Intestinal Microbiota

In general, studies of the effects of antibiotics on gut community taxonomic composition have found diminished levels of bacterial diversity, stereotypic declines and expansions in the relative abundances of certain taxa, and some degree of recovery in most individuals but persistent effects in others, and antibiotic- and individual host-specific effects (15). Antibiotics with strong and broad activity against anaerobes, for example clindamycin, have typically caused the longest-lasting effects on gut community expansion (16). Jakobsson et al. studied the impact of seven day courses of clarithromycin, metronidazole, and omeprazole on fecal taxonomic composition and found broad taxonomic compositional effects with rapid but only partial recovery in some cases and persistent effects at least four years after exposure (16). Dethlefsen et al. found that five days of ciprofloxacin influenced the abundance of about a third of the bacterial taxa in the gut and decreased taxonomic richness within days of initial exposure (17). Nearly complete recovery was seen in most cases by four weeks after exposure, although some compositional effects lasted for six months (17).

Changes to the highly co-evolved microbial community architecture leads to changes in resource availability and species-species interactions, opening niches available for pathogenic intrusion and leading to the loss of colonization resistance (15). This loss of colonization resistance contributes to the growth of opportunistic pathogens like *C. difficile*, which is now the

most frequently reported nosocomial pathogen in the US (18). Antibiotic treatment also selects for antibiotic-resistant community members, enriching the presence of resistance genes in the microbiome (15).

2.5- Primary Functions of the Intestinal Microbiota

2.5.1- Metabolism:

The gut microbiota largely derives their nutrients from dietary carbohydrates. Fermentation of the carbohydrates that escaped proximal digestion and indigestible oligosaccharides by colonic organisms such as *Bacteroides, Roseburia, Bifidobacterium, Fecalibacterium,* and *Enterobacteria* result in the synthesis of short chain fatty acids such as butyrate, propionate and acetate, which are rich sources of energy for the host (19). In addition to carbohydrate metabolism the gut microbiota has also been shown to play a role in lipid and protein metabolism (19). The gut microbiota also synthesizes vitamin K and several components of vitamin B (19).

2.5.2- Promotion of Protective Immunity:

The profound effects of the commensal microbiota on intestinal and immune cell development have best been highlighted by the phenotype of germ-free (GF) mice (20). One of the first deficiencies observed in GF mice was a profound reduction of secretory immunoglobulin A (IgA) levels in the intestine and subsequent mono-association of these mice with various bacteria was shown to lead to an increased IgA expression (20, 21). In addition to numerous defects in antibody production, GF mice display various morphological tissue defects in their intestines and these developmental impairments are attenuated following the introduction of gut bacteria (20). Investigations on GF mice also revealed cellular defects in intestinal

epithelial and lamina propria lymphocytes as well as in mesenteric lymph nodes (20). Normal functioning of intestinal epithelial cells, including the expression of microbial recognition receptors, defensins, and antimicrobial peptides (AMPs), was shown to be impaired in GF animals compared to their conventionally raised counterparts (20, 22).

Detailed investigations on certain members of the intestinal microbiota served to unravel mechanisms by which commensal bacteria induce immune tolerance (20). For example, investigations on segmented filamentous bacteria (SFB) revealed that these Gram-positive bacteria are sufficient to promote T helper 17 cells (Th17) development in the small intestinal lamina propria (20, 23). Th17 effector cytokines enhance epithelial cell tight junctions, induce mucin production, and have been associated with induction of AMPs (20). Colonization of GF mice with SFB resulted in the induction of multiple AMP genes, for example, RegIIIγ (20, 23). Even though Th17 cells are crucial for protecting the host against pathogenic infection, it should be noted that these cells also display an inflammatory potential as observed in different murine models of autoimmune diseases, particularly in experimental autoimmune encephalomyelitis (EAE) (20, 24).

In addition to Th17 cells, the intestinal microbiota has been shown to affect the development and functioning of regulatory T cells (Treg). Tregs are a special subset of T cells that prevent other immune cells from attacking the body's own tissues (25). Recently, certain strains within *Clostridia* clusters XIVa, IV, and XVIII were shown to induce Treg responses in the colon (20, 26). Another prominent human commensal, *Bacteroides fragilis*, was found to direct the development of $FoxP3^+$ Tregs via the immunomodulatory molecule polysaccharide A (20, 27). Monocolonization of GF mice with *B. fragilis* was accompanied by an increased

suppressive Treg capacity and the induction of an anti-inflammatory cytokine profile emerging from $FoxP3^+$ T cells in the gut (20, 27).

2.6- Norovirus and the Intestinal Microbiota

There is some evidence suggesting that the intestinal microbiota plays a protective role in norovirus infection. Antibiotic-treated mice infected with murine norovirus have shown reduced antiviral serum IgG levels 35 days post infection compared to microbially colonized mice (28, 29). Additionally, a higher abundance of *Lactobacillus* due to probiotic-fermented milk ingestion correlates with quicker recovery from human norovirus-induced fever (28, 30). Similarly, a higher abundance of *Lactobacillus* following experimental vitamin A treatment correlated with inhibition of murine norovirus infection (28, 31).

Conversely, there is also evidence that the intestinal microbiota may facilitate norovirus infection. Antibiotic-treated mice have been shown to have reduced acute viral titers in the distal ileum, mesenteric lymph nodes and colon compared with control mice, which reflects a decrease in viral replication in vivo following antibiotic treatment (28, 32). Germ free mice have also demonstrated a reduction in the amount of infectious virus shed in the feces compared to colonized hosts (28, 33). Finally, murine norovirus failed to establish persistent infection in antibiotic-treated mice, a phenotype that could be fully rescued by fecal transplantation from colonized mice (28, 29).

