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Antibiotic Prescribing Practices and Health Outcomes: Stunting and Rotavirus Vaccine Seroconversion among NIDI Infants at Hospital Los Andes in El Alto, Bolivia

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Antibiotic Prescribing Practices and Health Outcomes: Stunting and Rotavirus Vaccine Seroconversion among NIDI Infants at Hospital Los Andes in El Alto, Bolivia

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An abstract of
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Abstract

Antibiotic Prescribing Practices and Health Outcomes: Stunting and Rotavirus Vaccine Seroconversion among NIDI Infants at Hospital Los Andes in El Alto, Bolivia

Background: Antibiotics prescription rates are growing at exponential rates, including in infants. Antibiotic exposure early in life has been hypothesized as a potential factor in both low vaccine response and risk of stunting through the changes in gut microbiota that antibiotic exposure may induce (Mahfuz et al., 2017; Sommer & Backhed, 2013; Velasquez, Parashar, & Jiang, 2017)

Goal: This study aims to explore the potential relationship between physician antibiotic prescribing behavior as a measure of early antibiotic exposure and the health outcomes stunting and rotavirus vaccine seroconversion among infants 6 months of age at Hospital Los Andes in El Alto, Bolivia.

Methods: In 2013, Bolivian and US collaborators conducted a longitudinal comprehensive study-- *Nutrición, Inmunología, y Diarrea Infantil* (NIDI) at two Bolivian hospitals. In 2017, medical records of 135 infants at six months previously enrolled in the NIDI study at Hospital Los Andes were examined to collect additional data on with antibiotic prescription as a measure of exposure. To statistically analyze the potential relationships, logistic regression and chi-square analysis was completed with data retrieved from both the NIDI study and medical records of infants at Hospital Los Andes. The health outcomes, stunting and Rotavirus vaccine seroconversion data were extracted from the prior NIDI database.

Results: Of the patient records analyzed, 24% of infants were prescribed antibiotics by 6 months of age. There was a significant association observed between having been prescribed antibiotics at or before 6 months of age and stunting (odds ratio: 2.4, 95% confidence interval: 1.05–5.48, P= 0.04). There was no significant relationship observed between having had antibiotics prescribed and Rotavirus vaccine seroconversion at 6 months of age (odds ratio = 0.68, 95% confidence interval: 0.29–1.57, P=0.36).

Discussion and Conclusion: The effect of antibiotic exposure on developing infants from birth to 6 months of age are still unknown. One potential mechanism may include the imbalance of gut microbiota, which may impact immune responses and increase the incidence of diarrhea causing nutritional deficiencies, such as stunting. The results of this analysis suggest a continued need to investigate the effects of early-life antibiotic exposure, as antibiotics in infants 6 months of age is associated with an increased prevalence of stunting.

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ACRONYMS AND ABBREVIATIONS

Appx	Appendix
CAIA	*Centro de Atención Integral para Adolescentes* (Center for Integrated Care for Adolescents)
MPH	Master of Public Health
NIDI	*Nutrición, Inmunología, y Diarrea Infantil* (Infant Nutrition, Diarrhea, and Immunology)
RV	Rotavirus
SES	Socioeconomic Status
UNICEF	United Nations Children's Fund
UMSA	*Universidad Mayor de San Andrés*
WHO	World Health Organization

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Introduction

The reduction of childhood mortality is of utmost importance for public health worldwide. The yearly global toll of child deaths is still over 7 million and in 2010, 4.879 million died of infectious causes (Liu et al., 2012). Diarrheal illnesses are among the highest contributing factors to childhood mortality with over 800 000 of these fatalities were attributed diarrheal disease (Kotloff et al., 2013). Rotavirus (RV), one of the most common diarrheal pathogens, disproportionately affect children in the developing world likely due to malnutrition and associates nutritional deficiencies (Tickell et al., 2017) (WHO, 2010). In Latin America, prevalence of diarrhea is often over 30% (Coa, 2009). Diarrhea may have complicated ties to treatments early in life such as antibiotics. Antibiotics are of public health concern because the longitudinal effects that are still being researched and exposure to children early in life.

Antibiotics tend to be among the most overused and overprescribed medications (Tekleab, Asfaw, Weldetsadik, & Amaru, 2017; WHO, 2002). Due to low resource settings such as in Latin American countries like Bolivia, children under 5 years of age are at a greater risk for infectious diseases such as diarrhea, malnutrition, and poor vaccine responses. Antibiotics are the most often inappropriately prescribed treatment medication for infections in children under five (Tekleab et al., 2017; WHO, 2002). The effects of this early exposure are not yet fully understood particularly in developing countries such as Bolivia.

Bolivia, specifically, is an ideal study site for antibiotic exposure due to stunting prevalence, diarrhea prevalence, infant mortality, rotavirus vaccine implementation, and low vaccine performance. For stunting, 27% of Bolivian children under 5 are considered stunted (UNICEF, 2013). Prevalence of diarrhea in some states of Bolivia are as high as 36% for children under 5 (George et al., 2014). Diarrheal infections are among the highest contributing

factors to childhood mortality, ranking third leading cause of death for children under 5 in Bolivia (George et al., 2014). In Bolivia, 39 out of every 1000 children born in the country for 2013, died before their 5th year of life (UNICEF, 2014). In Bolivia, the rotavirus vaccine was introduced in August of 2008 (Inchauste et al., 2017). Since its introduction, there has been a 40% decrease in rotavirus related hospitalizations among children under 5 (Inchauste et al., 2017). Despite this progress, which can be quantified through observed decreasing infant mortality rates in Bolivia, the efficacy on the vaccine is still found to be lower than other countries in Latin America (Inchauste et al., 2017).

Potential mechanisms regarding prevalence of stunting and variation in vaccine performance may be due to gut microbial composition that can be directly affected by antibiotic exposure. There have been increasing reports of high antibiotic prescriptions in South America creating concern for the health implications inappropriate antibiotic use may affect (Urbiztondo et al., 2017). Although there is a lack of research-based data on the use of antibiotics, of what is available, the data is inconclusive (Rogawski et al., 2015). Thus, there is a need to explore antibiotic exposure and their potential relationship with health outcomes stunting and RV vaccine seroconversion in children under 5 living in Bolivia.

The city of El Alto is a distinctive population within the La Paz Department of Bolivia. Sitting at an elevation of 13,500 ft above sea level on a barren *altiplano* overlooking La Paz, El Alto is one of the fastest growing urban areas in Latin America (Studies, 2011). It is also the largest city in Latin America inhabited by indigenous people (Studies, 2011). The region has a high prevalence of infant malnutrition, high incidence of diarrheal infections, and a high infant mortality rate, thus making it a site of interest for research on reducing infant mortality (Coa, 2009) (UNICEF, 2014).

Since 2005, there have been US and Bolivian organizations collaborating on research regarding maternal and child health in El Alto. Stakeholders on both the US and Bolivian sides have conducted investigations and aided the Bolivian government in implementing national vaccination programs. US counterparts are Dr. Leon's research group from Emory University in Atlanta, GA and Bolivian counterparts are *Universidad Mayor de San Andrés* (UMSA) in La Paz, Bolivia and the *Centro de Atención Integral para Adolescentes* (CAIA) also in La Paz, Bolivia. Most recently, collaborations conducted a longitudinal comprehensive study-- *Nutrición, Inmunología, y Diarrea Infantil* (NIDI)— focusing on nutritional status, rotavirus vaccine immunogenicity, and other health outcomes such as stunting (Aceituno et al., 2017). The study included a cohort of 350 mother-infant pairs at two secondary hospitals in El Alto (Aceituno et al., 2017). For the purpose of this paper, only Hospital Los Andes was measured for a population size of 180 active mother-infant pairs.

The goal of this study is to explore potential relationships between antibiotic prescribing behavior as a measure of early antibiotic exposure and the health outcomes stunting and RV vaccine seroconversion among infants 6 months of age at Hospital Los Andes in El Alto, Bolivia. To achieve this, the project analyzed quantitative data collected from two sources. The prior NIDI study for data on infant stunting, rotavirus vaccine doses, and seroconversion. The second source was from data collected in June 2017 on antibiotics prescribed to infants in the NIDI cohort at Hospital Los Andes. Sources were merged and streamlined to address three central aims:

1. To quantify antibiotic practices among NIDI population using descriptive statistics
2. To explore potential relationship between antibiotic prescriptions and stunting as a health outcome
3. To explore potential relationship between antibiotic prescriptions and seroconversion as a health outcome.

Literature Review

Antibiotic prescribing practices as a form of antibiotic exposure in early childhood may influence two health outcomes, stunting and rotavirus vaccine seroconversion (Mahfuz et al., 2017; Owino et al., 2016). The focus of this thesis is to assess potential mechanisms that may connect antibiotics to stunting and seroconversion occurrence. The conceptual framework illustrates what factors may lead to stunting (Appx 2) and seroconversion (Appx 3) in early childhood. These factors are interrelated and affect multiple systems focusing on gut health and nutritional status. The multiple factors influencing seroconversion are still poorly understood (Velasquez et al., 2017). In both conceptual frameworks, infection is a key element, not measured in this study, that leads to antibiotic prescriptions (Appx 4). In addition, the factors in both frameworks overlap with both health outcomes. This literature review will discuss each factor and pathway as they explore potential relationships between the exposure, antibiotic prescriptions and the outcomes, stunting and seroconversion.

Antibiotics

Antibiotics are being prescribed exponentially worldwide (Tekleab et al., 2017; WHO, 2002). There has been a recent push in research efforts to understand the underlying effects of antibiotic exposure early in life (Tekleab et al., 2017). World Health Organization (WHO) estimates that globally at least 50% of all medication are prescribed, used, and dispensed inappropriately (WHO, 2002). Attributing to the surge of antibiotic prescriptions are lack of knowledge, unrestricted access, pharmaceutical incentives, unclear or unenforced guidelines, and overworked healthcare professionals (WHO, 2002). This international issue is particularly true for the developing world where antibiotics are more frequently and inappropriately used (Tekleab et al., 2017). Health effects of over prescription has shown there are connections

between antibiotics, an increased risk to inflammatory diseases, and impairments to the gut microbiome termed dysbiosis (Shi et al., 2018).

