Effect of Antimicrobial Exposure on *Clostridioides difficile (C. difficile)* Colonization of the Infant Gut Microbiota in the Canadian Healthy Infant Longitudinal Development (CHILD) Birth Cohort

by

Chinwe Vivien Obiakor

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Clinical Epidemiology

School of Public Health
University of Alberta

Abstract

Introduction: Antimicrobial exposure in early life has been associated with gut microbiota dysbiosis and development of allergic diseases in childhood. In adults and older children, *C. difficile* is the major pathogen responsible for antibiotic-induced diarrhea but the effect of colonization with this bacterium in infants is unclear. About 30% of infants are colonized with *C. difficile* without the presence of clinical symptoms, infection or diarrhea. However, colonization with this bacterium in infancy has been linked to development of asthma and allergic diseases later in life. The aim of this study was to determine the separate and cumulative impact from antibiotics and environmental antimicrobials (household cleaning products) on *C. difficile* colonization in infants.

Methods: This study population comprises of a representative sample of mothers and infants who were successfully enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. Infant antimicrobial exposure was obtained from hospital birth chart (maternal intrapartum antibiotic (IAP) and newborn intravenous antibiotic) and standardized questionnaires (infant oral antibiotic and household cleaning product use). Exposure to household cleaning products was based on frequency of use and split at median into lower and higher use. Fecal samples were collected at 3 and 12 months after home assessment. Analysis of *C. difficile* was performed using quantitative polymerase chain reaction (qPCR) with appropriate primers. Logistic regression analysis was used to determine the crude and adjusted association between antimicrobial exposure and *C. difficile* colonization.

Results: In this study, 44% of infants were indirectly exposed to antibiotics via maternal IAP, 8% directly received an oral or intravenous antibiotic and 47% lived in households with higher cleaning products use by 3 months. Infants were classified into 4 groups depending on

antimicrobial exposure: no antibiotics and lower cleaning products use (NALC), any antibiotics and lower cleaning products use (AALC), no antibiotics and higher cleaning products use (NAHC), any antibiotics and higher cleaning products use (AAHC). At 3 months, *C. difficile* colonization was significantly higher in AALC, NAHC and AAHC infants compared to NALC infants. After adjusting for covariates, the increased odds of *C. difficile* colonization remained significant in the AAHC infants (aOR 1.50 95% CI 1.03-2.17; p=0.032). At 12 months, *C. difficile* colonization was significantly higher in only the AALC and AAHC infants compared to NALC infants in both crude and adjusted analysis (aOR: 1.36 95% CI 1.02-1.83; p=0.035 for AALC and aOR: 1.37 95% CI 1.00-1.86; p=0.043 for AAHC)

Conclusion: Colonization with *C. difficile* as well as antimicrobial exposure in early infancy has been linked to increased risk of asthma and allergy. Our study suggests that cumulative exposure to antibiotics and higher household cleaning products use is not without consequence. Hence, the effect of antimicrobial exposure on the infant gut should be considered because colonization with *C. difficile* may be a marker for future health outcomes.

Preface

This thesis is an original work by Chinwe V. Obiakor. This thesis has been written in a paper format according to the guidelines of the Faculty of Graduate Studies and Research at the University of Alberta.

This thesis consists of an introduction with literature review (Chapter 1) followed by one study (Chapter 2) and a concluding chapter (Chapter 3). The statistical analysis and first draft of the manuscript in Chapter 2 are my own work.

Chapter 1 consists of a literature review on antimicrobial exposure from antibiotics and household cleaning products, infant gut microbiota and allergic diseases. This is followed by study objectives, sample size calculation and overview of study. Part of Section 1.2.1.1 and 1.2.3.2 was published as sections in a review paper "The association between early life antibiotic use and allergic disease in young children: recent insights and their implications" in "Expert Review of Clinical Immunology" (Obiakor et al. 2018;14(10):841–55. Available from: https://www.tandfonline.com/doi/full/10.1080/1744666X.2018.1521271). I carried out the database search for studies on infant antibiotic exposure and development of childhood allergic diseases and contributed to about 60% of the manuscript. Kozyrskyj AL. was the supervisory author. All authors contributed to the writing of the manuscript.

In Chapter 2, results of the main research questions are presented. In this chapter, the separate and cumulative effect of antimicrobial exposure on *C. difficile* colonization at 3 and 12 months were examined in the CHILD (Canadian Healthy Infant Longitudinal Development) birth cohort. A version of this chapter is in preparation for submission to an appropriate Journal as "Early Life Antimicrobial Exposure is Associated with *Clostridioides difficile* Colonization in Infants".

In the final chapter, Chapter 3, general discussion and conclusions are presented. This chapter highlights an assessment of bias, clinical significance of research findings, general strengths and limitations of the study and implications for future research.

Acknowledgements

I want to use this opportunity to appreciate Almighty God for providing me knowledge, strength and wisdom.

I owe the successful completion of my thesis to my supervisor, Dr. Anita Kozyrskyj for accepting and encouraging me throughout this journey. I am immensely grateful for her kind words, motherly advice and providing financial assistance. I would also like to appreciate and thank my committee members, Dr. Dean Eurich and Dr. Bonita Lee for sharing their knowledge, providing guidance and support during my master's program.

Many thanks to my lab mates – Brittany Matenchuk, Cara McLean, Kelsea Drall, Mon Tun, Sarah Bridgman, Jennifer Petrie, Liane Kang, Carmen Tessier, Khanh Vu, Aaron Van der Leek, Hein Tun and Bolin Chen – for their friendship and support. This project will not be possible without the participants and investigators of the CHILD (Canadian Healthy Infant Longitudinal Development) study who provided data and coordinated data collection respectively, thank you.

To my parents and siblings, thank you for your endless support and encouragement. My special thanks to my sister, Onyinye Omanukwue for providing feedback and support throughout my master's program.

Finally, I would like to thank my love and best friend, Mr. Yves Ibeme for his support from the beginning till the realization of this research project and throughout my graduate program.

Financial support was provided by The Lung Association, Alberta & NWT and Canadian Institutes for Health Research (CIHR).

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Background

It is interesting that something we never gave much thought to may play a role as important as other organs in our body. With the advance of modern sequencing techniques, our understanding of the importance of human microbiome has greatly increased and the perspective of viewing microbes as only pathogenic has shifted to recognize their significance (1). Previous culture-based methods for analyzing the microbiome were limited to only a subset of easily cultured species and limited our ability to identify and analyze many unculturable microbes (2). Similar to their ubiquitous nature in the environment, microbial populations thrive on various sites of the body such as the skin, vagina, oral cavity and gastrointestinal tract depending on conditions such as pH, oxygen level, nutrient availability, humidity and temperature (3). However, majority of the human microbiome is found in the gut and mostly comprises of several bacterial species. Under normal conditions, a symbiotic relationship is maintained whereby the organisms benefit nutrient and protection while aiding humans in digestion, metabolism and regulation of immune functions. To add complexity, we require these different microbes performing various functions in the right amount to benefit our body.

The "Hygiene Hypothesis" was first proposed in 1989 by David Strachan when he observed a negative association between having older siblings and the development of hay fever i.e. increased exposure to infections from older siblings helped in the development of a healthy immune system and provided a form of protection against allergy (4). More recently, the increasing rate of allergic diseases has been explained by the "Microbiota Disappearing Hypothesis" which suggests that various aspects of modern practices such as clean water, birthing process, pollution and antibiotic use have all contributed to the disturbance of members of the gut microbiota (5). The microbiota disappearing hypothesis places more emphasis on current practices and alteration of the balance of gut microbiota as the probable cause of immune dysregulation and development of allergic diseases. These practices affect exposures to the right microbes which in turn results to absence of the symbiotic relationship with our intestinal occupants and health benefits that we previously enjoyed. Our ability to properly characterize the gut microbiome and examine factors that affect its development may provide answers to several unanswered questions in epidemiological research.

1.2 Literature Review

1.2.1 Antimicrobial Exposure

1.2.1.1 Trends in Antibiotic Use from Birth to Childhood

Penicillin was the first antibiotic to be discovered in 1928 and remains effective against many bacterial infections caused by *Staphylococci* and *Streptococci* (6). In addition to penicillin antibiotics, cephalosporins and macrolides are commonly dispensed to children in modern medicine practice. Global antibiotic consumption has risen substantially (7), though, in North America, antibiotic use in infants below 1 year has significantly declined (8,9). Frenk et al. (9) reported a decrease in antibiotic use from 15.5% to 8.7% between 1999-2002 and 2011-12 mostly due to decline in commonly prescribed antibiotics, but the use of broad-spectrum antibiotics remains. Broad spectrum antibiotics are commonly administered to preterm neonates for the prevention and treatment of sepsis. Majority of preterm births occur due to intrauterine infections; hence intravenous antibiotics are used as a prophylactic measure to prevent morbidity or mortality (10).

In children, antibiotics are usually prescribed to treat respiratory tract infections such as otitis media (ear infection), laryngitis, sinusitis, pharyngitis and bronchiolitis even though some of these are of viral origin or self-limiting. Except in cases of penicillin allergy, clinical practice guidelines do not recommend broad spectrum macrolide and third-generation cephalosporin antibiotics as first-line treatment for common childhood respiratory and urinary tract infections (11,12). Due to increasing antimicrobial resistance and no apparent benefit, prophylactic antibiotic treatment of urinary tract infections in children is also not recommended (12). Despite these, Vaz et al. (13) found that respiratory tract infections accounted for more than 75% of outpatient antibiotic prescriptions for children, of which otitis media was the most common diagnosis, especially in children between 3 and 24 months of age. Regional differences in antibiotic treatment of hospitalized children are also of concern and may be a sign of emerging antimicrobial resistance. Versporten et al. (14) observed that broad-spectrum antibiotics, mainly third generation cephalosporins, were prescribed for children in Eastern Europe, Southern Europe, Asia, North America, and Latin America while narrow-spectrum antibiotics like benzylpenicillin, amoxicillin, and gentamycin were commonly administered in other regions. Antibiotic stewardship programs and numerous campaigns to improve adherence to prescribing guidelines have been implemented over the last few decades to promote treatment with narrow

spectrum antibiotics active against targeted pathogens. Despite their success, antibiotics remain overprescribed.

1.2.1.2 Maternal Intrapartum Antibiotic Prophylaxis

Maternal intrapartum antibiotic prophylaxis (IAP) is usually administered to pregnant women during labour or delivery to prevent early onset *Group B Streptococcus (GBS)* infection in newborns or to prevent wound infection from caesarean section (CS) in mothers (15). *Streptococcus agalactiae* or GBS, a gram-positive bacterium is a significant cause of neonatal sepsis and mortality worldwide (16). Up to 30% of women are colonized with GBS during pregnancy and can vertically transmit this bacterium to the newborn during vaginal delivery. However, most colonized newborns remain asymptomatic with less than 1% developing early onset GBS disease (17). Presence of two or more risk factors increases the likelihood of neonatal sepsis from GBS. In addition to maternal intrapartum GBS colonization during pregnancy, other risk factors include GBS bacteriuria at any time of the current pregnancy, having a previous infant with invasive GBS disease, prolonged (≥ 18h) rupture of membrane and maternal intrapartum fever (≥38ºC) (18). Worldwide disparity in IAP recommendation and administration exists. Some countries practice a risk-based approach based on clinical symptoms whereby IAP is administered during labour if women present with known risk factors, some countries practice a screening based approach whereby women are screened for GBS after a rectovaginal swab and given IAP during labour if positive, some countries implement both approaches while other countries have no recommendations regarding IAP (15).

In Canada, current guidelines recommend screening pregnant women at 35 to 37 weeks and administering IAP during labour to positive women or those with other risk factors (GBS bacteriuria or previous infant with GBS disease) (18). Penicillin G or ampicillin is the drug of choice while cefazolin is given to penicillin allergic women at low risk of anaphylaxis. Clindamycin, erythromycin or vancomycin may also be used in rare cases of risk of anaphylaxis to first line drugs. Screening approach for GBS remains controversial as about 30% of positive women screened at 35 to 37 weeks revert to negative before labor (17). More so, a large proportion of women colonized in labour have healthy neonates, so this approach increases antibiotic exposure and places a higher financial burden on the health system. On the other hand, IAP administration is based on the clinical risk assessment approach in some parts of Europe

which records lower antibiotic exposure. IAP exposure occurs in 13% of vaginal delivery in Denmark with risk assessment approach (19) while that of Canada is about 27% of vaginal deliveries (20). A 2014 Cochrane review of 3 randomized controlled trials (RCTs) found no conclusive evidence for the administration of IAP for GBS. Although ampicillin or penicillin for IAP reduced the occurrence of early onset GBS in the newborn, there were no differences in newborn mortality between treatment and no treatment groups (21).

Neonates are also routinely exposed to intrapartum antibiotics during caesarean section delivery. According to the Society of Obstetricians and Gynecologists of Canada (SOGC), pregnant women should receive cefazolin antibiotic before emergency or scheduled caesarean section (22). To achieve adequate tissue and serum level antibiotic concentration, cefazolin is usually administered prior to skin incision and cord clamping. While this may increase protection against infections in the mother, it also exposes the newborn to antibiotics. Antibiotic exposure to an infected neonate has its advantages but may pose some risks in healthy neonates. Early life antibiotics has been linked to amoxicillin-resistant late onset *Escherichia coli* infections during infancy (23), as well as gut microbiota dysbiosis which may affect future health outcomes (24–26). Some of these antibiotics can cross the placenta to the foetus before and during birth so the slightly increased benefits to the mother should be weighed against any potential risks of long-term harm to the newborn.

1.2.1.3 Household Cleaning Products

Discovery of the germ theory and disease transmission in the early 20th century promoted greater cleaning with soap and water to prevent infection. Standards to cleaning have evolved over the years and is linked to socio-cultural factors that influence human behaviour. To make the environment cleaner, emphasis has shifted to the increased use of cleaning products in the home (27). Like other common household items, cleaning products (hand soap, disinfectants, detergents, bleach, etc.) are sold in large quantities globally and are frequently used in the home (28). Disinfectants are active against a broad range of microorganisms while detergents are mainly used to remove dirt, dust, grease or fats. All-purpose cleaners, bathroom cleaners, glass cleaners, window and kitchen cleaners are some generalized names of products commonly used in household cleaning. Some of these products contain active chemical ingredients which can be inhaled and affect respiratory health (29,30).

Volatile organic compounds (VOCs) are low-molecular-weight organic chemicals found in air fresheners, insecticides, deodorizers and spray cleaners which are inhaled after the use of these products (31). In addition, some products contain synthetic compounds which leave toxic residues on the users, household surfaces or even kitchen utensils. Synthetic chemicals may mimic natural hormones like oestrogen and either activate or block oestrogen cell receptors causing significant harm (32). Most of these chemicals have not been properly tested in the real world while some are known pollutants, irritants and carcinogens (33). While it is important to wash away dirt, there is evidence showing potential harm in products designed for personal and home hygiene but many people are unaware of the extent of harm due to use of some chemicals in the products they use to clean themselves or their homes (34). Altman et al. (35) reported that women were unaware that the presence of industrial chemicals in their blood stream was linked to exposure to daily household cleaning products.

1.2.1.4 Antimicrobial Resistance

The persistent misuse and overuse of antibiotics are rendering them less effective as the targeted bacteria mutate and develop resistance to these drugs. The burden of increasing antibiotic or in general antimicrobial resistance (AMR) significantly impacts public health, global development and financial burden (36). Infections resulting from resistant organisms are harder to treat, more expensive to treat and prolong hospital length of stay. Of concern, widespread antibiotic overuse increases selective pressure for multi-drug resistant organisms. In developing countries, resistance to first line antibiotics for sepsis such as ampicillin and penicillin occurs in about 35% of infants while resistance to second line cephalosporins is increasing (37). Infants with sepsis from resistant organisms are at an increased risk of morbidity and mortality because the administered antibiotics may not be effective against the pathogen. Globally, approximately 214,000 deaths in neonates annually are due to resistant organisms (38). Antibiotic resistance genes are undesirable even in commensal organisms due to the potential to transfer these genes to pathogenic organisms. Yassour et al. (39) used whole genome sequencing (WGS) to identify specific genes in addition to antibiotic resistance genes following exposure to antibiotics in children aged 2 months to 3 years. They observed an increase in *Klebsiella pneumonia* beta-lactamase resistance genes after treatment with penicillin in one infant and the same trend for *Escherichia coli* (*E. coli*) resistance genes in another infant.

Organisms become resistant to the action of antimicrobials either through genetic mutation or acquisition of resistance genes contained within the chromosomal DNA and in rare cases plasmids or phages. The genetic factors are usually present for bacteria but acquisition peaks in the presence of selective pressure from antibiotics. The acquired resistance can then be transferred via horizontal gene transfer, direct cell-to-cell transfer (conjugation) or uptake of DNA released during cell lysis (transformation) (40). The resistance genes act by either altering the proteins targeted by antibiotics e.g. methicillin resistant *Staphylococcus aureus* (MRSA) or directly destroying the antibiotics e.g. penicillin resistance *E. coli* (41). Some bacteria can pump out antibiotics through the action of efflux pumps thereby preventing the required action on the body cells (42). Neonates admitted to the neonatal intensive care unit (NICU) have a great risk of acquiring resistant organisms from the hospital environment. Transmission from health-care workers, visitors, hospital surfaces and even medical devices have been reported (43). In some cases, resistance genes are acquired from the mother during labour, delivery or breastfeeding. Of note, some infants harbor antibiotic resistance even without direct antibiotic exposure.

Infants tend to have more similar antibiotic resistant genes (ARGs) with their mothers so maternal use of antibiotics which create room for resistance genes transfer should be considered. Paradoxically, the cessation of breastfeeding before 6 months has been associated with increased likelihood of ARG in infant gut microbiome (44). One explanation is that breastmilk contains sugars digestible by specific bacteria like *Bifidobacterium* that are less likely to carry ARGs and reduces abundance of Enterobacteriaceae prone to ARG carriage. To support recommendations by world health organization (WHO) (45), the benefits of exclusive breastfeeding on infant health also include reducing ARGs that can make infections from bacteria carrying these genes harder to treat. The high prevalence of antimicrobial exposure from antibiotics and cleaning products as well as the burden of antimicrobial resistance, coupled with our increasing understanding of the microbiome role in human health makes it important to understand short- and long-term effects of repeated exposure on the infant gut microbiota.

1.2.2 The Human Gut Microbiome/Microbiota

The human microbiome is the suite of microorganisms and their genes that reside on and within the human body (2). The complex and dynamic gut microbiome is the most researched and harbors trillions of mostly bacteria species and their genes which exceed our human genes

more than 100 times (46). The most common and abundant phyla which are from the prokaryotic domain include Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Verrucomicrobia. Two other domains have been identified; archaea (mostly *Methanospera* genus) and eukaryote (mostly *Saccharomyces* genus or fungi) (47). The gut or intestinal microbiota is involved in the regulation of several host metabolic pathways, enabling an interactive host-microbiota metabolic, signaling, and immune-inflammatory axes working to physiologically connect the gut, liver, muscle, and brain (48). The gut microbiota also assists in digestion – by breaking down food substances to release nutrients to the host – and provides colonization resistance to prevent invasion by pathogens. In the absence of disturbances from external factors, beneficial bacteria remain abundant, occupy most of the microbiological niches, compete for nutrients and keep pathogens at bay. For example, lactic acid producing bacteria and *Bifidobacteria* decrease the pH of the large intestine creating an unfavorable environment for common pathogens (49). Until recently, the gut microbiota and resistome (collection of antibiotic resistance genes encoded in a microbial community) was largely characterized by culture or PCR-based experiments (2,50). The use of modern sequencing techniques has led to a greater understanding of the gut microbiota and an increasing appreciation of the relationship between gut microbiota and health.

In adults, the gut microbiota is usually stable and exists in harmony with its host, but the developing infant gut microbiota can be affected by several factors surrounding birth and may not easily overcome disturbance from external factors. Hence, restoration of the gut microbiota in adults especially after antibiotic exposure is common (51). In infants, the microbiome is delicate and unstable so any disturbances that affect gut microbes may lead to partial recovery or the establishment of microbes that don't maintain the ideal environment (52). The early establishment of the gut microbiota also helps in immune stimulation, so disruptions to the less resilient infant microbiota from external factors may have implications on future health outcomes. Exposure to antibiotics, caesarean section delivery, formula feeding, and hospitalization are some factors that affect or delay colonization of the infant gut microbiota (52,53). Delayed colonization of the infant gut microbiota with the ideal microbes and alterations interfere with the development of "immunologic tolerance". When this happens, the immunosuppressive mechanism by regulatory T cells (Tregs) is disrupted and failure to balance T helper cells (Th1, Th2 and Th17) predisposes an infant to immune or inflammatory diseases (54). The importance of the gut microbiota has also been studied using mouse models which

have shown that germ free or antibiotic treated mice possess varying health outcomes or behaviour compared to their wild-type mice counterparts (55). Cox et al. (56) demonstrated that low dose penicillin given to mice pups led to gut microbiota alterations and permanent abnormalities in metabolism and immunity. Murine model experiments have also shown dysregulated antibody response to common human infant vaccines following early life exposure to antibiotics (57).

1.2.2.1 Succession of the Gut Microbiota in Early Life

Early life colonization of the infant gut is influenced by several environmental and host factors (58). The developing infant gut microbiota has been identified as a "critical window" of development which is constantly changing till about 2-3 years when the microbiota starts resembling that of an adult. Before attaining an adult like composition by the age of 3, the infant gut microbiota can be classified into 3 phases; developmental phase (3 to 14 months), transitional phase (15 to 30 months) and stable phase (31 to 46 months) (59). Colonization of the infant gut begins with facultative anaerobes and aerotolerant microbes like *Enterobacteria* from the Proteobacteria phylum followed by strict or obligate anaerobes like *Bifidobacteria* and *Bacteroides* (58,60). It is suggested that the first colonizers gradually consume the oxygen in the gut thereby reducing the oxidation-reduction potential which permits the growth and dominance of strict anaerobes. The initial inoculation of the infant gut microbiota comprises of mostly commensal organisms that colonize and establish a stable niche, a period which coincides with the onset of breastfeeding (53). Breastmilk contains human milk oligosaccharides (complex sugars) which serve as an energy source mostly utilized by *Bifidobacteria* and few members from the Firmicutes and Bacteroidetes phyla. The ideal growth trajectory is normally observed in full-term vaginally delivered exclusively breastfed babies but exposures or interventions such as antibiotics, formula feeding and caesarean delivery tend to disturb this process (52,60).

The natural acquisition of gut microbes is first compromised by caesarean delivery which bypasses exposure to microbes in the birth canal and/or maternal antibiotic exposure (61,62). The next important determinant of infant gut microbiota is diet. In the first 4 months of life, formula fed infants tend to have a greater diversity albeit lower species richness than exclusively breast-fed infants because the sugars present in formula can be utilized by more microbes (63). The cessation of breastfeeding and transition to solids coincides with an increase in alpha

diversity of the infant gut microbiota (64). The complementary introduction of solids provides different food substrates and nutrients which also promotes increase in Firmicutes and Bacteroidetes with a concurrent decrease in Proteobacteria and Actinobacteria similar to that of the adult microbiota (61). In a US based cohort of 43 infants, the gut microbiota from the first month of life was dominated by facultative anaerobes from the Enterobacteriaceae family followed by strict anaerobes such as *Bifidobacterium, Bacteroides and Clostridium*. With the onset of solid foods at 6 months, more diverse species from the Clostridiales family began to be observed, with the microbiota finally stabilizing at about 2 to 3 years (60). Other studies have also reported high levels of Enterobacteriaceae in the first days of life followed by *Bifidobacteria, Collinsella* or *Bacteroides* by 6 months of age (65). Characterization of stool samples of older infants aged 9 to 18 months revealed more diverse microbiota and increase in the relative abundance of families in Firmicutes such as Lachnospiraceae, Ruminococcaceae, Eubacteriaceae, Rikenellaceae and decrease in Actinobacteria (Actinomycetaceae and Bifidobacteriaceae), Enterobacteriaceae, Lactobacillaceae, Enterococcaceae and Carnobacteriaceae (64). Generally, the most dominant phyla observed in the colonization of the infant gut microbiota are Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria.

1.2.2.2 *Clostridioides difficile* Colonization of the Infant Gut

Clostridiodes difficile (*C. difficile*) formerly known as *Clostridium difficile* is a gram-positive spore forming bacteria which has toxigenic and non-toxigenic strains. Acquisition of *C. difficile* usually occurs in the hospital environment but is now common in the community. The rate of *C. difficile* colonization ranges from 25 to 30% in neonates below 1 month, 10 to 25% in infants 1 to 12 months and approaches 5% in children above 1 year, similar to that observed in adults (66). The spontaneous eradication after infancy is due to competition with other microbes that are better adapted to the gut environment and can out compete *C. difficile*. Davis et al. (67) observed an increase in diversity, Bacteroidetes and Firmicutes in fecal samples collected from 5.5 to 17 months; a period that coincides with weaning and introduction to solids suggesting the adult like microbiota was able to suppress *C. difficile*.

In adults and older children, *C. difficile* is the most common cause of antibiotic-induced diarrhea and its infection can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon. In addition, it can progress from asymptomatic colonization to

infection when favourable conditions are present. These conditions include disturbance of intestinal commensals by external factors, initial colonization with toxigenic *C. difficile*, and release of toxins A or B from the toxigenic strains (66). Colonization in infants below 2 years is usually asymptomatic and not well understood. It has been suggested that many infants do not manifest clinical symptoms from *C. difficile* for several reasons: immature or lack of toxin receptors to bind and activate toxins, protective mechanisms from breastmilk such as toxin neutralizing antibodies, and colonization resistance from an intact gut microbiota against *C. difficile* colonization (68). Although infants do not present with symptoms, colonization in early life has been associated with development of asthma and atopic diseases in childhood (69).

Evidence on maternal to newborn transmission of *C. difficile* is limited. *C. difficile* colonization is low in adults and even lower in pregnant women (70). One study reported a colonization rate of 89% in newborns whose mothers were vaginally colonized compared to 56% in newborns whose mothers were not colonized (71). The risk of fecal-oral transmission of *C. difficile* during vaginal delivery is also low. Similarly, only one study has reported evidence of fecal-oral transmission (72). These findings have not been replicated suggesting that vertical transmission of *C. difficile* during vaginal delivery is unlikely. The most probable route of *C. difficile* acquisition in neonates is from the home or hospital environment (52) since rectal or vaginal swabs from pregnant women rarely confirm presence of this microbe (73). Considering this, inoculation with the ideal microbes during delivery is important because they educate the immune system and provide colonization resistance against *C. difficile* and other pathogens. Compared to vaginally born infants not exposed to maternal IAP, those born to mothers administered IAP are not exposed to some beneficial vaginal or fecal microbes that have been depleted due to the antibiotics used (74).

Antimicrobial exposure can diminish competing gut commensals and provide room for *C. difficile* colonization, overgrowth and toxin production. *Lactobacilli* and *Bifidobacteria* have been reported to work in reverse to *C. difficile* by reducing the tendency for developing antibiotic-induced diarrhea; which is one reason they are considered probiotic (75,76). There is limited evidence regarding implications of antibiotic or antimicrobial exposure and *C. difficile* in infants. Higher abundance of *Clostridium* and its family Peptostreptococcaceae have been reported in newborns given intravenous (IV) antibiotics (77). Similarly, Azad et al. (62) reported

a higher abundance of *Clostridium* in vaginally delivered and emergency caesarean delivered infants exposed to maternal IAP at 3 months. A pilot study of 74 full term infants also reported increased abundance of *Clostridium* in infants exposed to maternal IAP which was influenced by duration of IAP administration (78). Very recently, Tapiainen et al. (24) reported differences in gut microbiota from perinatal antibiotics which were still evident at 6 months. The relative abundance of *Clostridium* increased while that of *Bacteroides* decreased in infants exposed to direct IV antibiotics, maternal IAP or both compared to infants with no antibiotic exposure. Hence, altered transmission of beneficial microbes that are depleted due to maternal IAP may predispose an infant to *C. difficile* colonization.