2.7- Rotavirus and the Intestinal Microbiota

There is likewise mixed evidence regarding potential risks or protective effects intestinal microbiota may serve in rotavirus infections. Probiotics have been shown to reduce the duration of viral diarrhea and administration of *Lactobacillus rhamnosus GG* reduces rotavirus shedding

(34). Soluble factors from commensal bacteria have also been shown to block rotavirus infection in intestinal epithelial cells in vitro (35). Meanwhile, other studies have shown that antibiotic-treated mice infected with rotavirus have reduced levels of viral genomes in intestinal tissues and delayed viral shedding compared to controls (28, 36). This study also found that the incidence and duration of diarrhea caused by rotavirus infection in suckling mice was reduced by antibiotic treatment (28, 36). Lastly, this study showed that antibiotic-treated mice had a substantially higher antiviral antibody response compared to microbially colonized controls. A higher antiviral antibody response was also observed in this study among germ free mice compared to conventionally housed mice (28, 36).

3. Prevalence of Antibiotic Use and Misuse in the United States

In the United States in 2014, healthcare providers prescribed 266.1 million antibiotic prescriptions- equivalent to 835 antibiotic prescriptions per 1000 persons (37). The prescription rate for oral antibiotics in the US in 2014 was slightly lower for people less than 20 years of age (778 antibiotic prescriptions per 1000 persons) compared to people greater than or equal to 20 years of age (838 antibiotic prescriptions per 1000 persons) (37). The antibiotic prescription rate was higher among females (999 prescriptions per 1000 persons) compared to males (659 prescriptions per 1000 persons) (36). The southern United States had the highest oral antibiotic prescription rate in 2014 (923 prescriptions per 1000 persons), followed by the Midwest (897 prescriptions per 1000 persons), Northeast (866 prescriptions per 1000 persons), and the western United States had the lowest prescription rate (615 prescriptions per 1000 persons) (37). By antibiotic class, Penicillins and Macrolides were the most commonly prescribed oral antibiotics in the US in 2014 (191 and 154 prescriptions per 1000 persons, respectively), followed by Cephalosporins (112 prescriptions per 1000 persons), Fluoroquinolones (103 prescriptions per

1000 persons), and B-lactams (74 prescriptions per 1000 persons) (37). It has been estimated that at least 30% of antibiotics prescribed in the outpatient setting are unnecessary, meaning that no antibiotic was needed at all (38). Total inappropriate antibiotic use, inclusive of unnecessary use and inappropriate selection, dosing and duration, may approach 50% of all outpatient antibiotic use (38).

3.1- Antibiotic Use in the Management of Acute Gastroenteritis

The use of antibiotic therapy in children with AGE is controversial (39). Although treatment may shorten the course of some diarrheal illnesses (e.g. shigellosis), most bacterial diarrheas are self-limited and will be resolved before the causative organism is identified; thus treatment using oral rehydration solution is the preferred approach to managing AGE in children (39). For some bacteria, such as noninvasive *Salmonella* species, antibiotic treatment may prolong the carrier period after the symptoms have resolved (39). For other bacteria, such as *Campylobacter jejuni* and *Yersinia enterocolitica*, the efficacy of antibiotics in hastening recovery is doubtful (39). As mentioned earlier, empiric antibiotic therapy may lead to the development of *C. difficile*-associated enterocolitis and a worsening of symptoms (39).

4. Conclusions

The global and domestic burden of disease due to acute gastroenteritis is tremendous, with the most prevalent pathogens being norovirus and rotavirus. Recent research, primarily done either in vitro or using a mouse model, has suggested that the intestinal microbiota plays a role in both norovirus and rotavirus infection. However, the exact role the microbiota plays remains unclear since some studies suggest that the intestinal microbiota is protective and others suggest that the microbiota facilitates infection by norovirus and rotavirus. The intestinal

microbiota has important functions in metabolism and immunomodulation, and disruption has been linked to many diseases including inflammatory bowel disease, rheumatoid arthritis, type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, and asthma. Antibiotics have long been known to cause significant and lasting disruptions to the intestinal microbiota and are the most commonly prescribed medication in the United States. It is estimated that at least 30% of outpatient antibiotic prescriptions are unnecessary, meaning that no antibiotics were needed. Inappropriate antibiotic use has become a major focus in public health with the dramatic and alarming increase in the prevalence of antibiotic resistant bacteria.

Despite the growing body of evidence that the microbiota plays a role in infection with norovirus and rotavirus as well as its role in metabolism and immunomodulation we were unable to find any studies which specifically assessed prior antibiotic use as a risk factor for norovirus or rotavirus-associated acute gastroenteritis. We were also unable to find any studies which evaluated the association between prior antibiotic use and the severity of acute gastroenteritis.

5. Study Aims

The primary goals of this study were to (1) evaluate prior antibiotic use as a risk factor for AGE among children, specifically that caused by norovirus or rotavirus and (2) assess the association between prior antibiotic use and the severity of acute gastroenteritis among children. Through this study, we sought to gain a better understanding of the role the intestinal microbiota plays in acute gastroenteritis among children, specifically that caused by norovirus or rotavirus. This study used data from the New Vaccine Surveillance Network (NVSN), specifically from the Vanderbilt University School of Medicine site from December 1, 2014 to November 30, 2015.

Chapter II: Manuscript

Abstract

Background: Among children under 5 years of age in the U.S., acute gastroenteritis (AGE) accounts for >1.5 million outpatient visits, 200,000 hospitalizations and approximately 300 deaths each year. Most AGE in children is caused by viruses, with norovirus and rotavirus being the most prevalent pathogens. Despite growing biologic evidence that the intestinal microbiota is involved in norovirus and rotavirus infections, there is a lack of epidemiologic studies which evaluate the relationship between antibiotic use and AGE caused by these pathogens.