At a clinical level, antibiotic exposure can lead to direct changes in the gut microbiome (Brüssow, 2015; Issa & Moucari, 2014). Changes can affect the function of the gut microbiome such as vitamin production, immune function, and nutrient distribution (Desselberger, 2017; Xiaomei Cong & Henderson, 2016). In both animal and human studies, antibiotic exposure has shown significant effects in changes to composition of the gut microbiome (Brüssow, 2015; Cho et al., 2012). Infants exposed to antibiotics show drastically reduced biodiversity of the gut microbiome (Xiaomei Cong & Henderson, 2016). Effects of these gut changes in early childhood may affect both stunting and vaccine seroconversion. Gut microbiota may play central roles in nutrition and immunogenicity.

Gut microbiome

Healthy gut microbiota is essential for development and homeostasis (Velasquez et al., 2017). In infants, development of gut microbiome composition is analogous to growth, immunogenicity, and even neurodevelopment (Desselberger, 2017; Xiaomei Cong & Henderson, 2016). Factors directing the development of gut microbiota early in life are birth mechanism, sex of infant, hospital environment, feeding, medications, genetics, and other early life experiences, such as infections (Xiaomei Cong & Henderson, 2016). For example, vaginal versus cesarean methods of birth and hospital conditions display considerably different intestinal flora (Xiaomei Cong & Henderson, 2016). Further, breast feeding specifically impacts microbial composition and immunogenicity (Velasquez et al., 2017). Medications such as antibiotics, previously described, disturb microbiome composition by reducing biodiversity and genetics play a factor in which commensal bacteria proliferate over others (Xiaomei Cong & Henderson, 2016). Early life experiences such as environmental factors have also impacted gut microbiome composition

particularly in places with high prevalence for infectious diseases (Xiaomei Cong & Henderson, 2016). In low-resource settings, environmental conditions such as sanitation and infrastructure exacerbate infant health and contribute to differences between industrial and developing countries. For children in developing countries, there are pronounced differences in gut microbiome composition than their counterparts in developed countries (Brüssow, 2015). The children in developing countries exhibited a more activated gut due to the diversity, variability, and sensitivity to environmental exposures (Uchiyama, Chassaing, Zhang, & Gewirtz, 2014; Velasquez et al., 2017).

Differences in gut microbiota among children globally may come down to nutritional status and rate of infections. Part of the gut microbiota's function early in life may be critical to supporting the growth and development even in conditions of nutritional deficiency (Xiaomei Cong & Henderson, 2016). Nutritional deficiencies may arise from chronic food insecurity that induces malabsorption of nutrients often leading to malnutrition (Brüssow, 2015). Malnutrition affects one in 3 people and is a root element in causes of childhood deaths (Brüssow, 2015; UNICEF, 2016). In the developing world, 3.5% of children under 5 are severely malnourished, 20% are underweight, and 32% exhibit stunting (Brüssow, 2015). Contributing to malnutrition in a two-way relationship are rate of infections (Brüssow, 2015). Enteric and respiratory infections have been specifically implicated. High rates of both infection and malnutrition influence gut microbiome as seen in children from developing countries.

Malnutrition

Malnutrition is a chronic worldwide problem contributing to global burden of disease and child mortality. As children under 5 are still in development, the effects malnutrition and associated nutritional deficiencies can have on the brain and body are devastating (Black et al., 2008; Xiaomei Cong & Henderson, 2016). Consequences of impairment of growth and

development can be seen through regional distribution of negative health outcomes such as stunting (Black et al., 2008).

Children living with malnutrition, particularly in the developing countries with limited resources and sanitation, show high prevalence of intestinal disturbances categorized as environmental enteric dysfunction (EED) (Owino et al., 2016). EED contributes to malnutrition and growth failure in children by causing chronic gut inflammation, nutrient malabsorption, intestinal leakages, and dysbiosis, an imbalance of gut microbes (Owino et al., 2016). Growth failure is termed "stunted" when the individual's height is at least two standard deviations below average by age standardized by WHO (WHO, 2017). Although a multifaceted global concern, research has found chronic inflammation, recurrent infections, and nutrient deficiencies to be highly associated with stunting in children (Owino et al., 2016).

Health outcome: Stunting

Negative health outcomes from malnutrition such as stunting contribute significantly to childhood mortality and morbidity worldwide. Globally, 26% of children under 5 have stunted growth, 11% of these children living in Latin America (Aguayo, Badgaiyan, & Paintal, 2015). There is a higher prevalence of childhood stunting in low income countries which is reflective on of the environmental conditions, nutritional status, and disease stressors (Black et al., 2008). Stunting can have longitudinal issues including decreased cognition function, educational performance, stature, productivity, and body mass (Aguayo et al., 2015). Lessening the gap between the industrial and developing countries may aid in lowering childhood mortality rates and burden of disease.

Infections and stunting

Increased incidence of infectious diseases also contribute to malnutrition and impaired growth leading to stunting (Black et al., 2008). Pneumonia and diarrhea are highlighted infections that have been implicated in the infection-malnutrition relationship (Brüssow, 2015). In Appx 2, infections are an overcasting factor for the multiple pathways leading to stunting. Antibiotics are prescribed when infections arise thus leading to dysbiosis which in turn may lead diarrhea and nutrient malabsorption. Malnutrition occurs in cases of chronic malabsorption which in early childhood emerges as stunting. The gut microbiome in malnourished and stunted children is also different than in other children. Differences are likely due to due to the integral function of the gut microbiome. These functions are suspected in nutrition, development, and may lead to increased incidence of infections particularly enteric infections (Brüssow, 2015).

Repeated enteric infections such as diarrhea is one of the factors leading to stunting (Appx 2). The odds of stunting multiply after each diarrheic episode in developing countries (Black et al., 2008). Access to care, economic development, and environmental conditions were among the factors exacerbating diarrheal incidence among children under 5 (Kotloff et al., 2013). Dysbiosis also contributes to diarrheal incidence. Reducing pathogens in the gut microbiome may be key in reducing the negative effects the infection-malnutrition cycle has on stunting (Brüssow, 2015; Owino et al., 2016).

Antibiotics and Stunting

Another factor leading to stunting is antibiotic exposure (Appx 2). Antibiotic's effects on the health outcome, childhood stunting, is still poorly understood. Pediatric literature contains contradictory results in regards to antibiotic exposure and growth (Brüssow, 2015; Gough et al., 2014). In some studies, antibiotics were shown to have growth promoting effects in children in low income countries (Gough et al., 2014). Growth is strongly associated with nutrition and in

countries where malnutrition is especially prevalent, antibiotics acted as a protective factor in nutrition (Gough et al., 2014). In these studies, antibiotics prevented infections in children thus reducing the incidence and severity of malnutrition (Gough et al., 2014). In other studies, antibiotics increased the incidence of diarrhea in children, a known predictor for poor growth and nutrient malabsorption (Rogawski et al., 2015). Therefore, the net effects of antibiotics on growth is unclear and likely part of a multifactor explanation where nutrition and nutrient absorption are central features.

Antibiotic treatments that reduce diarrhea may ultimately lead to a reduction of childhood stunting (Gough et al., 2014). Antibiotics that successfully treat diarrhea minimize the damage that diarrhea has on nutrition absorption and growth failure leading to stunting (Gough et al., 2014). Antibiotic interventions that showed growth among the children likely reduced the risk of infection and pathogenic diversity in the gut microbiome (Brüssow, 2015) It is difficult to conclude whether growth in antibiotic-treated-malnourished-children was due to specific growth-promoting factors or anti-infection effects (Brüssow, 2015). Moderate to severe diarrheal cases, defined in this study as sunken eyes, loss of skin turgor, intravenous rehydration administered or prescribed, dysentery, or admission to hospital with diarrhea, are attributed rotavirus (RV), cryptosporidium, Enterotoxic *Escherishia coli* (ETEC), and Shigella (Kotloff et al., 2013). Because of the variety of pathogenic agents that can cause diarrhea both viral and bacterial in nature, methods in prevention to aid in treatment are being explored (Collaborators*, 2015).

Bacterial Diarrhea

Bacterial diarrhea remains prevalent and is among the pathogenic causes in mortality among children under 5. Bacterial species that have detrimental effects particularly on susceptible children are Cryptosporidium, ETEC, and Shigella. These bacterial agents cause infections and children with severe malnutrition are up to 12 times at a higher risk of death

(Tickell et al., 2017). In Latin America, bacterial diarrheal disease occurs in pockets due to diversity in population socioeconomic status (SES) and geography.

Severe cases of bacterial diarrhea may be treated with antibiotics although such treatments have side effects for which long-term implications are unknown. The main treatments for bacterial diarrhea are oral rehydration solution, intravenous fluid, and zinc (Desselberger, 2017; Mahfuz et al., 2017). Oral gentamicin was once used as an alternative thought to be a good alternative in treating severe cases of diarrhea however after many studies, oral gentamicin showed little to no effect (Black, 1993). Nevertheless, both Gentamicin and Ciproflaxin are most commonly prescribed in severe cases of diarrhea (Black, 1993).

Viral Diarrhea

Viral diarrheal infections, however, are not treatable via antibiotics. Rotavirus is of particular note as one of the leading pathogens responsible for roughly 5% of child deaths annually (Mary M. Agócs, Nadia Teleb, & Jon R. Gentsch, 2014). The World Health Organization has coordinated the RV Surveillance Network since 2008 due to the burden of disease it presented on children (Mary M. Agócs et al., 2014). Estimates in 2013 indicated approximately 215,000 children under 5 died from RV (WHO, 2016). The global burden of disease presented by RV makes it the leading pathogen of deaths related to diarrheal infections (Collaborators*, 2015).