1.2.3 Antimicrobial Exposure, Gut Microbiota and Allergic Diseases

Antimicrobials alter the developmental trajectory of the infant gut microbiota. In general, antibiotics especially broad spectrum affects both commensal and pathogenic bacteria. This lack of specificity also means disruption of the balance of intestinal microbes, eradication and unintended loss of keystone taxa for maintaining homeostasis, loss of diversity and blooms of pathogens (25). Hence, we need to start weighing the benefits against the risks from antibiotic therapy. Asthma is the most common chronic respiratory disease in young children worldwide (79). In 2016, 10% of Canadian children and youth were living with asthma (80) with over 6,000 hospitalizations occurring between 2015 and 2016 (81). Atopic dermatitis, the most common inflammatory skin disease and food allergy which is on the rise also pose significant clinical and economic burden (82,83). Early life antibiotic exposure has been associated with infant gut microbiota dysbiosis and increased risk of childhood allergic diseases such as asthma, atopy, eczema, food sensitization and allergic rhinitis (26,84). Frequent use of environmental antimicrobials (household cleaning products and hypochlorite bleach) may lead to air pollution and inhalation of harmful particles associated with persistent wheeze, asthma, allergic rhinitis and respiratory symptoms in young children (30). Maternal asthma, air pollution and exposure to cigarette smoke are risk factors that have been associated with childhood asthma (85), but it is now evident that gut microbiota dysbiosis may be related to asthma development (86). In sum, reviews of epidemiological studies have suggested that early life colonization of the infant gut is associated with development of allergic diseases in childhood and gut microbiota dysbiosis may pave way for allergic diseases, antimicrobial resistant organisms and immunologic diseases (25,86).

1.2.3.1 Epidemiological Studies on Antimicrobial Exposure and Infant Gut Microbiota

Randomized controlled trials (RCTs) "the gold standard of epidemiological studies" have reported on the association between oral antibiotics and infant gut microbiota. A single dose of oral azithromycin in children who had never received antibiotics was shown to produce significant changes in the gut microbiota of the treatment group, although, this effect was not long lasting. Overall, azithromycin led to a decrease in diversity and depletion of more bacterial genera; which allowed the growth of more gram-positive anaerobes five days after treatment (87). Another RCT in 6 to 11-month-old Indian infants reported a modest effect from a 3-day course of oral azithromycin on gut microbiota composition. Fecal samples analyzed on day 14 post treatment showed a decrease in the relative abundance of Proteobacteria phylum (mostly *Escherichia coli*) and *Akkermansia mucinophila* the most abundant member of the Verrucomicrobia phylum (88). In addition, a double-blind placebo controlled RCT with oral azithromycin for 3 days found only differences in *Bifidobacterium* abundance between treatment and placebo groups which did not have long-lasting effects. Azithromycin produced obvious effects on the gut microbiota 14 days post randomization which were no longer evident at 4 years of age (89).

Several cohort studies have also reported on the association between antibiotics and infant gut microbiota. Fouhy and colleagues were the first to use high-throughput sequencing techniques and showed that intravenous ampicillin and gentamicin directly administered to newborn led to gut microbiota dysbiosis in the first weeks of life. Newborns who received antibiotics had higher proportions of Proteobacteria (mostly genera from Enterobacteriaceae) and lower proportions of *Bifidobacterium* and *Lactobacillus* at 4 weeks compared to those not given antibiotics. At 8 weeks, *Bifidobacterium* and *Lactobacillus* had recovered but Proteobacteria and *Clostridium* genus still remained higher in the antibiotic treated newborns (77). In a Japanese cohort, broad spectrum cephalosporins administered to newborns at risk of infection influenced the initial development of the gastrointestinal tract microbes. The results showed that diversity and *Bifidobacterium* was lower in antibiotic treated newborns at 3 days of age but returned to similar levels as the non-antibiotic newborns by 1 month whereas the levels of *Enterococcus* remained significantly higher in the antibiotic treated newborns up to 1 month of age (90). Another study showed that intravenous amoxicillin/ceftazidime in 15 preterm infants affected the gut microbiota composition depending on the duration of antibiotic exposure. Both short and

long duration were associated with decrease in *Bifidobacterium* up to 3 weeks while long duration produced additional effects up to 6 weeks. Members of Enterobacteriaceae family were sensitive to antibiotic treatment but *Enterococcus* remained abundant until 2 weeks of cessation of antibiotic therapy (10).

Maternal antibiotics may also influence the infant gut microbiota through prenatal exposure during pregnancy, perinatal exposure during labour or postnatal exposure during breastfeeding. Antibiotics administered especially before or during labour affect non-target bacterial populations and alter the mother's microbiota thereby preventing transfer of the ideal inoculum at birth (78). In a sample of 198 newborns, the gut microbiota profile of those whose mothers received IAP showed reduction in Bacteroidetes with greater abundance of *Clostridium* and *Enterococcus*; characteristics which have been associated with gut dysbiosis and development of atopic disease in early childhood (62). The authors also reported reduced microbiota richness at 3 months in vaginally delivered infants with IAP exposure compared to vaginally delivered infants without IAP exposure. At 1 year, only infants delivered via emergency CS with IAP exposure still had reduced abundance of Bacteroidetes as well as increased abundance of Proteobacteria and Firmicutes compared to vaginally delivered infants with no IAP exposure (62). On the other hand, Mazzola et al.(91) reported significant differences between IAP exposed group and control group at 1 week but not at 1 month of age. More so, these differences were only observed in breastfed infants where the IAP group had lower abundance of Actinobacteria (*Bifidobacterium*) and higher abundance of Proteobacteria (*Escherichia*). Using a metagenomic approach, Aloisio et al. (92) noted that a decease in microbial diversity, relative abundance of Actinobacteria (*Bifidobacterium*) and Bacteroidetes in addition to an increase in the relative abundance of Proteobacteria (Enterobacteriaceae family) occurred in newborns of mothers who had received IAP. Members of Proteobacteria phylum are resistant to many antibiotics and with the depletion of sensitive microbes they can occupy the open ecological niche. In a prospective birth cohort of 74 infants, those born to mothers given IAP during labor or delivery had a different microbial profile indicated by a delayed colonization with *Bifidobacterium* and persistence of *Escherichia* in comparison to infants whose mothers did not receive IAP. At the phylum level, IAP exposure led to a delay in the colonization by Actinobacteria, Firmicutes and prolonged persistence of Proteobacteria. In fact, longer duration

of IAP exposure had more impact on gut microbiota composition but did not seem to last past 12 weeks of age in most infants (78).

Research focusing on the association between household cleaning products and the infant gut microbiota is limited. Depending on their mechanism of action, cleaning products can be classified into various groups although some of these overlaps (93). In animal models, piglets exposed to continuous aerosols from disinfectants in their pen have been shown to have disturbed gut microbiota composition compared to unexposed piglets (94). Tun et al. (95) reported that frequent use of disinfectants in a cohort of 757 infants was associated with higher abundance of Lachnospiraceae and lower abundance of *Haemophilus* while frequent use of eco-friendly products was associated with lower abundance of Enterobacteriaceae. Increasing exposure to cleaning products and the harmful chemicals they contain places significant impact on health and is an emerging public health concern. The production of disinfectants and detergents usually involves vigorous technological manipulations giving rise to chemical products which may not be best suited for indoor use. The effect of these chemicals are even more dangerous to the developing infant that spend most time indoors (96). Many cleaning products commonly used in the home also contain antimicrobial agents which is concerning because these products may contribute to the burden of increasing antimicrobial resistance (97).

1.2.3.2 Epidemiological Studies on Antimicrobial Exposure and Allergic Diseases

There is ample evidence linking early life antibiotic exposure to the development of asthma and other allergic diseases. The first meta-analysis to support this association was published in 2006, where infant antibiotic exposure gave an overall pooled odds ratio (OR) of 2.05 (95% CI 1.41-2.99) with subsequent childhood asthma (98). Two systematic reviews published in 2011 also reported similar results. Murk et al. (99) found that infant antibiotic exposure increased the risk of childhood asthma by 52% while Penders et al. (26) reported an increased risk of 27% for asthma or wheeze. With respect to atopic dermatitis, Tsakok et al.'s (100) systematic review showed an increased risk of 41% with infant antibiotic exposure in the meta-analysis of 17 studies published from 1998 to 2010. Two studies from a Swedish birth cohort reported different results based on asthma phenotype. Perinatal antibiotics increased the risk of atopic asthma at 8 years (OR: 3.00 95% CI 1.50-6.00) and 12 years (OR: 2.22 95% CI 1.20-4.20). However, this association was not observed for non-atopic asthma (asthma without

allergic sensitization) (101,102). For food allergy, Batool et al. (103) reported that postnatal antibiotics was associated with a two-fold likelihood of this allergic condition. In addition, Mitre et al. (104) analyzed the health-care database records of almost 800,000 children and found associations between postnatal antibiotics and increased risk of several allergic conditions ranging from asthma, food allergy, anaphylaxis, atopic dermatitis, and medication allergy. Timing or duration of antibiotic exposure may also contribute to increased risk. Newborns whose mothers were exposed to intrapartum antibiotics were found to have an increased risk of atopic dermatitis only when the exposure lasted more than 24 hours (105). For more detailed review of early life antibiotic exposure and childhood allergic diseases, refer to section 2 in the Obiakor et al. (106) publication.

There is increasing evidence associating cleaning products with increasing asthma prevalence in developed countries (107,108). A large population study showed an increased risk of new-onset asthma in domestic and professional cleaners which was associated with chemical ingredients such as chlorine bleach and other disinfectants (107). In their systematic review, Vincent et al. (108) assessed the association between exposure to occupational and domestic cleaning products and development of asthma. They found a positive association between the use of cleaning products and asthma but no report on the implicating ingredients. In a cohort of 400 children, prenatal use of cleaning and scented products was associated with asthma prone symptoms like nocturnal cough but not current asthma (109). Frequent use of household cleaning products has also been associated with an increased risk of rhinitis in primary school children. In a Chinese cohort, increasing use of cleaning products was associated with a 21% increased odds of occasional rhinitis and 36% increased odds of frequent rhinitis in childhood (30). The underlying mechanism for this association is unknown but is thought to occur via contact or inhalation of chemical ingredients (e.g. propylene glycol and glycol ethers, alkyl phenol ethoxylates, volatile organic compounds, ethylene diamine acetic acid and nitrilotriacetic acid) contained in the cleaning products.

1.2.3.3 Epidemiological Studies on Gut Microbiota and Allergic Diseases

Gut microbiota composition was first linked to allergic diseases when infants from Sweden (high prevalence of allergy) and Estonia (low prevalence of allergy) were compared. It was observed that Swedish infants were more likely to be colonized by *C. difficile* whereas

Estonian infants were more likely to be colonized by *Lactobacillus* and *Eubacteria* (110). In Swedish infants, a lower diversity and abundance of *Bacteroides* at 1 month of age was also associated with atopic eczema at 2 years (111). More so, investigators from the KOALA birth cohort reported that higher levels of *C. difficile* was associated with the development of atopy. Fecal samples collected from 957 infants when they were 1 month were analyzed using quantitative real time PCR to identify gut microbes associated with atopic manifestations such as eczema and wheeze at 2 years of age. Infants colonized with *C. difficile* had a 40%, 75% and 54% increased odds of developing eczema, recurrent wheeze and allergic manifestations respectively; implicating *C. difficile* as a potential biomarker for atopic diseases (112). The same authors reported an increased risk of eczema, allergy, food sensitization and atopy by age 6 and 7. In their study, colonization with *C. difficile* at 1 month increased the risk of asthma more than 2 folds (OR 2.06; 95% CI 1.16 to 3.64) at 6 to 7 years of age (69).

The CHILD study has also found that early patterns of gastrointestinal colonization disturbances predispose a child to higher risk of allergy development i.e. children at high risk of developing asthma possess reduced abundance of important microbes which may protect against asthma (113). Using next-generation illumina sequencing to profile the gut of 166 infants, CHILD authors found a higher ratio of Enterobacteriaceae/Bacteroidaceae at 3 months of age to predict food sensitization at 1 year (114). In a nested case-control study, asthmatic children diagnosed by 4 years compared with healthy controls had different microbial phenotype in their gut microbiota samples collected when they were 3 months. The gut microbiota was characterised by decreased abundance of *Lachnospira* and increased abundance of *Clostridium* specie in the asthmatic children (115). Similarly, Lee et al. (116) reported that *C. difficile* colonization or infection was associated with later childhood development of allergic diseases in an Asian cohort. The loss of diversity and depletion of beneficial bacteria following exposure to gut microbiota disturbing factors can promote overgrowth of pathogenic bacteria like *C. difficile* and interfere with development of immunologic tolerance. With the current evidence, it is difficult to establish a cause relation between specific bacterial taxa and development of allergic diseases due to heterogeneity in study design, different sampling points, methods used to characterize gut microbiota, inter-individual variability and clinical practices in diagnosing atopic and allergic conditions.

1.2.4 Other Factors Affecting the Infant Gut Microbiota

1.2.4.1 Birth Method

Birth method is an important determining factor of gut microbiota acquisition and vaginal transfer of microbes from the mother to newborn is crucial in microbial succession. During pregnancy, the vaginal microbiota undergoes changes with other body processes in preparation for the birth of the newborn. The vaginal tract becomes increasingly colonized with *Lactobacillus* and members from the order of Clostridiales, Bacteroidales and Actinomycetales but decreases in diversity and richness (117). Vaginally born infants are first colonized with microbes similar to those found in the vaginal tract such as *Lactobacillus* and *Prevotella* whereas caesarean section (CS) delivered infants are first colonized with microbes resembling those found in the skin such as *Staphylococcus* and *Corynebacterium* (118). The fecal microbiota from vaginally born infants start to resemble their mothers as early as at 3 days old but such similarities is not observed in CS born infants. In fact, several studies have reported absence or delayed colonization of *Bacteroides* as a signature of caesarean birth (60,61,119,120). Infants delivered via CS also show a reduced complexity and diversity of gut microbiota and are initially less colonized with health promoting bacteria like *Bifidobacterium*. More so, CS delivered infants have been observed to be more frequently colonized by members of *Clostridium* sensu stricto (cluster I) and *C. difficile* (52,118,119). Bokulich et al. (60) reported notable differences between caesarean and vaginally born infants characterized by depletion of *Bacteroides* and enrichment of Clostridiales and Enterobacteriaceae in CS delivered infants. Backhed et al. (61) also found less colonization by *Bifidobacterium* and *Bacteroides* in caesarean delivered infants and more colonization by members of the gut species resembling that of an adult microbiota. Epidemiological studies have also implicated CS delivery as a contributing factor to future health outcomes. Using mediation analysis, Van Nimwegen et al. (69) showed that long term effects of CS included development of childhood asthma and was mediated by *C. difficile* colonization in early life. The different gut microbiota composition between caesarean and vaginally delivered babies as well as potential health impacts emphasizes the influence of birthing practices and protective effect of natural birth.

1.2.4.2 Breastfeeding

Breastmilk is another important contributor to the development of the infant gut microbiota. Breastmilk is a vital part of nutrition and its absence in an infant's diet may have

implications on future health outcomes (121). According to WHO, breastmilk should be given exclusively for the first 6 months after which it should be used supplement other food in the first 2 years of life (45). Breastfeeding not only protects the neonate/infant against infections but may also protect against breast cancer in the nursing mother (121). More so, it contributes to the positive development of the infant gut microbiota by providing human milk oligosaccharides (HMOs) which are easily utilized by the earlier gut colonizers and helps to maintain balance of gut microbes (53). Oligosaccharides are among the most abundant constituents of human milk after lactose and lipids. Human milk also consists of antimicrobial entities such as secretory immunoglobulin A (SIgA) which provides passive immunity from the mother to infant to fight against certain pathogens the mother has encountered as well as lactoferrin and lysozyme (122). Lactoferrin and lysozyme are glycosylated proteins that act as first line of defence and protect against pathogen invasion or infection of the gut microbiota.

Human milk was previously thought to be sterile but is now suggested to comprise its own microbiome in addition to protein, lipids and oligosaccharides necessary for the nourishment of the suckling infant (122). Characterization of human milk colostrum (first milk produced) revealed *Weisella, Leuconostoc, Staphylococcus, Streptococcus, and Lactococcus*, while breast milk samples collected within the first months showed *Veillonella, Leptotrichia, and Prevotella* as the dominant taxa. Several epidemiological studies have reported that breastmilk plays a vital role in the developing neonatal/infant gut microbiota. In comparison to exclusively formula fed or mixed fed infants, exclusively breastfed infants have a gut microbiota with lower diversity because very few microbes can utilize HMOs present in breastmilk (63). Formula contains a mix of carbohydrates and micronutrients allowing colonization with more microorganisms like *Enterococcus, Clostridium, Enterobacteria* and *Atopobium* cluster which are not necessarily beneficial in early life (123). In addition, formula feeding may allow for increased *C. difficile, E. coli and Bacteroides fragilis* colonization in comparison with exclusive breastfeeding as reported in 1-month old infants who had never received antibiotics (52). *Bifidobacterium* has been consistently reported to dominate the gut of breastfed infants and is considered a beneficial microbe that protects against pathogen colonization (53,62). Species in *Bifidobacteria* (some better than others) can utilize and ferment HMOs to produce lactate and acetate thereby increasing the acidity of the intestine and inhibiting growth of pathogens that find the environment unsuitable (124). As *Bifidobacteria* benefits and utilizes HMOs, it in turn

produces substrates that can be utilized by other microbes such as *Lactobacillus rhamnosus* (*L. rhamnosus*) through cross-feeding (125). At the species level, higher levels of *Bifidobacterium bifidum, Bifidobacterium breve*, *L. rhamnosus* and lower levels of *E. coli*, *Ruminococcus torques* and *Roseburia intestinalis* have been reported in breastfed infants (59). Barely a week after cessation of breastmilk, Davis et al. (67) observed an increased alpha diversity, fecal pH, relative abundance of *Bacteroides*, *Blautia* and *Ruminococcus* with a decrease in the relative abundance of *Bifidobacterium*, *Lactobacillus* and *Enterobacteria.* This implies that the maturation of the gut microbiota may be driven by absence of breastmilk rather than introduction to other foods.

1.2.4.3 Gestational Age at Birth

The establishment of the infant gut microbiota is also influenced by gestational age at birth. Preterm infants (less than 37 weeks of gestation) have a different gut microbiota composition, delayed colonization of *Bifidobacteria* and higher abundance of *Clostridia* compared to term infants (38 to 42 weeks of gestation) (126). More so, preterm infants are faced with other health challenges related to immune, respiratory and neurological functions. They are generally more likely to spend longer time in the hospital environment, be put on artificial respiration and continually exposed to antibiotics to prevent neonatal sepsis (127). Prematurity affects the gut microbiota composition which is further influenced by perinatal antibiotic use. Hence, it is difficult to determine if the different gut microbiota composition observed in this population is from being premature and/or the combination of above factors related to prematurity. In addition to delayed colonization with *Bifidobacteria*, preterm neonates have been observed to have decreased colonization with *Bacteroides* and increased colonization with Enterobacteriaceae, *Enterococcus* and other pathogenic microbes compared to full-term neonates (128,129). In their study of 36 preterm exclusively formula fed babies, Zhu et al. (127) noted that Proteobacteria and Firmicutes were the most abundant phylum at 1 week. *Enterococcus* and *Klebsiella* dominated the microbiota of infants administered piperacillin-tazobactam while *Streptococcus* and *Klebsiella* were most abundant in the penicillin-moxalactam group. Bacteroides; though generally low and Actinobacteria were also more abundant in both antibiotic groups compared to the antibiotic free group (127). Prematurity which is strongly correlated with hospitalization also results in higher rates of *C. difficile* colonization (52). Colonization with *C. difficile* was reported to be highest among premature infants, 64% of whom were born before

36 weeks. This is not surprising as hospital environment is one of the most common places to acquire *C. difficile* and other antibiotic resistant organisms (73). The factors associated with prematurity as well as gut microbiota disturbances makes the premature gut more unstable than that of full-term infants. This may be associated with a delay in transitioning and establishment of an adult-like microbiota and further affect future health outcomes (53).

1.2.4.4 Hospitalization and Home Environment

Nosocomial spread of microbes and pathogens is prevalent in the neonatal intensive care unit (NICU). It is not uncommon to isolate pathogenic bacteria and resistant bacteria (e.g. methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas*, *Clostridium*) from hospital equipment or even hands and clothing of hospital staff (130). Approximately 3% of the general population are colonized with the opportunistic pathogen, *Pseudomonas aeruginosa* compared to 20% in hospitalized patients (131). In Canada, most hospital acquired infections (HAI) are due to MRSA or *C. difficile* infection. Many populations of *C. difficile* increase in NICU environment and may increase the risk of other intractable infections. Infants born prematurely or via CS are even at greater risk because they often stay longer in the hospital and receive more antibiotics that can select for resistant organisms. Kato et al. (132) reported that 61% of infants in the NICU were colonized with *C. difficile* with almost all isolates from the 30 patients being identical and non-toxigenic. Penders et al. (52) showed that infants born via caesarean section in the hospital were more likely to be colonized with *C. difficile* and less likely to be colonized with *Bifidobacterium* and *Bacteroides fragilis* compared to infants vaginally delivered at home. They also showed that infants born at home were less likely to develop atopic disease in childhood than those born in the hospital and this association was mediated by *C. difficile* colonization (69).

The presence of pets or other children at home have been associated with changes in the infant gut microbiota. It is usually suggested that the influence of pets and siblings on the gut microbiota is in line with the “hygiene hypothesis’’ in that they increase exposure to environmental microbes in early life which primes the immune system and helps in the development of the infant gut microbiota (123,133). A study in 6-month old infants revealed lower abundance of *Lactobacillus reuteri* to be associated with presence of pets or older siblings (123). A systematic review of 6 studies reported that infants with no older siblings were more

likely to have a decreased abundance of *Lactobacillus* and *Bacteroides* and an increased abundance of *Clostridium* (134). In the CHILD cohort, having a pet at home was associated with increased microbiota richness, diversity, abundance of Clostridiaceae and *Veillonella* whereas an opposing trend was observed with having an older sibling (133).

1.2.4.5 Maternal Stress and Depression

Evidence directly linking maternal depression or stress to gut microbiota dysbiosis is lacking, but a few have observed an association between maternal stress and offspring infection leading to antibiotic use. In a Danish cohort, women who had experienced stressful life events such as death of a loved one during pregnancy were more likely to have an offspring developing infections during infancy and childhood (135). Hence, there may be greater prescribing of antibiotics in the early weeks of life to treat the infections thereby affecting gut microbiota. Women distressed during pregnancy also tend to have higher usage of prenatal antibiotics due to increased susceptibility to infections (136). In another study, Beijers et al. (137) reported that emotions surrounding pregnancy such as birthing a child with disabilities and increased maternal prenatal anxiety were associated with respiratory infections in the infant and subsequent use of antibiotics during infancy. Mechanisms via which prenatal psychosocial distress predispose to increased offspring infections is unknown but is suspected to be through secretion of cortisol which is regulated by the maternal HPA axis and the placenta (138). In a Dutch cohort, maternal prenatal stress was positively correlated with *Escherichia, Serratia, Enterobacter* and negatively correlated with *Lactobacillus, Lactoccus, Aerococcus* and *Bifidobacteria* (139).

1.2.5 Interventions to Restore Gut commensals / Prevent Dysbiosis

Antibiotics should be prescribed when there is certainty of a bacterial infection; and the use of narrow spectrum antibiotics that directly target the specific pathogens without affecting gut commensals should be encouraged. This may not always be feasible especially when there is risk of mortality or morbidity in life-threatening infections and the causative organism is unknown. Broad-spectrum antibiotics are usually administered to arrest infection from unknown or multiple pathogens. In this case, research on interventions such as probiotics and prebiotics to restore gut commensals or reverse dysbiosis should be considered (125). Due to the potential detrimental effects of maternal IAP on the neonatal microbiome, more focus should also be

placed on implementing strategies such as maternal vaccination against GBS to protect vulnerable newborns from invasive GBS disease (140).

1.2.6 Summary

It is evident that antimicrobial exposure affects the symbiotic relationship between humans and their microbes. Although lifesaving when treating a potentially fatal infection, antimicrobial interventions have notable impacts on the infant gut microbiota and more targeted therapy should be optimized. In addition, antimicrobials may be a contributing factor to the burden of childhood allergic diseases. In adults and older children, *C. difficile* is responsible for most antibiotic associated diarrhea and may progress to more life-threatening infections. Much thought has not been given to colonization of this microbe in infants because of the absence of clinical symptoms in this population. However, evidence suggest that this microbe may predispose colonized infants to future allergic diseases (69). Of concern, the influence of cumulative antimicrobial exposure from antibiotics and household cleaning products on *C. difficile* colonization is unknown.

1.3 Purpose Statement, Objectives and Hypotheses

The purpose of this thesis was to examine the association between early life antimicrobial exposure and *Clostridioides difficile (C. difficile)* colonization of the gut microbiota in infants. The first objective of this study was to determine the separate effect of antibiotics and household cleaning products on *C. difficile* colonization at 3 and 12 months. The second objective was to determine the cumulative effect of antibiotics and household cleaning products on *C. difficile* colonization at 3 and 12 months.

With reference from the literature, the first research hypothesis was that infants with higher antimicrobial exposure will have different *C. difficile* colonization and gut microbiota composition compared to those with the least antimicrobial exposure. The second research hypothesis was that exposure to both antibiotics and higher cleaning products use will produce a cumulative effect on *C. difficile* colonization of the infant gut microbiota.

1.4 Sample Size Calculation

Antibiotic exposure has been previously associated with gut microbiota dysbiosis and increased *Clostridium* colonization. To determine the effective sample size for this analysis, a statistically significant difference in proportions of *Clostridium* abundance was compared.

$$\text{Sample size per group needed based on proportions } = \text{ n} = 2\text{PI}^2(\frac{\bar{\text{p}}(1-\bar{\text{p}})}{(\text{p}_1-\text{p}_2)^2})$$

PI= Power Index

To determine the sample size with a 2-sided α of 0.05 and β of 0.20 (power=80%), the Power Index (PI) will be: 1.96 + 0.84 = 2.80

$\bar{\text{p}} = (\text{p}_1 + \text{p}_2)/2$

PI = 1.96 (0.05α, two-tailed) + 0.84(0.20β, two-tailed) = 2.80

$\text{p}_1 =$ *Clostridium* abundance in antibiotic group = 7% = 0.07

$\text{p}_2 =$ *Clostridium* abundance in no antibiotic group = 2% = 0.02

$\text{n} = 2(2.8)^2(\frac{\overline{0.045}(1-\overline{0.045})}{(0.07-0.02)^2}) \approx$ 308 per group

Estimated proportions were obtained from a previous study by Fouhy et al. (77) where *Clostridium* abundance was different between newborns exposed and not exposed to intravenous antibiotics. A colonization rate of 7% was observed for newborns exposed to antibiotics while that of the reference group was 2%. Based on this, the estimated required sample size assuming power = 80% and a two-sided alpha = 5% was determined to be approximately 308 infants per exposure group.

1.5 Overview of Study

1.5.1 Study Population

This study population comprises of a representative sample of mothers and infants who were successfully enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. CHILD cohort is a longitudinal, general population birth cohort composed of 3624 families recruited at four sites across Canada (Vancouver, Edmonton, Manitoba, Toronto), when the mother was pregnant. Mothers were enrolled between 2008 and 2012. Table 1.1 shows the

CHILD study inclusion/exclusion criteria. CHILD study participants were recruited from obstetrics & gynaecology clinic, clinic waiting rooms, booths at trade shows, and media (141). Additional inclusion criteria for this study was availability of mother's and infant's information on all antimicrobial exposure and infant 3- and 12-month fecal samples collected and sequenced.