Methods: We analyzed AGE cases and healthy controls from the Vanderbilt University School of Medicine site of the New Vaccine Surveillance Network from December 1, 2014 to November 30, 2015. Four outcome groups were defined: overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE. Multiple logistic regression was performed to evaluate the association between prior antibiotic use and the four AGE outcomes as well as with AGE severity.

Results: The percentage of patients with reported antibiotic use in the 3 months prior to enrollment was similar across the four AGE outcomes (overall: 21.1%, norovirus-associated: 23.4%, rotavirus-associated: 26.0%, and non-norovirus/non-rotavirus: 22.2%), and was higher than reported among healthy controls (9.4%). Compared to healthy controls, overall AGE cases were 4.1 (95% confidence interval [CI]: 1.6, 10.3) times more likely to have antibiotic use in the 3 weeks prior to enrollment and 2.7 (95% CI: 1.7, 4.3) times more likely to have antibiotic use within 3 months prior to enrollment. Similar results were found for the other specific AGE outcomes. For the overall AGE group, the odds of antibiotic use in the 3 months prior to illness onset was 4.4 (95% CI: 1.7, 3.4) times higher for inpatient compared to outpatient cases and 2.2 (95% CI: 1.5, 3.2) times higher for emergency department compared to outpatient cases.

Conclusions: This study provides evidence that prior antibiotic use among children in Davidson County, Tennessee was associated with increased odds of AGE, irrespective of etiology, and this association was stronger for more recent antibiotic use. Prior antibiotic use was also associated with more severe AGE. These data may suggest that intestinal microbiota play a protective role in AGE among children, providing additional support for the judicious use of antibiotics.

Introduction

Worldwide, diarrheal diseases are a leading cause of pediatric morbidity and mortality, with 1.5 billion episodes and 1.5-2.5 million deaths estimated to occur annually among children <5 years of age (2). Among children under 5 years of age in the United States, acute gastroenteritis (AGE) accounts for >1.5 million outpatient visits, 200,000 hospitalizations, approximately 300 deaths, and a cost of around 1 billion dollars each year (2). Most AGE cases of known etiology among children are caused by viruses (~70%), with norovirus and rotavirus being the most prevalent pathogens (3). Each year in the United States, norovirus causes an estimated 19-21 million cases of AGE, leading to 1.7-1.9 million outpatient visits and 400,000 emergency department visits, primarily among young children. Norovirus also contributes to an estimated 56,000-71,000 hospitalizations and 570-800 deaths each year in the United States, mostly among young children and the elderly (6). Rotavirus was the leading cause of severe diarrhea among infants and young children in the United States before the vaccine was introduced in 2006. Prior to the introduction of the vaccine, among children younger than 5 years of age in the US, rotavirus led to more than 400,000 doctor visits, more than 200,000 emergency room visits, 55,000-70,000 hospitalizations, and 20-60 deaths each year (7).

There is some evidence suggesting that the intestinal microbiota plays a protective role in norovirus infection. Antibiotic-treated mice infected with murine norovirus have shown reduced antiviral serum IgG levels 35 days post infection compared to microbially colonized mice (28, 29). Additionally, a higher abundance of *Lactobacillus* due to probiotic-fermented milk ingestion correlates with quicker recovery from human norovirus-induced fever (28, 30). Similarly, a higher abundance of *Lactobacillus* following experimental vitamin A treatment correlated with inhibition of murine norovirus infection (28, 31).

Conversely, there is also evidence that the intestinal microbiota may facilitate norovirus infection. Antibiotic-treated mice have been shown to have reduced acute viral titers in the distal ileum, mesenteric lymph nodes and colon compared with control mice, which reflects a decrease in viral replication in vivo following antibiotic treatment (28, 32). Germ free mice have also demonstrated a reduction in the amount of infectious virus shed in the feces compared to colonized hosts (28, 33). Finally, murine norovirus failed to establish persistent infection in antibiotic-treated mice, a phenotype that could be fully rescued by fecal transplantation from colonized mice (28, 29).

There is likewise mixed evidence regarding potential risks or protective effects intestinal microbiota may serve in rotavirus infections. Probiotics have been shown to reduce the duration of viral diarrhea and administration of *Lactobacillus rhamnosus GG* reduces rotavirus shedding (34). Soluble factors from commensal bacteria have also been shown to block rotavirus infection in intestinal epithelial cells in vitro (35). Meanwhile, other studies have shown antibiotic-treated mice infected with rotavirus have reduced levels of viral genomes in intestinal tissues and delayed viral shedding compared to controls (28, 36). This study also found that the incidence and duration of diarrhea caused by rotavirus infection in suckling mice was reduced by antibiotic treatment (28, 36). Lastly, this study showed that antibiotic-treated mice had a substantially higher antiviral antibody response compared to microbially colonized controls. A higher antiviral antibody response was also observed in this study among germ free mice compared to conventionally housed mice (28, 36).

Although it is difficult to discern the overall role played by the intestinal microbiota in norovirus and rotavirus infection from these studies, there is evidence suggesting the microbiota is involved in some capacity. However, despite the biologic evidence that the intestinal

microbiota is involved in norovirus and rotavirus infections, there is a lack of epidemiologic studies which evaluate the relationship between antibiotic use and AGE caused by these pathogens. The primary goals of this study were to (1) evaluate prior antibiotic use as a risk factor for AGE among children, specifically that caused by norovirus or rotavirus and (2) assess the association between prior antibiotic use and the severity of AGE among children.

Methods

Surveillance System

Since 2006, the New Vaccine Surveillance Network (NVSN) has conducted active, population-based surveillance for AGE in children. Seven U.S. medical centers currently participate with the Centers for Disease Control and Prevention (CDC) in active sentinel surveillance. The seven currently participating sites in 2017 are in Washington, California, Missouri, Texas, New York, Ohio, and Tennessee (49). This study used data from the Vanderbilt University School of Medicine, located in Nashville, Tennessee (Davidson County) from December 1, 2014 to November 30, 2015.