Rotavirus Prevention

Treatment for RV related diarrheal infections have shifted to focus on preventative methods, vaccination (Mary M. Agócs et al., 2014). In 2006, two live attenuated vaccines were developed, Rotarix® and RotaTeq® (Desselberger, 2017). Since 2009, WHO has placed RV vaccines on recommended national immunization programs (Mary M. Agócs et al., 2014). The public health impact RV vaccines have had since its introduction has made significant strides in

lowering the burden of enteric infections and diarrhea-related deaths in children worldwide (WHO, 2010).

Globally, RV vaccine efficacy trials have provided insightful results that illustrate a variation in vaccine performance. In Sub Saharan Africa, RV vaccine efficacy was found to be 39.35 effective against severe rotavirus gastroenteritis (George E Armah, 2010). In Latin America, a similar study was done where the vaccine efficacy was calculated at 80.5% (Linhares et al., 2008) and in the US, a study found a vaccine efficacy of 90% (Vesikari et al., 2007). Thus, high income countries, mass vaccinations for RV are 80-90% effective in preventing severe RV-associated disease contrasted with low income countries at 40-60% efficacy (Desselberger, 2017). Through immunoassays, infants were measured to verify the immune response to the vaccine (George E Armah, 2010). Of those that were vaccinated, RV cases are confirmed through lab tested stool samples and compared to the study group that received a placebo vaccine (Vesikari et al., 2007). Identifying immunological response factors and mechanisms are crucial in understanding varying vaccine performance geographically.

Although overall RV vaccines have positively impacted child health, vaccine underperformance tends to occur in low-income countries (Hoest et al., 2014). There are many factors in the life of a child that could account for the differences between developed and developing countries. Potential contributors include passive transfer of maternal antibodies, gut microbiota, and malnutrition (Desselberger, 2017). These are important factors when considering geographical differences in immune responses and seroconversion rates among infants to RV vaccine.

Health Outcome: RV Vaccine Seroconversion

Varying immune responses to RV infections illustrate how and what factors affect whether seroconversion occurs upon vaccination (Appx 3). There are two reactions that RV infections elicit in children, non-virus-specific innate and virus-specific acquired. An innate non-virus-specific response is upon exposure, activation of specific stimulatory genes that produce transcription factors converting cells into an "antiviral state" (Desselberger, 2017). RV can block the production of these transcription factors and thus prevent the host cell and immune response. RV has nonstructural genes specifically, NSP1, that interrupt this and thus evade the normal cellular immune response (Angel, Franco, & Greenberg, 2012). Some protection has been observed in cohort studies in Mexico and Guinea-Bissau illustrating how the initial episodes of RV was more severe than any recurrent episodes (Angel et al., 2012). Age of infection was also seen as factor in how protective recurrent infections may be. Early exposure (< 6 months of age) may prompt a delayed immune response for vaccination as the ability to induce antibodies for RV is age specific (Angel et al., 2012). This may contribute to the lower efficacy of natural infections and vaccinations in low-income countries with higher prevalence rates (Angel et al., 2012). In the virus-specific acquired immune response, the body recognizes and responds to infection by secreting humoral antibodies and T-cells specific to RV antigens (Desselberger, 2014). These antibodies and T-cells can neutralize RV antigens which somewhat correlates to protection against RV disease. These interactions are still being studied and are not fully understood.

Maternal Antibodies

A potential factor, maternal antibodies, may play important roles in the development of an infant's immune system affecting the success of seroconversion in RV vaccines. In RV endemic areas of the world, infants are easily exposed. The early exposure catches infants when

their immune systems both innate and acquired are still immature (Desselberger, 2017). Initial exposure may occur through breastfeeding, the passive transport of maternal antibodies (Desselberger, 2017). There is contradicting data regarding whether passive transfer affect vaccine efficacy. Some studies suggest that abstaining from breastfeeding around the time of vaccination does not significantly impact an infant successfully seroconverting (Desselberger, 2017). Other studies suggested maternal antibody transfer through both breastfeeding and during natural birth could be associated with interfering in the infant's seroconversion after RV vaccination (Angel et al., 2012; Uchiyama et al., 2014).

Gut microbiota and Seroconversion

Another potential factor, gut microbiota, may play a role in the immunological response for RV vaccine (Appx 3). Data is limited in the gut's role in immunogenicity but animal studies have shown how the composition of the gut microbiota affects both the development and function of the humoral immune system (Sommer & Backhed, 2013). Development of the immune system is dependent on microorganisms as they stimulate the formation of lymphoid structures and modulate immune cells (Sommer & Backhed, 2013).

For RV vaccine seroconversion, highly variable gut microbiota could influence poor vaccine performance (Uchiyama et al., 2014). Studies have shown differences in gut microbiota between seroconverted infants and non-seroconverted infants (Velasquez et al., 2017). As the gut microbiome plays a central role in immune system development, live vaccines such as both RV vaccines could also impact efficacy in infants when the gut microbiome is under development (Velasquez et al., 2017). Recent investigations have also correlated a factor in dysbiosis, malnutrition, especially micronutrient deficiencies to poor vaccine performance (Desselberger, 2017; Hoest et al., 2014). Activated gut microbiota from malnutrition, diarrhea, micronutrient deficiencies, or even enteric infections complicate immunogenicity as a whole. Diarrhea-caused

inflammation results from assaults to the gut microbiome and may affect vaccine performance (Appx 3)(Velasquez et al., 2017).

Antibiotics and Seroconversion

Dynamics within gut microbiota could also be related to antibiotic usage. One dose of antibiotic is sufficient to make changes in the gut microbiome (Shi et al., 2018). In some studies, the antibiotics prescribed inactivated the gut by controlling pathogens, reducing inflammation, and decreasing the number of infections (Uchiyama et al., 2014). The inactivated gut allowed for a more durable RV antibody response (Uchiyama et al., 2014). In other studies, antibiotics increased the risk of diarrhea (Issa & Moucari, 2014; Rogawski et al., 2015).

In an animal study published in China, a mouse model was used to measure the extent to which antibiotic exposure altered intestinal bacterial and immune cell homeostasis (Shi et al., 2018). Results confirmed that antibiotic exposure caused alterations to gut bacteria causing inflammation (Shi et al., 2018). Conversely, antibiotic exposure may limit acute and or persistent infections (Velasquez et al., 2017). A reduction in infections promoting gut health which may have a direct effect on vaccine efficacy among children in low income settings (Velasquez et al., 2017).

Study Location: Bolivia

Diarrhea and stunting in Bolivia remain prominent issues that has merited studies and contributes to the global burden of disease with serious health outcomes. There have been many studies done in this population regarding diarrhea prevalence and report illustrating how acute and chronic diarrhea impacts children under 5. In Bolivia, diarrhea ranks 3rd leading cause of morbidity and mortality in children under 5 (George et al., 2014). Before 2008, there was a sharp rise in incidence of diarrhea for children with a prevalence of 36.2% in some parts of the country (George et al., 2014). The Bolivian government responded to the sharp increase in diarrheal

infections among children in 2008 by introducing rotavirus (RV1) nationally (Inchauste et al., 2017). Presently, Bolivia has made significant strides in the betterment for child health. However, when compared to neighboring countries in Latin America, Bolivia is among the countries with the highest burden of disease. The under-five mortality rate is 39 out of 1000 births, compared to the regional rate of 15 per 1000. The prevalence for childhood stunting in Latin America is 16.1% and in Bolivia, 18.1% (Black et al., 2008; UNICEF, 2016).

Developing countries like Bolivia are making significant progress; however, children-under-five are still dying from preventative illnesses such as diarrhea and malnutrition. In response, there has been a surge of studies in Bolivia. In addition to rotavirus impacting rates of diarrhea, malnutrition was also found to be a determining factor in RV infection in developing countries (Desselberger, 2017). Malnutrition and infections such as RV have a two-way relationship where each impact the other (Brüssow, 2015). The health outcomes that are affected by this synergistic relationship are stunting and RV vaccine seroconversion. Moderate and severe forms of stunting as of 2012 affect 27.1% of Bolivians (UNICEF, 2013). Bolivia is an ideal study location because of high child under 5 mortality-rates, high incidence of diarrheal infection, rates of malnutrition, and nationally funded vaccination implementation programs for rotavirus vaccine (Aceituno et al., 2017). Thus, there is a need to explore antibiotic exposure and their potential relationship with health outcomes stunting and RV vaccine seroconversion in children under 5 living in Bolivia.

Significance

The findings of this exploratory project will contribute to understanding the complexity of external factors with regards to antibiotics prescribed to infants, infant stunting, and efficacy of the rotavirus vaccine. With the increasing global trend of antibiotic prescriptions and lack of

research available, this analysis will provide data of the rate and kinds of antibiotics prescribed to infants. Exploring the hypothesis of antibiotics role and its negative effects on infant health, this analysis can provide insight on prescribing practices that can translate to future research for interventions to modify or standardize how antibiotics are prescribed for infants. Stunting is also prevalent in Bolivia and the findings of this project can provide more information on how antibiotics may be related to this health outcome. With delayed or no seroconversion from RV vaccine still occurring in Bolivia, this project's finding can aid in exploring potential factors that are affecting the seroconversion of infants from RV vaccine. From a public health perspective, the assessment of antibiotics in relation to stunting and seroconversion will illuminate potential pathways ending in negative health outcomes. Narrowing down the variables affecting stunting and seroconversion can allow for researchers and physicians to make systematic changes in the treatments for patients particularly children under five.