Table 1.1. CHILD Study Inclusion/Exclusion Criteria

Inclusion Criteria	**Exclusion Criteria**
➢ Pregnant women aged 18 years or older (>19 years in Vancouver) ➢ English literacy ➢ Lives in proximity (<50km) to delivery hospital ➢ Plans to give birth at a designated recruitment centre ➢ Willing to give informed consent for study procedures ➢ Infants born at 35 weeks or older ➢ Families able to provide name, address and contact of two alternate individuals	➢ Children born with major congenital abnormalities or respiratory distress syndrome (RDS) ➢ Expecting to move away within one year of recruitment ➢ Children from invitro fertilization (IVF) ➢ Children from multiple pregnancy ➢ Infants born before 35 weeks ➢ Children who didn't spend at least 80% of nights in their study home

*Modified from Subbarao et al. (142)

1.5.2 Study Design

Mothers in the CHILD study completed questionnaires during pregnancy and when their child was 3 and 12 months of age (www.childstudy.ca). Relevant quantitative and clinical data have been previously and prospectively collected for the CHILD birth cohort. Mothers were asked to complete comprehensive and validated questionnaires regarding infant use of antibiotics and the family's use of household cleaning products post delivery. Data on maternal intrapartum antibiotic prophylaxis (IAP), newborn antibiotic treatment, infant sex, birth mode, gestational age, birthweight were obtained from hospital birth chart. Questionnaires were also used to collect information on family characteristics (e.g. maternal age, maternal mental health, smoke exposure, siblings), infant characteristics (e.g. health, nutrition) and environmental

characteristics (e.g. furry pets, location). Infant stool samples at 3 and 12 months were collected after home assessment.

1.5.3 Study Variables

The exposure variables in this study were antibiotics (maternal intrapartum antibiotic (IAP) or infant antibiotic) and environmental antimicrobials (postnatal use of household cleaning products). Maternal IAP and newborn intravenous antibiotic were obtained from hospital birth chart while infant oral antibiotic and household cleaning product use were obtained from questionnaires administered at 3 months. Mothers were asked if their offspring had been given any medications to obtain data on antibiotic use. Mothers were also asked about their use of household cleaning products from a list of 26 cleaning products such as bleach, detergents, disinfectants, cleaners etc. Please see Appendix B for a detailed list. The outcome variable in this study was *C. difficile* colonization (yes or no) at 3 and 12 months.

Birth method was obtained from hospital birth chart and categorized into vaginal delivery, elective (scheduled) caesarean section and emergency caesarean section. All caesarean delivery was with IAP according to standard practice in North America. Birthweight (in grams) and gestational age (in weeks) were obtained as continuous variables but categorized into clinically important groups where birthweight was grouped as <2500g, 2500–4000g, >4000g while gestational age was grouped as <39 weeks and ≥ 39 weeks. Infant sex was determined from hospital birth chart. Breastfeeding status obtained from 3 months home visit was categorized as exclusively breastfed, mixed fed (breastfeeding and formula) and exclusively formula fed while breastfeeding at 12 months was left as yes or no. Solid food introduction was also obtained based on if solid food was introduced before 6 months. Location was defined as the study cite where participants resided in during enrolment. Furry pet ownership was obtained from questionnaires about the home environment at 3 months. This was defined as any furry pets living in the home at or before the 3-month home visit. Having an older sibling(s) was obtained from questionnaires administered at 18+ weeks gestation and defined as any living children. Any smoke exposure was a combination of prenatal smoking and smoke exposure after delivery and was defined as maternal smoking during pregnancy or anyone in the home smoking when the mother was pregnant or after the delivery of the baby. Maternal age (in years) was obtained as a continuous variable and categorized into 18–29, 30–39, and ≥40 due to non-linearity with

C. difficile. Maternal postnatal antibiotic use was obtained from questionnaire on medication use after birth till when the child was 3 months. Maternal prenatal and postnatal depression symptoms were measured using the 20-item Center for Epidemiologic Studies Depression Scale (CES-D) (143). Women self reported how often they experienced various depressive cognitions and behaviors during the past week. Responses were given a score ranging from 0 (None of the time; less than 1 day) to 3 (Most or all the time; 5-7 days). Responses were summed, with higher scores indicating higher depressive symptoms (min=0, max=60). CES-D scores of 16 or greater represent significant risk for clinical depression. Maternal stress was determined through a 10-item version of the perceived stress scale, in which mothers reported various behaviours and were given a mean score using 17 as the cut off. For this study, both maternal prenatal/postnatal depression and prenatal/postnatal stress were grouped as binary variables (yes or no).

1.5.4 Ethical Considerations

This study aligns with ethics requirements for research on human subjects: respect for persons, concern for patient welfare and justice. In addition, this study is a secondary analysis utilizing data from a previously established cohort study examining multiple research questions (www.childstudy.ca). Mothers enrolled in the CHILD study agreed to and provided informed consent at recruitment and follow up. All personal identifying information were treated as confidential during analysis and the same will be done in publication of study results. This secondary analysis did not introduce any violation to agreements. During analysis, all study data were saved on a protected server to prevent loss, alteration and sharing of valuable study data. The study data were kept for a specified period and then destroyed in accordance to standard practice. When interpreting the research findings, biased language was not used against persons for their sex, sexual orientation, ethnicity or racial group. Participation in this study did not place participants in a position of increased vulnerability. Finally, this study only retrieved necessary participant information specific to the research questions and the data were not shared with a third party or utilized for other research purposes.

1.6 References

1. Zeissig S, Blumberg RS. Life at the beginning: Perturbation of the microbiota by antibiotics in early life and its role in health and disease. Nat Immunol. 2014;15(4):307–10.

2. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH Human Microbiome Project. Genome Res. 2009;19(12):2317–23.

3. Ursell L, Clemente J, Rideout J, Gevers D, Caporaso G, Knight R. The interpersonal and intrapersonal diversity of humanassociated microbiota in key body sites. J Allergy Clin Immunol. 2012;129(5):1204–8.

4. Strachan DP. Hay fever, hygiene, and household size. Br Med J. 1989;299:1259–60.

5. Hunter P. The changing hypothesis of the gut. EMBO Rep. 2012;13(6):498–500.

6. Flemming A. The discovery and development of penicillin. The Medical press. 1999. p. 1–11.

7. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014;14(8):742–50.

8. Ting JY, Synnes A, Roberts A, Deshpandey A, Dow K, Yoon EW, et al. Association between antibiotic use and neonatal mortality and morbidities in very low-birth-weight infants without culture-proven sepsis or necrotizing enterocolitis. JAMA Pediatr. 2016;170(12):1181–7.

9. Frenk SM, Kit BK, Lukacs SL, Hicks LA, Gu Q, Public S, et al. Trends in the use of prescription antibiotics: NHANES 1999-2012. 2016;71(1):251–6.

10. Zwittink RD, Renes IB, van Lingen RA, van Zoeren-Grobben D, Konstanti P, Norbruis OF, et al. Association between duration of intravenous antibiotic administration and early-life microbiota development in late-preterm infants. Eur J Clin Microbiol Infect Dis. 2018;37(3):475–83.

11. Saux N Le, Robinson JL, Society CP. Canadian Pediatric Society Position Statement:

Management of acute otitis media in children six months of age and older. Pediatr Child Heal. 2016;21(1):1–8.

12. Robinson JL, Finlay JC, Lang ME, Bortolussi R, Bridger NA, Martin S, et al. Urinary tract infection in infants and children: Diagnosis and management. Paediatr Child Heal. 2014;19(6):315–9.

13. Vaz LE, Kleinman KP, Raebel MA, Nordin JD, Lakoma MD, Dutta-Linn MM, et al. Recent trends in outpatient antibiotic use in children. Pediatrics. 2014;133(3):375–85.

14. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H, Calle GM, et al. The worldwide antibiotic resistance and prescribing in european children (ARPEC) point prevalence survey: Developing hospital-quality indicators of antibiotic prescribing for children. J Antimicrob Chemother. 2016;71(4):1106–17.

15. Le Doare K, O'Driscoll M, Turner K, Seedat F, Russell NJ, Seale AC, et al. Intrapartum Antibiotic Chemoprophylaxis Policies for the Prevention of Group B Streptococcal Disease Worldwide: Systematic Review. Clin Infect Dis. 2017;65(Suppl 2):S143–51.

16. Madrid L, Seale AC, Kohli-Lynch M, Edmond KM, Lawn JE, Heath PT, et al. Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(Suppl 2):S160–72.

17. Seedat F, Stinton C, Patterson J, Geppert J, Tan B, Robinson ER, et al. Adverse events in women and children who have received intrapartum antibiotic prophylaxis treatment: A systematic review. BMC Pregnancy Childbirth. 2017;17:247.

18. Jefferies AL. Management of term infants at increased risk for early-onset bacterial sepsis. Paediatr Child Heal. 2017;22(4):223–8.

19. Stokholm J, Schjørring S, Pedersen L, Bischoff AL, Følsgaard N, Carson CG, et al. Prevalence and predictors of antibiotic administration during pregnancy and birth. PLoS One. 2013;8(12):1–7.

20. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, Kozyrskyj AL. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: A population-based study. J Matern Neonatal Med. 2015 Jul 3;28(10):1190–5.

21. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database Syst Rev. 2014;2014(6).

22. van Schalkwyk J, Van Eyk N. Antibiotic prophylaxis in obstetric procedures. J Obstet Gynaecol Can. 2010;32(9):878–92.

23. Didier C, Streicher MP, Chognot D, Campagni R, Schnebelen A, Messer J, et al. Late-onset neonatal infections: Incidences and pathogens in the era of antenatal antibiotics. Eur J Pediatr. 2012;171(4):681–7.

24. Tapiainen T, Koivusaari P, Brinkac L, Lorenzi HA, Salo J, Renko M, et al. Impact of intrapartum and postnatal antibiotics on the gut microbiome and emergence of antimicrobial resistance in infants. Sci Rep. 2019;9(1):1–11.

25. Vangay P, Ward T, Gerber J, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. Cell Host Microbe. 2015;17(5):553–64.

26. Penders J, Kummeling I, Thijs C. Infant antibiotic use and wheeze and asthma risk: A systematic review and meta-analysis. Eur Respir J. 2011;38(2):295–302.

27. Nazaroff WW, Weschler CJ. Cleaning products and air fresheners: Exposure to primary and secondary air pollutants. Atmos Environ. 2004;38(18):2841–65.

28. Dimitroulopoulou C, Lucica E, Johnson A, Ashmore MR, Sakellaris I, Stranger M, et al. EPHECT I: European household survey on domestic use of consumer products and development of worst-case scenarios for daily use. Sci Total Environ. 2015;536:880–9.

29. Herr M, Just J, Nikasinovic L, Foucault C, Le Marec AM, Giordanella JP, et al. Influence of host and environmental factors on wheezing severity in infants: Findings from the PARIS birth cohort. Clin Exp Allergy. 2012;42(2):275–83.

30. Lao XQ, Ho KF, Wong CC, Tian LW. Respiratory health effects of household cleaning products on Hong Kong school children. Hong Kong Med J. 2019;25(1):24–6.

31. Wolkoff P, Schneider T, Kildesø J, Degerth R, Jaroszewski M, Schunk H. Risk in cleaning : chemical and physical exposure. Sci Total Environ. 1998;215:135–56.

32. McLachlan JA, Arnold SF. Environmental estrogens. Am Sci. 1996;84(5):452–61.

33. EWG. Pollution in People: Toxic Chemicals Found in Minority Cord Blood. Washington, DC. 2009 [cited 2019 Oct 3]. Available from: https://www.ewg.org/news/news-releases/2009/12/02/toxic-chemicals-found-minority-cord-blood

34. Ouimette AMY. Cleaning House : Considerations of Ecological Health and Sustainability in the Selection of Household Cleaning Products. 2011.

35. Altman RG, Morello-Frosch R, Brody JG, Rudel R, Brown P, Averick M. Pollution comes home and gets personal: Women's experience of household chemical exposure. J Health Soc Behav. 2008;49(4):417–35.

36. Padiyara P, Inoue H, Sprenger M. Global Governance Mechanisms to Address Antimicrobial Resistance. Infect Dis Res Treat. 2018;11:1–4.

37. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: Efficacy of WHO's currently recommended antibiotics - Systematic review and meta-analysis. Arch Dis Child. 2013;98(2):146–54.

38. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, et al. Access to effective antimicrobials: A worldwide challenge. Lancet. 2016;387(10014):168–75.

39. Yassour M, Vatanen T, Siljander H, Hämäläinen A. Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability. Sci Transl Med. 2016;8(343).

40. Sun D. Pull in and push out: Mechanisms of horizontal gene transfer in bacteria. Front Microbiol. 2018;9(SEP):1–8.

41. Jacoby GA. AmpC B-Lactamases. Clin Microbiol Rev. 2009;22(1):161–82.

42. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, et al. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol. 2018;16(9):523–39.

43. Ramirez CB, Cantey JB. Antibiotic resistance in the neonatal intensive care unit. Neoreviews. 2019;20(3):e135–44.

44. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al.

Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat Commun. 2018;9(1):1–11.

45. WHO. Global Strategy for Infant and Young Child Feeding. Geneva. 2003 [cited 2019 Oct 3]. p. 1–37. Available from: https://www.who.int/nutrition/publications/infantfeeding/9241562218/en/

46. Lozupone CA, Stombaugh J, Gordon JI, Jansson J, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220–30.

47. Wampach L, Heintz-Buschart A, Hogan A, Muller EEL, Narayanasamy S, Laczny CC, et al. Colonization and succession within the human gut microbiome by archaea, bacteria, and microeukaryotes during the first year of life. Front Microbiol. 2017;8:1–21.

48. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-Gut Microbiota Metabolic Interactions. Science. 2012;336:1262–8.

49. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9(5):313–23.

50. Jauréguy F, Carton M, Panel P, Foucaud P, Butel MJ, Doucet-Populaire F. Effects of intrapartum penicillin prophylaxis on intestinal bacterial colonization in infants. J Clin Microbiol. 2004;42(11):5184–8.

51. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol. 2018;3:1255–65.

52. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(2):511–21.

53. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev. 2017;81(4):1–67.

54. Omenetti S, Pizarro TT. The Treg/Th17 axis: A dynamic balance regulated by the gut

microbiome. Front Immunol. 2015;6:1–8.

55. Al-Asmakh M, Zadjali F. Use of germ-free animal models in microbiota-related research. J Microbiol Biotechnol. 2015;25(10):1583–8.

56. Cox LM, Yamanishi S, Sohn J, Alekseyenko A V., Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705–21.

57. Lynn MA, Tumes DJ, Choo JM, Sribnaia A, Blake SJ, Leong LEX, et al. Early-Life Antibiotic-Driven Dysbiosis Leads to Dysregulated Vaccine Immune Responses in Mice. Cell Host Microbe. 2018;23(5):653-660.e5.

58. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Heal Dis. 2015;26:1–17.

59. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018;562(7728):583–8.

60. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 2016;8(343):1–14.

61. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17(5).

62. Azad M, Konya T, Persaud R, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. BJOG An Int J Obstet Gynaecol. 2016;123(6):983–93.

63. Azad M, Konya T, Maughan H, Guttman D, Field C, Chari R, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. Cmaj. 2013;185(5):385–94.

64. Laursen MF, Andersen LBB, Michaelsen KF, Mølgaard C, Trolle E, Bahl MI, et al. Infant Gut Microbiota Development Is Driven by Transition to Family Foods Independent of Maternal Obesity. mSphere. 2016;1(1):1–16.

65. Dogra S, Sakwinska O, Soh SE, Ngom-Bru C, Brück WM, Berger B, et al. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. MBio. 2015;6(1):1–9.

66. Lees EA, Miyajima F, Pirmohamed M, Carrol ED. The role of Clostridium difficile in the paediatric and neonatal gut — a narrative review. Eur J Clin Microbiol Infect Dis. 2016;35(7):1047–57.

67. Davis MY, Zhang H, Brannan LE, Carman RJ, Boone JH. Rapid change of fecal microbiome and disappearance of Clostridium difficile in a colonized infant after transition from breast milk to cow milk. Microbiome. 2016;4:1–10.

68. Pant C, Deshpande A, Altaf MA, Minocha A, Sferra TJ. *Clostridium difficile* infection in children: a comprehensive review. Curr Med Res Opin. 2013;29(8):967–84.

69. Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol. 2011;128(5):948-955.e3.

70. Cózar-Llistó A, Ramos-Martinez A, Cobo J. Clostridium difficile Infection in Special High-Risk Populations. Infect Dis Ther. 2016;5(3):253–69.

71. Tabaqchalsih S, O'farrell E, Nasha JQ, Wilks M. Vaginal Carriage and Neonatal Acquisition of Clostridium difficile. J Med Microbiol. 1984;18:47–53.

72. Matsuki S, Ozaki E, Shozu M, Inoue M, Shimizu S, Yamaguchi N, et al. Colonization by Clostridium difficile of neonates in a hospital, and infants and children in three day-care facilities of Kanazawa, Japan. Int Microbiol. 2005;8(1):43–8.

73. Jangi S, Lamont JT. Asymptomatic colonization by clostridium difficile in infants: Implications for disease in later life. J Pediatr Gastroenterol Nutr. 2010;51(1):2–7.

74. Nogacka A, Salazar N, Suárez M, Milani C, Arboleya S, Solís G, et al. Impact of

intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. Microbiome. 2017;5(1):1–10.

75. Corrêa NBO, Péret Filho LA, Penna FJ, Lima FMLS, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. J Clin Gastroenterol. 2005;39(5):385–9.

76. Plummer S, Weaver MA, Harris JC, Dee P, Huter J. Clostridium difficile pilot study: Effects of probiotic supplementation on the incidence of C. difficile diarrhoea. Int Microbiol. 2004;7(1):59–62.

77. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother. 2012;56(11):5811–20.

78. Stearns JC, Simioni J, Gunn E, McDonald H, Holloway AC, Thabane L, et al. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. Sci Rep. 2017;7:16527.

79. Network GA. The Global Asthma Report Asthma affects. 2018.

80. StatCan. Asthma by Age Group. [cited 2019 Oct 5]. Available from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310009608

81. Canadian Institute for Health Information. Asthma Hospitalizations Among Children and Youth in Canada: Trends and Inequalities Chartbook. 2018. Available from: https://www.cihi.ca/sites/default/files/document/asthma-hospitalization-children-2018-chartbook-en-web.pdf

82. Williams H, Stewart A, von Mutius E, Cookson W, Anderson HR. Is eczema really on the increase worldwide? J Allergy Clin Immunol. 2008;121(4).

83. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41–58.

84. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis. Allergy. 2018;73(5):971–86.

85. Lavigne É, Bélair MA, Duque DR, Do MT, Stieb DM, Hystad P, et al. Effect modification of perinatal exposure to air pollution and childhood asthma incidence. Eur Respir J. 2018;51:1701884.

86. Johnson CC, Ownby DR. The infant gut bacterial microbiota and risk of pediatric asthma and allergic diseases. Transl Res. 2017;179:60–70.

87. Doan T, Arzika AM, Ray KJ, Cotter SY, Kim J, Maliki R, et al. Gut microbial diversity in antibiotic-naive children after systemic antibiotic exposure: A randomized controlled trial. Clin Infect Dis. 2017;64(9):1147–53.

88. Parker EPK, Praharaj I, John J, Kaliappan SP, Kampmann B, Kang G, et al. Changes in the intestinal microbiota following the administration of azithromycin in a randomised placebo-controlled trial among infants in south India. Sci Rep. 2017;7(1):1–9.

89. Wei S, Mortensen MS, Stokholm J, Brejnrod AD, Thorsen J, Rasmussen MA, et al. Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: A double-blind, randomized, placebo-controlled trial. EBioMedicine. 2018;38:265–72.

90. Tanaka S, Kobayashi T, Songjinda P, Tateyama A, Tsubouchi M, Kiyohara C, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol Med Microbiol. 2009;56(1):80–7.

91. Mazzola G, Murphy K, Ross RP, Di Gioia D, Biavati B, Corvaglia LT, et al. Early gut microbiota perturbations following intrapartum antibiotic prophylaxis to prevent group B streptococcal disease. PLoS One. 2016;11(6):1–11.

92. Aloisio I, Quagliariello A, De Fanti S, Luiselli D, De Filippo C, Albanese D, et al. Evaluation of the effects of intrapartum antibiotic prophylaxis on newborn intestinal microbiota using a sequencing approach targeted to multi hypervariable 16S rDNA regions. Appl Microbiol Biotechnol. 2016;100(12):5537–46.

93. Bello A, Quinn MM, Perry MJ, Milton DK. Quantitative assessment of airborne exposures

generated during common cleaning tasks: A pilot study. Environ Heal A Glob Access Sci Source. 2010;9(1):1–10.

94. Schmidt B, Mulder IE, Musk CC, Aminov RI, Lewis M, Stokes CR, et al. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One. 2011;6(12):e:28284.

95. Tun MH, Tun HM, Mahoney JJ, Konya TB, Guttman DS, Becker AB, et al. Postnatal exposure to household disinfectants, infant gut microbiota and subsequent risk of overweight in children. Cmaj. 2018;190(37):E1097–107.

96. Bornehag C, Hagerhed-Engman L, Sigsgaard T, Janson S, Aberg N, DBH-studyGroup. Dampness at home and its association with airway, nose and skin symptoms among 10 851 preschool children in Sweden: A cross sectional study. Indoor Air. 2005;2005(15):48–45.

97. Levy SB. Antibacterial household products: Cause for concern. Emerg Infect Dis. 2001;7(3):512–5.

98. Marra F, Lynd L, Coombes M, Richardson K, Legal M, FitzGerald JM, et al. Does antibiotic exposure during infancy lead to development of asthma? A systematic review and metaanalysis. Chest. 2006;129(3):610–8.

99. Murk W, Risnes KR, Bracken MB. Prenatal or Early-Life Exposure to Antibiotics and Risk of Childhood Asthma: A Systematic Review. Pediatrics. 2011;127(6):1125–38.

100. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review. Br J Dermatol. 2013;169(5):983–91.

101. Goksör E, Alm B, Pettersson R, Möllborg P, Erdes L, Åberg N, et al. Early fish introduction and neonatal antibiotics affect the risk of asthma into school age. Pediatr Allergy Immunol. 2013;24(4):339–44.

102. Stromberg Celind F, Vasileiadou S, Alm B, Goksor E. Antibiotics in the first week of life were associated with atopic asthma at 12 years of age. Acta Paediatr Int J Paediatr. 2018;1–7.

103. Batool T, Reece PL, Schulze KM, Morrison KM, Atkinson SA, Anand SS, et al. Prenatal and early-life predictors of atopy and allergic disease in Canadian children: Results of the Family Atherosclerosis Monitoring In earLY life (FAMILY) Study. J Dev Orig Health Dis. 2016;7(6):665–71.

104. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association between use of acid-suppressive medications and antibiotics during infancy and allergic diseases in early childhood. JAMA Pediatr. 2018;172(6).

105. Wohl DL, Curry WJ, Mauger D, Miller J, Tyrie K. Intrapartum Antibiotics and Childhood Atopic Dermatitis. J Am Board Fam Med. 2015;28(1):82–9.

106. Obiakor C V., Tun HM, Bridgman SL, Arrieta M-C, Kozyrskyj AL. The association between early life antibiotic use and allergic disease in young children: recent insights and their implications. Expert Rev Clin Immunol. 2018;14(10):841–55.

107. Zock JP, Vizcaya D, Le Moual N. Update on asthma and cleaners. Curr Opin Allergy Clin Immunol. 2010;10(2):114–20.

108. Vincent MJ, Parker A, Maier A. Cleaning and asthma: A systematic review and approach for effective safety assessment. Regul Toxicol Pharmacol. 2017;90:231–43.

109. Bably M, Arif AA, Post A. Prenatal use of cleaning and scented products and its association with childhood asthma, asthma symptoms, and mental health and developmental comorbidities. J Asthma. 2019:1–6.

110. Sepp E, Julge K, Vasar M, Naaber P, Björksten B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. Acta Paediatr Int J Paediatr. 1997;86(9):956–61.

111. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol. 2012;129(2):434–40.

112. Penders J, Thijs C, Van Den Brandt PA, Kummeling I, Snijders B, Stelma F, et al. Gut microbiota composition and development of atopic manifestations in infancy: The KOALA birth cohort study. Gut. 2007;56(5):661–7.

113. Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015;7(307).

114. Azad MB, Konya T, Guttman DS, Field CJ, Sears MR, Hayglass KT, et al. Infant gut microbiota and food sensitization: Associations in the first year of life. Clin Exp Allergy. 2015;45(3):632–43.

115. Stiemsma LT, Arrieta M-C, Dimitriu PA, Cheng J, Thorson L, Lefebvre DL, et al. Shifts in Lachnospira and Clostridium sp. in the 3-month stool microbiome are associated with preschool age asthma. Clin Sci. 2016;130(23):2199–207.

116. Lee SH, Gong YN, Ryoo E. Clostridium difficile colonization and / or infection during infancy and the risk of childhood allergic diseases. 2017;60(5):145–50.

117. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One. 2012;7(6):e36466.

118. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107(26):11971–5.

119. Adlerberth I, Strachan DP, Matricardi PM, Ahrné S, Orfei L, Åberg N, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol. 2007;120(2):343–50.

120. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by Caesarean section. Gut. 2014;63(4):559–66.

121. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475–90.

122. Cabrera-Rubio R, Collado C, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of

delivery. Am J Clin Nutr. 2012;96:544–51.

123. Martin R, Makino H, Yavuz AC, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS One. 2016;11(6):1–30.

124. Taft DH, Liu J, Maldonado-Gomez MX, Akre S, Huda MN, Ahmad SM, et al. Bifidobacterial Dominance of the Gut in Early Life and Acquisition of Antimicrobial Resistance. mSphere. 2018;3(5):1–24.

125. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. Microbiome. 2018 Oct;6(1):182.

126. Ferraris L, Butel MJ, Campeotto F, Vodovar M, Rozé JC, Aires J. Clostridia in premature neonates' gut: Incidence, antibiotic susceptibility, and perinatal determinants influencing colonization. PLoS One. 2012;7(1):e30594.

127. Zhu D, Xiao S, Yu J, Ai Q, He Y, Cheng C, et al. Effects of One-Week Empirical Antibiotic Therapy on the Early Development of Gut Microbiota and Metabolites in Preterm Infants. Sci Rep. 2017;7(1):1–10.

128. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. Microbiome. 2017;5(1):1–18.

129. Arboleya S, Sánchez B, Solís G, Fernández N, Suárez M, Hernández-Barranco AM, et al. Impact of prematurity and perinatal antibiotics on the developing intestinal microbiota: A functional inference study. Int J Mol Sci. 2016;17(5):1–14.

130. Patient Safety Network. Health care-associated infections. Agency for Healthcare Research and Quality. 2019 [cited 2019 Oct 7]. Available from: https://psnet.ahrq.gov/primer/health-care-associated-infections

131. Stoodley BJ, Thom BT. Observations on the Intestinal Carriage of Pseudomonas Aeruginosa. J Med Microbiol. 1970;3:367–75.

132. Kato H, Kato N, Watanabe K, Ueno K, Ushijima H, Hashira S, et al. Application of typing by pulsed-field gel electrophoresis to the study of Clostridium difficile in a neonatal intensive care unit. J Clin Microbiol. 1994;32(9):2067–70.

133. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Sears MR, et al. Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. Allergy, Asthma Clin Immunol. 2013;9(15):1–9.

134. Chan CWH, Wong RS, Law PTW, Wong CL, Tsui SKW, Tang WPY, et al. Environmental factors associated with altered gut microbiota in children with eczema: A systematic review. Int J Mol Sci. 2016;17:1–14.

135. Nielsen NM, Hansen AV, Simonsen J, Hviid A. Prenatal stress and risk of infectious diseases in offspring. Am J Epidemiol. 2011;173(9):990–7.

136. Field T, Hernandez-Reif M, Diego M. Risk factors and stress variables that differentiate depressed from nondepressed pregnant women. Infant Behav Dev. 2006;29(2):169–74.

137. Beijers R, Jansen J, Riksen-Walraven M, De Weerth C. Maternal prenatal anxiety and stress predict infant illnesses and health complaints. Pediatrics. 2010;126(2).

138. Beijers R, Buitelaar JK, de Weerth C. Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. Eur Child Adolesc Psychiatry. 2014;23(10):943–56.

139. Zijlmans MAC, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology. 2015;53:233–45.

140. Heath PT. Status of vaccine research and development of vaccines for Staphylococcus aureus. Vaccine. 2016;34:2876–9.

141. Maghera A, Kahlke P, Lau A, Zeng Y, Hoskins C, Corbett T, et al. You are how you recruit: A cohort and randomized controlled trial of recruitment strategies. BMC Med Res Methodol. 2014;14:114.

142. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR, et al. The Canadian Healthy Infant Longitudinal Development (CHILD) study: Examining developmental origins of allergy and asthma. Thorax. 2015;70(10):998–1000.

143. Lewinsohn PM, Seeley JR, Roberts RE, Allen NB. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. Psychol Aging. 1997;12(2):277–87.

Chapter 2: Early Life Antimicrobial Exposure is Associated with *Clostridioides difficile* Colonization in Infants

Authors: Chinwe V. Obiakor[1], Jaclyn Parks[2], Tim K. Takaro[2], Hein M. Tun[3], Nadia Morales-Lizcano[4], Theodore B. Konya[4], David S. Guttman[5], Allan B. Becker[6], Meghan B. Azad[6], Diana L. Lefebvre[7], Piushkumar J. Mandhane[8], Theo J. Moraes[9], Malcom R. Sears[10], Stuart E. Turvey[11], Padmaja Subbarao[9], James A. Scott[4] and Anita L. Kozyrskyj[1,8]

Author Affiliations: [1]School of Public Health, University of Alberta, Canada

[2]Faculty of Health Sciences, Simon Frasier University, Canada

[3]School of Public Health, University of Hong Kong, Hong Kong

[4]Dalla Lana School of Public Health, University of Toronto, Canada

[5]Department of Cell & Systems Biology, University of Toronto, Canada

[6]Department of Pediatrics & Child Health, University of Manitoba, Canada

[7]McMaster University, Canada

[8]Department of Pediatrics, University of Alberta, Canada

[9]Department of Pediatrics, University of Toronto, Canada

[10]Department of Medicine, McMaster University, Canada

[11]Department of Pediatrics, University of British Columbia, Canada

2.1 Introduction

Global antibiotic consumption has risen substantially (1). An overlooked source of infant antibiotic exposure is the administration to mothers during childbirth. According to the Society of Obstetricians and Gynecologists of Canada (SOGC) guidelines, pregnant women should receive cefazolin antibiotic before skin incision in emergency or scheduled caesarean section (2). In addition, current guidelines recommend screening pregnant women at 35 to 37 weeks and administering intrapartum antibiotic prophylaxis (IAP) during labour to women positive for *Group B Streptococcus* (GBS) or those with other risk factors (GBS bacteriuria or previous infant with GBS disease) (3). In accordance with these recommendations, up to 40% of newborns are exposed to maternal IAP for prevention of early onset GBS or during caesarean section delivery (4). Furthermore, about 2 to 5% of vaginally delivered newborns receive intravenous antibiotics after birth for suspected treatment of neonatal sepsis (4,5). In the first year, oral antibiotics are mainly prescribed to treat respiratory infections such as otitis media (ear infection), laryngitis, sinusitis, pharyngitis, bronchitis even though some of these are of viral origin or self-limiting. Standards to cleaning have also evolved over the years and is linked to socio-cultural factors that influence human behaviour. To make the environment cleaner, emphasis has shifted to the increased use of cleaning products in the home, contributing to the burden of antimicrobial exposure (6).

Antimicrobial exposure during infancy is not without consequences. Epidemiological studies have shown that early life antimicrobial exposure is associated with disruption of gut microbiota (7–10) and future asthma and allergic diseases (11,12). In infants, colonization of the gut microbiota is also strongly influenced by birth method (13,14) and feeding (14,15). Multiple courses of antibiotics have a greater influence on the composition of infant gut microbiota (16) but cumulative antimicrobial exposure from additional sources such as household cleaning products have not been studied. *Clostridiodes difficile* (*C. difficile*) formerly known as *Clostridium difficile* is a gram-positive spore forming bacteria which has toxigenic and non-toxigenic strains. In adults and older children, *C. difficile* is the major pathogen responsible for antibiotic-induced diarrhea and can cause symptoms ranging from diarrhea to life-threatening inflammation of the colon. Although the colonization rate is high (about 30%) in infants below 1 year, the role of *C. difficile* in this age group remains uncertain as most colonized infants do not

manifest clinical symptoms of infection (17). However, infants colonized with this microbe may have an increased risk of asthma and allergic diseases later in childhood (18,19).

Antimicrobial exposure can diminish competing gut commensals and provide room for *C. difficile* colonization, overgrowth and toxin production. The presence of *C. difficile* in infants has also been suggested as a marker for reduced colonization resistance and delayed gut microbiota maturation (18,20). Previous studies reporting on the association between antimicrobials and *C. difficile* in infants is lacking. Fouhy et al. (21) reported a higher abundance of *Clostridium* and its family Peptostreptococcaeace in newborns given intravenous (IV) antibiotics while Azad et al. (10) reported an increased abundance of *Clostridium* in caesarean delivered infants exposed to maternal IAP. In a recent study of 149 term infants, the authors observed an increased abundance of *Clostridium* in infants exposed to direct IV antibiotics, maternal IAP or both compared to those without any antibiotic exposure (7). On the contrary, Tun et al. (9) reported lowered abundance of *Clostridium* in the gut microbiota of infants living in homes with higher use of disinfectants. These studies propose clear effects of antimicrobial exposure in infants but are limited to reporting at the genus level.

Therefore, the aim of our study was to determine the separate and cumulative impact from antibiotics and environmental antimicrobials (i.e. household cleaning products) on *C. difficile* colonization and how these antimicrobial exposures modify the gut microbiota. This area of research is currently understudied and not fully understood.

2.2 Methods

2.2.1 Study design

This study includes a subsample of 1,429 infants at 3 months and 1,728 infants at 12 months from the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort of the Edmonton, Winnipeg and Vancouver sites (www.childstudy.ca). Women were enrolled during pregnancy from 2008 to 2012. Written informed consent was obtained from the mother at enrollment. This study was approved by the Ethics Committee of the University of Alberta, the University of Manitoba Human Research Ethics Board and the University of British Columbia/Children's and Women's Health Centre of British Columbia Research Ethics Board.

2.2.2 Exposures

Relevant quantitative, clinical and demographic data have been previously and prospectively collected for the CHILD birth cohort. This study examined antimicrobial exposures before or at 3 months. Data on maternal intrapartum antibiotic prophylaxis (IAP) and newborn antibiotic treatment were obtained from hospital birth chart. At 3 months post-partum, mothers completed validated questionnaires regarding infant use of antibiotics and the family's use of household cleaning products (www.childstudy.ca). For the household cleaning products, mothers were asked about their use of household cleaning products from a list of 26 cleaning products. Please see Appendix B for a detailed list. The frequency of use for each product was assigned a score: 0 for never, 1 for less than a month, 2 for monthly, 3 for weekly and 4 for daily. The score for each respondent was summed to obtain a total score. The total scores were split at the median into two groups of higher and lower use of household cleaning products in order to make comparisons of effect sizes during analyses. In order to explore the cumulative effect of antibiotics and household cleaning products, 4 groups were created: no antibiotics & lower cleaning products use (NALC), any antibiotics & lower cleaning products use (AALC), no antibiotics & higher cleaning products use (NAHC) and any antibiotics and higher cleaning products use (AAHC).

2.2.3 Fecal microbiota analysis

Infant stool samples (fresh or frozen) were collected at approximately 3 and 12 months after home assessment or brought to the clinic. Samples were stored and transported at -80ºC prior to analysis. Fecal samples were characterized using illumina MiSeq using the bacterial 16S rRNA hypervariable V4 region, as previously described (22). Analysis of *C. difficile* was performed using quantitative polymerase chain reaction (qPCR) with appropriate primers, as previously described (10). Universal 16S primers and probes from Nadkarni et al. (23), was used to quantify total bacterial charge of the samples. Primers and probe efficiency were determined by standard curve procedure by making five 1:10 serial dilutions of *C. difficile* ATCC 9689D-5 genomic DNA starting at 1ng/uL. For each plate a non-template control was used. An efficiency between 90 and 110 % and an R^2 equal or higher than 0.9 for the primers and probes combination were used as quality control parameters for each run. QIIME pipeline was used to group microbiota into taxonomic order and summarize OTU data within infant fecal samples.

2.2.4 Statistical Analysis

Statistical analysis was conducted using STATA 13 statistical software. *C. difficile* colonization at 3 and 12 months was analyzed as a binary variable (yes or no). Pearson chi-square test was used to determine the association between antimicrobial exposure and demographic variables. Fisher's exact test were used to determine the crude association between demographic variables and *C. difficile* colonization. Statistical significance was set at $p<0.05$. Logistic regression analysis was used to determine the crude and adjusted association between antimicrobial exposure and *C. difficile* colonization. Adjusted and stratified analysis were performed to account for confounding variables and effect modifiers respectively. Purposeful model building as described by Hosmer and Lemeshow (24) was used to create the final model. Variables were also purposefully chosen based on prior knowledge from the literature. Variables were initially selected if significant with *C. difficile* at $p<0.10$ using fisher's exact test or were clinically important. Confounding variables for the exposure of interest were assessed and kept in the model if the regression coefficients changed by >15%. Interaction terms were analyzed in the final model and included at $p\leq0.05$. Variables that either changed the regression coefficients of the exposure of interest by more than 15% or were significant at $p<0.05$ with *C. difficile* colonization in the multiple logistic regression remained in the final model.

2.3 Results

2.3.1 Study Population and CHILD Cohort

Fecal samples were collected from 1,429 infants at 3 months (mean age 3.6 ± 1.04 months) and 1,728 infants at 12 months (mean age 12.2 ± 1.48 months). Of note, the 12-month sample was larger because more stool samples were easier to collect and analyze at that time point. Our sample of 1,429 mother-infant pairs at 3 months and 1,728 mother-infant pairs at 12 months did not differ from the overall CHILD cohort (Table 1).

Table 1. Distribution (%) of Antibiotic Exposure, Household Cleaning Product Use and Other Covariates at Two Time Points: 3 Months and 12 Months; Compared to the Entire CHILD Cohort at 3 Sites (Edmonton, Winnipeg, Vancouver)

Column percentages	**3 Month Sample % N=1,429**	**12 Month Sample % N=1,728**	**CHILD Cohort at 3 Sites % N=2,150**
Any antibiotics*			
No	51	50	50
Yes	49	50	50
House cleaning product use			
Lower	53	54	53
Higher	47	46	47
Birth method			
Vaginal	78	75	76
Caesarean	22	25	24
Breastfeeding			
No	18	14	15
Yes	82	86	85
Infant sex			
Male	54	52	52
Female	46	48	48
Smoke exposure			
No	82	83	82
Yes	18	17	18

Notes: *Exposure to maternal intrapartum antibiotic or infant antibiotic by 3 Months

2.3.2 Study Population and Antimicrobial Exposure

In our cohort, 44% of infants were indirectly exposed to antibiotics via maternal IAP and 8% directly received an oral or IV antibiotic resulting to 49% (705/1,429) of infant antibiotic exposure by 3 months. 47% (677/1,429) of infants lived in households with higher cleaning products use by 3 months while direct infant antibiotics increased to 21% by 12 months of age resulting to 57% (986/1,728) of infant antibiotic exposure by 12 months (Figure 1).

Infants were classified into 4 groups depending on antimicrobial exposure at 3 months: no antibiotics and lower cleaning products use (**NALC**; n=406; 28%), any antibiotics and lower cleaning products use (**AALC**; n=346; 24%), no antibiotics and higher cleaning products use (**NAHC**; 318; 22%) and any antibiotics and higher cleaning products use (**AAHC**; n=359; 25%)

or cumulative exposure. Table 2 describes the population characteristics distribution according to the 4 groups of antimicrobial exposure. Table S1 (Appendix A) describes the population characteristics distribution according to antibiotics or household cleaning products use. Overall, 77% of infants were vaginally delivered while 55% of infants were exclusively breastfed at 3 months. Approximately half (49%) of the infants were still breastfed by 12 months. All caesarean section (CS) deliveries were with antibiotic prophylaxis in accordance with Canadian practice guidelines leading to greater antimicrobial exposure ($p<0.001$). A higher proportion of mixed fed or exclusively formula fed infants lived in homes with higher cleaning products use and had greater antimicrobial exposure ($p<0.001$). Infants of younger mothers were exposed to higher cleaning products use while infants with no older siblings were more likely to be exposed to antibiotics and had higher antimicrobial exposure ($p<0.001$). Differences were also observed for gestational age, location, having a furry pet at home and smoke exposure according to antimicrobial exposure (Table 2).

2.3.3 Study Population and *C. difficile* Colonization

3 Months

In our study, 31% (445/1,429) of infants were colonized with *C. difficile* at 3 months. In a subsample of vaginally delivered infants, not exposed to antibiotics, living in households with lower cleaning products use and exclusively breastfed, *C. difficile* colonization reduced to 20% compared to 33% in the other infants ($p<0.001$) (Figure 2). *C. difficile* colonization (yes or no) according to population characteristics is shown in Table 3. *C. difficile* colonized 24% of NALC infants, 30% of AALC infants, 32% of NAHC infants and 39% of infants AAHC (Figure 3). Overall, *C. difficile* colonization was influenced by antimicrobial exposure ($p<0.001$). Several other factors influencing *C. difficile* colonization at 3 months were birth method ($p=0.002$), breastfeeding status at 3 months ($p<0.001$), having a furry pet at home ($p=0.005$), having an older sibling ($p=0.034$), smoke exposure ($p<0.001$), maternal age ($p<0.001$) and maternal prenatal depression ($p=0.001$) in the crude analysis.

The crude analysis from both Fisher's exact test and simple logistic regression showed that antimicrobial exposure was significantly associated with *C. difficile* colonization at 3 months. Compared to the NALC infants the odds of *C. difficile* colonization was 38% higher (OR:1.38 95% CI:1.00-1.91; $p=0.047$) in the AALC infants, 52% higher (OR:1.52 95% CI 1.10-

2.11; p=0.011) in the NAHC infants and 103% higher (OR:2.03 95% CI 1.49-2.78; p<0.001) in the AAHC infants (Table 4). Multiple logistic regression analysis was performed to identify variables significantly associated with *C. difficile* colonization and determine the adjusted odds of *C. difficile* colonization (yes/no) in relation to antimicrobial exposure. Variables were selected to be included in the final model using purposeful model building as described by Hosmer and Lemeshow (24). The directed acyclic graph (DAG) model was further employed to increase understanding (Appendix Figure S4) (25). There was an interaction between cumulative antimicrobial exposure and maternal prenatal depression on *C. difficile* colonization at 3 months. The interaction result is reported in section 2.3.4. The crude and adjusted models for 3-month analysis are shown in table 4.

In model 1, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for maternal and birth characteristics such as maternal age, birth method and gestational age. The odds of *C. difficile* colonization significantly increased for the NAHC infants (aOR: 1.48 95% CI 1.06-2.06; p=0.020) and AAHC infants (aOR: 1.67 95% CI 1.16-2.41; p=0.006) but not AALC infants compared to the NALC infants (Table 4). Individual adjustment was done for each variable to identify why the odds of *C. difficile* colonization in the AALC infants lost significance (Appendix Table S3). Birth method was the confounder responsible for the observed association with only antibiotic exposure in the crude analysis. In model 2, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for postnatal characteristics such as breastfeeding status at 3 months, location, having a furry pet at home, having an older sibling and smoke exposure. Compared to the NALC infants, the odds of *C. difficile* colonization significantly increased in the AAHC infants (aOR: 1.45 95% CI 1.04-2.03; p=0.027). The increased odds of colonization with *C. difficile* was marginally significant in the AALC infants (aOR: 1.37 95% CI 0.97-1.92; p=0.068) while there was no significant association for infants in the NAHC infants (Table 4). Individual adjustment revealed breastfeeding as the confounder responsible for the observed association with only higher cleaning products use in the crude analysis (Appendix Table S3).

In the final model, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for variables gotten from purposeful model building i.e. maternal age, birth method and breastfeeding status at 3 months. Although location was associated with both

antimicrobial exposure and *C. difficile* colonization, it was excluded from the final model because of correlation with maternal age and to prevent over adjustment. The odds of colonization with *C. difficile* was significantly higher only for infants in the cumulative exposure (AAHC) group (aOR: 1.50 95% CI 1.03-2.17; p=0.032) compared to the NALC infants. The odds of colonization with *C. difficile* was also higher in the AALC infants and NAHC infants compared to NALC infants but did not attain statistical significance. In the final model, the odds of colonization with *C. difficile* was significantly higher in infants whose mother was younger (18-29 years), infants born via emergency CS and infants that were not exclusively breastfed by 3 months (Table 4).

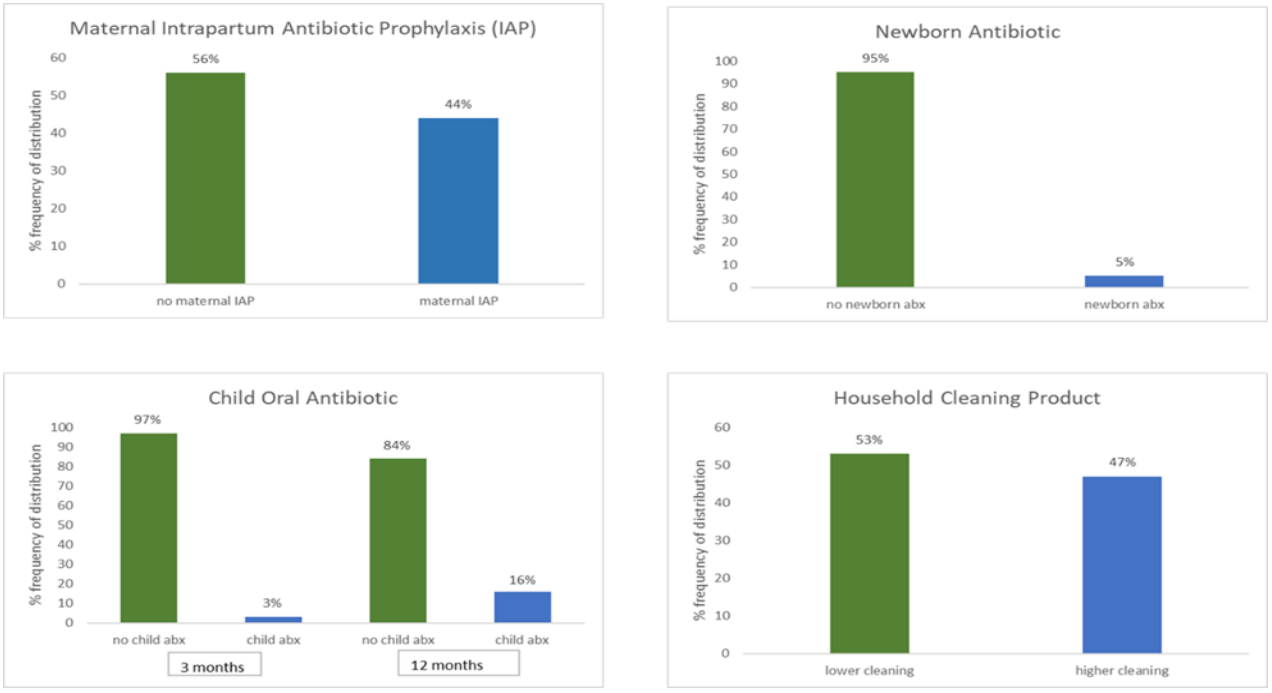
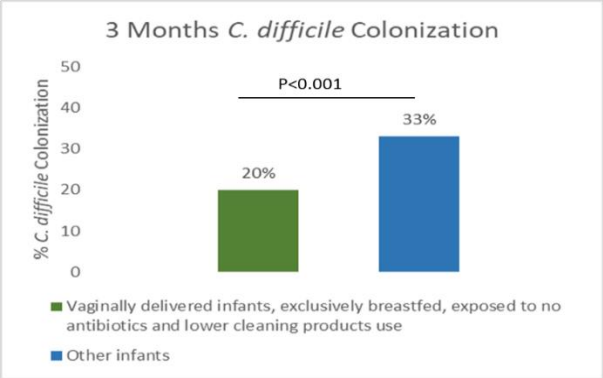


Figure 1. Frequency Distribution by Exposure in this Study Sample (abx: antibiotic)



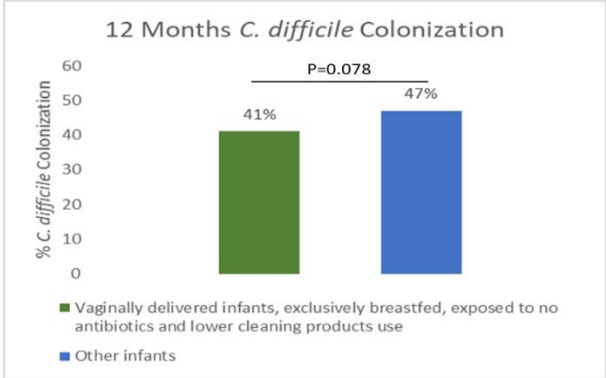


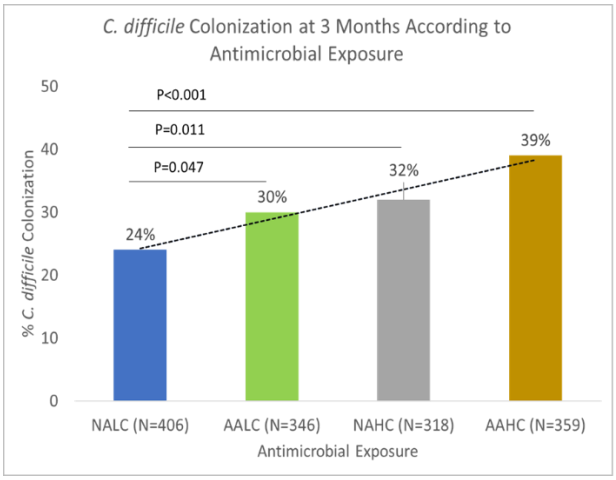
Figure 2. Colonization Rates of *C. difficile* in Vaginally Delivered Infants, Exclusively Breastfed, not Exposed to Antibiotics and Lower Cleaning Products Use Compared to Other Infants

Table 2. Population Characteristics According to Antimicrobial Exposure at 3 months (3 Month Sample; N=1,429)

Column percentages	**Total[a]**	**NALC* N (%)**	**AALC* N (%)**	**NAHC* N (%)**	**AAHC* N (%)**	**P value**
Birth mode						
Vaginal	1,096	406 (100%)	211 (61%)	317 (100%)	162 (46%)	**<0.001**
CS-elective	134	0	52 (15%)	0	82 (23%)	
CS-emergency	194	0	82 (24%)	0	112 (31%)	
Birthweight						
<2500g	31	12 (2%)	8 (3%)	4 (1%)	9 (3%)	0.402
2500 – 4000g	1,186	340 (84%)	286 (84%)	273 (87%)	287 (81%)	
>4000g	194	55 (14%)	44 (13%)	38 (12%)	57 (16%)	
Gestational age						
<39 weeks	372	73 (18%)	107 (31%)	73 (23%)	119 (34%)	**<0.001**
≥39 weeks	1,052	333 (82%)	238 (69%)	245 (77%)	236 (66%)	
Infant Sex						
Male	766	206 (51%)	196 (56%)	161 (51%)	203 (57%)	0.172
Female	663	200 (49%)	150 (43%)	157 (49%)	156 (43%)	
Breastfeeding at 3 Months						
Exclusive	791	262 (65%)	209 (61%)	153 (48%)	167 (47%)	**<0.001**
Mixed	384	98 (24%)	88 (26%)	85 (27%)	113 (32%)	
Formula	251	45 (11%)	48 (13%)	80 (25%)	78 (22%)	

Location						
Edmonton	388	94 (24%)	65 (19%)	106 (33%)	123 (34%)	**<0.001**
Vancouver	445	135 (33%)	147 (43%)	58 (18%)	105 (29%)	
Winnipeg	596	177 (43%)	134 (39%)	154 (48%)	131 (36%)	
Furry pets						
No	777	258 (64%)	205 (59%)	258 (48%)	205 (45%)	**<0.001**
Yes	648	146 (36%)	141 (41%)	166 (52%)	195 (55%)	
Older siblings						
No	712	189 (47%)	194 (56%)	125 (39%)	204 (57%)	**<0.001**
Yes	712	216 (53%)	150 (44%)	193 (61%)	153 (43%)	
Smoke exposure						
No	1160	352 (87%)	290 (85%)	251 (80%)	267 (76%)	**<0.001**
Yes	248	51 (13%)	50 (15%)	62 (20%)	85 (24%)	
Maternal age						
<30	503	148 (36%)	105 (30%)	137 (43%)	113 (31%)	**<0.001**
30 – 39	874	241 (59%)	220 (64%)	177 (56%)	236 (66%)	
≥40	52	17 (4%)	21 (6%)	4 (1%)	10 (3%)	
Maternal antibiotic (No IAP)[b]						
No	1,250	363 (90%)	295 (86%)	282 (90%)	310 (87%)	0.226
Yes	167	40 (10%)	48 (14%)	32 (10%)	47 (13%)	
Maternal prenatal depression						
No	1,072	315 (78%)	258 (75%)	232 (74%)	267 (75%)	0.532
Yes	338	86 (21%)	84 (25%)	81 (26%)	87 (25%)	
Maternal prenatal stress						
No	542	172 (43%)	130 (38%)	104 (33%)	136 (38%)	0.072
Yes	868	229 (57%)	212 (62%)	209 (67%)	218 (62%)	

Notes: *Antimicrobial Exposure (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use); [a]Total may not add up due to missing data; [b]maternal postnatal antibiotics after birth till 3 months; IAP: Intrapartum Antibiotic Prophylaxis; P value calculated using Pearson chi-square test, significant in **Bold.**



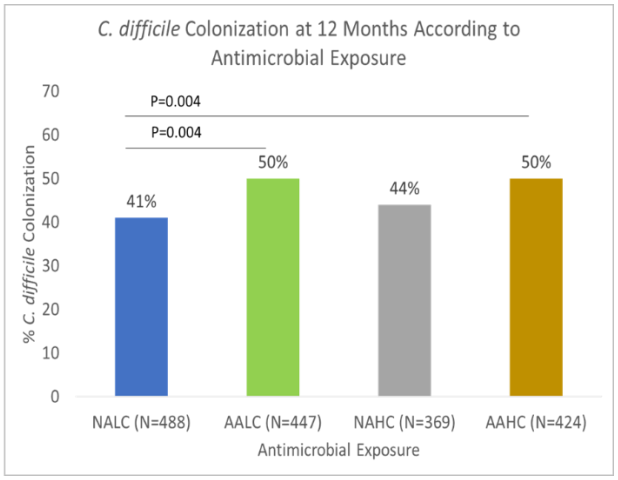


Figure 3. *C. difficile* Colonization According to Antimicrobial Exposure

Table 3. *C. difficile* Colonization at 3 Months & 12 Months According to Population Characteristics

	***C. difficile* Colonization at 3 Months Total N=1,429**			***C. difficile* Colonization at 12 Months Total N=1,728**		
Row percentages	**Total[a]**	***C. difficile* Yes, N (%)**	**P value (X^2 exact)**	**Total[a]**	***C. difficile* Yes, N (%)**	**P value (X^2 exact)**
Any antibiotics[b]						
No	724	200 (28%)	**0.004**	857	362 (42%)	**0.001**
Yes	705	245 (35%)		871	435 (50%)	
Cleaning products[c]						
Lower use	752	202 (29%)	**<0.001**	935	421 (45%)	0.333
Higher use	677	243 (36%)		793	376 (47%)	
Antimicrobial exposure*						
NALC	406	97 (24%)	**<0.001**	488	198 (41%)	**0.009**
AALC	346	105 (30%)		447	223 (50%)	
NAHC	318	103 (32%)		369	164 (44%)	
AAHC	359	140 (39%)		424	212 (50%)	
Birth mode						
Vaginal	1,096	314 (29%)	**0.002**	1297	584 (45%)	0.248
CS-elective	134	50 (37%)		175	86 (49%)	
CS-emergency	194	77 (40%)		252	126 (50%)	
Birthweight						

<2500g	31	9 (29%)	0.053	42	27 (64%)	**0.011**
2500 – 4000g	1,186	355 (30%)		1,440	670 (46%)	
>4000g	194	75 (39%)		225	90 (40%)	
Gestational age						
<39 weeks	372	115 (31%)	1.000	455	226 (50%)	0.070
≥39 weeks	1,057	326 (31%)		1,267	566 (45%)	
Infant Sex						
Male	766	255 (33%)	0.067	896	378 (42%)	**0.001**
Female	663	190 (29%)		832	419 (50%)	
Breastfeeding at 3 Months						
Exclusive	791	182 (23%)	**<0.001**	1072	488 (46%)	0.717
Mixed	384	142 (37%)		420	201 (48%)	
Formula	251	121 (48%)		435	108 (46%)	
Breastfeeding at 12 Months						
No	-	-	-	873	415 (46%)	0.306
Yes	-	-		822	370 (45%)	
Solids before 6m						
No	-	-	-	488	218 (45%)	0.485
Yes	-	-		1227	572 (47%)	
Location						
Edmonton	388	164 (42%)	**<0.001**	406	175 (43%)	0.300
Vancouver	445	97 (22%)		529	255 (48%)	
Winnipeg	596	184 (31%)		793	367 (46%)	
Furry pets						
No	777	217 (28%)	**0.005**	936	443 (47%)	0.244
Yes	648	226 (35%)		787	350 (44%)	
Older siblings						
No	712	240 (34%)	**0.034**	884	462 (52%)	**<0.001**
Yes	712	202 (28%)		839	333 (40%)	
Smoke exposure						
No	1,160	332 (29%)	**<0.001**	1,422	657 (46%)	0.697
Yes	248	103 (42%)		285	128 (45%)	

Maternal age						
<30	503	195 (39%)	**<0.001**	576	268 (47%)	0.916
30 – 39	874	238 (27%)		1,094	501 (46%)	
≥40	52	12 (23%)		58	28 (48%)	
Maternal antibiotic (No IAP)[d]						
No	1,250	388 (31%)	0.929	1,507	685 (45%)	0.102
Yes	167	51 (31%)		207	107 (52%)	
Maternal prenatal depression						
No	1,072	307 (29%)	**0.001**	1329	613 (46%)	0.907
Yes	338	129 (38%)		379	173 (46%)	
Maternal postnatal depression (6 Months)						
No	-	-	-	1335	610 (46%)	0.245
Yes	-	-		336	166 (49%)	
Maternal prenatal stress						
No	542	160 (30%)	0.375	706	326 (46%)	0.922
Yes	868	276 (31%)		1002	460 (46%)	
Maternal postnatal stress (6 Months)						
No	-	-	-	732	335 (46%)	0.621
Yes	-	-		940	442 (47%)	

Notes: *Antimicrobial Exposure by 3 months (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use); [a]Total may not add up due to missing data; [b]Exposure to maternal intrapartum antibiotic or infant antibiotic by 3 Months; [c]household cleaning product use by 3 months; [d]maternal postnatal antibiotics after birth till 3 months; IAP: Intrapartum Antibiotic Prophylaxis; P value calculated using Fisher's exact test, significant in **Bold.**

12 Months

In our study, 46% (797/1,728) of infants were colonized with *C. difficile* at 12 months. In a subsample of vaginally delivered infants, not exposed to antibiotics, living in households with lower cleaning products use and exclusively breastfed, *C. difficile* colonization reduced to 41% compared to 47% in the other infants (p=0.078) (Figure 2). The distribution of *C. difficile* colonization (yes or no) according to population characteristics is shown in Table 3. *C. difficile* colonized 41% of NALC infants, 50% of AALC infants, 44% of NAHC infants and 50% of AAHC infants (Figure 3). Overall, *C. difficile* colonization at 12 months was influenced by antimicrobial exposure (p=0.009). All covariates that were significantly associated with *C. difficile* at 3 months were no longer associated at 12 months except for having an older sibling (p<0.001). Other factors associated with *C. difficile* colonization at 12 months were birthweight (p=0.011) and infant sex (p<0.001) in the crude analysis.