Enrollment of Subjects

AGE cases were enrolled if they were between the ages of 14 days and 18 years and were hospitalized, visited the emergency department (ED) or the outpatient clinic at the Vanderbilt University School of Medicine from December 1, 2014 through November 30, 2015 with diarrhea (≥3 episodes within 24 hours) or vomiting (≥1 episode within 24 hours), whose illness duration was ≤10 days, and with informed consent from a parent or guardian. Enrolled subjects were screened for pre-existing conditions and excluded from eligibility if they had such indications including a non-infectious cause of diarrhea, a history of immune deficiency,

previous enrollment for the same AGE episode, or transfer from another hospital. Healthy controls were children younger than 18 years and older than 14 days who were systematically enrolled on arrival at scheduled well-child visits at the outpatient clinic of the Vanderbilt University School of Medicine during the same time period as the AGE case enrollment and had no reported clinical immunodeficiency or symptoms of AGE 14 days before enrollment (44, 45).

Specimen Collection and AGE Outcome Definitions

Whole stool specimens were obtained within 10 days of symptom onset. Norovirus testing was performed by the Tennessee Department of Health using real-time quantitative reverse transcription polymerase chain reaction (real time-qRT-PCR) and rotavirus testing was done at the Vanderbilt University School of Medicine using enzyme-linked immunosorbent assay (EIA). Four AGE outcome groups were defined for this study: overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE. The overall AGE group represents patients who were enrolled in the study as AGE cases regardless of testing results. Norovirus-associated AGE was defined as those who were enrolled as AGE cases and tested positive for norovirus by real time-qRT-PCR and negative for rotavirus by EIA. Rotavirus-associated AGE was defined as those who were enrolled as AGE cases and tested positive for rotavirus by EIA and negative for norovirus by real time-qRT-PCR. The non-norovirus/non-rotavirus AGE group consists of patients who were enrolled as AGE cases, but tested negative for both norovirus and rotavirus. Individuals positive for both rotavirus and norovirus were not considered as a separate outcome group, but are represented in the overall AGE group. The control group was defined as children who were enrolled as healthy controls and who tested negative for both norovirus and rotavirus.

Demographic and clinical data were systematically collected through parent/guardian interviews at the time of enrollment. The primary exposure of interest, prior antibiotic use, was also obtained through parent interviews as a dichotomous variable indicating whether the child received antibiotics by mouth, IV, or injection in the past 3 months before onset of this illness (3 months prior to enrollment for healthy controls). The time since last antibiotic dose (in days) was also collected for those who had reported antibiotic use in the 3 months prior to illness onset (or enrollment for healthy controls).

Descriptive Analyses

All data analysis was conducted using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). A univariate analysis was performed to describe the distribution of age, sex, race, ethnicity, insurance type, clinical setting, and prior antibiotic use for the four defined AGE outcome groups (overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE) and healthy controls. Bivariate analyses were conducted to examine the associations between potential confounding demographic and epidemiologic variables with norovirus-associated AGE and prior antibiotic use status. Potential confounding variables were identified for all AGE outcomes, but differences in potential models were negligible for the outcome of interest. For the sake of comparability, a single model form was selected: the norovirus-associated AGE group. The variables considered included age at enrollment, sex, race, ethnicity, household income, insurance type, contact with an AGE affected person inside the household, contact with an AGE affected person outside the household, contact with someone who has traveled outside the U.S. in the past 7 days, travel outside the U.S. in the past 7 days, daycare attendance, season of enrollment (October-April vs. other), whether the child was ever breastfed, and whether the child is currently breastfeeding. Differences in these

variables by disease outcome and exposure status were analyzed using chi-square tests for significance.

Modeling Strategy

Multiple logistic regression was performed to calculate odds ratios and 95% confidence intervals for the association between antibiotic use in the 3 months prior to enrollment and overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE. The model identification process was performed for norovirus-associated AGE and was checked for the other outcomes by comparing the odds ratios obtained using the exposure-only model and the full model selected for norovirus-associated AGE. If the odds ratio of the exposure-only model was within 10% of the odds ratio found using the model identified for norovirus-associated AGE, then the norovirus-associated AGE model was used for that outcome. Multiple logistic regression was also performed using different cutoffs for days since last antibiotic dose prior to enrollment (less than or equal to 3 weeks, greater than 3 weeks, and unknown time frame) to further assess the relationship between proximity of antibiotic use and the four defined AGE outcomes.

To address the potential issue of reverse causality, in which people may have taken antibiotics in response to AGE symptoms, the final model was run for overall AGE excluding those who had indicated antibiotic use within 7 or 14 days prior to enrollment and examined the resulting odds ratios and 95% confidence intervals. An additional analysis was performed which excluded norovirus-associated AGE cases having cycle threshold (CT) values greater than 31. CT values, given by real time qRT-PCR, are inversely related to viral load; so limiting the analysis to include cases with lower CT values may make it more likely that the norovirus

detected was the actual cause of the AGE symptoms, as opposed to the antibiotics themselves. A CT value of 31 was previously shown to be an appropriate cut-off for diagnosing norovirus-associated illness (42).

To evaluate the potential association between AGE severity and prior antibiotic use, multiple logistic regression was performed using care level at enrollment as the outcome. Two separate analysis were performed, one comparing inpatient to outpatient visits and the other comparing ED to outpatient visits. Outpatient visits were selected as the reference group for the analysis because they represent the least severe and largest number of cases. Age was included in the models *a priori* as a potential confounding variable.

The association between antibiotic use in the 3 months prior to illness onset and AGE severity was also assessed using modified Vesikari scores (MVS), which were calculated from clinical information using a pre-established 20 point scoring system (43). The modified Vesikari scoring system is a type of clinical severity scale which has been validated to measure the severity of acute gastroenteritis in pediatric populations in the United States. Modified Vesikari scores were evaluated as dichotomous variable using the median value for the overall AGE group as the cut-point. Age was also included in these models *a priori* as a potential confounding variable.