Methods

Goal

The goal of this project was to assess antibiotic prescribing practices for young infants and determine the relationship between potential antibiotics and stunting seroconversion of RV vaccine from infants that were part of the NIDI study at Hospital Los Andes in El Alto, Bolivia. The assessment included describing and identifying antibiotic prescribing practices for infants less than 6 months by medical practitioners at Hospital Los Andes. The quantitative assessment included quantifying stunting, RV vaccine seroconversion in the Bolivian infants less than 6 month, and exploring potential relationship between antibiotics prescribed and the two health outcomes: stunting and seroconversion. This project was part of an existing approved Emory IRB protocol: IRB00056127.

Study project population and site

The study site for the NIDI longitudinal birth-cohort study was in El Alto, Bolivia, a sprawling Andean metropolis, which had a population in 2016 of 902,000 where 3 out of every 10 adults living in the department of La Paz are residents of El Alto (Estadistica, 2016) (Studies, 2011) (Aceituno et al., 2017). El Alto is one of the highest Latin American cities at 13,500ft above sea level on the *altiplano* overlooking La Paz,and has a predominately indigenous population. Hospital Los Andes is one of the two hospitals in El Alto where mother-infant pairs were recruited from for the NIDI study. Of the 350 mother-infant pairs from the prior NIDI study that ended in March 2015, 180 infants attended Hospital Los Andes (Aceituno et al., 2017). Medical charts were found and reviewed from the Registry Department at Hospital Los Andes for 152 infants of the 180 infants recruited from this hospital. Data abstracted included

identifying visits of interest defined as any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea as reason of visit.

Data Collection

Chart Abstraction Tool. A one-page chart abstraction form was developed in collaboration with Hospital Los Andes and partners at Emory University (Appx.1). The form's final version was translated to Spanish. The form was in open-ended format to facilitate and encompass the complexity of each medical chart. It had two main sections. The first section was for identifiers including name, date of birth, medical record number, ABO status, and sex. The second section was for visits of interests predetermined to be any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea as reason of visit. The chart abstraction tool was pilot tested for improvements on the first five medical records resulting in amending the format of the tool and eliminating unnecessarily variables. An independent reviewer, which was a recent medical graduate from Bolivia and familiar with terminology, was hired to consult on reading medical charts and aid in filling out forms owing to specialized knowledge in Bolivian hospital chart organization and terminology.

Medical charts. Medical charts were obtained from the Registration department at Hospital Los Andes. Hospital staff were given a list of names and dates of birth of NIDI participants. Of the 180 active NIDI participants recruited from Hospital Los Andes for hospitalizations, outpatient, and well-child visits, only 135 infants had data from both the prior NIDI study and medical records at Hospital Los Andes.

Chart abstractions. The author, practicum partner, medical consultant, and Post-Doc fellow collected data on site from May 2017 to July 2017. Approximately 5-10 medical charts were given daily and each medical chart was independently reviewed twice by two individuals. A form was filled out for each visit to Los Andes Hospital with the following criteria for a visit:

any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea as reason of visit. To minimize errors, two independent abstractions were performed by two researchers and then compared for discrepancies. If discrepancies occurred, chart was reviewed a third time.

Data Analysis

Database creation. We created a database to enter data. The database was based out of Excel version 16.9. We did double data entry to clean and standardize the database. The final database was then imported into SAS 9.4.

Merging databases. The focal data analysis was done by merging from two sources, the chart abstraction database and NIDI study database. Due to the size of the NIDI study database, the following variables of interest were extracted for the final merged database: infant age at visit 6, infant date of birth, date of first dose of Rotatrix vaccine, date of second dose of Rotatrix vaccine, infant stunted at visit 6 or below (measurement done by NIDI staff at time of visit using WHO guidelines), study ID number, visit number, maternal age, maternal education, baby male, relationship status, infant seroconverted, baby stunted at visit 6, wealth index quintile factor. The variables to be analyzed were approved by NIDI database creator RM Burke. The merged result contains visits prior to the sixth visit of the main NIDI database to correspond to RV vaccine seroconversion and stunting measured at this date. Observations were manually inspected to ensure proper merge alignment.

Analysis. Data merging and statistical analysis was done using SAS 9.4. The project analysis was organized into 3 different aims and used a combination of descriptive analysis and statistical modeling to answer each aim. For Aim 1, descriptive statistics were used to identify proportions of antibiotics prescribed and demographic information. For Aim 2 and Aim 3, a

combination of descriptive statistics and logistic regression was used to identify any potential relationship between antibiotic prescriptions and the health outcomes, stunting (Aim 2) and seroconversion (Aim 3). Chi square was utilized for both categorical variables stunting and seroconversion to identify any significance in distribution. Statistical significance in models had a p-value of 0.05 and include an adjusted and unadjusted model in which the adjusted model controlled for potential confounders that were sex of infant and SES (wealth index variable). No multicollinearity was found between the variables.

Results

135 prior NIDI study infants were retrieved from the medical chart records at Hospital Los Andes. The data collected for the infants were from birth to 6 months of age. The range of births for the infants was between April 2013 and May 2014. The goal of this project is to explore antibiotic prescriptions at Hospital Los Andes in El Alto, Bolivia and identify relationships between antibiotic prescribing practices, stunting, and rotavirus vaccine underperformance as measured by RV vaccine seroconversion.

I. Characteristics of Mother-Infant pairs

To assess characteristics of the population for this study and aid in future studies, demographic data was collected for a total of 135 NIDI mother-infant pairs. Through descriptive statistics, we found an even distribution in the sex of the infant (Table 1). Twenty four percent of the infants had been prescribed at least one antibiotic course at or before 6 months of life. Other variables, that can be found in table 1, illustrate the ABO status (blood type) and maternal demographics. For maternal demographics, the median age was 25 and the majority resided with their partners (table1). Most mothers had received at least some secondary education and were evenly distributed in their SES (wealth index quintile variable) (Table 1).

Table 1: Characteristics Table of unique Mother-Infant Pairs N=135

Characteristic	Frequency (N)	Percentage
Sex of infant		
Male	72	53%
Female	63	47%
Prescribed Antibiotic by 6 months of age		
Yes	33	24%
No	102	76%
ABO status	N=62	
O+	60	45%
A+	2	1%
Missing	73	54%
Maternal Age (yrs)		
Median (25^{th}-75^{th})	25 (22-30)	
Maternal relationship status		
Living with partner	117	87%
Living without partner	18	13%
Maternal Education		
Primary or less	19	14%
At least some secondary	89	66%
At least some superior	27	20%
Wealth Index Quintile	N=115	
1	26	23%
2	22	19%
3	24	21%
4	21	18%
5	22	19%

II. Antibiotics and Infants

To classify infant visits in relation to antibiotic prescriptions given at Hospital Los Andes, we collected data of visits between birth and age 6 months. Visits were defined as: any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea. The histogram (figure 1) shows visit 1 to be the most common among the infants. In table 2, of the antibiotic classes prescribed, penicillin was the most common class prescribed across all visits. In order to identify what specific antibiotic classes were being prescribed by visit, we measured antibiotic class by number of visits for the infants that were prescribed antibiotics (figure 2). The

majority of antibiotics were prescribed to those who attended the hospital 2 times and multiple classes were prescribed to them at those individual visits. Although most infants attended the Hospital once and were not prescribed antibiotics, the infants that were prescribed antibiotics tended to come to the hospital more than once.

Figure 1: Histogram of number of visits to Hospital Los Andes before Infants 6months old

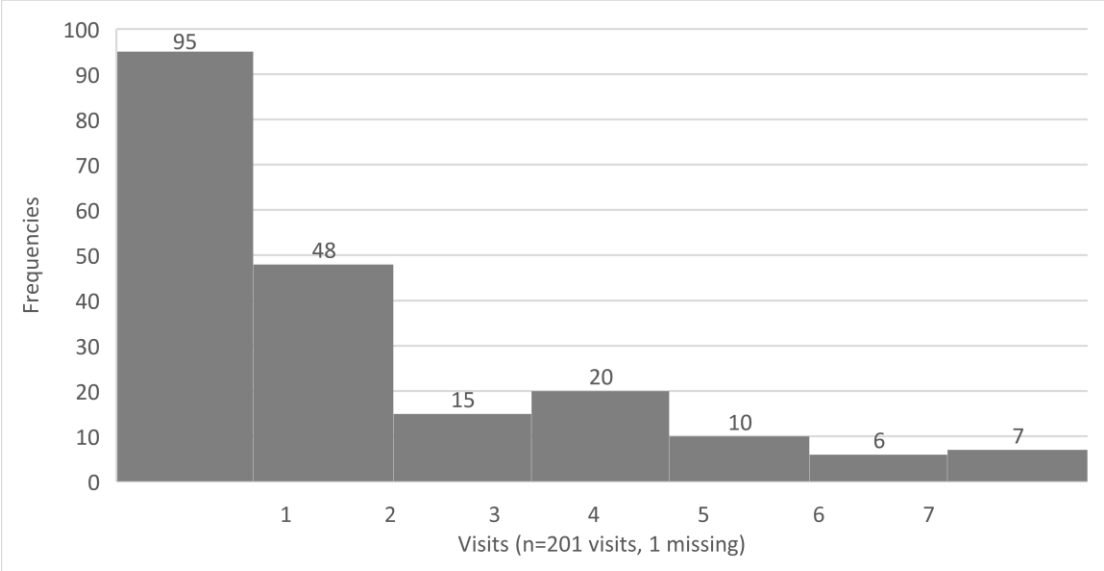
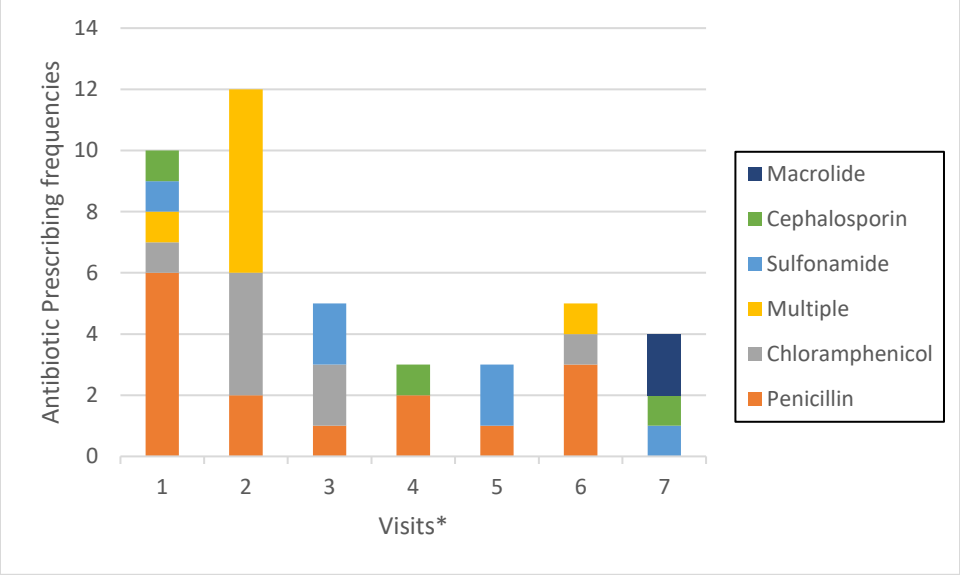