The crude analysis from both Fisher's exact test and simple logistic regression showed that antimicrobial exposure from antibiotics was significantly associated with *C. difficile* colonization at 12 months. In comparison with NALC infants, the odds of colonization with *C. difficile* was 46% higher for both AALC infants (OR:1.46 95% CI 1.12-1.88; p=0.004) and AAHC infants (OR:1.46 95% CI 1.12-1.90; p=0.004). The odds of colonization with *C. difficile* was not different for NAHC infants compared to NALC infants showing higher use of cleaning products alone did not significantly affect *C. difficile* at 12 months in the crude analysis (Table 5). We also performed multiple logistic regression similar to the 3 months analysis to determine the adjusted odds of *C. difficile* colonization at 12 months in relation to antimicrobial exposure. Variables were selected to be included in the final model using purposeful model building as described by Hosmer and Lemeshow (24). The DAG model was further employed to increase understanding (Appendix Figure S5) (25). There was an interaction between cumulative antimicrobial exposure and infant sex on *C. difficile* colonization at 12 months. Interaction between antibiotics as well as cumulative antimicrobial exposure and siblings was also observed. The interaction results are reported in section 2.3.4. The crude and adjusted models for 12-month analysis are shown in table 5.

Many covariates were no longer significant with *C. difficile* colonization at 12 months, but we included birth method, gestational age, breastfeeding and pet ownership in the initial

model building because they are factors previously reported to affect the infant gut microbiota composition (14,26,27). In model 1, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for birth characteristics such as birth method and gestational age. The odds of *C. difficile* colonization significantly increased for the AALC infants (aOR: 1.44 95% CI 1.08-1.92; p=0.012) and AAHC infants (aOR: 1.47 95% CI 1.09-1.99; p=0.012) but not NAHC infants compared to the NALC infants (Table 5). In model 2, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for postnatal characteristics such as breastfeeding at 12 months, having a furry pet at home, having an older sibling and smoke exposure. Compared to the NALC infants, the odds of *C. difficile* colonization significantly increased for AALC infants (aOR: 1.34 95% CI 1.02-1.75; p=0.030) and AAHC infants (aOR: 1.37 95% CI 1.04-1.80; p=0.023) (Table 5).

In the final model, the association between antimicrobial exposure and *C. difficile* colonization was adjusted for variables gotten from purposeful model building i.e. birth method, breastfeeding at 12 months and having an older sibling. The odds of *C. difficile* colonization remained significantly higher in both the AALC infants (aOR: 1.36 95% CI 1.02-1.83; p=0.035) and the AAHC infants (aOR: 1.37 95% CI 1.00-1.86; p=0.043) compared to the NALC infants. In order to account for antibiotics given after 3 months to 12 months we performed sensitivity analysis by adjusting for infants who received antibiotics after 3 months up to 12 months as well as excluding infants that received antibiotics after 3 months up to 12 months. Similar results were obtained (Appendix Table S4). In the final model, the odds of colonization with *C. difficile* was significantly lower in infants with older siblings. There were no other significant association with *C. difficile* colonization at 12 months (Table 5).

Table 4. Crude and Adjusted Logistic Regression for Antimicrobial Exposure and *C. difficile* colonization at 3 months

	Crude (unadjusted)		**Model 1 (adjusted for maternal and birth characteristics**		**Model 2 (adjusted for postnatal characteristics)**		**Model 3 (adjusted for maternal, birth and postnatal characteristics)**		**Final Model**	
	OR (95% CI)	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Antimicrobial Exposure* (ref=NALC)										
AALC	1.38 (1.00-1.91)	**0.047**	1.23 (0.86-1.76)	0.238	1.37 (0.97-1.92)	0.068	1.25 (0.86-1.81)	0.226	1.21 (0.84-1.73)	0.293
NAHC	1.52 (1.10-2.11)	**0.011**	1.48 (1.06-2.06)	**0.020**	1.13 (0.79-1.60)	0.481	1.15 (0.81-1.63)	0.417	1.27 (0.90-1.78)	0.166
AAHC	2.03 (1.49-2.78)	**<0.001**	1.67 (1.16-2.41)	**0.006**	1.45 (1.04-2.03)	**0.027**	1.31 (0.89-1.93)	0.159	1.50 (1.03-2.17)	**0.032**
Block 1: Maternal and birth characteristics										
Maternal age (ref= 30-39)										
18-29	1.60 (1.34-2.13)	**<0.001**	1.79 (1.41-2.27)	**<0.001**			1.54 (1.19-2.01)	**0.001**	1.71 (1.34-2.18)	**<0.001**
≥40	0.80 (0.41-1.55)	0.513	0.79 (0.40-1.55)	0.505			0.88 (0.44-1.76)	0.720	0.86 (0.43-1.71)	0.688
Birth method (ref=vaginal)										
CS-elective	1.42 (1.02-2.15)	**0.039**	1.44 (0.93-2.22)	0.095			1.26 (0.80-1.99)	0.309	1.28 (0.83-1.99)	0.251
CS-emergency	1.63 (1.19-2.24)	**0.002**	1.54 (1.06-2.24)	**0.022**			1.49 (1.01-2.21)	**0.044**	1.49 (1.02-2.18)	**0.038**
Gestational age (ref≤39weeks)										
≥39 weeks	1.00 (0.77-1.29)	0.979	1.08 (0.83-1.41)	0.550						

Block 2: Postnatal characteristics										
Breastfeeding[a] (ref=Exclusive)										
Mixed	1.96 (1.50-2.55)	**<0.001**			1.81 (1.37-2.39)	**<0.001**	1.81 (1.37-2.39)	**<0.001**	1.88 (1.43-2.46)	**<0.001**
Formula	3.11 (2.31-4.19)	**<0.001**			2.57 (1.87-3.54)	**<0.001**	2.50 (1.81-3.44)	**<0.001**	2.71 (1.99-3.69)	**<0.001**
Location (ref=Vancouver)										
Edmonton	2.62 (1.94-3.55)	**<0.001**			2.41 (1.74-3.33)	**<0.001**	2.21 (1.59-3.07)	**<0.001**		
Winnipeg	1.60 (1.20-2.12)	**0.001**			1.40 (1.03-1.90)	**0.029**	1.25 (0.91-1.72)	0.155		
Furry pet (ref=No)										
Yes	1.38 (1.10-1.73)	**0.005**			1.12 (0.88-1.43)	0.343				
Older sibling (ref=No)										
Yes	0.77 (0.62-0.97)	**0.030**			0.75 (0.58-0.95)	**0.021**	0.81 (0.63-1.05)	0.119		
Smoke exposure (ref=No)										
Yes	1.77 (1.33-2.35)	**<0.001**			1.38 (1.02-1.87)	**0.036**	1.33 (0.98-1.80)	0.065		

Notes: *Antimicrobial Exposure by 3 months (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use);[a]Breastfeeding status at 3 months; OR: odds ratio, aOR: adjusted odds ratio; Significant P value in **Bold.**

Table 5. Crude and Adjusted Logistic Regression for Antimicrobial Exposure and *C. difficile* colonization at 12 months

	Crude (unadjusted)		**Model 1 (adjusted for birth characteristics)**		**Model 2 (adjusted for postnatal characteristics)**		**Final Model**	
	OR (95% CI)	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**	**aOR (95% CI)**	**P value**
Antimicrobial Exposure* (ref=NALC)								
AALC	1.46 (1.12-1.88)	**0.004**	1.44 (1.08-1.92)	**0.012**	1.34 (1.02-1.75)	**0.030**	1.36 (1.02-1.83)	**0.035**
NAHC	1.17 (0.89-1.54)	0.256	1.16 (0.88-1.53)	0.272	1.13 (0.85-1.51)	0.371	1.11 (0.84-1.48)	0.442
AAHC	1.46 (1.12-1.90)	**0.004**	1.47 (1.09-1.99)	**0.012**	1.37 (1.04-1.80)	**0.023**	1.37 (1.00-1.86)	**0.043**
Block 1: Birth characteristics								
Birth method (ref=vaginal)								
CS-elective	1.17 (0.86-1.61)	0.305	0.90 (0.63-1.28)	0.575			1.13 (0.78-1.63)	0.511
CS-emergency	1.22 (0.93-1.59)	0.148	0.97 (1.71-1.33)	0.892			0.86 (0.63-1.18)	0.374
Gestational age (ref≤39weeks)								
≥39 weeks	0.81 (0.66-1.01)	0.067	0.85 (0.68-1.06)	0.170				
Block 2: Postnatal characteristics								
Breastfeeding[a] (ref=Yes)								
No	1.10	0.297			1.09	0.369	1.08	0.399

	(0.91-1.34)				(0.89-1.33)		(0.89-1.32)	
Furry pet (ref=No)								
Yes	0.89 (0.73-1.07)	0.236			0.84 (0.68-1.02)	0.091		
Older sibling (ref=No)								
Yes	0.60 (0.49-0.72)	**<0.001**			0.60 (0.49-0.73)	**<0.001**	0.59 (0.48-0.73)	**<0.001**
Smoke Exposure (ref=No)								
Yes	0.94 (0.73-1.22)	0.690			0.91 (0.70-1.19)	0.533		

Notes: *Antimicrobial Exposure by 3 months (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use);[a]Breastfeeding at 12 months; OR: odds ratio, aOR: adjusted odds ratio; Significant P value in **Bold.**

2.3.4 Stratified Analysis and Modification of Antimicrobial Effects on *C. difficile* Colonization

We performed stratified analysis by important covariates to reduce heterogeneity and further explore the effect of antimicrobial exposure on *C. difficile* colonization as well as identify potential effect modifiers.

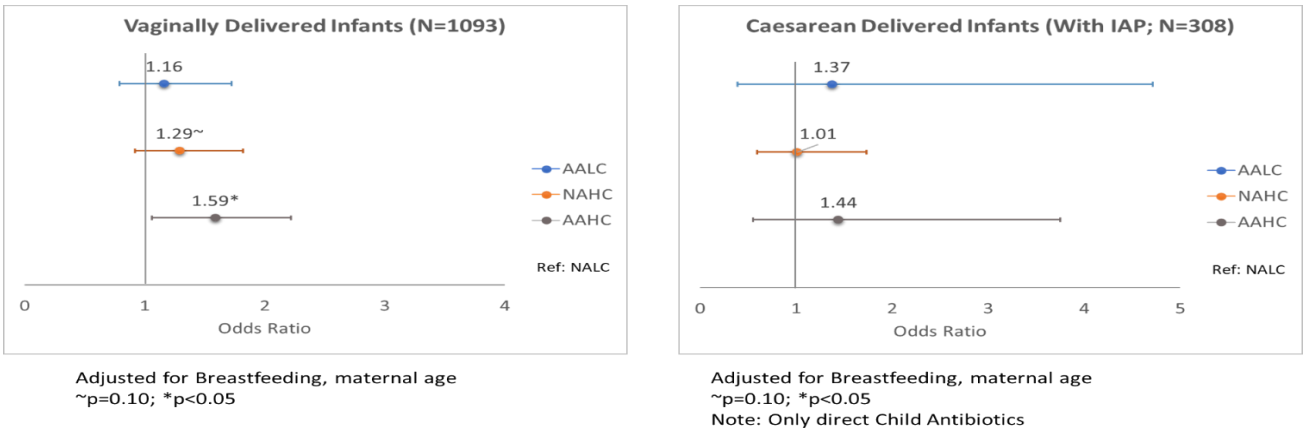
2.3.4.1 Antimicrobial Effect on *C. difficile* Colonization at 3 Months is Independent of Birth Mode and Modified by Maternal Prenatal Depression

In a subset of vaginally delivered infants, the odds of colonization with *C. difficile* was higher for AAHC infants (aOR: 1.59 95% CI 1.06-2.38) compared to NALC infants. The effect of any antibiotic exposure could not be examined in caesarean delivered infants because all were exposed to antibiotics via maternal IAP. Another analysis examining the additional effect from only direct infant antibiotics and household cleaning products use in caesarean delivered infants showed no association with *C. difficile* colonization at 3 months (Figure 4). Although the global interaction between antimicrobial exposure and prenatal depression was not significant (p=0.11), stratified analysis was done because of marginal significance with only antibiotics (p=0.08) (Appendix Table S5). In infants whose mothers experienced prenatal depression, those with cumulative exposure from antibiotics and higher cleaning products use (AAHC) had a 133% increased odds of *C. difficile* colonization (aOR: 2.33 95% CI 1.11-4.83; p=0.024) compared to those with no antibiotics and lower cleaning products use (NALC). This association was not observed for infants whose mothers did not experience prenatal depression (Appendix Figure S1)

2.3.4.2 Antimicrobial Effect on *C. difficile* Colonization at 12 Months is Modified by Infant Sex and Having an Older Sibling

Similar to 3 months, analysis in a subset of vaginally delivered infants showed increased odds of *C. difficile* colonization at 12 months for AAHC infants compared to NALC infants (aOR: 1.62 95% CI 1.15-2.29; p=0.005) (Figure 5). The effect of any antibiotic exposure could not be examined in caesarean delivered infants because all were exposed to antibiotics via maternal intrapartum antibiotics. Another analysis examining the additional effect from only direct infant antibiotics and household cleaning products use in caesarean delivered infants showed no association with *C. difficile* colonization at 12 months. Significant differences were observed in the stratified analysis by infant sex. Of note, significant interaction with antimicrobial exposure was not observed in the global test (p=0.21) but was observed between

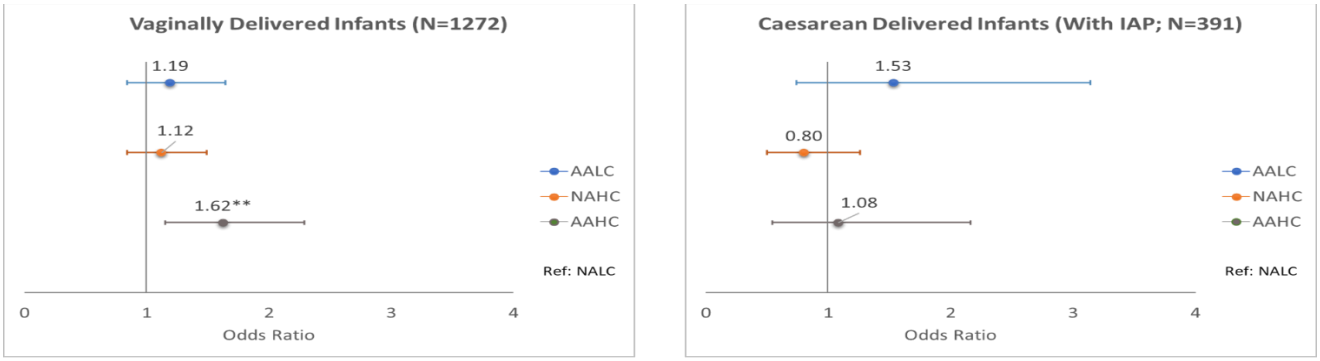
any antibiotics and infant sex (p=0.042) (Appendix Table S5). In males, the odds of *C. difficile* colonization at 12 months was higher for AALC infants (aOR: 1.49 95% CI 0.99-2.24; p=0.055) and AAHC infants (aOR: 1.56 95% CI 1.01-2.24; p=0.044) compared to NALC infants. This association was not observed in females (Appendix Figure S2). Antimicrobial effect on *C. difficile* colonization also varied between infants having an older sibling or not. In infants without an older sibling, the odds of colonization with *C. difficile* was 58% higher (aOR: 1.58 95% CI 1.07-2.35; p=0.021) and 77% higher (aOR: 1.77 95% CI 1.16-2.70; p=0.008) for the AALC and AAHC infants respectively. This association was not observed in infants with an older sibling (Appendix Figure S3).



Adjusted for Breastfeeding, maternal age
~p=0.10; *p<0.05

Adjusted for Breastfeeding, maternal age
~p=0.10; *p<0.05
Note: Only direct Child Antibiotics

Figure 4. Stratified Analysis by Birth Method for Antimicrobial Exposure and *C. difficile* Colonization at 3 Months



Adjusted for Breastfeeding, Siblings
~p=0.10; *p<0.05; **p<0.01

Adjusted for Breastfeeding, Siblings
~p=0.10; *p<0.05; **p<0.01
Note: Only direct Child Antibiotics

Figure 5. Stratified Analysis by Birth Method for Antimicrobial Exposure and *C. difficile* Colonization at 12 Months

2.3.5 Antimicrobial Exposure and *C. difficile* Colonization is Associated with Other Gut Microbes

Infant gut microbiota composition differed across groups of antimicrobial exposure at 3 months and to a lesser extent at 12 months. Of note, the most changes occurred in the cumulative exposure group (AAHC) where the highest risk of *C. difficile* colonization was observed. At 3 months, the relative abundance of Bifidobacteriaceae and Bacteroidaceae decreased while the relative abundance of Clostridaceae, Lachnospiraceae, Veillonellaceae and Enterobacteriaceae increased in the AALC and AAHC infants compared to the NALC infants. In the NAHC infants, the relative abundance of Lachnospiraceae and Ruminococcaceae increased compared to NALC infants (Appendix Figure S4). At 12 months, the most obvious changes were lower abundance of Bacteroidaceae in the AALC and AAHC infants compared to NALC infants, although Clostridaceae, Lachnospiraceae, Veillonellaceae and Enterobacteriaceae were still higher. In the NAHC infants, the relative abundance of Bifidobacteriaceae and Clostridaceae reduced compared to NALC infants (Appendix Figure S6).

2.4 Discussion

2.4.1 Main Findings

In our study, *Clostridioides difficile* (*C. difficile*) colonization of the infant gut microbiota differed according to antimicrobial exposure and was greatest in the cumulative exposure group compared to the reference group unexposed to antibiotics with lower cleaning products use. Increased colonization at 3 months persisted till 12 months but was mainly due to indirect (maternal IAP) antibiotic exposure. *C. difficile* was specifically chosen because of its reported association with asthma and allergic diseases. This microbe has also been identified as a marker for reduced colonization resistance and delayed gut microbiota maturation (18,20).

2.4.2 Interpretation

In our study, we examined and compared the separate and cumulative effect of antibiotics and household cleaning products on *C. difficile* colonization of the infant gut microbiota according to 4 groups: no antibiotics and lower cleaning products use (NALC), any antibiotics and lower cleaning products use (AALC), no antibiotics and higher cleaning products use (NAHC), any antibiotics and higher cleaning products use (AAHC). Infants were considered exposed to antibiotics if their mothers received intrapartum antibiotics (IAP) during vaginal or

caesarean delivery, or the infant directly received oral or intravenous (IV) antibiotics. Exposure to household cleaning products was based on frequency of use and split at the median into higher and lower cleaning products use.

Previous studies have evaluated the separate effect of maternal IAP or postnatal antibiotics on the infant gut microbiota (7,10,14,21). Fouhy et al. (21) were the first to use next generation sequencing techniques to examine the gut microbiota impact of IV ampicillin and gentamicin given to newborns. Differences in gut microbiota observed at 4 weeks persisted till 8 weeks and were driven by higher abundance of *Clostridium* and Proteobacteria, which is similar to our results. Likewise, researchers from the Baby & Mi cohort reported increased *Clostridium* abundance before 12 weeks in infants of mothers who received IAP (28). They also noted lower alpha diversity, delayed colonization and decreased *Bifidobacterium* abundance in the same infants which was influenced by length of IAP administration. The recently published study by Tapiainen et al. (7) reported differences from both IAP exposure and IV antibiotics in the infant gut microbiota compared to control group which were still observed at 6 months. The relative abundance of *Clostridium* was increased while *Bacteroides* was decreased in infants exposed to antibiotics. Consistent with what others have reported (7,21,28–30), our sub analysis of the gut microbiota at the family level showed reduction of Bifidobacteriaceae and Bacteroidaceae with antibiotic exposure as well as increase in Clostridaceae, Lachnospiraceae, Veillonellaceae and Enterobacteriaceae at both 3 and 12 months. Colonization of more Proteobacteria (a member of Enterobacteriaceae family) may be a signal for gut dysbiosis and inflammation (31), while reduction of important gut microbes provides room for *C. difficile* colonization and overgrowth.

Evidence on household cleaning products and the infant gut microbiota is limited. Many household cleaning products contain harmful chemicals with active ingredients ranging from alcohols, peroxides and halides to antimicrobial chemicals like triclosan and quaternary ammonium compounds (QAC) (32). In this study, we observed differences in *C. difficile* colonization at 3 months in infants exposed to higher cleaning products use which did not persist till 12 months. The CHILD cohort previously reported increasing abundance of Lachnospiraceae and *Ruminococcus* at 3 months associated with frequent use of household cleaning products and in particular those classified as disinfectants (9). In this study, analysis of gut microbes at the family level revealed higher abundance of Lachnospiraceae and Ruminococceae at 3 months in

infants exposed to higher cleaning products use which did not persist till 12 months. In addition to antibiotic exposure, continuous sublethal concentrations of cleaning disinfectants can give rise to resistant bacteria. Our results further demonstrate the combined effect of any antibiotic exposure and additional antimicrobial exposure from household cleaning products on *C. difficile* colonization of the infant gut microbiota. The effect of cumulative antimicrobial exposure on *C. difficile* colonization at 3 months which persisted till 12 months; though not as strong demonstrates that the collateral damage inflicted on the gut microbiota is not rapidly repaired. Our results support ongoing efforts to reduce antimicrobial exposure during infancy and especially in the neonatal period and find restorative or alternative interventions (e.g. probiotics, prebiotics, maternal GBS vaccination) to avoid harmful effect of antimicrobials on gut microbiota and future health.

Future health impacts from antimicrobial exposure in infancy has been reported but the mechanisms via which these exposures act are yet to be understood. In experimental models, mice exposed to antibiotics before weaning have disturbed fecal microbiota compared to mice not given antibiotics while germ free mice have impaired immune function and increased levels of immunoglobulin E (IgE) than their wild type counterparts (33,34). Previous epidemiological studies have shown that antimicrobials are associated with gut microbiota dysbiosis and shown that children with allergic diseases have different gut microbiota colonization compared to those without allergy (19,35,36). In addition, colonization with *C. difficile* has been associated with increased risk of atopic manifestations in childhood (18,37,38). In the KOALA birth cohort, colonization with *C. difficile* at 1 month was associated with an increased risk of wheeze, eczema and asthma at 7 years (18). Lee et al. (37) also reported that infants presenting with *C. difficile* colonization or infection have a higher risk of developing allergic diseases in early childhood. The CHILD study previously reported that colonization with another *Clostridium* specie; *C. neonatale* at 3 months was associated with development of preschool age asthma (38).

In our sub analysis of vaginally delivered infants, the cumulative effect of antimicrobial exposure on *C. difficile* colonization persisted even after adjusting for other covariates, showing the association was independent of caesarean section effect. We performed this analysis because the high correlation between birth method and maternal IAP may create bias towards the null when adjusting for birth method. In Canada, IAP is recommended for all CS deliveries so it is

difficult to determine if gut microbiota dysbiosis observed in CS born infants is from lack of exposure to vaginal microbes or lack of exposure to beneficial microbes sensitive to the antibiotics or both. Caesarean delivery and maternal IAP may have independent or additive effects on the infant gut microbiota (39). Our results examining effect of only direct infant antibiotics showed that it did not significantly affect of *C. difficile* colonization at both 3 and 12 months. Analogous to what others have reported (13,40,41), we found that infants born via caesarean section (CS) had an increased risk of *C. difficile* colonization. Penders et al. (41) showed that infants born via CS are more frequently colonized with *C. difficile* compared to vaginally delivered infants. However, we observed this association in only infants born via emergency CS. Most women undergoing emergency CS delivery planned to give birth vaginally and may have been positive for GBS. Hence, combined exposure to antibiotics for GBS and CS delivery may have increased the amount or duration of antimicrobial exposure to the newborn.

Several epidemiological studies have identified breastfeeding as an important contributor to the infant gut microbiota (14,15,41). In our study, exclusively breastfed infants were less likely to be colonized with *C. difficile* at 3 months than mixed fed infants or exclusively formula fed infants. This is in line with what we and other studies that reported on lower abundance of Clostridiales in breastfed infants (10,42). Fecal samples from breastfed infants are also characterized by enrichment of *Bifidobacteria* which is reported to be negatively correlated with *Clostridium* (10,43). Although the gut microbiota of exclusively breastfed babies is less diverse than mixed fed or exclusively formula fed babies before 6 months, it is dominated by more health promoting microbes which utilize HMOs (natural prebiotics) (15,44). Nogacka et al. (45) found that the effects of maternal IAP were more evident in formula fed than exclusively breastfed infants. Their population of formula fed infants probably comprised of mixed fed and exclusively formula fed since they were only specific for exclusive breastfeeding. In contrast, Mazzola et al. (30) reported greater effects from maternal IAP on *C. difficile* colonization in exclusively breastfed infants compared to mixed fed infants. We did not observe effect modification of antimicrobial exposure on *C. difficile* colonization by breastfeeding. Introduction to solids which correlates with cessation of breastfeeding increases the diversity of the infant gut microbiota to resemble that of an adult. Introduction of solids has been shown to be protective against *C. difficile* colonization (46), but we did not observe this association in our study.