Results

Univariate Analysis

There were a total of 1143 AGE cases and 236 healthy controls in this study. Of the 1143 AGE cases, 864 had norovirus and rotavirus testing performed, of which 205 (23.7%) were classified as norovirus-associated AGE, 96 (11.1%) as rotavirus-associated AGE, 542 (62.7%) as

non-norovirus/non-rotavirus AGE, and 21 (2.4%) were positive for both norovirus and rotavirus. Median age, sex, race, ethnicity, clinical setting, and insurance type were similar across the four AGE outcome groups and the healthy controls (Table 1). The percentage of patients with reported antibiotic use in the 3 months prior to enrollment was similar across the four AGE outcome groups (overall AGE: 21.1%, norovirus-associated AGE: 23.4%, rotavirus-associated AGE: 26.0%, and non-norovirus/non-rotavirus AGE: 22.2%), and was higher than reported among healthy controls (9.4%).

Bivariate Analysis and Model Selection

From the bivariate analysis, race and contact with an AGE affected person outside the household were identified as potential confounders to be included in the initial logistic regression model because they met the *a priori* inclusion criteria ($p \leq 0.25$) for both norovirus-associated AGE status and prior antibiotic use status (Table 2 and Supplemental Table 2). Specific information on the model selection process is described in the supplemental materials section.

Prior Antibiotic Use and Defined AGE Outcomes

The odds of antibiotic use in the 3 months prior to enrollment was 2.7 (95% confidence interval [CI]: 1.7, 4.3) times higher for AGE cases overall compared to healthy controls, controlling for race and AGE contact outside the household (Table 3). Similar adjusted odds ratios (aOR) were found for norovirus-associated AGE (aOR: 3.2, 95% CI: 1.8, 5.6), rotavirus-associated AGE (aOR: 3.3, 95% CI: 1.7, 6.6), and non-norovirus/non-rotavirus AGE (aOR: 2.9, 95% CI: 1.7, 4.7). Using non-norovirus/non-rotavirus AGE as the referent group, adjusted odds ratios of 1.1 (95% CI: 0.7, 1.6) and 1.0 (95% CI: 0.6, 1.8) were obtained for norovirus and rotavirus, respectively.

For the association between antibiotic use less than or equal to 3 weeks before enrollment and overall AGE, we obtained an aOR of 4.1 (95% CI: 1.6, 10.3). Similar adjusted odds ratios were obtained for norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-norovirus AGE (Table 3). For those who had reported antibiotic use greater than 3 weeks before enrollment or unknown time since enrollment, we obtained adjusted odds ratios of 1.4 (95% CI: 0.6, 3.0) and 3.1 (95% CI: 1.5, 6.4), respectively, for overall AGE. Similar adjusted odds ratios were obtained for the other AGE outcomes.

For overall AGE, excluding patients who had reported antibiotic use less than or equal to 7 or 14 days prior to enrollment resulted in adjusted odds ratios of 2.1 (95% CI: 1.3, 3.5) and 2.2 (95% CI: 1.3, 3.8), respectively. For norovirus-associated AGE, limiting the analysis to include only cases with CT values less than 31 resulted in an aOR of 3.6 (95% CI: 2.0, 6.5).

Prior Antibiotic Use and AGE Severity

The proportion of patients who used antibiotics in the 3 months prior to illness onset was higher among inpatient (39.1%) and ED cases (23.8%) compared to outpatient cases (13.3%) for the overall AGE group. For the overall AGE group, the odds of antibiotic use in the 3 months prior to illness onset was 4.4 (95% CI: 1.7, 3.4) times higher for inpatient compared to outpatient visits, controlling for age, race, and AGE contact outside the household. Comparing ED to outpatient visits, an aOR of 2.2 (1.5, 3.2) was obtained for the overall AGE outcome.

The median MVS for the overall AGE group was 8 (IQR: 6-10) (Supplemental Table 3). Using multiple logistic regression, it was found that among AGE cases overall, the odds of antibiotic use 3 months before illness onset was 2.1 (95% CI: 1.5, 2.8) times higher for patients with a MVS greater than 8 compared to those having a score less than 8.

Discussion

This study provides evidence that prior antibiotic use among children in Davidson County, Tennessee was associated with increased odds of AGE, irrespective of etiology, and this association was stronger for more recent antibiotic use. Additionally, we found that antibiotic use in the three months prior to illness onset was associated with more severe AGE outcomes, regardless of the specific etiology. These data suggest that the intestinal microbiota may play an overall protective role in AGE among children, as disruption of this microbiota through antibiotic use increases the odds of AGE.

The exact biologic mechanism by which the intestinal microbiota protects against AGE is not known, but the consistency of the association observed between prior antibiotic use and the four AGE outcomes considered (overall AGE, norovirus-associated AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE) suggests a nonspecific protective mechanism. One possibility is that antibiotic use, leading to disruption of the intestinal microbiota, causes reduced local antiviral antibody production. The intestinal microbiota has been shown to affect localized secretory IgA production (21).

The association between prior antibiotic use and AGE was stronger when antibiotics were used within 3 weeks of enrollment compared to use within 3 months. This finding is biologically plausible, given that antibiotics have the greatest impact on intestinal microbiota during the actual course of antibiotic administration, which typically last 7–10 days (48). Antibiotics can also have long term compositional effects on the intestinal microbiota. For example, Dethlefsen et al. found that a five day course of ciprofloxacin led to complete microbial recovery in most cases by four weeks post exposure, but some compositional effects lasted for six months (17).

These long term compositional effects may be reflected by the observed association between antibiotic use and AGE even at 3 months before enrollment.