Table 2. Antibiotic class prescribed by number of infant visits to Hospital at or before Infants 6 months old

Antibiotic Class Prescribed	**Visits[1]**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
No Antibiotic	85	36	10	17	7	1	3	159
Penicillin	6	2	1	2	1	3	0	15
Chloramphenicol	1	4	2	0	0	1	0	8
Multiple[2]	1	6	0	0	0	1	0	8
Sulfonamide	1	0	2	0	2	0	1	6
Cephalosporin	1	0	0	1	0	0	1	3
Macrolide	0	0	0	0	0	0	2	2
Total observations	95	48	14	20	10	6	7	201

[1]Visits= any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea as reason of visit at or before the 6th NIDI visit date.
[2]Multiple class defined in table 3

Figure 2. Graph of Antibiotics Prescribed (n=42), excluding No Antibiotic category, by visit[1]



[1]Visits= any visit where an antibiotic was prescribed, nutritional supplement prescription, or diarrhea as reason of visit at or before the 6th NIDI visit date.

To identify the time of prescription for the infants at or before 6 months of age, antibiotics were divided into time frames around the two RV vaccine doses given as part of the prior NIDI study. Vaccine 1 (RV dose 1) was given at NIDI visit 2 where infants were at or around 2 months of age and Vaccine 2 (RV dose 2) was given at NIDI visit where infants were at or around 6 months of age (Aceituno et al., 2017). Distinct antibiotics courses for the purpose of this study are separated by visit. Antibiotics were consistently prescribed across the time frames (Table 3). Of the antibiotics prescribed, most were in the Penicillin class (36%) or multiple antibiotic classes (19%) prescribed at the same time (Table 3) compared to antibiotic classes, chloramphenicol, sulfonamide, cephalosporin, macrolide, and aminoglycoside. Antibiotics are being prescribed to infants 6 months and younger; this indicates that early exposure does occur and should be evaluated.

Table 3: Antibiotic Characteristics by name and class for infants prescribed Antibiotics at or before 6 months old

	Total no.	Before Vaccine 1 No. (%)	Between Vaccine 1 and Vaccine 2 No. (%)	After Vaccine 2[1] No. (%)
Antibiotic Class Prescriptions (n)	42	12 (29%)	13 (31%)	17 (40%)
Penicillin class	15 (36%)	3 (25%)	3 (23%)	9 (53%)
Amoxicillin	12	1	3	8
Ampicillin	6	6	0	0
Dicloxacillin	1	0	0	1
Penicillin	2	3	0	0
Chloramphenicol (class)	8 (19%)	2 (17%)	3 (23%)	3 (18%)
Multiple*	8 (19%)	6 (50%)	2(15%)	0
Ampicillin+ Gentamicin	4	4	0	0
Ampicillin+ Gentamicin+ Penicillin	1	1	0	0
Ampicillin +Gentamicin+ Cotrimoxazole	1	1	0	0
Ceftriaxone+Erithromycin	1	0	1	0
Cefotaxime+ Chloramfenicol	1	0	1	0
Sulfonamide class	6 (14%)	0	3(23%)	3 (18%)
Cotrimoxazole	6	1	3	3
Cephalosporin class	3 (7%)	1 (8%)	0	2(12%)
Cefadroxil	1	0	0	1
Cefixime	1	1	0	0
Cefotaxime	1	0	0	0
Ceftriaxone	1	0	0	1
Macrolide class	2 (5%)	0	2 (15%)	0
Claritromycin	2	0	2	0
Erithromycin	1	0	0	0
Aminoglycoside class (Gentamicin)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[1]After Vaccine 2= any antibiotic prescribed after vaccine 2 and at or before the 6th NIDI visit date

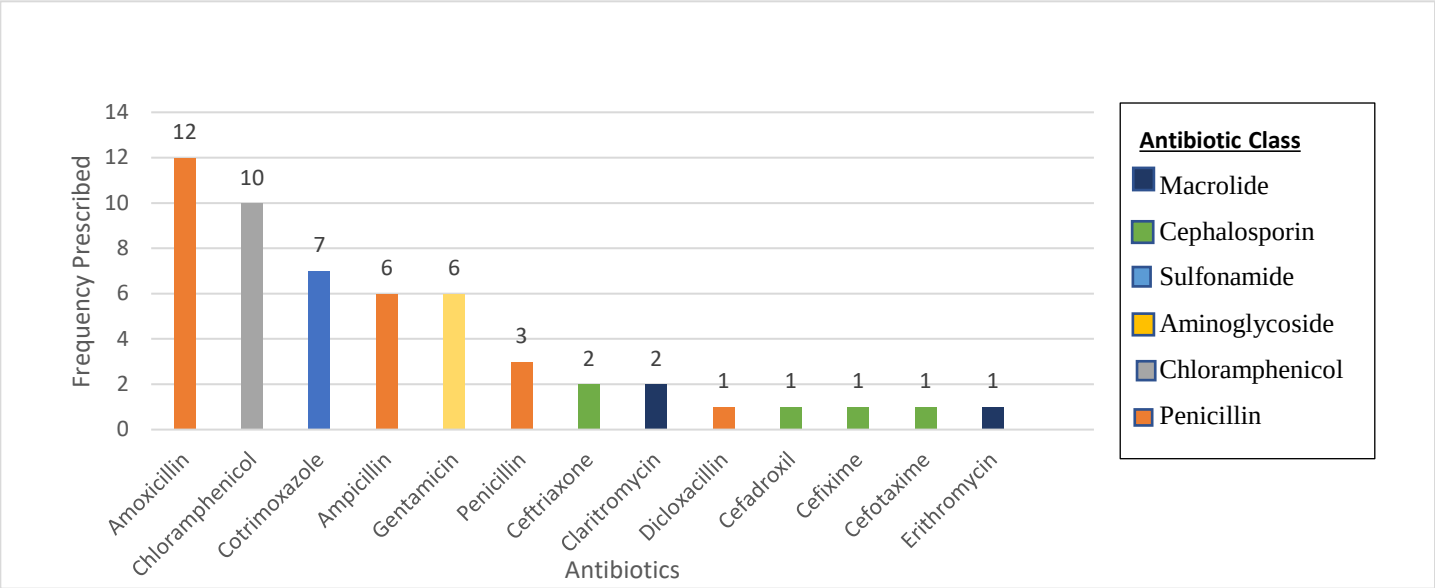
To understand the frequency of antibiotics and general diagnostics for infants, we evaluated diagnostic data collected in the medical records among the infants prescribed antibiotics (24% of total infants). Diagnoses of infants when an antibiotic was prescribed were respiratory infections, conjunctivitis, diarrhea, antibiotic not prescribed by physician, sepsis, dermal infections, urinary tract infections, congenital syphilis, ear infection, and meningitis (table 4). Nine percent of antibiotics in medical charts were self-reported by caregiver and not prescribed by physician. Thirty three percent of infants diagnosed with antibiotics had multiple diagnoses. In cases of multiple diagnoses, data collected from medical charts did not specify which antibiotic corresponded with which diagnosis. Fifteen percent of visits that prescribed antibiotics resulted in having labs done. Amoxicillin was most frequently prescribed to infants (figure 3). Only 12.5% of antibiotics were from infants coming to the hospital with diarrhea as a chief complaint. Thirty two percent of infants attending Hospital Los Andes came in for respiratory infections (table 4).