We observed that antimicrobial exposure increased the likelihood of *C. difficile* colonization at 3 months in infants whose mothers experienced prenatal depression, suggesting prenatal depression modifies the relationship. It is still unclear if maternal depression directly influences infant gut microbiota composition, but some studies have linked stressful life events during pregnancy to increased risk of infections in women and their offspring (47,48). Hence, prenatal depression or stress may not be directly linked to gut microbiota acquisition but subsequent use of antibiotics to treat infections will have effect on the gut microbiota. In addition, one study showed that maternal prenatal stress was positively correlated with *Escherichia, Serratia, Enterobacter* and negatively correlated with *Lactobacillus, Lactoccus, Aerococcus and Bifidobacteria* (49). Depression also affects maternal mental health status and depressed mothers have been reported to be less likely to breastfeed which may predispose an infant to greater risk of infections (50).

Antimicrobial effect on *C. difficile* colonization at 12 months differed according to infant sex and according to if the infant had an older sibling or not. In the stratified analysis by infant sex, antibiotics alone and in combination with higher household cleaning products use increased the risk of *C. difficile* colonization in males but not females. Sex effect on gut microbiota composition is rarely studied but males have been reported to have higher bacterial colonization while females are first colonized by *Lactobacillus ruminis*, *Lactobacillus gasseri* and *Lactobacillus reuteri* (15). Infant sex might also play a role in *C. difficile* colonization before 1 year, however, evidence is lacking. In our study, having an older sibling significantly influenced *C. difficile* colonization at 12 months, where infants having an older sibling were less likely to be colonized. In accordance with our results, a systematic review of 6 studies reported a decreased abundance of *Clostridium* in infants with an older sibling (51). Having an older sibling also influenced the association between antimicrobial exposure and *C. difficile* colonization at 12 months. In infants without an older sibling i.e. firstborns, antibiotics alone and combined with higher cleaning products use increased the likelihood of *C. difficile* colonization at 12 months. In infants with an older sibling, antimicrobial exposure had no effect on *C. difficile* colonization at 12 months, suggesting a protective effect from having an older sibling. The presence of other children at home have been associated with changes in the infant gut microbiota. The influence of siblings on the infant gut microbiota is suggested to be in line with the “hygiene hypothesis”

in that they increase exposure to early gut colonizers that prime the immune system and provide colonization resistance against pathogens and *C. difficile* (10,15).

2.4.3 Strengths and Limitations

Our study has several strengths, including the application of high throughput sequencing and qPCR to profile gut microbiota *C. difficile* colonization, in a prospective birth cohort with representative and large sample size. To the best of our knowledge, this study is the first to examine the influence of household cleaning products and their cumulative effect with antibiotics on *C. difficile* colonization of the infant gut microbiota. Unique to our study is the capture of both perinatal IV and postnatal oral antibiotics.

Our findings should be interpreted considering some limitations. Exposure status to cleaning products were dependent on self-reported questionnaire and we were not able to determine specific ingredients contained in the cleaning products or perform analysis based on chemical composition. We did not also report on the transient nature or stable colonization of *C. difficile* since our sample collection was limited to two time points.

2.5 Conclusion

In Canada, oral antibiotic use in infants is low but exposure to intrapartum antibiotics given to the mother is increasing, owing to current recommendations for GBS and growing prevalence of caesarean delivery (4). Our study suggests that cumulative exposure to antibiotics and higher household cleaning products use is not without consequence. Previous studies have linked early life antimicrobial exposure to development of childhood asthma and allergic diseases but the mechanism for this association is unknown; *C. difficile* colonization and/or gut microbiota composition may or may not be a pathway. Further studies are required to replicate these findings in other populations and determine the impact on future health outcomes. It is also important to develop interventions to replenish beneficial infant gut microbiota, such as the use of probiotics and prebiotics (52) to minimize adverse effects of antimicrobials when they cannot be avoided.

2.6 References

1. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014;14(8):742–50.

2. van Schalkwyk J, Van Eyk N. Antibiotic prophylaxis in obstetric procedures. J Obstet Gynaecol Can. 2010;32(9):878–92.

3. Jefferies AL. Management of term infants at increased risk for early-onset bacterial sepsis. Paediatr Child Heal. 2017;22(4):223–8.

4. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, Kozyrskyj AL. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: A population-based study. J Matern Neonatal Med. 2015 Jul 3;28(10):1190–5.

5. Fjalstad J, Stensvold H, Bergseng H, Simonsen G, Salvesen B, Rønnestad A, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. Pediatr Infect Dis J. 2016;35:1–6.

6. Nazaroff WW, Weschler CJ. Cleaning products and air fresheners: Exposure to primary and secondary air pollutants. Atmos Environ. 2004;38(18):2841–65.

7. Tapiainen T, Koivusaari P, Brinkac L, Lorenzi HA, Salo J, Renko M, et al. Impact of intrapartum and postnatal antibiotics on the gut microbiome and emergence of antimicrobial resistance in infants. Sci Rep. 2019;9(1):1–11.

8. Wei S, Mortensen MS, Stokholm J, Brejnrod AD, Thorsen J, Rasmussen MA, et al. Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: A double-blind, randomized, placebo-controlled trial. EBioMedicine. 2018;38:265–72.

9. Tun MH, Tun HM, Mahoney JJ, Konya TB, Guttman DS, Becker AB, et al. Postnatal exposure to household disinfectants, infant gut microbiota and subsequent risk of overweight in children. Cmaj. 2018;190(37):E1097–107.

10. Azad M, Konya T, Persaud R, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. BJOG An Int J Obstet Gynaecol.

2016;123(6):983–93.

11. Ni J, Friedman H, Boyd BC, McGurn A, Babinski P, Markossian T, et al. Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. BMC Pediatr. 2019;19(1):1–8.

12. Obiakor C V., Tun HM, Bridgman SL, Arrieta M-C, Kozyrskyj AL. The association between early life antibiotic use and allergic disease in young children: recent insights and their implications. Expert Rev Clin Immunol. 2018;14(10):841–55.

13. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107(26):11971–5.

14. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 2016;8(343):1–14.

15. Martin R, Makino H, Yavuz AC, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS One. 2016;11(6):1–30.

16. Korpela K, Zijlmans MAC, Kuitunen M, Kukkonen K, Savilahti E, Salonen A, et al. Childhood BMI in relation to microbiota in infancy and lifetime antibiotic use. Microbiome. 2017;5(1):1–9.

17. Lees EA, Miyajima F, Pirmohamed M, Carrol ED. The role of Clostridium difficile in the paediatric and neonatal gut — a narrative review. Eur J Clin Microbiol Infect Dis. 2016;35(7):1047–57.

18. Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol. 2011;128(5):948-955.e3.

19. Penders J, Thijs C, Van Den Brandt PA, Kummeling I, Snijders B, Stelma F, et al. Gut microbiota composition and development of atopic manifestations in infancy: The KOALA birth cohort study. Gut. 2007 May;56(5):661–7.

20. Penders J, Stobboringh EE, Van Den Brandt PA, Van Ree R, Thijs C. Toxigenic and non-toxigenic Clostridium difficile: Determinants of intestinal colonisation and role in childhood atopic manifestations. Gut. 2008;57(7):1025–6.

21. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother. 2012;56(11):5811–20.

22. Azad MB, Konya T, Guttman DS, Field CJ, Sears MR, Hayglass KT, et al. Infant gut microbiota and food sensitization: Associations in the first year of life. Clin Exp Allergy. 2015;45(3):632–43.

23. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology. 2002;148(1):257–66.

24. Hosmer D, Lemeshow S. Applied Logistic Regression. Second Edi. New York: Wiley; 2000. p.1–397.

25. Textor J, Hardt J. DAGitty: A graphical tool for analyzing causal diagrams. Epidemiology. 2011;22(5):745.

26. Ferraris L, Butel MJ, Campeotto F, Vodovar M, Rozé JC, Aires J. Clostridia in premature neonates' gut: Incidence, antibiotic susceptibility, and perinatal determinants influencing colonization. PLoS One. 2012;7(1):e30594.

27. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Sears MR, et al. Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. Allergy, Asthma Clin Immunol. 2013;9(15):1–9.

28. Stearns JC, Simioni J, Gunn E, McDonald H, Holloway AC, Thabane L, et al. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. Sci Rep. 2017;7:16527.

29. Coker MO, Hoen AG, Dade E, Lundgren S, Li Z, Wong AD, et al. Specific class of

intrapartum antibiotics relates to maturation of the infant gut microbiota : a prospective cohort study. BJOG An Int J Obstet Gynaecol. 2019;1–11.

30. Mazzola G, Murphy K, Ross RP, Di Gioia D, Biavati B, Corvaglia LT, et al. Early gut microbiota perturbations following intrapartum antibiotic prophylaxis to prevent group B streptococcal disease. PLoS One. 2016;11(6):1–11.

31. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33(9):496–503.

32. Bondi CAM. Applying the precautionary principle to consumer household cleaning product development. J Clean Prod. 2011;19(5):429–37.

33. Cox LM, Yamanishi S, Sohn J, Alekseyenko A V., Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705–21.

34. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe. 2013;14(5):559–70.

35. Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015;7(307).

36. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol. 2012;129(2):434–40.

37. Lee SH, Gong YN, Ryoo E. Clostridium difficile colonization and / or infection during infancy and the risk of childhood allergic diseases. 2017;60(5):145–50.

38. Stiemsma LT, Arrieta M-C, Dimitriu PA, Cheng J, Thorson L, Lefebvre DL, et al. Shifts in Lachnospira and Clostridium sp. in the 3-month stool microbiome are associated with preschool age asthma. Clin Sci. 2016;130(23):2199–207.

39. Lemas DJ, Yee S, Cacho N, Miller D, Cardel M, Gurka M, et al. Exploring the

contribution of maternal antibiotics and breastfeeding to development of the infant microbiome and pediatric obesity. Semin Fetal Neonatal Med. 2016;21(6):406–9.

40. Adlerberth I, Strachan DP, Matricardi PM, Ahrné S, Orfei L, Åberg N, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol. 2007;120(2):343–50.

41. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(2):511–21.

42. Hesla HM, Stenius F, Jäderlund L, Nelson R, Engstrand L, Alm J, et al. Impact of lifestyle on the gut microbiota of healthy infants and their mothers - the ALADDIN birth cohort. FEMS Microbiol Ecol. 2014;90(3):791–801.

43. Tanaka S, Kobayashi T, Songjinda P, Tateyama A, Tsubouchi M, Kiyohara C, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol Med Microbiol. 2009;56(1):80–7.

44. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17(5).

45. Nogacka AM, Salazar N, Arboleya S, Suárez M, Fernández N, Solís G, et al. Early microbiota, antibiotics and health. Cell Mol Life Sci. 2018;75(1):83–91.

46. Davis MY, Zhang H, Brannan LE, Carman RJ, Boone JH. Rapid change of fecal microbiome and disappearance of Clostridium difficile in a colonized infant after transition from breast milk to cow milk. Microbiome. 2016;4:1–10.

47. Field T, Hernandez-Reif M, Diego M. Risk factors and stress variables that differentiate depressed from nondepressed pregnant women. Infant Behav Dev. 2006;29(2):169–74.

48. Nielsen NM, Hansen AV, Simonsen J, Hviid A. Prenatal stress and risk of infectious diseases in offspring. Am J Epidemiol. 2011;173(9):990–7.

49. Zijlmans MAC, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. Maternal

prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology. 2015;53:233–45.

50. Bascom EME, Napolitano MA. Breastfeeding Duration and Primary Reasons for Breastfeeding Cessation among Women with Postpartum Depressive Symptoms. J Hum Lact. 2016;32(2):282–91.

51. Chan CWH, Wong RS, Law PTW, Wong CL, Tsui SKW, Tang WPY, et al. Environmental factors associated with altered gut microbiota in children with eczema: A systematic review. Int J Mol Sci. 2016;17:1–14.

52. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. Microbiome. 2018 Oct;6(1):182.

Chapter 3: General Discussion and Conclusion

3.1 Assessment of Bias

3.1.1 Selection Bias

Selection bias occurs when the sample is not representative of the population due to the way participants were enrolled or followed up in a study. This bias is reduced because CHILD cohort is a prospective birth cohort where participants were recruited from multiple sites using various means such as clinics, booths at tradeshows, fax and by phone calls. However, most CHILD study participants were from urban residences, educated and had higher allergy rates than the general population (22.2% vs 9.2%) which may limit generalizability of study (external validity). CHILD study also has high retention rate at study sites of approximately 92% at 12 months of age (1). Attrition bias, a type of selection bias may arise from missing data leading to poor adjustment for some variables; however, comparisons between this study samples and the overall CHILD cohort showed no differences (Table 1). One limitation in sample collection is that breastfed infants tend to have more watery stool samples; which made it difficult to obtain enough quantity from diaper and limited our ability to use all breastfed infant samples in microbial profiling assays. This resulted in sequencing of more stool samples from formula and partially breast-fed infants. However, qPCR doesn't require large sample and was enough for the *C. difficile* assays.

3.1.2 Measurement Bias

Measurement bias occurs from poor measurement or inconsistencies of the study outcome between groups of interest. It deals with the validity of information obtained on study participants. This bias is minimized because CHILD study used the same validated questionnaires, database source and sample collection methods to obtain information on all participants. Another strength of this study is the prospective collection of study data which minimizes problems associated with retrospective collection. Recall bias from errors in measuring exposure variables was also avoided due to the retrieval of birth method, maternal IAP and newborn antibiotic from hospital charts. However, using hospital chart or database to retrieve information increases chances of non-differential misclassification which leads to bias toward the null. An internal validity test was done for the questionnaire on household cleaning

products by the research assistant to look at products reported by the mothers during the home visit and has also been used in Seattle Healthy Homes study.

Measurement bias in using questionnaires may arise when interviewer or participants are aware of study exposures and outcomes. Demographics such as maternal smoking status and certain socioeconomic status indicators may be under-reported or misreported due to societal pressures but is not expected to be differential in study groups. Questionnaires collected at multiple time points were also assessed for changes in response. Miscommunication and language barriers can also lead to inaccurate reporting. CHILD exclusion criteria involved exclusion of participants that were unable to read or write in English to minimize errors from language barriers. Microbiota sequencing in CHILD involves using a mixture of qPCR and 16S rRNA measurements to make findings more robust. For this study, bias from real time qPCR methods is a possibility but is reduced due to the way results were analyzed. *C. difficile* was quantified from the 16S region and one lab technician ran the qPCR using specific primers and probes to reduce measurement bias. False positives (below a specified level of detection) were removed and *C. difficile* genomes that exceeded total bacteria were identified as errors.

3.1.3 Confounding Bias

Confounding bias is one of the most important issues with establishing causality especially in observational studies. Confounding occurs when an association between the exposure and outcome is masked by or partially or totally accounted for by a third extraneous variable. This bias was addressed by establishing an a priori hypothesis to effectively determine the association between antimicrobial exposure and *C. difficile* colonization of the infant gut microbiota. Covariates were collected in CHILD validated questionnaires and adjusted for when performing statistical analysis. Given that CHILD was not primarily designed to answer this study's research question, some covariates such as maternal microbiome (stool and vaginal) and maternal infection status were not collected. Important covariates for this research question were obtained and those related to both exposure and outcome in the final model were adjusted for using multiple logistic regression analysis. To reduce heterogeneity, account for variables not properly adjusted due to collinearity and identify effect modifiers, stratified analysis was also performed. Although, birth mode was adjusted for, another analysis in only vaginal delivered infants showed that cumulative antimicrobial exposure from antibiotic and household cleaning products affected

C. difficile colonization independent of CS. However, due to the study design and because the proposed study is a secondary analysis, residual confounding specific to the previously outlined research questions are of concern.

3.2 Strengths and Limitations of the Study

3.2.1 Strengths

Our study has several strengths, including the application of high throughput sequencing and qPCR to profile gut microbiota *C. difficile* colonization in a birth cohort with a representative and large sample size. High throughput sequencing is desirable for complete identification of bacterial taxa but is limited to genus while qPCR enables identification up to species level. This study is the first to examine the influence of household cleaning products and their cumulative effect with antibiotics on *C. difficile* colonization of the infant gut microbiota. Although *C. difficile* has been reported in relation to antibiotics, most studies are in the adult population while those in infants are of smaller sample size. Our large sample enabled us to examine separate and cumulative effect on *C. difficile* colonization from antibiotics and household cleaning products as well as perform multivariable adjustment and stratified analysis to identify confounders and effect modifiers. We first examined effect of maternal IAP and direct infant antibiotics before classifying infants as exposed to any antibiotics or not. Exposure to household cleaning products was based on frequency of use where participants were grouped into higher or lower cleaning products use. Our unadjusted and adjusted analysis showed that antimicrobials influenced *C. difficile* colonization of the infant gut microbiota.

Temporality is a strength of our study in relation to *C. difficile* colonization at 12 months, since exposures were assessed at 3 months. CHILD study design enables prospective collection of information from the same people over time to measure changes in outcome associated with the exposure. Our exposures for this study occurred before or at 3 months, and maternal IAP; which had the strongest effect on *C. difficile* colonization occurred at birth. We were also able to collect and adjust for antibiotics given after 3 months which did not significantly change the results. We analyzed *C. difficile* as our main outcome because it has been previously identified as a biomarker for gut dysbiosis in infants and subsequent development of allergic diseases.

3.2.2 Limitations

Our findings should also be interpreted considering some limitations. Study exposure variables (household cleaning products use, infant oral antibiotics) and some important covariates (breastfeeding, having an older sibling) were measured by self-administered questionnaire. Household cleaning products use, and infant oral antibiotics use were obtained at 3 months during stool collection so recall and measurement bias may be a problem. The cross-sectional nature of household cleaning products use, infant oral antibiotics and *C. difficile colonization* at 3 months makes it difficult to determine direction. However, while it is possible for *C. difficile* colonization to have been altered by another factor, it is unlikely that *C. difficile* colonization altered these exposures. In addition, we were not able to determine specific ingredients contained in the cleaning products and perform analysis based on chemical composition.

Our study results may not be generalizable to the whole Canadian population because CHILD study participants were predominantly recruited from urban area. However, 80% of Canadian population lives in urban area (1). We did not report on the transient nature or stable colonization since our sample collection was limited to two time points. We were not able to differentiate toxigenic and non-toxigenic strains due to nature of methods used for sequencing. We did not report on the class or spectrum of direct antibiotic prescribed because only a small proportion (8%) of infants were exposed before 3 months. In addition, previous studies in Canadian cohort have shown most prescription for infant antibiotics are ampicillin/amoxicillin while maternal IAP for GBS is penicillin and antibiotic for CS is cefazolin (2–4).

3.3 Knowledge Translation

This study will benefit the study participants and global community by contributing to the body of knowledge surrounding antimicrobial exposure and potential health impacts. The results will also be useful to many researchers and physicians. The findings have been reported in a thesis manuscript that will be published to contribute to the limited literature on this topic; and the results will be available to the public. Other goals include presenting results at various research conferences and in the media/news. By understanding the effect of early life antimicrobial exposures, we can determine the impact of these factors on gut microbiota; and identify potential microbes that can predict future allergic diseases in children.

3.4 Clinical Relevance

Early life colonization of the infant gut microbiota is crucial for the maturation of the immune system and is influenced by environmental and host factors. Exposure to antibiotics, method of birth and infant diet are the most common factors known to affect infant gut microbiota acquisition. There is little evidence showing the additional antimicrobial effect from the use of household cleaning products on the infant gut microbiota.

Significant associations between antimicrobials and *C. difficile* colonization in this study support evidence of early life factors that influence infant gut microbiota. The impact of these factors and consequential changes may influence development of allergic diseases in childhood. Hence, our results add to the existing literature to help inform physician and parent decisions around the use of antibiotics and household cleaning products and would be beneficial in clinical practice.

3.5 Implications for Future Research

Many questions are yet to be answered. The infant gut microbiota is increasingly appreciated as an important contributor to immunity and health development. Further research is needed to explore the relationship between antimicrobials, gut microbiota and health outcomes (e.g. allergic and immunologic diseases). Studies are also required to assess the mechanisms and interrelationship between antimicrobial exposure, *C. difficile* and other important gut species in relation to gut microbiota dysbiosis. These questions could be first explored under controlled experiments using animal models; however, the challenge is extrapolating results to humans due to differences in physiology and growth trajectory. In addition, studies that explore alternate interventions to maternal IAP (e.g. GBS vaccination) or replenishment of gut microbiota (e.g. probiotics and prebiotics) are needed.

3.6 Conclusion

Early life development is critical and factors that affect development may have irreversible impacts on future health. In this thesis, the separate and cumulative effect of antibiotics and household cleaning products on *C. difficile* colonization was reported in relation to composition of infant gut microbiota. Our results showed increasing colonization with *C. difficile* with higher antimicrobial exposure. Findings from this thesis will provide a population-based evidence and

contribute to the literature on antimicrobials and their effect on infant gut microbiota composition.

3.7 References

1. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR, et al. The Canadian Healthy Infant Longitudinal Development (CHILD) study: Examining developmental origins of allergy and asthma. Thorax. 2015;70(10):998–1000.

2. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, Kozyrskyj AL. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: A population-based study. J Matern Neonatal Med. 2015 Jul 3;28(10):1190–5.

3. Stearns JC, Simioni J, Gunn E, McDonald H, Holloway AC, Thabane L, et al. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. Sci Rep. 2017;7:16527.

4. van Schalkwyk J, Van Eyk N. Antibiotic prophylaxis in obstetric procedures. J Obstet Gynaecol Can. 2010;32(9):878–92.

BIBLIOGRAPHY

1. Zeissig S, Blumberg RS. Life at the beginning: Perturbation of the microbiota by antibiotics in early life and its role in health and disease. Nat Immunol. 2014;15(4):307–10.

2. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH Human Microbiome Project. Genome Res. 2009;19(12):2317–23.

3. Ursell L, Clemente J, Rideout J, Gevers D, Caporaso G, Knight R. The interpersonal and intrapersonal diversity of humanassociated microbiota in key body sites. J Allergy Clin Immunol. 2012;129(5):1204–8.

4. Strachan DP. Hay fever, hygiene, and household size. Br Med J. 1989;299:1259–60.

5. Hunter P. The changing hypothesis of the gut. EMBO Rep. 2012;13(6):498–500.

6. Flemming A. The discovery and development of penicillin. The Medical press. 1999. p. 1–11.

7. Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, et al. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. Lancet Infect Dis. 2014;14(8):742–50.

8. Ting JY, Synnes A, Roberts A, Deshpandey A, Dow K, Yoon EW, et al. Association between antibiotic use and neonatal mortality and morbidities in very low-birth-weight infants without culture-proven sepsis or necrotizing enterocolitis. JAMA Pediatr. 2016;170(12):1181–7.

9. Frenk SM, Kit BK, Lukacs SL, Hicks LA, Gu Q, Public S, et al. Trends in the use of prescription antibiotics: NHANES 1999-2012. 2016;71(1):251–6.

10. Zwittink RD, Renes IB, van Lingen RA, van Zoeren-Grobben D, Konstanti P, Norbruis OF, et al. Association between duration of intravenous antibiotic administration and early-life microbiota development in late-preterm infants. Eur J Clin Microbiol Infect Dis. 2018;37(3):475–83.

11. Saux N Le, Robinson JL, Society CP. Canadian Pediatric Society Position Statement:

Management of acute otitis media in children six months of age and older. Pediatr Child Heal. 2016;21(1):1–8.

12. Robinson JL, Finlay JC, Lang ME, Bortolussi R, Bridger NA, Martin S, et al. Urinary tract infection in infants and children: Diagnosis and management. Paediatr Child Heal. 2014;19(6):315–9.

13. Vaz LE, Kleinman KP, Raebel MA, Nordin JD, Lakoma MD, Dutta-Linn MM, et al. Recent trends in outpatient antibiotic use in children. Pediatrics. 2014;133(3):375–85.

14. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H, Calle GM, et al. The worldwide antibiotic resistance and prescribing in european children (ARPEC) point prevalence survey: Developing hospital-quality indicators of antibiotic prescribing for children. J Antimicrob Chemother. 2016;71(4):1106–17.

15. Le Doare K, O'Driscoll M, Turner K, Seedat F, Russell NJ, Seale AC, et al. Intrapartum Antibiotic Chemoprophylaxis Policies for the Prevention of Group B Streptococcal Disease Worldwide: Systematic Review. Clin Infect Dis. 2017;65(Suppl 2):S143–51.

16. Madrid L, Seale AC, Kohli-Lynch M, Edmond KM, Lawn JE, Heath PT, et al. Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(Suppl 2):S160–72.

17. Seedat F, Stinton C, Patterson J, Geppert J, Tan B, Robinson ER, et al. Adverse events in women and children who have received intrapartum antibiotic prophylaxis treatment: A systematic review. BMC Pregnancy Childbirth. 2017;17:247.

18. Jefferies AL. Management of term infants at increased risk for early-onset bacterial sepsis. Paediatr Child Heal. 2017;22(4):223–8.

19. Stokholm J, Schjørring S, Pedersen L, Bischoff AL, Følsgaard N, Carson CG, et al. Prevalence and predictors of antibiotic administration during pregnancy and birth. PLoS One. 2013;8(12):1–7.

20. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, Kozyrskyj AL. Perinatal antibiotic exposure of neonates in Canada and associated risk factors: A population-based study. J Matern Neonatal Med. 2015 Jul 3;28(10):1190–5.

21. Ohlsson A, Shah VS. Intrapartum antibiotics for known maternal Group B streptococcal colonization. Cochrane Database Syst Rev. 2014;2014(6).

22. van Schalkwyk J, Van Eyk N. Antibiotic prophylaxis in obstetric procedures. J Obstet Gynaecol Can. 2010;32(9):878–92.

23. Didier C, Streicher MP, Chognot D, Campagni R, Schnebelen A, Messer J, et al. Late-onset neonatal infections: Incidences and pathogens in the era of antenatal antibiotics. Eur J Pediatr. 2012;171(4):681–7.

24. Tapiainen T, Koivusaari P, Brinkac L, Lorenzi HA, Salo J, Renko M, et al. Impact of intrapartum and postnatal antibiotics on the gut microbiome and emergence of antimicrobial resistance in infants. Sci Rep. 2019;9(1):1–11.

25. Vangay P, Ward T, Gerber J, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. Cell Host Microbe. 2015;17(5):553–64.

26. Penders J, Kummeling I, Thijs C. Infant antibiotic use and wheeze and asthma risk: A systematic review and meta-analysis. Eur Respir J. 2011;38(2):295–302.

27. Nazaroff WW, Weschler CJ. Cleaning products and air fresheners: Exposure to primary and secondary air pollutants. Atmos Environ. 2004;38(18):2841–65.

28. Dimitroulopoulou C, Lucica E, Johnson A, Ashmore MR, Sakellaris I, Stranger M, et al. EPHECT I: European household survey on domestic use of consumer products and development of worst-case scenarios for daily use. Sci Total Environ. 2015;536:880–9.

29. Herr M, Just J, Nikasinovic L, Foucault C, Le Marec AM, Giordanella JP, et al. Influence of host and environmental factors on wheezing severity in infants: Findings from the PARIS birth cohort. Clin Exp Allergy. 2012;42(2):275–83.

30. Lao XQ, Ho KF, Wong CC, Tian LW. Respiratory health effects of household cleaning products on Hong Kong school children. Hong Kong Med J. 2019;25(1):24–6.

31. Wolkoff P, Schneider T, Kildesø J, Degerth R, Jaroszewski M, Schunk H. Risk in cleaning : chemical and physical exposure. Sci Total Environ. 1998;215:135–56.