The observed associations between prior antibiotic use and AGE were also robust to approaches evaluating the potential effects of antibiotic-associated diarrhea and reverse causality. Antibiotics are known to cause diarrhea directly in about 10–15% of patients (40). This issue is exacerbated by the fact that detection of norovirus by RT-PCR can sometimes occur in asymptomatic individuals; thus, determining the cause of AGE symptoms in patients that had prior antibiotic use and evidence of norovirus infection can be difficult. This is less of a concern for rotavirus-associated AGE cases because the EIA technique detects rotavirus at levels which are more likely to cause disease (41). To address this potential problem, we performed an additional analysis which included only norovirus-associated AGE cases with cycle threshold (CT) values less than 31, which are indicative of higher viral loads. From this analysis, we obtained an adjusted odds ratio similar to what was obtained for the association between antibiotic use in the 3 months prior to enrollment and norovirus-associated AGE, indicating that prior antibiotic use is associated with norovirus-associated AGE. However, it is worth noting that the utility of using CT values is limited because there is no clear, well-defined cut-off for disease causing levels of norovirus (42).

There also exists the potential for reverse causality, by which people may have taken antibiotics in response to symptoms of acute gastroenteritis being experienced. We tried to address this by excluding patients who had reported antibiotic use less than 1 or 2 weeks prior to enrollment and found that the association was reduced, but persisted in the absence of these patients, indicating that the overall association was not driven entirely by potential reverse causality.

Strengths and Limitations

This study had a number of strengths. First, the use of healthy controls who were negative for norovirus and rotavirus was an ideal comparison group for this analysis. Second, the New Vaccine Surveillance Network has strict inclusion criteria for both AGE cases and healthy controls making it much less likely for inclusion of non-infectious AGE cases or those with a history of immune deficiencies. Third, the data had a high level of completeness with regard to demographic and clinical variables as well as the prior antibiotic use. Finally, the sample size used for the study was large, lending to more precise estimates and greater power.

This study had a few limitations that should also be considered. First, it is possible that the association between prior antibiotic use and AGE can be explained by residual confounding due to some underlying health condition. For example, antibiotic use may be an indicator for people who are sick more frequently, potentially due in part to some unknown genetic or immunologic deficiency. Patients with a known history of immune deficiency were excluded from the study, however, this may not completely address the issue. Second, prior antibiotic use was self-reported by parent and may thus be subject to recall bias. Additionally, about 40% of those who reported antibiotic use in the 3 months prior to enrollment couldn't recall the number of days since last dose, limiting our ability to draw conclusions on the effect of time since antibiotic use. Third, we lacked information on the class, dose, and duration of antibiotics used, limiting the specificity of our analysis and conclusions. Lastly, the cases captured by the surveillance system likely represent the more serious end of the illness spectrum, as they were serious enough to seek healthcare. This bias towards more severe AGE cases limits the generalizability of the result to medically–attended AGE and may not be applicable to milder AGE in the community.

Future Directions

Future studies could consider the dose, type/class (narrow vs. broad spectrum), and duration of antibiotic use and may increase the specificity of evidence behind the observed association between prior antibiotic use and acute gastroenteritis among children. Additional studies could utilize medical records to ascertain information on antibiotic use as this would reduce the potential for recall bias and may lead to more complete information on when the antibiotics were taken in relation to illness onset. Our findings provide further justification for the judicious use of antibiotics, especially among children. Reducing the unnecessary use of antibiotics will not only help stem the rising tide of antibiotic resistant bacteria and reduce the number of *C. difficile* infections, but may also help reduce the burden of AGE among children.

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Tables

Table 1: Selected patient characteristics of defined acute gastroenteritis (AGE) outcomes and healthy controls- New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

	AGE outcome				
Patient Characteristic	**Overall AGE (n=1143) n (%)**	**Norovirus-associated AGE (n=205) n (%)**	**Rotavirus-associated AGE (n=96) n (%)**	**Non-norovirus/non-rotavirus AGE (n=542) n (%)**	**Healthy Controls (n=236) n (%)**
Median Age in months (range)	26 (0-18 years)	18 (1 month-15 years)	26 (1 month-17 years)	22 (1 month-18 years)	22 (1 month-17 years)
Sex					
Male	585 (51.2)	90 (43.9)	52 (54.2)	283 (52.2)	127 (46.2)
Race					
White	667 (59.2)	136 (66.3)	58 (60.4)	330 (60.9)	154 (65.3)
Black	424 (37.1)	57 (27.8)	36 (37.5)	194 (35.8)	76 (32.2)
Other/unknown	42 (3.7)	12 (5.9)	2 (2.1)	18 (3.3)	6 (2.5)
Ethnicity					
Hispanic	465 (40.7)	97 (47.3)	40 (41.7)	230 (42.4)	106 (44.9)
Clinical Setting					
Inpatient	46 (4.1)	8 (4.0)	5 (5.4)	28 (5.2)	N/A
ED	689 (61.4)	106 (52.5)	62 (67.4)	329 (61.4)	N/A
Outpatient	387 (34.5)	88 (43.6)	25 (27.2)	179 (33.4)	236 (100%)
Insurance Type					
Private	115 (10.1)	24 (11.7)	12 (12.5)	52 (9.6)	24 (10.2)
Public	996 (87.1)	177 (86.3)	79 (82.3)	476 (87.8)	207 (87.7)
None/unknown	32 (2.8)	4 (2.0)	5 (5.2)	14 (2.6)	5 (2.1)
Used antibiotics in 3 months prior to enrollment	239 (21.1)	47 (23.4)	25 (26.0)	119 (22.2)	22 (9.4)
≤ 3 weeks prior to enrollment	91 (38.1)	17 (36.2)	6 (24.0)	54 (45.4)	5 (22.7)
> 3 weeks prior to enrollment	51 (21.3)	11 (23.4)	6 (24.0)	24 (20.2)	8 (36.4)
Unknown time	96 (40.2)	19 (40.4)	13 (52.0)	40 (33.6)	9 (40.9)