Table 4. Diagnostic characteristics among infants (N=33) prescribed Antibiotics

Diagnosing illness	**No. (%)**
Respiratory Infections[1]	17 (32%)
Conjunctivitis[2]	7 (13%)
Diarrhea	7 (13%)
Not prescribed by physician	5 (10%)
Sepsis	5 (10%)
Dermal Infections	4 (8%)
Urinary Tract Infections	2 (4%)
Congenital syphilis	2 (4%)
Ear Infection	2 (4%)
Meningitis	1 (2%)
Total	52

[1]Respiratory infections in table 4 correspond to cough, cold, acute respiratory infection (Infección Respiratoria Aguda, IRA), laryngitis, and pharyngitis
[2]Eye drops, not oral antibiotic

Figure 3: Graph of antibiotics prescribed to Infants(N=33) by frequency and class



III. Stunting

To investigate the effects antibiotic prescriptions has on stunting, data were compared of a sample of infants (n=135) that participated in the prior NIDI study (N=350) using Chi-Square and Logistic Regression. Stunting was measured against cumulative antibiotic prescriptions for infants 6 months old. If the infant was measured as stunted in any visit throughout the NIDI study prior to infants 6 months of age, then the infant was considered stunted. Logistic regression was calculated in an adjusted and unadjusted model. The adjusted model controlled for sex of infant and wealth index factor of the household (SES). SES was controlled due to evidence from the literature holding SES as a risk factor in stunting (Robert E. Black, 2016). Logistic regression was done on 2 datasets, the first was frequency of antibiotic and stunting in infants 6 months of age, the second was whether antibiotic was ever prescribed and stunting in infants 6 months of age. The investigation found that twenty eight percent of infants in the project were stunted at some point from birth to 6 months of age (Table 5). Of those that were stunted, 37% had

received at least one antibiotic at or before 6 months of age (table 5). Penicillin was the most prescribed class among all infants with specific emphasis among stunted infants where the preference for penicillin prescriptions was the highest (figure 4). There was a significant relationship between antibiotic prescription and stunting before 6 months of age (Chi Sq= 4.40, p=0.036) (LogReg OR=2.4 P=0.04) (Table 6). Stunting in this study appears to be a significantly affected by antibiotic exposure early in life.

The odds of stunting are 2.4 times as high for infants prescribed at least one antibiotic compared to those infants not prescribed any. There is a significant relationship between antibiotic prescriptions and stunting.

Table 5. Association between antibiotic prescription before six months of age and stunting for Infants (N=135)

Stunting	Any Antibiotic Prescription		
	Yes No. (%)	No No. (%)	Total/N=135 No.
Yes	14 (37%)	24 (63%)	38/135 (28%)
No	19 (20%)	78 (80%)	97/135 (72%)
Total	33	102	135

Figure 4: Association between antibiotic class before six months and stunting in infants prescribed antibiotics (Infant N=33, antibiotic class n=42)

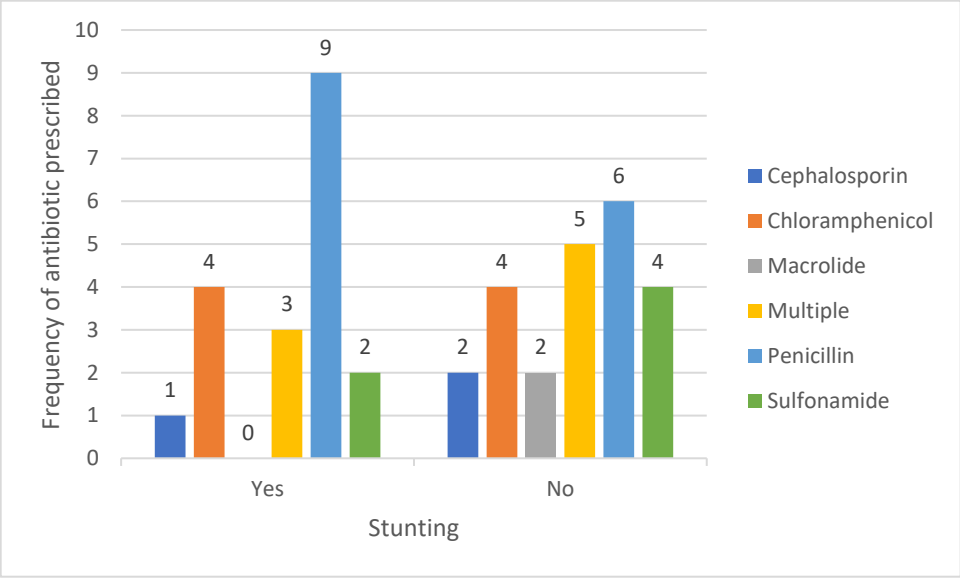


Table 6. Logistic Regression Model: Characteristics associated with Stunting and number of antibiotics prescribed in the medical charts at Hospital Los Andes to NIDI infants at or before the 6th NIDI visit

Exposure: Antibiotic prescription	Outcome: Stunting			
	Crude Odds Ratio (95% CI)	P-value	Adjusted Odds Ratio[1] (95% CI)	P-value
Infant stunting and antibiotics[2]	1.48 (0.89-2.46)	0.13	1.85 (1.01-3.24)	0.033
Grouped antibiotics[3]	2.4 (1.05-5.48)	0.04	3.5 (1.37-8.98)	0.009

[1]Adjusted: Sex of infant and wealth index factor
[2]Dataset with antibiotic frequency= continuous numerical variable
[3]Dataset with antibiotic frequency= binomial, Y/N

IV. Seroconversion

To investigate the effects antibiotic prescriptions have on seroconversion, data were compared of a sample of infants (n=135) that participated in the prior NIDI study (N=350). Seroconversion was measured against cumulative antibiotic prescriptions for infants 6 months old in both Chi-Square and Logistic Regression. If the infant was measured as seropositive at 6 months, then the infant was considered to have seroconverted to the RV vaccine. Logistic regression was calculated in an adjusted and unadjusted model. The adjusted model controlled for sex of infant and wealth index factor of the household (SES). Logistic regression was done on 2 datasets, the first was frequency of antibiotic and stunting, the second was whether antibiotic was ever prescribed and stunting in infants 6 months of age. The investigation found

that thirty one percent, 40 of 129, NIDI infants did not seroconvert following their RV vaccination (Table 7). Among the infants that did not seroconvert, 30% were prescribed at least one antibiotic at or before 6 months of age (Table 7). There was not a significant relationship between antibiotic prescription and RV vaccine seroconversion at 6 months of age (Chi-sq= 0.84 P=0.36) (Log Reg OR=0.68 P=0.36) (Table 8). There are no significant effects antibiotic prescriptions have on seroconversion.

Table 7. Association between antibiotic prescription before six months of age and RV vaccine seroconversion for Infants (N=129)*

	Any Antibiotic Prescription		
Seroconverted	Yes No. (%)	No No. (%)	Total No. (%)
Yes	20 (22%)	69 (78%)	89
No	12 (30%)	28 (70%)	40

*6 missing

Table 8. Characteristics associated with seroconversion and number of antibiotics prescribed in the medical charts at Hospital Los Andes to NIDI infants at or before the 6th NIDI visit

	Outcome: Seroconversion			
Exposure: Antibiotic Prescription	Crude Odds Ratio (95% CI)	P-value	Adjusted Odds Ratio[1] (95% CI)	P-value
Infant seroconversion and antibiotics*	0.88 (0.51-1.52)	0.65	0.97 (0.53- 1.8)	0.92
Grouped Antibiotics **	0.68(0.29-1.57)	0.36	0.76 (0.3-1.97)	0.58

[1]Adjusted: Sex of infant and wealth index factor
*Dataset with antibiotic frequency 0-3
**Dataset with antibiotic frequency grouped 0 or 1

Discussion

This project was designed to explore antibiotic prescriptions at Hospital Los Andes in El Alto, Bolivia and identify relationships between antibiotic prescribing practices, stunting, and rotavirus vaccine underperformance as measured by RV vaccine seroconversion. As there is little literature regarding specific antibiotic prescriptions both in frequency and class, descriptive analysis will be useful for future research in this population and Latin America. Overall, the study found that there was a significant relationship between stunting and antibiotic prescriptions. The literature however frequently implicates infection to negative health outcomes like stunting and delayed or no RV vaccine seroconversion (Black, 1993; Brüssow, 2015). Because this study did not measure infection, a confounder model illustrates its role in both the exposure, antibiotic prescription and the outcomes, stunting and seroconversion (Appx 4). Antibiotics are prescribed in the event of an infection and infections may impede body functions that are critical to growth and immunogenicity.

Stunting of infants at or before six months

The first key result was that there was a significant relationship found between stunting and frequency of antibiotics prescribed in infants after controlling for sex of infant and wealth index factor. Thirty seven percent of infants in this study exhibited both stunting and were prescribed at least one antibiotic in the first six months of life. A potential mechanism that could affect stunting in early childhood is the changes to gut microbiota that an antibiotic course may have (Aguayo et al., 2015; Desselberger, 2017; WHO, 2017). Although the literature on antibiotics and growth directly in humans is inconsistent, over prescription is common specifically for children under 5 who have underdeveloped microbiota (Gough et al., 2014; Issa & Moucari, 2014; Owino et al., 2016). Antibiotic exposure changes the make-up of the gut

microbiota causing some bacteria to overpopulate over others. Gut microbiota is critical to the human health that when imbalanced(dysbiosis) such as during cases of severe diarrhea, can have detrimental effects such as nutrient malabsorption and increase the odds of stunting (Black et al., 2008; Desselberger, 2017). Ultimately antibiotic exposure affects the gut microbiota in a way that impedes its function specifically related to nutrient absorption. Longitudinal dysfunction early in life may lead to severe growth problems such as stunting.

There has been a plethora of animal studies confirming antibiotic exposure's relationship to dysbiosis and the subsequent health effects including chronic diarrhea and inflammation (Cho et al., 2012; Shi et al., 2018). One study exposed mice to broad spectrum antibiotic, ampicillin and observed changes to gut microbiota. The mice were then treated with *Lactobacillus* to recover the biodiversity of the gut microbiome (Shi et al., 2018). After 2 weeks administration of ampicillin to mice, microbial diversity was decreased, the pouch at the start of the large intestine (cecum) was swollen, and gut permeability was increased (Shi et al., 2018). Results and conclusion of the study showed that antibiotic administration changed the gut microbiota significantly with a single dose of antibiotic being sufficient to induce dysbiosis. In contrast, probiotics like *Lactobacillus* species stabilized the gut (Shi et al., 2018).