32. McLachlan JA, Arnold SF. Environmental estrogens. Am Sci. 1996;84(5):452–61.

33. EWG. Pollution in People: Toxic Chemicals Found in Minority Cord Blood. Washington, DC. 2009 [cited 2019 Oct 3]. Available from: https://www.ewg.org/news/news-releases/2009/12/02/toxic-chemicals-found-minority-cord-blood

34. Ouimette AMY. Cleaning House : Considerations of Ecological Health and Sustainability in the Selection of Household Cleaning Products. 2011.

35. Altman RG, Morello-Frosch R, Brody JG, Rudel R, Brown P, Averick M. Pollution comes home and gets personal: Women's experience of household chemical exposure. J Health Soc Behav. 2008;49(4):417–35.

36. Padiyara P, Inoue H, Sprenger M. Global Governance Mechanisms to Address Antimicrobial Resistance. Infect Dis Res Treat. 2018;11:1–4.

37. Downie L, Armiento R, Subhi R, Kelly J, Clifford V, Duke T. Community-acquired neonatal and infant sepsis in developing countries: Efficacy of WHO's currently recommended antibiotics - Systematic review and meta-analysis. Arch Dis Child. 2013;98(2):146–54.

38. Laxminarayan R, Matsoso P, Pant S, Brower C, Røttingen JA, Klugman K, et al. Access to effective antimicrobials: A worldwide challenge. Lancet. 2016;387(10014):168–75.

39. Yassour M, Vatanen T, Siljander H, Hämäläinen A. Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability. Sci Transl Med. 2016;8(343).

40. Sun D. Pull in and push out: Mechanisms of horizontal gene transfer in bacteria. Front Microbiol. 2018;9:1–8.

41. Jacoby GA. AmpC B-Lactamases. Clin Microbiol Rev. 2009;22(1):161–82.

42. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, et al. Multidrug efflux pumps: structure, function and regulation. Nat Rev Microbiol. 2018;16(9):523–39.

43. Ramirez CB, Cantey JB. Antibiotic resistance in the neonatal intensive care unit. Neoreviews. 2019;20(3):e135–44.

44. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, et al.

Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat Commun. 2018;9(1):1–11.

45. WHO. Global Strategy for Infant and Young Child Feeding. Geneva. 2003 [cited 2019 Oct 3]. p. 1–37. Available from: https://www.who.int/nutrition/publications/infantfeeding/9241562218/en/

46. Lozupone CA, Stombaugh J, Gordon JI, Jansson J, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489(7415):220–30.

47. Wampach L, Heintz-Buschart A, Hogan A, Muller EEL, Narayanasamy S, Laczny CC, et al. Colonization and succession within the human gut microbiome by archaea, bacteria, and microeukaryotes during the first year of life. Front Microbiol. 2017;8:1–21.

48. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-Gut Microbiota Metabolic Interactions. Science. 2012;336:1262–8.

49. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9(5):313–23.

50. Jauréguy F, Carton M, Panel P, Foucaud P, Butel MJ, Doucet-Populaire F. Effects of intrapartum penicillin prophylaxis on intestinal bacterial colonization in infants. J Clin Microbiol. 2004;42(11):5184–8.

51. Palleja A, Mikkelsen KH, Forslund SK, Kashani A, Allin KH, Nielsen T, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. Nat Microbiol. 2018;3:1255–65.

52. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118(2):511–21.

53. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev. 2017;81(4):1–67.

54. Omenetti S, Pizarro TT. The Treg/Th17 axis: A dynamic balance regulated by the gut

microbiome. Front Immunol. 2015;6:1–8.

55. Al-Asmakh M, Zadjali F. Use of germ-free animal models in microbiota-related research. J Microbiol Biotechnol. 2015;25(10):1583–8.

56. Cox LM, Yamanishi S, Sohn J, Alekseyenko A V., Leung JM, Cho I, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell. 2014;158(4):705–21.

57. Lynn MA, Tumes DJ, Choo JM, Sribnaia A, Blake SJ, Leong LEX, et al. Early-Life Antibiotic-Driven Dysbiosis Leads to Dysregulated Vaccine Immune Responses in Mice. Cell Host Microbe. 2018;23(5):653-660.e5.

58. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Heal Dis. 2015;26:1–17.

59. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. Nature. 2018;562(7728):583–8.

60. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med. 2016;8(343):1–14.

61. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17:693–703.

62. Azad M, Konya T, Persaud R, Guttman DS, Chari RS, Field CJ, et al. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: A prospective cohort study. BJOG An Int J Obstet Gynaecol. 2016;123(6):983–93.

63. Azad M, Konya T, Maughan H, Guttman D, Field C, Chari R, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. Cmaj. 2013;185(5):385–94.

64. Laursen MF, Andersen LBB, Michaelsen KF, Mølgaard C, Trolle E, Bahl MI, et al. Infant Gut Microbiota Development Is Driven by Transition to Family Foods Independent of Maternal Obesity. mSphere. 2016;1(1):1–16.

65. Dogra S, Sakwinska O, Soh SE, Ngom-Bru C, Brück WM, Berger B, et al. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. MBio. 2015;6(1):1–9.

66. Lees EA, Miyajima F, Pirmohamed M, Carrol ED. The role of Clostridium difficile in the paediatric and neonatal gut — a narrative review. Eur J Clin Microbiol Infect Dis. 2016;35(7):1047–57.

67. Davis MY, Zhang H, Brannan LE, Carman RJ, Boone JH. Rapid change of fecal microbiome and disappearance of Clostridium difficile in a colonized infant after transition from breast milk to cow milk. Microbiome. 2016;4:1–10.

68. Pant C, Deshpande A, Altaf MA, Minocha A, Sferra TJ. *Clostridium difficile* infection in children: a comprehensive review. Curr Med Res Opin. 2013;29(8):967–84.

69. Van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol. 2011;128(5):948-955.e3.

70. Cózar-Llistó A, Ramos-Martinez A, Cobo J. Clostridium difficile Infection in Special High-Risk Populations. Infect Dis Ther. 2016;5(3):253–69.

71. Tabaqchalsih S, O'farrell E, Nasha JQ, Wilks M. Vaginal Carriage and Neonatal Acquisition of Clostridium difficile. J Med Microbiol. 1984;18:47–53.

72. Matsuki S, Ozaki E, Shozu M, Inoue M, Shimizu S, Yamaguchi N, et al. Colonization by Clostridium difficile of neonates in a hospital, and infants and children in three day-care facilities of Kanazawa, Japan. Int Microbiol. 2005;8(1):43–8.

73. Jangi S, Lamont JT. Asymptomatic colonization by clostridium difficile in infants: Implications for disease in later life. J Pediatr Gastroenterol Nutr. 2010;51(1):2–7.

74. Nogacka A, Salazar N, Suárez M, Milani C, Arboleya S, Solís G, et al. Impact of

intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates. Microbiome. 2017;5(1):1–10.

75. Corrêa NBO, Péret Filho LA, Penna FJ, Lima FMLS, Nicoli JR. A randomized formula controlled trial of Bifidobacterium lactis and Streptococcus thermophilus for prevention of antibiotic-associated diarrhea in infants. J Clin Gastroenterol. 2005;39(5):385–9.

76. Plummer S, Weaver MA, Harris JC, Dee P, Huter J. Clostridium difficile pilot study: Effects of probiotic supplementation on the incidence of C. difficile diarrhoea. Int Microbiol. 2004;7(1):59–62.

77. Fouhy F, Guinane CM, Hussey S, Wall R, Ryan CA, Dempsey EM, et al. High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother. 2012;56(11):5811–20.

78. Stearns JC, Simioni J, Gunn E, McDonald H, Holloway AC, Thabane L, et al. Intrapartum antibiotics for GBS prophylaxis alter colonization patterns in the early infant gut microbiome of low risk infants. Sci Rep. 2017;7:16527

79. Network GA. The Global Asthma Report Asthma affects. 2018.

80. StatCan. Asthma by Age Group. [cited 2019 Oct 5]. Available from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310009608

81. Canadian Institute for Health Information. Asthma Hospitalizations Among Children and Youth in Canada: Trends and Inequalities Chartbook. 2018. Available from: https://www.cihi.ca/sites/default/files/document/asthma-hospitalization-children-2018-chartbook-en-web.pdf

82. Williams H, Stewart A, von Mutius E, Cookson W, Anderson HR. Is eczema really on the increase worldwide? J Allergy Clin Immunol. 2008;121:947–54.

83. Sicherer SH, Sampson HA. Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. J Allergy Clin Immunol. 2018;141(1):41–58.

84. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis. Allergy. 2018;73(5):971–86.

85. Lavigne É, Bélair MA, Duque DR, Do MT, Stieb DM, Hystad P, et al. Effect modification of perinatal exposure to air pollution and childhood asthma incidence. Eur Respir J. 2018;51:1701884.

86. Johnson CC, Ownby DR. The infant gut bacterial microbiota and risk of pediatric asthma and allergic diseases. Translational Research. 2017, 170:60–70.

87. Doan T, Arzika AM, Ray KJ, Cotter SY, Kim J, Maliki R, et al. Gut microbial diversity in antibiotic-naive children after systemic antibiotic exposure: A randomized controlled trial. Clin Infect Dis. 2017;64(9):1147–53.

88. Parker EPK, Praharaj I, John J, Kaliappan SP, Kampmann B, Kang G, et al. Changes in the intestinal microbiota following the administration of azithromycin in a randomised placebo-controlled trial among infants in south India. Sci Rep. 2017;7(1):1–9.

89. Wei S, Mortensen MS, Stokholm J, Brejnrod AD, Thorsen J, Rasmussen MA, et al. Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: A double-blind, randomized, placebo-controlled trial. EBioMedicine. 2018;38:265–72.

90. Tanaka S, Kobayashi T, Songjinda P, Tateyama A, Tsubouchi M, Kiyohara C, et al. Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol Med Microbiol. 2009;56(1):80–7.

91. Mazzola G, Murphy K, Ross RP, Di Gioia D, Biavati B, Corvaglia LT, et al. Early gut microbiota perturbations following intrapartum antibiotic prophylaxis to prevent group B streptococcal disease. PLoS One. 2016;11(6):1–11.

92. Aloisio I, Quagliariello A, De Fanti S, Luiselli D, De Filippo C, Albanese D, et al. Evaluation of the effects of intrapartum antibiotic prophylaxis on newborn intestinal microbiota using a sequencing approach targeted to multi hypervariable 16S rDNA regions. Appl Microbiol Biotechnol. 2016;100(12):5537–46.

93. Bello A, Quinn MM, Perry MJ, Milton DK. Quantitative assessment of airborne exposures

generated during common cleaning tasks: A pilot study. Environ Heal A Glob Access Sci Source. 2010;9(1):1–10.

94. Schmidt B, Mulder IE, Musk CC, Aminov RI, Lewis M, Stokes CR, et al. Establishment of normal gut microbiota is compromised under excessive hygiene conditions. PLoS One. 2011;6(12):e28284.

95. Tun MH, Tun HM, Mahoney JJ, Konya TB, Guttman DS, Becker AB, et al. Postnatal exposure to household disinfectants, infant gut microbiota and subsequent risk of overweight in children. Cmaj. 2018;190(37):E1097–107.

96. Bornehag C, Hagerhed-Engman L, Sigsgaard T, Janson S, Aberg N, DBH-studyGroup. Dampness at home and its association with airway, nose and skin symptoms among 10 851 preschool children in Sweden: A cross sectional study. Indoor Air. 2005(15):48–45.

97. Levy SB. Antibacterial household products: Cause for concern. Emerg Infect Dis. 2001;7(3):512–5.

98. Marra F, Lynd L, Coombes M, Richardson K, Legal M, FitzGerald JM, et al. Does antibiotic exposure during infancy lead to development of asthma? A systematic review and metaanalysis. Chest. 2006;129(3):610–8.

99. Murk W, Risnes KR, Bracken MB. Prenatal or Early-Life Exposure to Antibiotics and Risk of Childhood Asthma: A Systematic Review. Pediatrics. 2011;127(6):1125–38.

100. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review. Br J Dermatol. 2013;169(5):983–91.

101. Goksör E, Alm B, Pettersson R, Möllborg P, Erdes L, Åberg N, et al. Early fish introduction and neonatal antibiotics affect the risk of asthma into school age. Pediatr Allergy Immunol. 2013;24(4):339–44.

102. Stromberg Celind F, Vasileiadou S, Alm B, Goksor E. Antibiotics in the first week of life were associated with atopic asthma at 12 years of age. Acta Paediatr Int J Paediatr. 2018;1–7.

103. Batool T, Reece PL, Schulze KM, Morrison KM, Atkinson SA, Anand SS, et al. Prenatal

and early-life predictors of atopy and allergic disease in Canadian children: Results of the Family Atherosclerosis Monitoring In earLY life (FAMILY) Study. J Dev Orig Health Dis. 2016;7(6):665–71.

104. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association between use of acid-suppressive medications and antibiotics during infancy and allergic diseases in early childhood. JAMA Pediatr. 2018;172(6).

105. Wohl DL, Curry WJ, Mauger D, Miller J, Tyrie K. Intrapartum Antibiotics and Childhood Atopic Dermatitis. J Am Board Fam Med . 2015;28(1):82–9.

106. Obiakor C V., Tun HM, Bridgman SL, Arrieta M-C, Kozyrskyj AL. The association between early life antibiotic use and allergic disease in young children: recent insights and their implications. Expert Rev Clin Immunol. 2018;14(10):841–55.

107. Zock JP, Vizcaya D, Le Moual N. Update on asthma and cleaners. Curr Opin Allergy Clin Immunol. 2010;10(2):114–20.

108. Vincent MJ, Parker A, Maier A. Cleaning and asthma: A systematic review and approach for effective safety assessment. Regul Toxicol Pharmacol. 2017;90:231–43.

109. Bably M, Arif AA, Post A. Prenatal use of cleaning and scented products and its association with childhood asthma, asthma symptoms, and mental health and developmental comorbidities. J Asthma. 2019:1–6. Available from: https://doi.org/10.1080/02770903.2019.1656229

110. Sepp E, Julge K, Vasar M, Naaber P, Björksten B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. Acta Paediatr Int J Paediatr. 1997;86(9):956–61.

111. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol. 2012;129(2):434–40.

112. Penders J, Thijs C, Van Den Brandt PA, Kummeling I, Snijders B, Stelma F, et al. Gut microbiota composition and development of atopic manifestations in infancy: The KOALA birth cohort study. Gut. 2007;56(5):661–7.

113. Arrieta MC, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Sci Transl Med. 2015;7(307).

114. Azad MB, Konya T, Guttman DS, Field CJ, Sears MR, Hayglass KT, et al. Infant gut microbiota and food sensitization: Associations in the first year of life. Clin Exp Allergy. 2015;45(3):632–43.

115. Stiemsma LT, Arrieta M-C, Dimitriu PA, Cheng J, Thorson L, Lefebvre DL, et al. Shifts in Lachnospira and Clostridium sp. in the 3-month stool microbiome are associated with preschool age asthma. Clin Sci. 2016;130(23):2199–207.

116. Lee SH, Gong YN, Ryoo E. Clostridium difficile colonization and / or infection during infancy and the risk of childhood allergic diseases. 2017;60(5):145–50.

117. Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One. 2012;7(6):e36466

118. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010;107(26):11971–5.

119. Adlerberth I, Strachan DP, Matricardi PM, Ahrné S, Orfei L, Åberg N, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol. 2007;120(2):343–50.

120. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by Caesarean section. Gut. 2014;63(4):559–66.

121. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: Epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387(10017):475–90.

122. Cabrera-Rubio R, Collado C, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of

delivery. Am J Clin Nutr. 2012;96:544–51.

123. Martin R, Makino H, Yavuz AC, Ben-Amor K, Roelofs M, Ishikawa E, et al. Early-Life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS One. 2016;11(6):1–30.

124. Taft DH, Liu J, Maldonado-Gomez MX, Akre S, Huda MN, Ahmad SM, et al. Bifidobacterial Dominance of the Gut in Early Life and Acquisition of Antimicrobial Resistance. mSphere. 2018;3(5):1–24.

125. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. Microbiome. 2018;6(1):182.

126. Ferraris L, Butel MJ, Campeotto F, Vodovar M, Rozé JC, Aires J. Clostridia in premature neonates' gut: Incidence, antibiotic susceptibility, and perinatal determinants influencing colonization. PLoS One. 2012;7(1):e30594

127. Zhu D, Xiao S, Yu J, Ai Q, He Y, Cheng C, et al. Effects of One-Week Empirical Antibiotic Therapy on the Early Development of Gut Microbiota and Metabolites in Preterm Infants. Sci Rep. 2017;7(1):1–10.

128. Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al. Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. Microbiome. 2017;5(1):1–18.

129. Arboleya S, Sánchez B, Solís G, Fernández N, Suárez M, Hernández-Barranco AM, et al. Impact of prematurity and perinatal antibiotics on the developing intestinal microbiota: A functional inference study. Int J Mol Sci. 2016;17(5):1–14.

130. Patient Safety Network. Health care-associated infections. Agency for Healthcare Research and Quality. 2019 [cited 2019 Oct 7]. Available from: https://psnet.ahrq.gov/primer/health-care-associated-infections

131. Stoodley BJ, Thom BT. Observations on the Intestinal Carriage of Pseudomonas Aeruginosa. J Med Microbiol. 1970; 3:367–75.

132. Kato H, Kato N, Watanabe K, Ueno K, Ushijima H, Hashira S, et al. Application of typing by pulsed-field gel electrophoresis to the study of Clostridium difficile in a neonatal intensive care unit. J Clin Microbiol. 1994;32(9):2067–70.

133. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Sears MR, et al. Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. Allergy, Asthma Clin Immunol. 2013;9(15):1–9.

134. Chan CWH, Wong RS, Law PTW, Wong CL, Tsui SKW, Tang WPY, et al. Environmental factors associated with altered gut microbiota in children with eczema: A systematic review. Int J Mol Sci. 2016;17:1–14.

135. Nielsen NM, Hansen AV, Simonsen J, Hviid A. Prenatal stress and risk of infectious diseases in offspring. Am J Epidemiol. 2011;173(9):990–7.

136. Field T, Hernandez-Reif M, Diego M. Risk factors and stress variables that differentiate depressed from nondepressed pregnant women. Infant Behav Dev. 2006;29(2):169–74.

137. Beijers R, Jansen J, Riksen-Walraven M, De Weerth C. Maternal prenatal anxiety and stress predict infant illnesses and health complaints. Pediatrics. 2010;126(2).

138. Beijers R, Buitelaar JK, de Weerth C. Mechanisms underlying the effects of prenatal psychosocial stress on child outcomes: beyond the HPA axis. Eur Child Adolesc Psychiatry. 2014;23(10):943–56.

139. Zijlmans MAC, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology. 2015;53:233–45.

140. Heath PT. Status of vaccine research and development of vaccines for Staphylococcus aureus. Vaccine. 2016;34:2876–9.

141. Maghera A, Kahlke P, Lau A, Zeng Y, Hoskins C, Corbett T, et al. You are how you recruit: A cohort and randomized controlled trial of recruitment strategies. BMC Med Res Methodol. 2014;14:111.

142. Subbarao P, Anand SS, Becker AB, Befus AD, Brauer M, Brook JR, et al. The Canadian Healthy Infant Longitudinal Development (CHILD) study: Examining developmental origins of allergy and asthma. Thorax. 2015;70(10):998–1000.

143. Lewinsohn PM, Seeley JR, Roberts RE, Allen NB. Center for Epidemiologic Studies Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. Psychol Aging. 1997;12(2):277–87.

144. Fjalstad J, Stensvold H, Bergseng H, Simonsen G, Salvesen B, Rønnestad A, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. Pediatr Infect Dis J. 2016;35:1–6.

145. Ni J, Friedman H, Boyd BC, McGurn A, Babinski P, Markossian T, et al. Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. BMC Pediatr. 2019;19(1):1–8.

146. Korpela K, Zijlmans MAC, Kuitunen M, Kukkonen K, Savilahti E, Salonen A, et al. Childhood BMI in relation to microbiota in infancy and lifetime antibiotic use. Microbiome. 2017;5(1):1–9.

147. Penders J, Stobboringh EE, Van Den Brandt PA, Van Ree R, Thijs C. Toxigenic and non-toxigenic Clostridium difficile: Determinants of intestinal colonisation and role in childhood atopic manifestations. Gut. 2008;57(7):1025–6.

148. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology. 2002;148(1):257–66.

149. Hosmer D, Lemeshow S. Applied Logistic Regression. Second Edi. New York: Wiley; 2000. 1–397 p.

150. Textor J, Hardt J. DAGitty: A graphical tool for analyzing causal diagrams. Epidemiology. 2011;22(5):745.

151. Coker MO, Hoen AG, Dade E, Lundgren S, Li Z, Wong AD, et al. Specific class of intrapartum antibiotics relates to maturation of the infant gut microbiota : a prospective cohort study. BJOG An Int J Obstet Gynaecol. 2019;1–11.

152. Shin NR, Whon TW, Bae JW. Proteobacteria: Microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015;33(9):496–503.

153. Bondi CAM. Applying the precautionary principle to consumer household cleaning product development. J Clean Prod. 2011;19(5):429–37.

154. Cahenzli J, Köller Y, Wyss M, Geuking MB, McCoy KD. Intestinal microbial diversity during early-life colonization shapes long-term IgE levels. Cell Host Microbe. 2013;14(5):559–70.

155. Lemas DJ, Yee S, Cacho N, Miller D, Cardel M, Gurka M, et al. Exploring the contribution of maternal antibiotics and breastfeeding to development of the infant microbiome and pediatric obesity. Semin Fetal Neonatal Med. 2016;21(6):406–9.

156. Hesla HM, Stenius F, Jäderlund L, Nelson R, Engstrand L, Alm J, et al. Impact of lifestyle on the gut microbiota of healthy infants and their mothers - the ALADDIN birth cohort. FEMS Microbiol Ecol. 2014;90(3):791–801.

157. Nogacka AM, Salazar N, Arboleya S, Suárez M, Fernández N, Solís G, et al. Early microbiota, antibiotics and health. Cell Mol Life Sci. 2018;75(1):83–91.

158. Bascom EME, Napolitano MA. Breastfeeding Duration and Primary Reasons for Breastfeeding Cessation among Women with Postpartum Depressive Symptoms. J Hum Lact. 2016;32(2):282–91.

APPENDICES

Appendix A

Table S1. Population Characteristics According to Antibiotics and Household Cleaning Products Use (3 Month Sample; N=1,429)

		Any Antibiotics (Maternal IAP and Direct Infant)			Household Cleaning Products Use		
Column percentages	**Total[a]**	**No N (%)**	**Yes N (%)**	**P value**	**Lower N (%)**	**Higher N (%)**	**P value**
Birth mode							
Vaginal	1,096	723 (100%)	373 (53%)	**<0.001**	617 (82%)	479 (71%)	**<0.001**
CS-elective	134	0 (0%)	134 (19%)		52 (7%)	82 (12%)	
CS-emergency	194	0 (0%)	194 (28%)		82 (11%)	112 (17%)	
Birthweight							
<2500g	31	11 (2%)	20 (3%)	0.141	18 (2%)	13 (2%)	0.758
2500 – 4000g	1,186	613 (86%)	573 (83%)		626 (84%)	560 (84%)	
>4000g	194	93 (13%)	101 (15%)		99 (13%)	95 (14%)	
Gestational age							
<39 weeks	372	146 (20%)	226 (32%)	**<0.001**	180 (24%)	192 (29%)	0.053
≥39 weeks	1,052	578 (80%)	474 (68%)		571 (76%)	481 (71%)	
Infant sex							
Male	766	367 (51%)	399 (57%)	**0.026**	402 (53%)	364 (54%)	0.915
Female	663	357 (49%)	306 (43%)		350 (47%)	313 (46%)	
Breastfeeding at 3 Months							
Exclusive	791	415 (57%)	376 (53%)	0.288	471 (63%)	320 (47%)	**<0.001**
Mixed	384	183 (25%)	201 (29%)		186 (25%)	198 (29%)	
Formula	251	125 (17%)	126 (18%)		93 (12%)	158 (23%)	
Location							
Edmonton	388	200 (28%)	188 (27%)	**<0.001**	159 (21%)	229 (34%)	**<0.001**
Vancouver	445	193 (27%)	252 (36%)		282 (38%)	163 (24%)	
Winnipeg	596	331 (46%)	265 (38%)		311 (42%)	285 (42%)	
Furry pets							
No	777	410 (57%)	367 (52%)	0.089	463 (62%)	314 (47%)	**<0.001**
Yes	648	312 (43%)	336 (48%)		287 (38%)	261 (53%)	

Older siblings							
No	712	314 (43%)	398 (57%)	**<0.001**	383 (51%)	329 (49%)	0.396
Yes	712	409 (57%)	303 (43%)		366 (49%)	346 (51%)	
Smoke exposure							
No	1160	603 (84%)	557 (80%)	0.069	642 (86%)	518 (78%)	**<0.001**
Yes	248	113 (16%)	135 (20%)		101 (14%)	147 (22%)	
Maternal age							
<30	503	285 (39%)	218 (31%)	**0.002**	253 (34%)	250 (37%)	**0.006**
30 – 39	874	418 (56%)	456 (65%)		461 (61%)	413 (61%)	
≥40	52	21 (3%)	31 (4%)		38 (5%)	14 (2%)	
Maternal antibiotic (No IAP)[b]							
No	1,250	645 (90%)	605 (86%)	**0.048**	658 (88%)	592 (88%)	1.000
Yes	167	72 (10%)	95 (14%)		88 (12%)	79 (12%)	
Prenatal depression							
No	1,072	547 (77%)	525 (75%)	0.618	573 (77%)	499 (75%)	0.318
Yes	338	167 (23%)	171 (25%)		170 (23%)	168 (25%)	
Prenatal stress							
No	542	276 (39%)	266 (38%)	0.870	302 (41%)	240 (36%)	0.079
Yes	868	438 (61%)	430 (62%)		441 (59%)	427 (64%)	

Notes: [a]Total may not add up due to missing data; [b]maternal postnatal antibiotics after birth till 3 months; IAP: Intrapartum Antibiotic Prophylaxis; P value calculated using Fisher's exact test, significant in **Bold.**

Table S2. Population Characteristics According to Antimicrobial Exposure at 3 months (12 Month Sample; N=1,728)

Column percentages	**Total[a]**	**NALC* N (%)**	**AALC* N (%)**	**NAHC* N (%)**	**AAHC* N (%)**	**P value**
Birth mode						
Vaginal	1,297	488 (100%)	245 (55%)	368 (100%)	196 (47%)	**<0.001**
CS-elective	175	0	77 (17%)	0	98 (23%)	
CS-emergency	252	0	125 (28%)	0	127 (30%)	
Birthweight						

<2500g	42	8 (2%)	12 (3%)	6 (2%)	16 (4%)	0.158
2500 – 4000g	1,440	407 (84%)	380 (86%)	315 (87%)	338 (81%)	
>4000g	225	67 (14%)	50 (11%)	44 (12%)	64 (15%)	
Gestational age						
<39 weeks	455	86 (18%)	142 (32%)	88 (24%)	139 (33%)	**<0.001**
≥39 weeks	1,267	402 (82%)	303 (68%)	281 (76%)	281 (67%)	
Infant sex						
Male	896	246 (50%)	241 (54%)	188 (51%)	221 (52%)	0.729
Female	832	242 (50%)	206 (46%)	181 (49%)	203 (48%)	
Breastfeeding at 3 Months						
Exclusive	1,072	348 (71%)	296 (66%)	202 (55%)	226 (53%)	**<0.001**
Mixed	420	101 (21%)	102 (23%)	92 (25%)	125 (29%)	
Formula	235	39 (8%)	48 (11%)	75 (20%)	73 (17%)	
Breastfeeding at 12 Months						
No	873	198 (41%)	198 (45%)	230 (64%)	247 (60%)	**<0.001**
Yes	822	282 (59%)	240 (55%)	132 (36%)	168 (40%)	
Solids before 6 months						
No	488	151 (31%)	151 (34%)	74 (20%)	112 (27%)	**<0.001**
Yes	1,227	336 (69%)	292 (66%)	292 (80%)	307 (73%)	
Location						
Edmonton	406	93 (19%)	82 (18%)	110 (30%)	121 (29%)	**<0.001**
Vancouver	529	157 (32%)	190 (43%)	64 (17%)	118 (28%)	
Winnipeg	793	238 (49%)	175 (39%)	195 (53%)	185 (44%)	
Furry pets						
No	936	300 (62%)	273 (61%)	165 (45%)	198 (47%)	**<0.001**
Yes	787	185 (38%)	174 (39%)	203 (55%)	225 (53%)	
Older siblings						
No	884	224 (46%)	264 (59%)	154 (42%)	242 (57%)	**<0.001**
Yes	839	263 (54%)	181 (41%)	214 (58%)	181 (43%)	
Smoke exposure						
No	1,422	424 (88%)	378 (85%)	291 (80%)	329 (79%)	**<0.001**