Table 2: Bivariate relationships between selected patient characteristics and outcome of norovirus-associated acute gastroenteritis (AGE) - New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

Patient Characteristics	Norovirus AGE (n=205) n (%)	Healthy Controls (n=236) n (%)	P-value[a]
Age			0.85
< 1 year	69 (33.7)	79 (33.5)	
1 year old	57 (27.8)	56 (23.7)	
2 years old	21 (10.2)	28 (11.9)	
3-5 years old	29 (14.2)	34 (14.4)	
6-18 years old	29 (14.2)	39 (16.5)	
Sex			0.63
Male	90 (43.9)	109 (46.2)	
Race			0.16
White	136 (66.3)	154 (65.3)	
Black	57 (27.8)	76 (32.2)	
Other/Unknown	12 (5.9)	6 (2.5)	
Ethnicity			0.61
Hispanic	97 (47.3)	106 (44.9)	
Household Income			0.62
Less than or equal to $25,000	104 (50.7)	116 (49.2)	
Greater than $25,000	51 (24.9)	68 (28.8)	
Unknown/no response	50 (24.4)	52 (22.0)	
Insurance Type			0.87
Private	24 (11.7)	24 (10.2)	
Public	177 (86.3)	207 (87.7)	
None/unknown	4 (2.0)	5 (2.1)	
Had contact with AGE affected person inside household	56 (27.3)	5 (2.1)	<0.0001
Had contact with AGE affected person outside household	33 (16.9)	0	<0.0001
Child traveled outside US in past 7 days	0	N/A	N/A
Had contact with someone who has traveled outside US in past 7 days	2 (1.0)	N/A	N/A
Child attends daycare	57 (27.8)	70 (29.7)	0.67
Season of Enrollment			<0.0001
October-April	175 (85.4)	142 (60.2)	
Child has been breastfed	154 (75.1)	182 (77.5)	0.57
Child is currently breastfeeding	29 (19.0)	38 (20.9)	0.66

a: The chi square statistic was used to assess differences in distribution of patient characteristics

Table 3: Multiple logistic regression models[a] for association of antibiotic use less than or equal to 3 weeks, greater than 3 weeks, unknown time, and less than or equal to 3 months prior to enrollment and selected acute gastroenteritis (AGE) outcomes- New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

	Time Since Last Antibiotic Dose			
AGE Outcome	**≤ 3 weeks aOR[b] (95% CI[c])**	**> 3 weeks aOR (95% CI)**	**Unknown time aOR (95% CI)**	**≤ 3 months aOR (95% CI)**
Overall AGE	4.1 (1.6, 10.3)	1.4 (0.6, 3.0)	3.1 (1.5, 6.4)	2.7 (1.7, 4.3)
Norovirus-associated AGE	4.6 (1.6, 13.2)	1.6 (0.6, 4.5)	3.8 (1.6, 9.1)	3.2 (1.8, 5.6)
Rotavirus-associated AGE	2.9 (0.8, 11.2)	1.4 (0.4, 5.3)	5.6 (2.2, 14.6)	3.3 (1.7, 6.6)
Non-norovirus/non-rotavirus AGE	5.3 (2.1, 13.6)	1.4 (0.6, 3.3)	2.7 (1.2, 5.9)	2.9 (1.7, 4.7)

a: models control for race and contact with an AGE affected person outside the household (gold standard model identified from norovirus-associated AGE modeling)
b: aOR= adjusted odds ratio
c: 95% CI= 95 % confidence interval

Chapter III: Public Health Implications

Among children under 5 years of age in the United States, acute gastroenteritis (AGE) accounts for >1.5 million outpatient visits, 200,000 hospitalizations, approximately 300 deaths, and a cost of around 1 billion dollars each year (2). Most AGE in children is caused by viruses (~70%), with norovirus and rotavirus being the most prevalent pathogens (3). Despite growing biologic evidence that the intestinal microbiota is involved in norovirus and rotavirus infections, there are a lack of epidemiologic studies which evaluate the relationship between antibiotic use and AGE caused by these pathogens. The primary goal of this study was to evaluate prior antibiotic use as a risk factor for AGE among children, specifically that caused by norovirus and rotavirus.

This study provides evidence that prior antibiotic use among children in Davidson County, Tennessee was associated with increased odds of AGE, irrespective of etiology, and this association was stronger for more recent antibiotic use. This finding provides further justification for the judicious use of antibiotics, especially among children. Reducing the unnecessary use of antibiotics will not only help stem the rising tide of antibiotic resistant bacteria and reduce the number of *C. difficile* infections, but may also help reduce the burden of AGE among children.

Future studies could consider the dose, type/class (narrow vs. broad spectrum), and duration of antibiotic use and may increase the specificity of evidence behind the observed association between prior antibiotic use and acute gastroenteritis among children. Additionally, further research on the intestinal microbiota may help elucidate the specific biologic mechanism behind the association of prior antibiotic use and AGE.

Appendices

Appendix A

Modeling Strategy

Variables included in the initial logistic regression model for norovirus-associated AGE were determined *a priori* as those having a Pearson's chi-square p-value of 0.25 or less for both illness and exposure status as identified in the bivariate analysis. Multicollinearity was assessed using condition indices (CIs) and variance decomposition proportions (VDPs) by running a COLIN macro in SAS. If a condition index was found to be above the cutoff value of 30, terms containing a VDP value of greater than 0.5 were removed in descending order until all condition indices were below the cutoff value. Two-way interaction was assessed by performing a likelihood ratio test for all interaction terms using a significance level of 0.05. Confounding was assessed using data-based criterion, which was defined as a 10% change in the odds ratio of the primary exposure variable, prior-antibiotic use, in the full model. If dropping a variable resulted in a greater than 10% change in the odds ratio from that of the full model, then that variable was considered to be a meaningful confounder and was retained in the model. Precision of the odds ratio for prior antibiotic use was also considered by calculating and comparing confidence interval ratios, which were obtained by dividing the upper bound of the 95% confidence interval by its lower bound. We assessed the goodness of fit for the final model using the Deviance test statistic and a significance level of 0.05.