Study design is another mechanism that potentially influenced the results of the study. This study did not assess infections or sickness of infants. Infants receive antibiotics when they are sick; it may be that the infants that are stunted were prescribed antibiotics more often because they are more prone to infection. There is a two-way relationship between malnutrition and infection (Brüssow, 2015). Stunting is related to deficiencies in nutrition but those deficiencies may be due to infants being sick more often and not getting adequate amount of nutrients. A sick child may or may not be eating well and therefore contributing to nutritional deficiencies that over time and persistent infections may lead to stunting.

In this study, both the chi-square and logistic regression analysis illustrated a significant relationship between the frequency of antibiotic prescription and stunting. In both chi-square and logistic regression analysis, there was significance between being prescribed an antibiotic and stunting at or before 6 months of age. We controlled for sex of infant and SES through the wealth index variable. In the literature, statistically, male babies and low SES have a tendency to exhibit stunting early in life (Black et al., 2008). Using data-based criterion method to measure confounding for sex of infant and SES, there was a -20% difference in dataset 1 and -29% difference in dataset 2 between the crude odds ratio and adjusted odds ratio (Appx 5). The percent difference suggests that there was joint confounding by sex of infant and SES for this analysis (Appx 4).

Seroconversion following two doses of RV vaccine

The second finding for this project was that no significant relationship was found between seroconversion and the frequency of antibiotics prescribed. Although 31% of the infants in the study did not seroconvert after both doses of RV vaccine, 30% among them were prescribed at least one antibiotic within the first 6 months of life. Some potential mechanisms for this result may be due to the study design and other causal pathways unrelated to antibiotic prescriptions. This study had a sample size of 135 infants from the prior NIDI study that had 350 infants. At a 95% confidence interval and 80% power, at least 169 infants were needed to detect a statistical difference in seroconversion. However, this study focused on one hospital that collected data for 135 infants. Using the Data criterion method to measure confounding for sex of infant and SES, there was a -9% difference in dataset 1 and -14% difference in dataset 2 between the crude and adjusted odds ratio (Appx 6). Joint confounding by sex of infant and SES was a factor for the second dataset only.

Antibiotic prescriptions and seroconversion are likely directly unrelated based on the literature and the results of this study. In our study design, antibiotic prescriptions were not measured at the time of vaccination. Further, antibiotic exposure was not a study question during the NIDI study when the vaccines were done. In addition, the data collected at the hospital pertaining to antibiotic prescriptions did not specify a window prior to the RV vaccinations. Antibiotic prescriptions in charts solely suggest that the physician prescribed them; usage of the antibiotics can only be implied. There was also self-reporting in the charts. Mothers may or may not have accurately disclosed all medications given to their infants. Antibiotics are readily available in Bolivia and mothers may not be aware when buying medication from their local pharmacy, specific names or types of medications. Medical charts are also limited to one location. As of 2014, Hospital Los Andes became a referral only secondary tier healthcare facility. Infants may have attended other healthcare centers and not disclosed medications or diagnoses when referred back to Hospital Los Andes.

Other potential mechanisms could be that there are other causal pathways for lower rates of seroconversion that this study did not measure. These causal pathways highlighted in the literature are maternal antibodies and seasonality of rotavirus (Desselberger, 2017; Velasquez et al., 2017). Maternal antibodies are hypothesized to interfere with infants mounting a proper immune response to RV vaccine (Desselberger, 2017). Breast feeding is one direct mechanism infants develop their immune system from maternal antibodies (Velasquez et al., 2017). Incidence of rotavirus in Bolivia is seasonal with spikes occurring during the rainy season (Inchauste et al., 2017; Velasquez et al., 2017). The timing of both vaccine and birth of infants in relation to RV season could influence seroconversion (Velasquez et al., 2017). There may be other underlying biological mechanisms of greater importance to vaccine underperformance that this study did not measure such as maternal antibodies and seasonality of RV.

Strength, Limitations, and Future Research

Strengths for this project include access to a comprehensive dataset from a longitudinal study, access to resources both in Bolivia and the US that collaborated in obtaining the data from the medical charts, and the variables RV vaccine administration and stunting. Access to the NIDI database was a strength for this study because it was a comprehensive longitudinal dataset that matched up directly to the infants used in this study. The data from NIDI was essential in providing details such as mother and infant demographics, dates of RV vaccine, and seroconversion status that was not existent in the medical charts found at Hospital Los Andes. Access to resources in Bolivia and the US was a strength because without the approval and collaboration from the Hospital, the data would not have been available for extraction. In addition, several investigators both in Bolivia and US were key in providing expert recommendations in how to frame and conduct the project. Finally, a strength for the study was that key variables were controlled. This project would not be possible without controlling for RV vaccine dose dates, seroconversion lab testing, and stunting.

Limitations for this project include questionable quality of data extracted from medical records, confounders in assumptions made regarding infant doctor visits, and constitutional changes to the healthcare system in Bolivia mid NIDI study. A main limitation of this project was the quality and analyzability of the data extracted from the medical charts at Hospital Los Andes. Medical charts were handwritten with each physician having their own codes and methodologies for filling out medical charts. It was difficult to read and thus prone to errors. Therefore C Torrez Quispe (MD) had to be hired to help decipher the codes used in the medical charts. To minimize errors, two independent abstractions were performed by two researchers and then compared for discrepancies. If discrepancies occurred, chart was reviewed a third time. The ambiguity of the medical charts made data collected difficult to not only extract but also analyze.

Further, antibiotic usage cannot be accurately measured from medical charts alone. Prescription of antibiotic does not guarantee that an infant took the antibiotics or if they completed the full course prescribed. There were also assumptions made when analyzing the data collected from the medical charts. There are many confounders in that mothers most likely went to different hospitals and health centers to receive care for their babies. The medical charts at Hospital Los Andes portray only a partial picture for what the infants experienced medically in the first 6 months of life. Finally, another limitation was a constitutional restructuration of the healthcare system in Bolivia in 2014 (Linera, 2014). The effects of the restructuration included an all-encompassing universal healthcare where individuals go to health centers as their primary care and hospitals like Hospital Los Andes become exclusively specialty healthcare facilities (Linera, 2014). Because this occurred in the middle of the NIDI study, mothers were transitioning to health centers for their infant's primary care needs. Medical charts may reflect this transition by missing infants that changed healthcare facilities at different rates.

Further investigation is necessary to continue to investigate the effect antibiotics have on health outcomes. There were some limitations to this analysis in what data was available for extraction however one suggestion would be look into different hospitals and health centers within a specific city or neighborhood to obtain more complete data on what antibiotics are prescribed. Because antibiotics are available over the counter, gathering data from local pharmacies regarding the kinds of antibiotics sold for a specific neighborhood could also be useful. Programmatically, medical chart writing should be standardized, Bolivian antibiotic treatment guidelines should be evaluated, and education modules implemented for mothers to have better understanding of potential interactions and risks antibiotics may have on children particularly in the first year of life.

Public Health Implications

The project's findings have important public health implications. For the results of this study, stunting is prevalent among Bolivian infants and seroconversion rates are lower. There are also implications to note for public health in Bolivia that through this study design, we were able to identify.

There are implications for the first key finding, the significant relationship between stunting and antibiotic prescriptions. Some implications include physician prescribing practices, nutritional status, educational modules in secondary education regarding pregnancy and infant health, and supporting interventions targeting nutrition of children. An implication from this study was the prescribing practices of physicians at Hospital Los Andes. Identifying whether treatments adhered to hospital regulation by physician would benefit the children's health. Also, further identifying national protocols of treatments for common diagnoses such as diarrhea and respiratory infections among children would aid in what medications are prescribed and when lab testing is needed. There were few cases of lab testing in this study and many prescriptions for antibiotics in this study.

For nutritional status implications, stunting appears to be directly related. More research should be done in this population addressing nutrient deficiencies and stunting. Antibiotics has been shown to affect nutrient absorption in the gut however going beyond that relationship, understanding why infants are prescribed antibiotics in the first place should be further researched. Infections among infants was exceedingly common in this study. An implication for public health in Bolivia would be to address the risk factors for high rates of infections among infants therefore reducing the frequency antibiotics are prescribed early in life. Although causal

pathways are still being researched, infections early in life are related to malnutrition and thus nutritional status.

Educational modules regarding maternal and infant nutrition would also benefit Bolivia overall. There are many interventions in Bolivia over the past decade that has significantly reduced children under five mortality rates to 39 deaths out of 1000 live births. A public health implication would be to incorporate nutritional education in secondary education as most mothers for this study obtained at least some secondary education. New mothers are a particular demographic that would be key to incorporate. Finally, another implication is to support interventions focusing on mother-infant nutrition. Bolivia and its international collaborators have and should continue to work with maternal and child health. This study's sample population was from one such intervention and the implications of the study illustrate the need of the community for nutritional outreach programs to continue.

For the second key finding in seroconversion, the implications are continued variabilities in vaccine performance. Although in this study, there was no significant relationship found between seroconversion and frequency of antibiotic prescribed, the seroconversion rate for the study population was 69%. 69% is relatively lower than the 80% vaccine efficacy average for Latin America (Linhares et al., 2008). Evidently, there are unidentified immunological interactions affecting this specific population. Public health implications for this may be to review RV vaccine schedules and identify trends in age and season. However, more research is needed to aid in increasing immunogenicity and identifying causal pathways. RV vaccine schedule is targeted at 2 months for the first dose due to early childhood exposure to RV. However, at this age, the infant's immune system is influenced greatly by maternal antibodies. Adding a third dose or waiting until some months later could aid in increasing seroconversion rates for children under 5 (Velasquez et al., 2017). This public health implication would need to

be population specific and a third dose in certain countries with low vaccine performance may benefit.

Another implication for this study is to research and identify other causal relationships affecting RV vaccine seroconversion rates. Antibiotic prescriptions were not significantly related to seroconversion however infant immunogenicity is still poorly understood. By investigating populations such as those in El Alto, Bolivia, investigators can facilitate the closing of health gaps in country and between countries. Specifically, the RV vaccine efficacy gaps that are still present between high and low income countries (Desselberger, 2017).