Yes	285	58 (12%)	65 (15%)	74 (20%)	88 (21%)	
Maternal age						
<30	576	171 (35%)	124 (28%)	142 (38%)	139 (33%)	**<0.001**
30 – 39	1,094	296 (61%)	298 (67%)	222 (60%)	278 (66%)	
≥40	58	21 (4%)	25 (6%)	5 (1%)	7 (2%)	
Maternal antibiotic (No IAP)[b]						
No	1,507	438 (91%)	384 (86%)	323 (89%)	362 (86%)	0.110
Yes	207	46 (10%)	60 (14%)	41 (11%)	60 (14%)	
Maternal prenatal depression						
No	1,329	387 (80%)	345 (78%)	282 (77%)	315 (75%)	0.356
Yes	379	95 (18%)	98 (22%)	83 (23%)	103 (25%)	
Maternal prenatal stress						
No	706	209 (43%)	179 (40%)	153 (42%)	165 (39%)	0.654
Yes	1,002	273 (57%)	264 (60%)	212 (58%)	253 (61%)	
Maternal postnatal depression						
No	1,335	376 (79%)	364 (84%)	292 (81%)	303 (75%)	0.012
Yes	336	100 (21%)	69 (16%)	67 (19%)	100 (25%)	
Maternal postnatal stress						
No	732	204 (43%)	189 (44%)	172 (48%)	167 (41%)	0.311
Yes	940	273 (57%)	244 (56%)	187 (52%)	236 (59%)	

Notes: *Antimicrobial Exposure (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use); [a]Total may not add up due to missing data; [b]maternal postnatal antibiotics after birth till 3 months; IAP: Intrapartum Antibiotic Prophylaxis; P value calculated using Pearson chi-square test, significant in **Bold.**

Table S3. Individual Adjustment for Covariates on Antimicrobial Exposure and *C. difficile* Colonization

		***C. difficile* colonization (3 months)**		***C. difficile* colonization (12 months)**	
		Odds Ratio	**P value**	**Odds Ratio**	**P value**
CRUDE OR					
	Antimicrobial Exposure* (Ref=NALC)				
	AALC	1.38 (1.00-1.91)	**0.047**	1.45 (1.12-1.88)	**0.004**
	NAHC	1.52 (1.10-2.11)	**0.011**	1.17 (0.89-1.54)	0.256
	AAHC	2.03 (1.49-2.78)	**<0.001**	1.46 (1.12-1.90)	**0.004**
ADJUSTED FOR					
Maternal age					
	AALC	1.45 (1.04-2.01)	**0.026**	1.45 (1.12-1.88)	**0.004**
	NAHC	1.47 (1.05-2.04)	**0.022**	1.17 (0.89-1.54)	0.256
	AAHC	2.11 (1.54-2.89)	**<0.001**	1.46 (1.12-1.90)	**0.004**
Maternal antibiotic (No IAP)[a]					
	AALC	1.33 (0.96-1.85)	0.080	1.47 (1.13-1.90)	**0.004**
	NAHC	1.49 (1.07-2.07)	**0.016**	1.17 (0.89-1.54)	0.253
	AAHC	2.01 (1.47-2.75)	**<0.001**	1.45 (1.11-1.89)	**0.005**
Birth method					
	AALC	1.21 (0.85-1.71)	0.284	1.47 (1.10-1.96)	**0.008**
	NAHC	1.53 (1.10-2.12)	**0.011**	1.17 (0.89-1.54)	0.242
	AAHC	1.67 (1.17-2.40)	**0.005**	1.49 (1.10-2.01)	**0.008**
Gestational age					
	AALC	1.38 (1.00-1.92)	**0.048**	1.40 (1.08-1.83)	**0.009**
	NAHC	1.53 (1.10-2.12)	**0.011**	1.16 (0.88-1.52)	0.286
	AAHC	2.02 (1.47-2.77)	**<0.001**	1.41 (1.08-1.84)	**0.010**
Infant sex					
	AALC	1.37 (0.99-1.89)	0.055	1.47 (1.14-1.91)	**0.003**
	NAHC	1.52 (1.10-2.12)	**0.011**	1.17 (0.89-1.54)	0.249
	AAHC	2.01 (1.47-2.75)	**<0.001**	1.47 (1.13-1.92)	**0.004**
Breastfeeding at 3 Months					
	AALC	1.34 (0.96-1.87)	0.076	1.46 (1.12-1.89)	**0.004**
	NAHC	1.29 (0.92-1.80)	0.138	1.17 (0.88-1.54)	0.265
	AAHC	1.76 (1.28-2.43)	**<0.001**	1.45 (1.11-1.89)	**0.005**
Breastfeeding at 12 Months					
	AALC	-	-	1.41 (1.09-1.84)	**0.009**
	NAHC	-	-	1.09 (0.82-1.44)	0.529

	AAHC	-	-	1.42 (1.08-1.85)	**0.010**
Location					
	AALC	1.49 (1.07-2.07)	**0.017**	1.44 (1.11-1.87)	**0.005**
	NAHC	1.37 (0.98-1.91)	0.063	1.20 (0.91-1.58)	0.194
	AAHC	1.92 (1.40-2.64)	**<0.001**	1.49 (1.14-1.93)	**0.003**
Furry pet					
	AALC	1.36 (0.98-1.88)	0.061	1.47 (1.13-1.90)	**0.004**
	NAHC	1.45 (1.04-2.02)	**0.026**	1.19 (0.90-1.58)	0.197
	AAHC	1.90 (1.39-2.61)	**<0.001**	1.49 (1.14-1.95)	**0.003**
Older sibling					
	AALC	1.34 (0.97-1.86)	0.073	1.38 (1.06-1.79)	**0.015**
	NAHC	1.54 (1.11-2.15)	**0.009**	1.18 (0.90-1.56)	0.224
	AAHC	1.95 (1.43-2.67)	**<0.001**	1.38 (1.06-1.80)	**0.016**
Smoke exposure					
	AALC	1.37 (0.99-1.90)	0.055	1.44 (1.11-1.87)	**0.006**
	NAHC	1.44 (1.03-2.01)	**0.029**	1.15 (0.87-1.52)	0.295
	AAHC	1.90 (1.38-2.61)	**<0.001**	1.46 (1.12-1.90)	**0.005**

Notes: *Antimicrobial Exposure by 3 months (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use); ª maternal postnatal antibiotics after birth till 3 months; IAP: Intrapartum Antibiotic Prophylaxis; P value calculated using logistic regression, significant in **Bold.**

Table S4. Sensitivity Analysis for Antimicrobial Exposure and *C. difficile* Colonization at 12 Months

	Adjusted OR† (95% CI)	**P value**	**Adjusted OR‡ (95% CI)**	**P value**
Antimicrobial Exposure* (ref=NALC)				
AALC	1.35 (1.00-1.83)	**0.043**	1.41 (1.02-1.94)	**0.034**
NAHC	1.12 (0.84-1.49)	0.438	1.06 (0.78-1.44)	0.694
AAHC	1.35 (0.99-1.85)	0.057	1.33 (0.95-1.88)	0.095

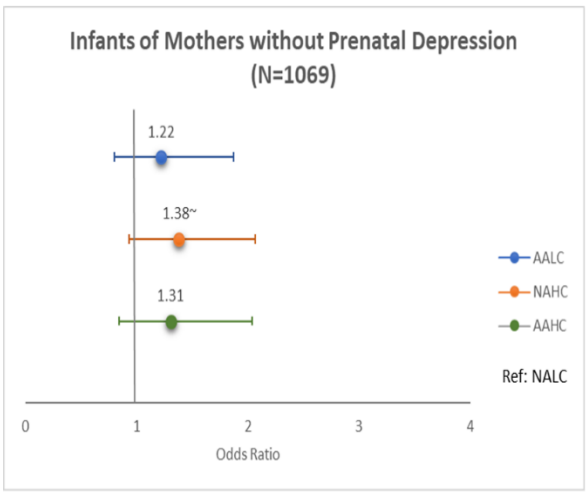
Notes: *Antimicrobial Exposure by 3 months (NALC: no antibiotics and lower cleaning products use, AALC: any antibiotics and lower cleaning products use, NAHC: no antibiotics and higher cleaning products use, AAHC: any antibiotics and higher cleaning products use); †Adjusted for antibiotics after 3 months and variables in final model, ‡ Excluding any infant that received antibiotics after 3 months and adjusted for variables in final model; Significant P value in **Bold.**

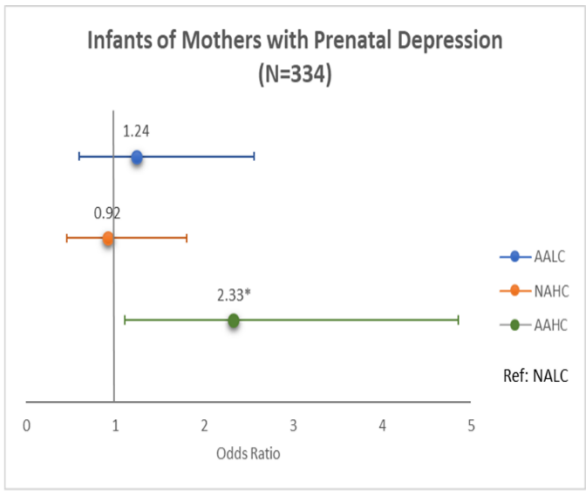
Table S5. Analysis for Interaction Between Exposures of Interest and Covariates in the Final Model for *C. difficile* Colonization at 3 and 12 Months

Interaction	P value (3 Months)	P value (12 Months)
Antibiotics & cleaning products	0.91	0.58
Antibiotics & breastfeeding	0.17	0.73
Antibiotics & birth mode	-	-
Antibiotics & maternal age	0.22	-
Antibiotics & prenatal depression	0.08	-
Antibiotics & Infant sex	0.14	**0.04**
Antibiotics & siblings	-	0.28
Cleaning products & breastfeeding	0.97	0.82
Cleaning products & birth mode	0.16	0.09
Cleaning products & maternal age	0.13	-
Cleaning products & prenatal depression	0.94	-
Cleaning products & infant sex	0.68	0.87
Cleaning products & siblings	-	0.12
Antimicrobial exposure & breastfeeding	0.58	0.98
Antimicrobial exposure & birth mode	0.15	0.07
Antimicrobial exposure & maternal age	0.32	-
Antimicrobial exposure & prenatal depression	0.11	-
Antimicrobial exposure & infant sex	0.52	0.18
Antimicrobial exposure & siblings	-	0.30

Notes: Antibiotics: exposure to maternal intrapartum antibiotic or infant antibiotic by 3 months;

Significant P value in **Bold.**

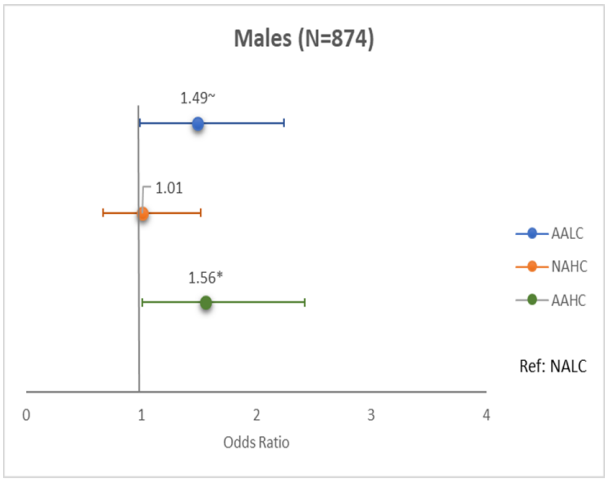


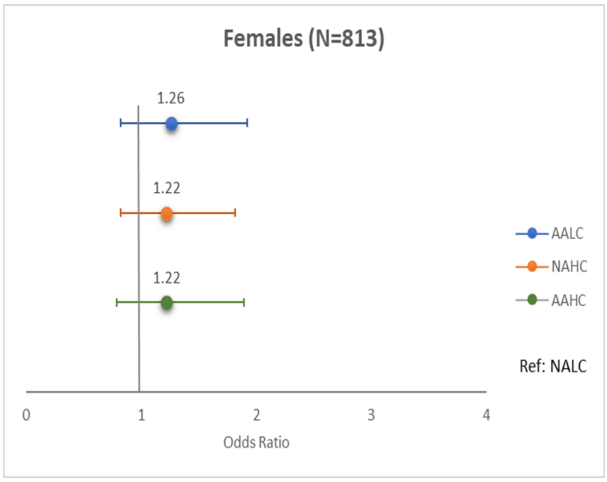


Adjusted for Breastfeeding, birth mode, maternal age; ~p=0.10; *p<0.05

Adjusted for Breastfeeding, birth mode, maternal age; ~p=0.10; *p<0.05

Figure S1. Stratified Analysis by Maternal Prenatal Depression for Antimicrobial Exposure and *C. difficile* Colonization at 3 Months

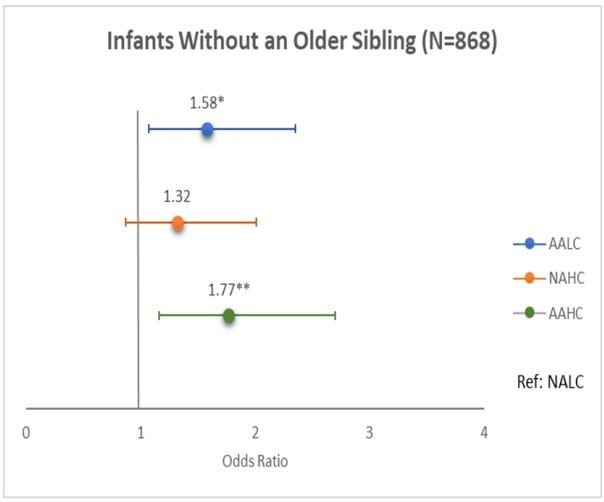


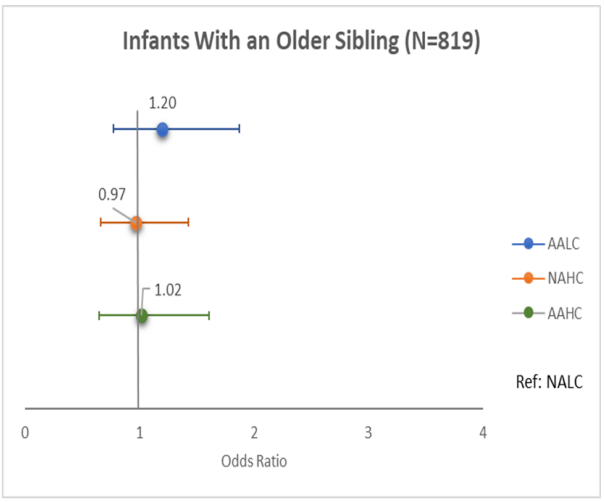


Adjusted for Breastfeeding, birth mode, siblings; ~p=0.10; *p<0.05

Adjusted for Breastfeeding, birth mode, siblings; ~p=0.10; *p<0.05

Figure S2. Stratified Analysis by Infant Sex for Antimicrobial Exposure and *C. difficile* Colonization at 12 Months





Adjusted for Breastfeeding, birth mode;
~p=0.10; *p<0.05; **p<0.01

Adjusted for Breastfeeding, birth mode;
~p=0.10; *p<0.05; **p<0.01

Figure S3. Stratified Analysis by Siblings for Antimicrobial Exposure and *C. difficile* Colonization at 12 Months

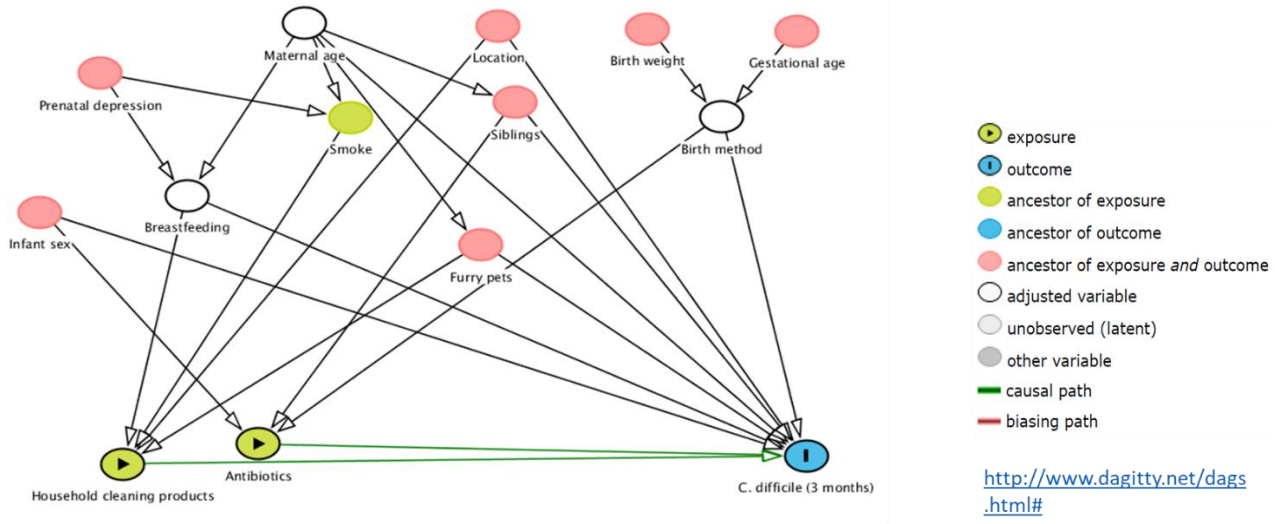


Figure S4. Schematic Representation of Early Life Factors Associated with Antimicrobial Exposure or *C. difficile* colonization at 3 Months

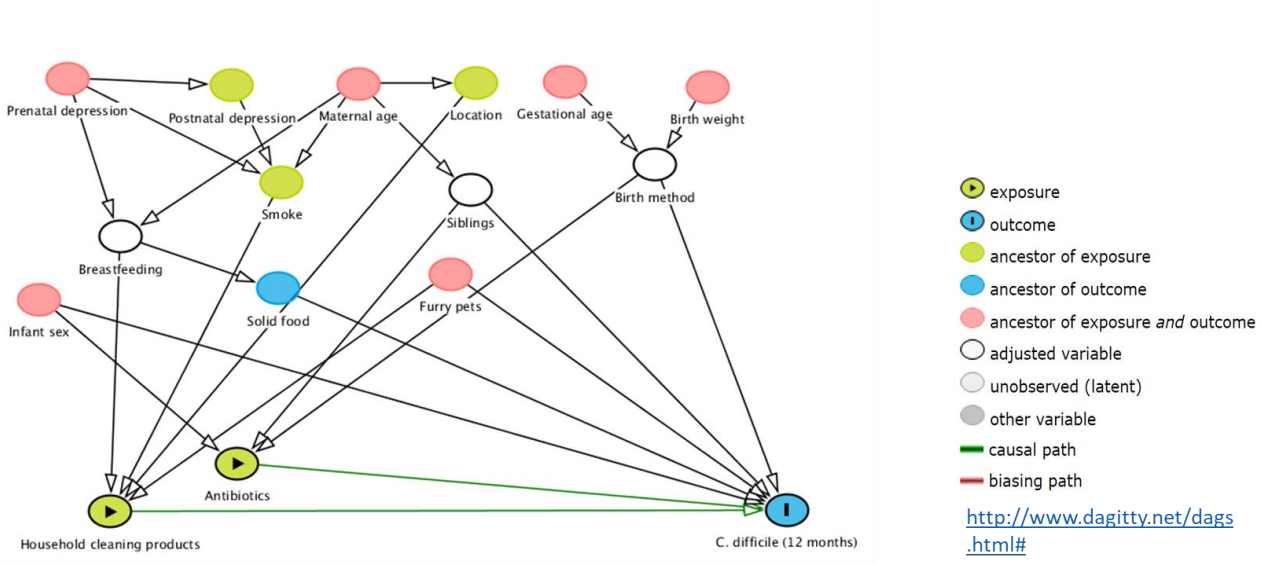


Figure S5. Schematic Representation of Early Life Factors Associated with Antimicrobial Exposure or *C. difficile* colonization at 12 Months

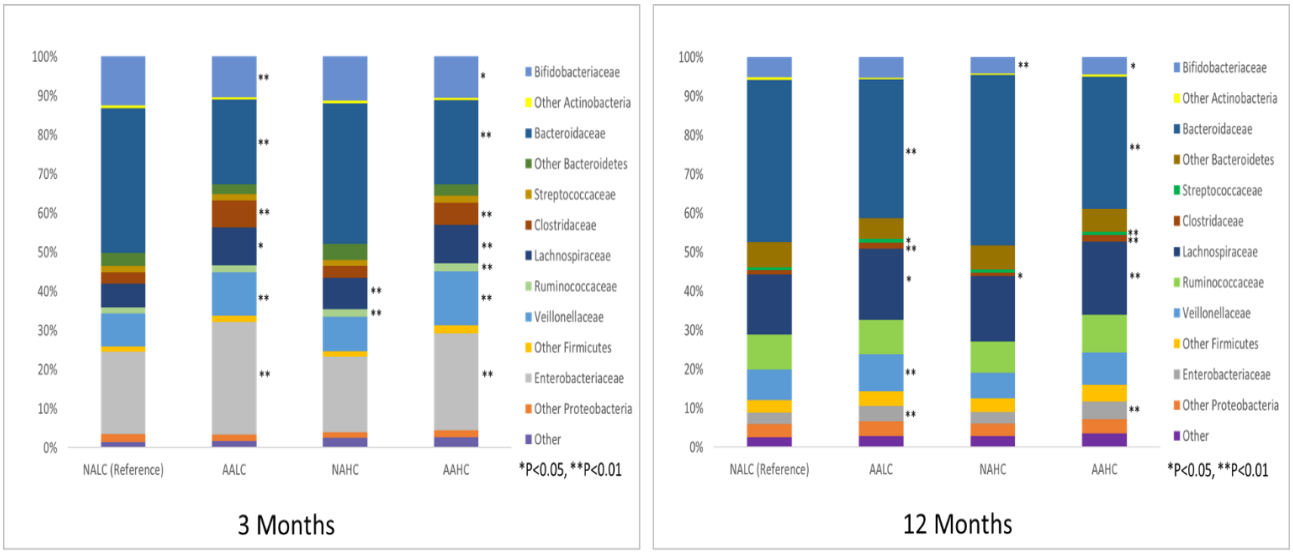


Figure S6. Stacked Bar Plots of Mean Relative Abundance of Dominant Taxa (Family Level) According to Antimicrobial Exposure; Comparisons were performed using Kruskal-Wallis test with Dunn's post hoc test. Positive false discovery rate (FDR) was used to adjust P values for multiple testing.

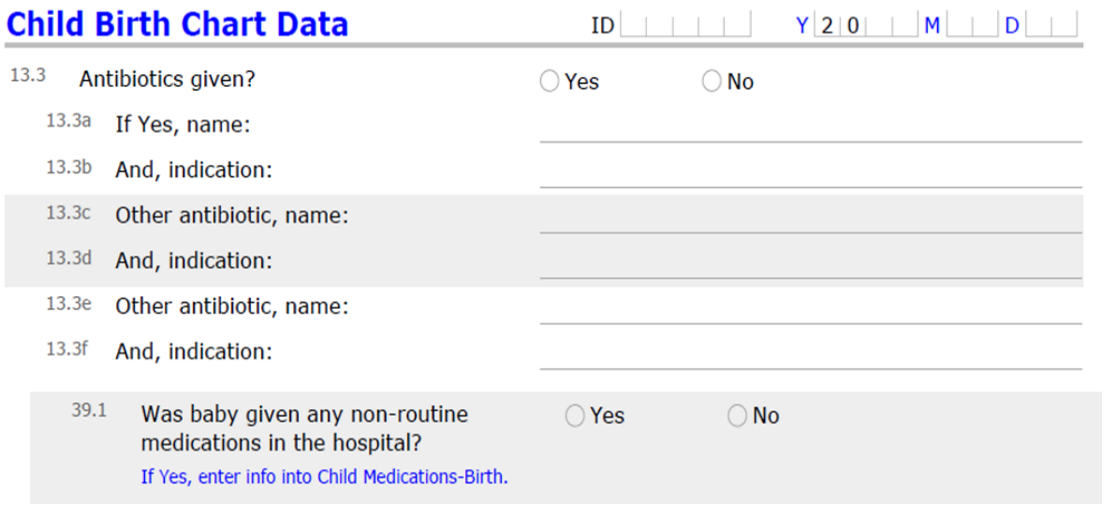
Appendix B

Table A1. Covariates Collected or not Collected/Unattainable

Covariates (collected)	Covariates (not collected/unattainable)
➢ Birth mode ➢ Birthweight ➢ Gestational age ➢ Infant sex ➢ Infant diet ➢ Infant age at stool collection ➢ Maternal age ➢ Maternal smoking ➢ Maternal postnatal antibiotics ➢ Location ➢ Breastfeeding status ➢ Furry pets ➢ Siblings ➢ Prenatal/postnatal stress ➢ Prenatal/postnatal depression	➢ Maternal Microbiome (vaginal, stool) ➢ Maternal diet ➢ Maternal Infection

Questionnaire examples

i) Maternal intrapartum antibiotic and newborn antibiotic



Child Birth Chart Data ID Y 2 0 M D

13.3 Antibiotics given? ○ Yes ○ No

13.3a If Yes, name:

13.3b And, indication:

13.3c Other antibiotic, name:

13.3d And, indication:

13.3e Other antibiotic, name:

13.3f And, indication:

39.1 Was baby given any non-routine medications in the hospital? ○ Yes ○ No

If Yes, enter info into Child Medications-Birth.

ii) Infant postnatal antibiotics

Child Medications - 3 Months ID Y 2 0 M D

1. Generic or Brand Name:
   - 1.1 DIN number:
   - 1.2 Type of Medication: (e.g., tablets, liquid, aerosol inhaler, cream, injection, etc.)
   - 1.3 Total Dose: (e.g., 10 mg, 250 mcg, etc.)
   - 1.4 Frequency: (e.g., once a day, twice a day, or when needed, etc.)
   - 1.5 Start Date: Y R 2 0 M D
   - 1.6 Quantity: (e.g., total number of tablets, total volume)
   - 1.7 Stop Date: Y R 2 0 M D
   - 1.8 Reason for use:

iii) Household cleaning products

50. Which of the following products have been USED in your home since your baby was born?
(Day=daily, Wk=weekly, Mth=monthly, <Mth=less than monthly, No=not used)

50.1	Liquid or solid air freshener:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.2	Spray air freshener:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.3	Plug-in deodorizer/air freshener:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.4	Floor Cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.5	Furniture polish:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.6	Floor polish:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.7	Dusting polish or spray:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.8	Drain cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.9	Hand dishwashing detergent:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.10	Dishwasher detergent:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.11	Bleach:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.12	Multi-surface cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.13	Silver or brass polish:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.14	Disinfectant in bedrooms:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.15	Disinfectant in home in general:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.19	Eco & organic cleaning product:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.20	Glass cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.21	Chemical hand cleaner (e.g., for grease):	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.22	Purell-type hand cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.23	Unscented laundry detergent:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.24	Scented laundry detergent:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.25	Fabric softener:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.26	Toilet bowl cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.27	Oven cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.28	Bathroom tile cleaner:	○ Day	○ Wk	○ Mth	○ <Mth	○ No
50.29	Solvents (e.g. nail polish/paint remover):	○ Day	○ Wk	○ Mth	○ <Mth	○ No