Model Selection

No major multicollinearity problems were detected for this initial logistic regression model. Two-way interaction was considered for prior antibiotic use by race and AGE contact outside the household, however, a likelihood ratio test for the interaction terms showed no

statistically significant interaction ($X^2_{df=1}$=0.018, p=0.89). Since the interaction terms were found not to be statistically significant they were eliminated from the model. Assessment of confounding using the 10% change in estimate approach revealed no meaningful confounding by race or contact with an AGE affected person outside the household as all model subsets had an adjusted odds ratio within 10% of the full model (gold standard) (Supplemental Table 1). Precision of the odds ratio estimates was also considered and there was no meaningful gain in precision comparing the full model (CI ratio= 3.2) to the model with only prior antibiotic use (CI ratio=3.0) (Supplemental Table 1). Since there was little loss in precision when controlling for these variables it was decided that the model containing race and contact with an AGE affected person outside the household would be considered as the final model for norovirus-associated AGE. The model was found to have good fit with a deviance statistic of 3.58 and a p-value of 0.83. Comparing the odds ratios obtained using this model to that obtained using an exposure only model for the different AGE outcomes found no meaningful differences, as all were within 10% of each other. Since no meaningful differences were detected, it was decided that the model containing race and contact with AGE outside the household would be also be used as the final model for overall AGE, rotavirus-associated AGE, and non-norovirus/non-rotavirus AGE.

Appendix B

Supplementary Tables

Table 1: Multiple logistic regression models for association of antibiotic use in 3 months prior to enrollment and norovirus-associated acute gastroenteritis (AGE)- assessment of confounding by race and contact with an AGE affected person outside the household using 10% change in estimate approach- New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

Variables in model	OR[a] for antibiotic use (95% CI[b])	Within 10% of OR_{GS}[c]	Cl ratio[d]
Antibiotic use, race, contact with AGE outside (Gold Standard model)	3.2 (1.8, 5.6)	N/A	3.2
Antibiotic use, contact with AGE outside	3.1 (1.7, 5.5)	Yes	3.2
Antibiotic use, race	3.01 (1.7, 5.2)	Yes	3.0
Antibiotic use	2.95 (1.7, 5.1)	Yes	3.0

a: OR= odds ratio
b: 95% CI= 95% confidence interval
c: OR_{GS}= odds ratio for the gold standard model
d: CI ratio obtained by dividing upper bound of 95% CI by lower bound

Table 2: Bivariate relationships between selected patient characteristics and exposure of antibiotic use in the 3 months prior to enrollment among norovirus-associated acute gastroenteritis (AGE) cases and healthy controls - New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

Patient Characteristics	Antibiotics used in past 3 months (n=69) n (%)	Antibiotics not used in past 3 months (n=367) n (%)	P-value[a]
Age			0.06
< 1 year	25 (36.2)	120 (32.7)	
1 year old	22 (31.9)	89 (24.3)	
2 years old	5 (7.3)	44 (12.0)	
3-5 years old	13 (18.8)	50 (13.6)	
6-18 years old	4 (5.8)	64 (17.4)	
Sex			0.66
Male	33 (47.8)	165 (45.0)	
Race			0.21
White	51 (73.9)	235 (64.0)	
Black	17 (24.6)	115 (31.3)	
Other/Unknown	1 (1.5)	17 (4.6)	
Ethnicity			0.99
Hispanic	32 (46.4)	170 (46.3)	
Household Income			0.67

Less than or equal to $25,000	31 (44.9)	186 (50.7)	
Greater than $25,000	21 (30.4)	97 (26.4)	
Unknown/no response	17 (24.6)	84 (22.9)	
Insurance Type			0.89
Private	8 (11.6)	38 (10.4)	
Public	60 (87.0)	321 (87.5)	
None/unknown	1 (1.5)	8 (2.2)	
Had contact with AGE affected person inside household	9 (13.0)	52 (14.2)	0.80
Had contact with AGE affected person outside household	8 (12.1)	24 (6.7)	0.13
Child traveled outside US in past 7 days	0	0	N/A
Had contact with someone who has traveled outside US in past 7 days	1 (2.1)	1 (0.7)	0.37
Child attends daycare	19 (27.5)	106 (28.9)	0.82
Season of Enrollment			0.64
October-April	51 (73.9)	261 (71.1)	
Child has been breastfed	55 (79.7)	278 (76.0)	0.50
Child currently breastfeeding	14 (25.5)	51 (18.4)	0.23

a: The chi square statistic was used to assess differences in distribution of patient characteristics

Table 3: Modified Vesikari scores (MVS) for different acute gastroenteritis (AGE) outcomes- New Vaccine Surveillance Network (NVSN), Vanderbilt University School of Medicine, Nashville, Tennessee, December 1, 2014- November 30, 2015

	Modified Vesikari Score (MVS)			
AGE Outcome	**MVS: 0-5 n (%)**	**MVS: 6-10 n (%)**	**MVS: 11-17 n (%)**	**Median MVS score (IQR)**
Overall AGE	274 (24.0)	590 (51.6)	279 (24.4)	8 (4)
Norovirus-associated AGE	42 (20.5)	113 (55.1)	74 (36.1)	8 (4)
Rotavirus-associated AGE	14 (14.6)	41 (42.7)	41 (42.7)	10 (4)
Non-norovirus/non-rotavirus AGE	145 (26.8)	279 (51.5)	118 (21.8)	8 (5)