Recommendations from this study design highlighted Medical record keeping and antibiotic access. The difficulty of extracting clean data from Bolivian medical charts indicates that, along with the constitutional reconstruction to national (Bolivian) healthcare, the method of recording data for patients needs to also be reconstructed. By creating a standardized format, medical charts can be easily read and transferred across facilities for higher quality health services. Ease of access to antibiotics in countries like Bolivia pose a public health concern. Firstly, purchasing antibiotics at any point without a prescription and medicating themselves or their children may have long term health risks. In this study, it was difficult to ascertain antibiotic usage because medical charts solely illustrated prescribed antibiotics and relied on self-reporting from mothers for any other medications given to infant. Antibiotic names are complicated and the likelihood of accurately remembering names and dosage is low. Creating barriers such as requiring a prescription for antibiotics may be needed to mitigate the risk of antibiotic overuse.

Conclusion

Antibiotic interactions and health outcomes are still poorly understood. This study of 135 NIDI infants found significance between whether antibiotics were prescribed and stunting. This relationship can be attributed to either sickly infants that are prescribed antibiotics more often because they are susceptible to infections or the biological effects that extended exposure to antibiotics may have on the immature body system found in infants. Susceptibility to infections are highly correlated to malnutrition (Black et al., 2008). Without proper nutrition, normal growth cannot occur (Black et al., 2008). There was no significance found between whether antibiotics were prescribed and seroconversion. The rate of RV vaccine seroconversion for NIDI infants in this study was 69%, a marked 11% difference from the average seroconversion rates in Latin America (Linhares et al., 2008). Seroconversion is an ubiquitous method of measuring vaccine efficacy (Desselberger, 2017). More research is needed to identify specific mechanisms in the biological model as well as controlling for the many extraneous variables such as but not limited to clear antibiotic data.

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Appendix 1. *Chart Abstraction Tool*

Nombres y Apellidos		Fec. Nac. (DD/MM/YYYY)	/ /
No de Hist. Clínica		Sexo	□ M □ F
ABO tipo de sangre	□ O □ A □ B □ AB □ No se encontró	Factor Rh	□ + (Pos) □ — (Neg) □ No se encontró

Número de Visitas:

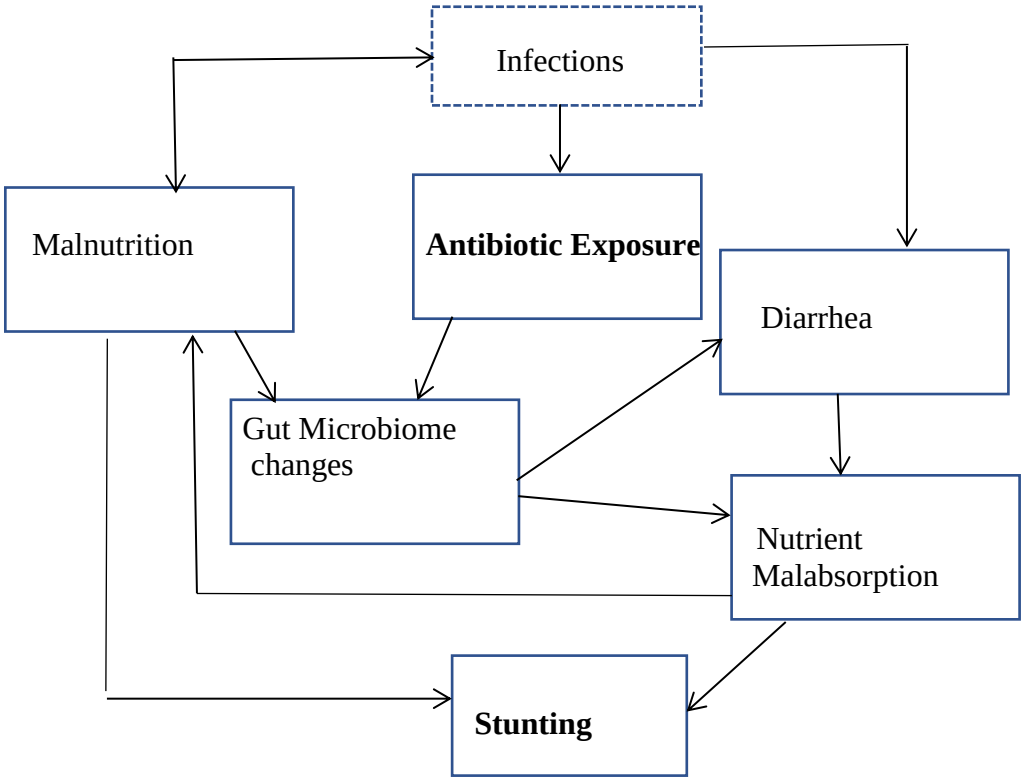
Controles	Enfermedades	Internaciones

Fecha de la primera visita_____ Fecha de la visita más reciente_____

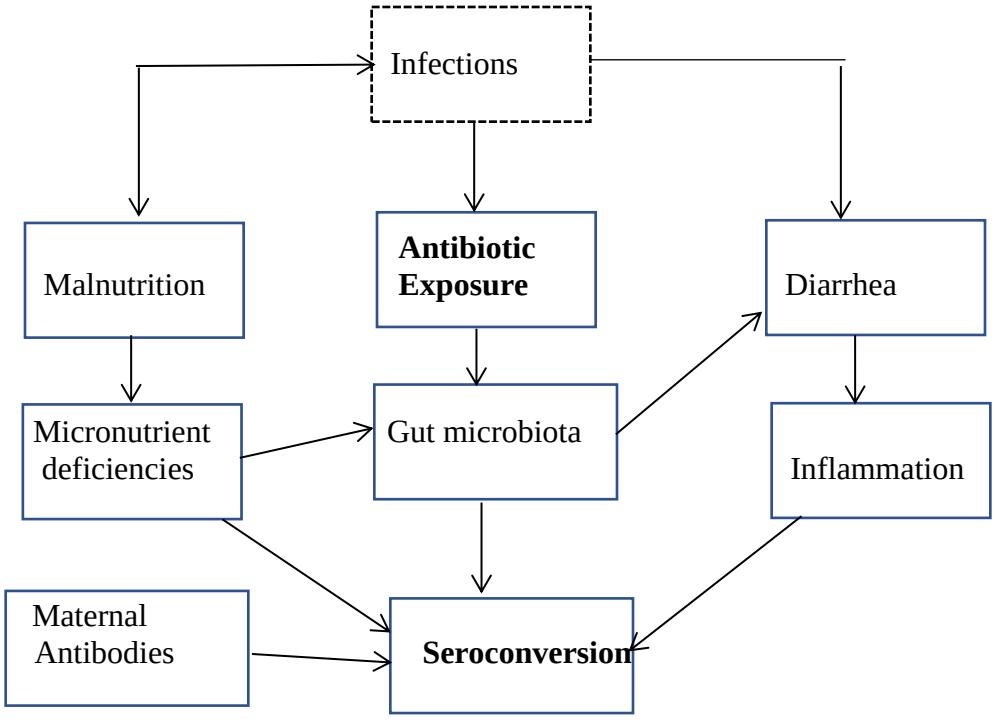
Visitas de Interés:

Fecha	**Temp**	**Dx**	**Rx**	**Lab**
		□ Diarrea □ Bajo Peso/Talla baja □ DNT Otro_____	□ Antibiótico_____ □ Chispitas □ Zinc □ Sales (ORS) □ Paracetamol □ Otro_____ □ Nada	Muestra de: _____ Resultado:
	Notas:			
		□ Diarrea □ Bajo Peso/Talla baja □DNT Otro_____	□ Antibiótico_____ □ Chispitas □ Zinc □ Sales (ORS) □ Paracetamol □ Otro_____ □ Nada	Muestra de: _____ Resultado:
	Notas:			
		□ Diarrea □ Bajo Peso/Talla baja □ DNT Otro_____	□ Antibiótico_____ □ Chispitas □ Zinc □ Sales (ORS) □ Paracetamol □ Otro_____ □ Nada	Muestra de: _____ Resultado:
	Notas:			
		□ Diarrea □ Bajo Peso/Talla baja □ DNT Otro_____	□ Antibiótico_____ □ Chispitas □ Zinc □ Sales (ORS) □ Paracetamol □ Otro_____ □ Nada	Muestra de: _____ Resultado:

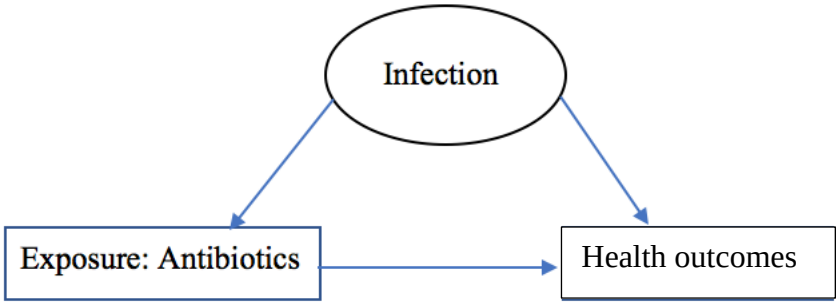
Appendix 2. Conceptual Framework for Stunting



Appendix 3. Conceptual Framework for Seroconversion



Appendix 4. Confounding Model
Confounder: Infection



Appendix 5. Data criterion method, Stunting
Assessing confounding: Stunting
% difference stunting dataset 1= (1.48-1.85)/1.85= -20%
% difference stunting dataset 2= (2.4-3.5)/3.5= -31%

Appendix 6. Data criterion method, Seroconversion
Assessing confounding: Seroconversion
% difference stunting dataset 1= (0.88-0.97)/0.97= -9%
% difference stunting dataset 2= (0.68-0.79)/0.79= -14%